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LUÍSA CIDÁLIA GALLO DE ALMEIDA

EFEITO DO RESVERATROL E DAS VITAMINAS C E E EM GLÂNDULAS PARÓTIDAS DE RATOS SUBMETIDOS À RADIOTERAPIA.

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Pontifícia Universidade Católica do Rio Grande do Sul

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EFEITO DO RESVERATROL E DAS VITAMINAS C E E EM GLÂNDULAS PARÓTIDAS DE RATOS SUBMETIDOS À RADIOTERAPIA

Dissertação apresentada à Faculdade de Odontologia da Pontifícia Universidade Católica do Rio Grande do Sul como parte dos requisitos para obtenção do título de Mestre em Odontologia, área de concentração em Estomatologia Clínica.

Orientadora: Fernanda Gonçalves Salum

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DEDICATÓRIA

Para mi querida amiga Jenny Salomé.
La vida es un soplo.

EPÍGRAFE

“Somos o que repetidamente fazemos. A excelência não é um feito, e sim um hábito.”

Aristóteles

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AGRADECIMENTOS

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RESUMO

A xerostomia e a hipossalivação são importantes sequelas da radioterapia em região de cabeça e pescoço uma vez que as glândulas salivares são extremamente sensíveis aos efeitos da radiação ionizante. O primeiro artigo desta dissertação trata-se de uma revisão de literatura na qual foram abordados métodos preventivos de xerostomia radioinduzida tais como o uso de fármacos agonistas colinérgicos, substâncias antioxidantes, acupuntura, terapia laser de baixa potência e transferência de glândula submandibular. O segundo artigo descreve um estudo experimental, realizado com o objetivo de avaliar e comparar o efeito radioprotetor da vitamina E, da associação das vitaminas C e E, bem como do resveratrol sobre alterações morfológicas induzidas pela radioterapia em glândulas parótidas de ratos. Setenta ratos Wistar, machos, adultos foram distribuídos em cinco grupos: grupo controle (1,0 mL/kg de solução salina); grupo controle irradiado (1,0 mL/kg de solução salina); grupo vitamina E (360 mg/kg, 200 UI/mL de vitamina E); grupo vitaminas C e E (360 mg/kg, 200 UI/mL de vitamina E e 100 mg/kg, 200 mg/mL de vitamina C); grupo resveratrol (100 mg/kg de resveratrol). Previamente à radioterapia, os animais receberam uma dose por dia do fármaco correspondente a cada grupo, durante três dias consecutivos, por meio de gavagem. Os animais foram submetidos à teleterapia por cobalto-60 em região de cabeça e pescoço (exceto o grupo-controle), em sessão única de 20 Gy. Os animais foram eutanasiados sete e trinta dias após a radioterapia e as glândulas parótidas foram dissecadas e preparadas para avaliação morfológica. Após sete dias não houve diferença significativa entre os grupos quanto à presença de desorganização acinar, vacuolação citoplasmática, alterações sugestivas de apoptose e pleomorfismo celular. Trinta dias após a radioterapia, nos grupos vitamina E e resveratrol a presença de vacuolação foi significativamente inferior em comparação ao grupo irradiado (p=0,015). Ainda neste período, o grupo resveratrol apresentou menos pleomorfismo nuclear em relação ao grupo irradiado (p=0,015). Além disso, os grupos vitamina E e resveratrol apresentaram área nuclear significativamente superior aos demais na avaliação de 30 dias (p=0,000). De acordo com os resultados apresentados é possível sugerir que a vitamina E e o resveratrol atenuaram os efeitos da radiação ionizante nas células acinares de parótidas de ratos. O resveratrol parece ter sido mais eficiente em comparação à vitamina E.

Palavras-chave: Glândulas salivares. Hipossalivação radioinduzida. Vitamina E. Resveratrol.

ABSTRACT

Xerostomia and hyposalivation are important sequelae of radiotherapy in the head and neck region since the salivary glands are extremely sensitive to the effects of ionizing radiation. The first article of this dissertation comprehends a literature review in which preventive methods of radiotherapy-induced xerostomia such as the use of cholinergic agonist drugs, antioxidant substances, acupuncture, low power laser therapy and submandibular gland transfer were discussed. The second article describes an experimental study with the objective of evaluating and comparing the radioprotective effect of vitamin E, the association of vitamins C and E, as well as resveratrol on morphological changes induced by radiotherapy in the parotid glands of rats. Seventy male Wistar rats were divided into five groups: control group (1.0 mL / kg of saline); Irradiated control group (1.0 mL / kg of saline solution); Vitamin E group (360 mg / kg, 200 IU / mL vitamin E); Vitamin C and E group (100 mg / kg, 200 mg / mL vitamin C and 360 mg / kg, 200 IU / mL vitamin E); Resveratrol group (100 mg / kg resveratrol). Prior to radiotherapy, the animals received one dose per day of the drug corresponding to each group, for three consecutive days, through gavage. The animals were submitted to cobalt-60 teletherapy in the head and neck region (except the control group), in a single session of 20 Gy. They were euthanized seven and thirty days after radiotherapy and the parotid glands were dissected and prepared for morphological evaluation. After seven days there was no significant difference between the groups regarding the presence of acinar disorganization, cytoplasmic vacuolation, changes suggestive of apoptosis and cellular pleomorphism. Thirty days after radiotherapy, in the vitamin E and resveratrol groups the presence of vacuolation was significantly lower in comparison to the irradiated group (p = 0.015). Also during this period, the resveratrol group presented less nuclear pleomorphism in relation to the irradiated group (p = 0.015). In addition, the vitamin E and resveratrol groups had a significantly higher nuclear area in the 30-day evaluation (p = 0.000). According to the results presented, it is possible to suggest that vitamin E and resveratrol attenuated the effects of ionizing radiation on rat parotid acinar cells. Resveratrol appears to have been more efficient compared to vitamin E.

Keywords: Salivary glands. Radiation-Induced Hyposalivation. Vitamin E. Resveratrol.

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

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2D	*Two Dimensional*
3DCRT	*Three Dimensional Conformal Radiation Therapy*
InGaAIP	*Aluminium Gallium Indium Phosphide*
CG	*Control group*
^{60}Co	*Cobalt 60*
CR	*Convetional Radiotherapy*
DNA	*Deoxyribonucleic Acid* (*Ácido Desoxirribonucleico)*
FDA	*US Food and Drug Administration*
Gy	*Gray*
IrradG	*Irradiated group*
IMRT	*Intensity-Modulated Radiotherapy*
IV	*Intravenous Injection*
LLLT	*Low-Level-Laser-Therapy*
MDA	*Methane Dicarboxylic Aldehyde*
RC	Radioterapia Convencional
ResG	*Resveratrol group*
ROS	*Reactive Oxygen Species*
SIRT1	Sirtuína-1, Sirtuin-1
SGT	*Submandibular Gland Transfer*
SOD	*Superoxide Dismutase*
TLBP	Terapia Laser de Baixa Potência
VitCEG	*Vitamin C and E group*
VitEG	*Vitamin E group*

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INTRODUÇÃO

1 INTRODUÇÃO

A radioterapia consiste na utilização de doses elevadas de radiação ionizante para o tratamento de neoplasias malignas. A radiação interage com os tecidos tumorais, atuando sobre proteínas, lipídeos e DNA nuclear, causando a morte ou incapacidade de replicação celular por meio da produção de radicais livres (SEGRETO; SEGRETO, 2000). A radioterapia pode ter indicação terapêutica primária no tratamento de neoplasias malignas da região de cabeça e pescoço, adjuvante à cirurgia e/ou à quimioterapia (MARINHO et al., 2012). De um modo geral, os pacientes irradiados em região de cabeça e pescoço apresentam efeitos adversos tais como mucosite, periodontite, trismo, osteorradionecrose e xerostomia (AHMED et al., 2009; EPSTEIN et al., 2012; KLEIN; LIVERGANT; RINGASH, 2014).

As glândulas salivares maiores são frequentemente envolvidas nos portais terapêuticos de radiação por estarem em proximidade com os sítios de tumores primários e cadeias linfáticas da região de cabeça e pescoço. Como consequência, passam por um processo de degeneração, resultando em hipossalivação e xerostomia (EISBRUCH et al., 2001; MONTERO; REYES; CIFUENTES; 2002; TROTTI, 2000; KONINGS; COPPES; VISSINK, 2005). A qualidade de vida desses pacientes é profundamente afetada, pois a hipossalivação aumenta a suscetibilidade à cáries, infecções orais oportunistas, disgeusia, disfonia, ardência bucal, entre outras (TROTTI, 2000; VISSINK et al., 2010; KLEIN; LIVERGANT; RINGASH, 2014).

Na teleterapia, forma de radioterapia mais utilizada em região de cabeça e pescoço, são usualmente aplicadas doses entre 50 e 70 Grays (Gy), fracionadas em 2 Gy ao dia, cinco vezes por semana (EPSTEIN et al., 2012) . Uma dose média de 25,8 Gy pode reduzir em 25% o funcionamento das glândulas parótidas em comparação ao estado inicial (BLANCO et al., 2005). Dentre as modalidades de radioterapia mais utilizadas em região de cabeça e pescoço, a radioterapia convencional 2D (RC) é a que apresenta os efeitos adversos mais significativos sobre o tecido glandular. A radioterapia de intensidade modulada (IMRT) e a radioterapia conformacional tridimensional (3DCRT) reduzem a dose de radiação nas estruturas sadias adjacentes ao tumor e, consequentemente, a toxicidade causada pela radiação ionizante (NUTTING et al., 2001; STONE et al., 2003; van RIJ et al., 2008; RUDAT et al., 2008; DUARTE et al., 2014; MARTA et al., 2014).

Macroscopicamente, as glândulas salivares irradiadas apresentam atrofia, com redução do seu volume e peso (NAGLER et al., 1998; MOTALLEBNEJAD et al., 2014). Sob o aspecto microscópico são encontradas alterações como desorganização acinar, perda e atrofia das células acinares, dano às mitocôndrias e às membranas celulares, hipovascularização, vacuolação citoplasmática, condensação nuclear e formação de tecido fibroso (COPPES; VISSINK; KONINGS, 2002; BORAKS et al., 2008; TUJI et al., 2010; XU et al., 2013; ABEDI et al., 2015).

Na literatura encontram-se estudos testando diferentes métodos de prevenção da xerostomia radioinduzida. Dentre estes se podem destacar as substâncias antioxidantes (RAMOS et al., 2006; RUDAT et al., 2008; ŞIMŞEK et al., 2012), agonistas seletivos dos receptores muscarínicos como a pilocarpina (HADDAD; KARIMI, 2002; BURLAGE et al., 2008) e o betanecol (JHAM et al., 2007), acupuntura (MENG et al., 2012), transferência da glândula submandibular (JHA et al., 2000; RIEGER et al., 2012) e radiação laser de baixa potência (ACAUAN et al., 2015).

Os antioxidantes podem ser classificados em enzimáticos como a superóxido dismutase e a catalase ou não enzimáticos como as vitaminas A (carotenoides), C (ácido ascórbico), E (tocoferol) e o selênio. Os antioxidantes previnem a formação de radicais livres, impedindo a sua ação sobre lipídeos, aminoácidos, dupla ligação dos ácidos graxos poli-insaturados e bases de DNA. Os antioxidantes também removem danos ao DNA, reparando lesões causadas pelos radicais livres e reconstituindo a membrana celular danificada (BIANCHI; ANTUNES, 1999).

A vitamina E é a principal vitamina lipossolúvel presente no plasma sanguíneo (CATANIA; BARROS; FERREIRA, 2009). É um componente comum dos óleos vegetais, encontrada na natureza basicamente em quatro formas α, β, γ e δ-tocoferol, sendo o α–tocoferol a forma biologicamente ativa mais amplamente distribuída nos tecidos e no plasma e também a mais pesquisada (BIANCHI; ANTUNES, 1999; CATANIA; BARROS; FERREIRA, 2009). Esta vitamina reage com os radicais peroxil, transformando-os em radicais alfa-tocoferoxil, o que interrrompe a cadeia de peroxidação lipídica. Com isso, a vitamina E acaba transformando-se no radical tocoferil, que necessita ser regenerado para recuperar o seu potencial. Nesse processo atuam o ácido ascórbico, a enzima glutationa e a coenzima Q10 (CATANIA; BARROS; FERREIRA, 2009; POCERNICH; BUTTERFIELD, 2012). Em estudo clínico realizado por Chitra e Shyamala Devi (2008), pacientes que durante a radioterapia receberam 400 UI/dia de vitamina E por via oral tiveram o fluxo salivar preservado após seis semanas. A análise bioquímica demonstrou que o pH, níveis de amilase salivar, potássio, proteína e sódio mantiveram-se próximos do normal.

A vitamina C ou ácido ascórbico é uma vitamina hidrossolúvel e termolábil. A vitamina C está presente em praticamente todos os tecidos animais sob duas formas: reduzida ou oxidada (ácido deidroascórbico) e é considerada um antioxidante primário ou preventivo, pois reage com o oxigênio, reduzindo o estresse oxidativo. Seres humanos e primatas são os únicos mamíferos que não conseguem sintetizá-la (MANELA-AZULAY et al, 2003; DU; CULLEN; BUETTNER, 2012). Além disso, ela auxilia no processo de regeneração da vitamina E, ajuda a proteger o organismo de infecções e é fundamental na integridade da parede dos vasos sanguíneos e na formação das fibras colágenas (ENWONWU, 1992; CATANIA; BARROS; FERREIRA, 2009). Em um estudo prospectivo, randomizado, duplo-cego, placebo-controlado, Chung et al. (2016) avaliaram o efeito radioprotetor das vitaminas C e E sobre as glândulas salivares em 45 pacientes irradiados (≥40 Gy) em região de cabeça e pescoço, que utilizaram 100 UI de vitamina E + 500 mg de vitamina C, duas vezes ao dia, iniciando uma semana antes da radioterapia até um mês após. Após cinco meses, os pacientes que utilizaram as vitaminas C e E recuperaram parte da função das glândulas salivares, apresentando menores índices de xerostomia.

O resveratrol é um composto natural polifenóico não-flavonóide que pode ser encontrado na casca do amendoim, no mirtilo, no vinho tinto, e principalmente na casca da uva tinta. Ele possui ação anti-inflamatória, anticancerígena, antioxidante, antienvelhecimento, vasodilatadora, neuroprotetora, entre outras (DIAS, 2009). O resveratrol é um ativador natural da sirtuína-1 (SIRT1), que atua na estabilidade genômica e no reparo, regulação do metabolismo e proliferação celulares. A SIRT1 tem sido relacionada à proteção contra o estresse oxidativo em enfermidades que envolvem desordens metabólicas, doenças cardiovasculares e neurodegenerativas (MARQUES; MARKUS; MORRIS, 2009; CHONG et al. 2012). Xu et al. (2013) investigaram o efeito radioprotetor do resveratrol em glândulas submandibulares de camundongos. Os animais foram tratados com dosagem de 20 mg/kg de resveratrol intraperitoneal durante três dias, previamente à radioterapia (15Gy). Após 24 horas e 30 dias, o fluxo salivar do grupo teste foi significativamente superior ao do grupo controle. Na análise histológica, o grupo irradiado que foi tratado previamente com resveratrol apresentou menos alterações morfológicas, com manutenção do tecido glandular, indicando que esta substância é capaz de prevenir alguns efeitos colaterais da radioterapia em glândulas salivares.

Segundo Conklin (2000), são produzidos radicais livres na destruição do tumor pela radioterapia, porém isso não impede a utilização de antioxidantes durante o tratamento oncológico. Os antioxidantes auxiliam a radioterapia, pois mantém os tecidos bem oxigenados, aumentando a sua eficiência. Sabe-se que massas tumorais de tamanho avantajado recebem menos aporte de oxigênio. Assim, o uso de antioxidantes poderia melhorar as condições desse tecido, aumentando a efetividade da radioterapia (CONKLIN, 2000; VALKO et al., 2006; MOSS, 2007).

Estudos científicos demonstram os efeitos benéficos dos antioxidantes na proteção dos tecidos frente à radiação ionizante em região de cabeça e pescoço. Considerando os danos às glândulas salivares causados pela radioterapia, causando hipossalivação e xerostomia, o presente estudo avaliou e comparou o efeito da vitamina E, da associação das vitaminas E e C, bem como do resveratrol sobre alterações morfológicas induzidas pela radioterapia em glândulas parótidas de ratos.

PROPOSIÇÃO

2 PROPOSIÇÃO

2.1 Objetivo Geral

Avaliar o efeito da vitamina E, da associação das vitaminas C e E e do resveratrol sobre alterações morfológicas induzidas pela radioterapia em glândulas parótidas de ratos.

2.2 Objetivos Específicos

- Realizar uma revisão de literatura abordando métodos preventivos de xerostomia radioinduzida.
- Avaliar e comparar, por meio de análise histológica, o efeito da vitamina E, da associação das vitaminas C e E e do resveratrol na morfologia das glândulas parótidas de ratos irradiados em região de cabeça e pescoço.

ARTIGO DE REVISÃO DE LITERATURA

3 ARTIGO DE REVISÃO DE LITERATURA

RADIOTHERAPY-INDUCED SALIVARY HYPOFUNCTION: AN UPDATE ON THE PREVENTIVE MECHANISMS

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RADIOTHERAPY-INDUCED SALIVARY HYPOFUNCTION: AN UPDATE ON THE PREVENTIVE MECHANISMS

PREVENTION OF RADIOINDUCED XEROSTOMIA

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ABSTRACT

Background: Head and neck radiotherapy is one of the main causes of salivary hypofunction. The salivary glands are extremely sensitive to ionizing radiation, presenting irreversible structural alterations, with alterations in salivary flow and composition.

Objective: The present study aimed to provide an update on the mechanisms of prevention of the radiotherapy-induced salivary hypofunction.

Materials and methods: A research was conducted in Medline / PubMed / Lilacs / Bireme databases using the terms radiotherapy, intensity-modulated radiotherapy, xerostomia, hyposalivation, radiation-protective agents, amifostine, bethanechol, pilocarpine, vitamin E, resveratrol, acupuncture, low level laser therapy and submandibular gland transfer. Pre-clinical studies in animal models, controlled clinical trials and meta-analysis were selected.

Results: Studies with bethanechol, pilocarpine and amifostine corroborate the prophylactic effect of these drugs on the salivary glands. However, they have side effects which contraindicate its use in several patients. Researches about acupuncture and LLLT show preservation of salivary flow in oncologic patients with no or little side effects. Patients who undergo the submandibular gland transfer technique previously to radiotherapy also have less incidence of xerostomia.

Conclusion: In general, the therapies presented in this review can help to prevent the symptoms caused by the hypofunction of the irradiated salivary glands. Nevertheless, at this point there are no techniques or drugs capable of completely preventing the development of radiotherapy-induced salivary hypofunctions. Controlled clinical trials are still needed in order to find therapeutical methods that preserve the glandular structure undergoing ionizing radiation, thus preventing hyposalivation and xerostomia.

Keywords: Salivary gland. Radioprotector. Xerostomia. Hyposalivation.

INTRODUCTION

The saliva is a fluid of extreme importance in the maintenance of oral homeostasis, being present in several biologic processes. It is composed in almost its totality by water and electrolytes, besides proteins such as immunoglobulins and mucins. This fluid acts in the lubrication of the mucosa, digestion, phonation, gustation, neutralization of acid components and in the immune systems through antimicrobial peptides against bacteria, fungi and viroses.[1-5] Hyposalivation is characterized by the decrease in the capacity of the salivary glands to secrete saliva, and xerostomia is defined as the subjective sensation of oral dryness. Such alterations can be caused by drugs, systemic diseases, head and neck radiotherapy, among other reasons.[6] Patients affected by salivary hypofunctions tend to show an atrophic buccal mucosa, as well as dysphonia, dysphagia, oral burning sensation and palate alterations. There is increased risk to oral candidiasis, caries lesions and periodontal alterations.[7-9] The diminished bactericidal effect of saliva favors the increase of population of the microorganisms responsible for these diseases.[8,10]

Head and neck radiotherapy is one of the main causes of salivary hypofunction.[11,12] The major salivary glands are usually included in the radiation portals due to the fact that they reside close to primary tumor sites and lymphatic chains of the head and neck region and frequently have their function impaired resulting in hyposalivation and xerostomia.[11,13-15] Among the radiotherapy methods most employed in head and neck region, conventional 2D radiotherapy (RC) is the one that presents the most significant side effects on the glandular tissue. Intensity-modulated radiotherapy (IMRT) and Three Dimensional Conformal Radiotherapy (3DCRT) reduce the radiation dose on the health structures close to the tumor and, consequently, the toxicity caused by the ionizing radiation.[13,16-20]

The salivary glands are extremely sensitive to ionizing radiation, presenting structural alterations which cause changes in salivary flow and composition.[21] These alterations are dose-dependent and can be irreversible.[21,22] Permanent hyposalivation is frequently associated to doses of ≥50 Gy, usually used for head and neck cancer treatment.[23] Irradiated patients often present salivary flow lower than users of drugs which cause xerostomia and of patients with Sjögren syndrome.[24]

Many studies have been investigating radiotherapy effects on the morphology of salivary glands. Among the microscopic and ultrastructural acute alterations there are hypovascularization, cytoplasmic vacuolation, pleomorphism, nuclear condensation, and damage to mitochondria and to cell membranes of acinar cells. Later there are phenomena such as inflammation and vacuolation, as well as duct dilatation, vascular congestion, decrease in the number of acinar cells and in the glandular volume, increase in adipose and fibrous tissues, with atrophy and parenchymatous degeneration.[25-30] The irradiated salivary glands can present a reduction of approximately 50% of their weight in comparison to non-irradiated glands.[21,25]

Studies have been conducted in order to find agents capable of preventing the sequelae caused by ionizing radiation on salivary glands.[30-35] Several modalities such as the use of antioxidant substances (vitamins and resveratrol), selective agonists of muscarinic receptors, submandibular gland transfer, acupuncture, low-level laser therapy [34,36-41] have been investigated in clinical and laboratory studies in prevention of radiotherapy-induced xerostomia.

The level of decrease in salivary flow post-radiotherapy is dose-dependent and this effect can be aggravated with time.[32] The best treatment for radiotherapy-induced hyposalivation is prevention since radiotherapy can cause irreversible damages to glandular tissues. Thus, the present study aimed to review and provide an update in mechanisms for prevention of radiotherapy-induced salivary hypofunction. A research was conducted in Medline / PubMed / Lilacs / Bireme databases using the terms radiotherapy, intensity-modulated radiotherapy, xerostomia, hyposalivation, radiation-protective agents, amifostine, bethanechol, pilocarpine, vitamin E, resveratrol, acupuncture, low level laser therapy and submandibular gland transfer. Pre-clinical studies in animal models, meta-analysis and controlled clinical trials with preventive character on salivary glands, published between 1995-2016 were selected and analyzed. Proper references of these papers were also reviewed.

RADIOPROTECTIVE DRUGS

In order to be considered radioprotective, the substance must have the capacity to protect the tissues without causing genetic alterations, mutations, damages to the immune system or toxicity.[29] Many drugs have been tested, among which the most quoted in literature are the cholinergic agonists, such as pilocarpine and bethanechol, and the antioxidant substances.

Bethanechol

Bethanecol is a parasympathomimetic drug, selective agonist of muscarinic receptors of the parasympathetic nervous system, which stimulates the secretory function.[37,42] This drug is widely employed in the treatment of postoperative urinary retention and has been studied in xerostomia treatment, showing good results.

When employed for xerostomia, the recommended dose is of 25-50mg twice to three times a day.[37,42,43]

Jham et al.[37] assessed the effect of bethanechol in the prevention of xerostomia in a clinical trial with 43 patients irradiated on the head and neck regions. One group used bethanechol, 25mg, three times a day, and the other used artificial saliva during the oncological treatment. The major salivary glands received an amount of at least 45 Gy of radiation. In the bethanechol group, the salivary flow not stimulated was of 0.30 ml/min, significantly different from the control group, which was of 0.18 min/ml ($p=0.03$). Furthermore, the participants of the bethanechol group reported less symptoms, with lower values in the xerostomia assessment ($p=0.05$).

In a double-blind study, bethanechol was used by 42 patients, from the begining until one month after radiotherapy. The 3DCRT and the IMRT techniques were used in both groups, bethanechol and control. The group that used bethanechol presented an improvement in saliva production both in resting ($p=0.008$) as well as under stimulation ($p=0.005$) in comparison to the placebo group. Besides, the prevalence of grade III xerostomia was lower ($p=0.004$) in the group that received bethanechol, even three months after the end of radiotherapy.[43]

The side effects reported by the patients that used bethanechol were facial burning sensation, muscle cramps, nervousness, diarrhea, nausea, lacriamation, frequent urination and excessive sweating.[37] Bethanechol is contraindicated in cases of hyperthyroidism, peptic ulcer, asthma, coronarian arterial disease, gastrointestinal diseases, bradicardia, hypotension, vasomotor instability, epilepsy and Parkinson disease.[44]

Pilocarpine

Pilocarpine is a drug which stimulates glandular function also through the activation of cholinergic muscarinic receptors.[11,42] Studies have demonstrated favorable results in xerostomia prevention in patients who undergo radiotherapy.[38,45,46] Pilocarpine must be ingested in small doses, usually of 5 mg from three to four times a day[47] with a maximum daily dose of 30 mg.[11] Its effect against hyposalivation is immediate, and can last up to four hours. Nonetheless, when used in patients post-radiotherapy of head and neck, its effect can take up to 12 weeks to occur.[11] Zimmerman et al.[45] observed that the patients that received this drug during radiotherapy and in the three months following it showed less symptoms of xerostomia.

Table 1 shows the clinical trials investigating the preventive effect of pilocarpine on salivary alterations in patients irradiated on head and neck regions.

Table 1. Clinical trials investigating the effects of pilocarpine in radiotherapy-induced xerostomia and hyposalivation prevention in patients with head and neck cancer.

Authors	Groups and Radiation Doses	Pilocarpine	Results
ZIMMERMAN et al.[45]	CR + Placebo (n=18) CR + Pilocarpine (n=22) 50- 74 Gy ≥45 Gy in parotid glands	5 mg, 4 X a day, orally, during radiotherapy and until 3 months after.	Less symptoms of xerostomia reported in the Pilocarpine group ($p<0.01$).
HADDAD; KARIMI[46]	CR + Placebo (n=29) CR + Pilocarpine (n=31) 45-70 Gy ≥40 Gy in parotid glands	5 mg, 3 X a day, orally, during radiotherapy and until 3 months after.	Six months after radiotherapy the Pilocarpine group presented better results with significant differences both in the subjective ($p=0.02$) as well as in the objective ($p=0.01$) xerostomia criteria analysis.
WARDE et al.[48]	CR + Placebo (n=65) CR + Pilocarpine (n=65) 50-70 Gy ≥50 Gy in parotid glands	5 mg, 3 X a day, orally, during radiotherapy and until 1 month after.	There was no significant difference between the groups. Pilocarpine did not decrease the incidence or the grade of xerostomia, neither improved quality of life of the patients during and following the CR.
BURLAGE et al.[38]	CR + Placebo (n=85) CR+ Pilocarpine (n=85) 46-70 Gy ≥40 Gy in parotid glands ≥40 Gy in submandibular glands	5 mg, 4 X a day, orally, two days before starting radiotherapy and until 14 days after.	There was no significant difference between the groups in the salivary flow. After six months, xerostomia scores were lower in the Pilocarpine group, but with no significant difference to the placebo group. After 12 months, the Pilocarpine group presented significant difference in the incidence of xerostomia ($p=0.017$) when the parotid glands received ≥40 Gy.

CR (conventional radiotherapy).

In a meta-analysis, Yang et al.[47] reviewed the use of pilocarpine as a method for radiotherapy-induced xerostomia prevention. The analyzed studies showed that pilocarpine increased the non-stimulated salivary flow in the first 12 months of follow-up. The authors concluded that pilocarpine can improve the quality of life of irradiated patients. However, since it is a cholinergic drug, patients can present side effects such as nausea, lacrimation, sweating, frequent urination, rhinitis, low intensity headache and gastrointestinal disturbs. Pilocarpine is contraindicated for patients with chronic diseases such as asthma, cardiopathies, epilepsy, angle-closure glaucoma, hypothyroidism, Parkinson disease and anyone using other adrenergic antagonist drugs or drugs with parasympathomimetic or anticholinergic effects.[42,46-49]

Amifostine

Amifostine has been researched since the 1950 decade for the prevention of damages to tissues adjacent to tumors treated through radiotherapy and chemotherapy, because it has antioxidant properties, acting against free radicals. The *American Society of Clinical Oncology (ASCO*) and the *US Food and Drug Administration (FDA)* recognized its radioprotective effect on healthy cells.[50] Table 2 shows clinical trials assessing the radioprotective effect of amifostine in patients with head and neck cancer.

Table 2. Clinical trials assessing the effect of amifostine in the prevention of radiotherapy-induced xerostomia and hyposalivation in patients with head and neck cancer.

Authors	**Groups and Radiation Doses**	**Amifostine**	**Results**
BUNTZEL et al.[51]	CR (n=14) CR + Amifostine (n=14) ≤60 Gy ≥75% of the parotid glands were included in the radiation field.	500mg/m^2 daily, IV, from 1st to 5th and from the 21st to the 25th days of chemotherapy.	100% of the CR group presented grade 2 xerostomia, while 12% of the RC + Amifostine group (p=0.0001) presented this score.
BRIZEL et al.[23]	CR (42 Gy) (n=150) CR (60 Gy) + Amifostine-(n=153) ≥75% of the parotid glands were included in the radiation field.	200 mg/m^2 daily, IV, 15-30 min before each session of CR.	Reduction in grade ≥2 xerostomia significantly superior in the amifostine group in comparison to the control group (p<.0001). The drug prevented hyposalivation without reducing CR efficacy.
BUENTZEL et al.[33]	CR + placebo (n=65) CR + Amifostine (n=67) 60-70 Gy ≥75% of the parotid glands were included in the radiation field.	200 or 300 mg/m^2, 30 min before each session of CR.	Xerostomia levels did not differ among groups. Acute grade ≥2 xerostomia: 39% in amifostine group *versus* 34% in placebo. Late grade ≥2 xerostomia: 39% in amifostine group *versus* 24% in placebo.
JELLEMA et al.[52]	CR + placebo (n=31) CR + Amifostine 3x/week (n=30) CR + Amifostine 5x/week (n=30) 40-70 Gy	200 mg/m^2, IV, 15-30 min before CR.	After six months the prevalence of xerostomia was significantly lower (p=0.03) in the amifostine 5x/week group in comparison to the others. However, after this period the patients started to show chronic symptoms of xerostomia and there was no significant difference among the groups that used amifostine (p=0.81).
RUDAT et al.[18]	CR (n=34) CR + Amifostine (n=35) IMRT (n=31) 60 Gy 60.7 Gy in parotid glands in the CR and CR + Amifostine 30.9 Gy in parotid glands in the IMRT group	200 ou 340 mg/m^2, IV, 15-30 min before each session of radiotherapy.	In the first 12 months only the IMRT group showed preservation of parotid function, with significant difference in comparison to the CR + Amifostine group (p<0.01) and CR group (p<0.001). However, after 13-45 months the same groups did not significantly differ in scintigraphy exam (p=0.07). The CR + Amifostine group significantly differed from the CR group in the period of 13 to 47 months after radiotherapy (p=0.02).

CR (conventional radiotherapy), IMRT (Intensity-modulated radiotherapy), IV (Intravenous injection).

The effect of amifostine in the prevention of salivary alterations was assessed through meta-analysis that included 1167 patients with head and neck cancer who underwent radiotherapy or radiochemotherapy. Amifostine prevented chronic and acute xerostomia in irradiated patients. In patients treated through chemotherapy and radiotherapy, amifostine prevented chronic xerostomia, but it was not effective on acute xerostomia.[53]

The side effects that patients using amifostine can present during oncologic treatment are arterial hypotension, nausea, vomits, asthenia, fever, mucositis, tachycardia, bronchitis, allergic reaction and *rash*.[33,52,54] The higher the applied dose, the more severe the side effects related to amifostine. Furthermore, chemotherapeutic drugs can increase this drug’s toxicity.[54] Patients allergic to products of *aminothiol* and *manitol* basis, pregnant or breast-feeding women, people older than 70 and younger than 18 years old, people that are dehydrated, who are using antidepressant drugs or drugs that trigger hypocalcemia, who present cardiovascular or cerebrovascular diseases, severe renal or hepatic impairment or hypocalcemia risk are contraindicated for the use of amifostine.[55,56] Although it is widely studied, amifostine still has not had its mechanism of action completely clarified.[57]

Vitamin E

Due to its high antioxidant power, vitamin E (α –Tocopherol) has been analyzed as a radioprotective agent in the small bowel, thyroid gland and salivary glands.[30,36,58-60] Ramos et al.[36] analyzed the radioprotective effect of vitamin E in salivary glands of irradiated rats (15 Gy). Vitamin E was administered in doses of 360 mg/kg, 24, 48 and 72 hours before radiotherapy. After 30 days of radiotherapy, a recuperation of salivary flow was observed in the irradiated animals that had received vitamin E, suggesting late protection over the salivary glands.

Gomes et al.[59] did not observe differences in the number of acinar cells between animals treated with vitamin E and placebo 30 days after the radiotherapy (15 Gy). On the other hand, Abedi et al.[30], in a study with rats, demonstrated that microscopic alterations in irradiated parotid glands, such as vacuolation, ductal dilatation, vascular congestion, lysis of acinar cells and fibrosis were less evident in the animals treated with vitamin E.

Chitra et al.[61] analyzed the salivary flow, pH levels, salivary amylase, sodium, potassium and salivary total protein concentration in the saliva of irradiated patients (n=89) who received vitamin E simultaneously to radiotherapy. Patients received 400 UI/day of vitamin E, orally, during three or six weeks (five days a week). There was no decrease in salivary flow in comparison to the beginning of the treatment after the three first weeks of radiation. On the other hand, the irradiated control group had a significant decrease of the salivary flow in the same period ($p<0.001$). Besides, the control patients presented lower levels of amylase and proteins, as well as pH level increase ($p<0.001$). After six weeks of radiotherapy, patients that used vitamin E presented higher levels of pH, salivary flow, potassium ($p<0.001$), salivary amylase activity ($p<0.01$), proteins and sodium ($p<0.05$), in comparison to the control group. In a prospective, randomized, double-blind, placebo-controlled study, Chung et al.[35] assessed the radioprotective effect of vitamins C and E on salivary glands in 45 patients with head and neck cancer. A pill containing 100 UI of vitamin E and 500 mg of vitamin C was given twice a day, beginning one week before radiotherapy until one month after. IMRT technique was used, the average radiation dose on the parotid glands was of 12 Gy for the control group and 14 Gy for the group that took the vitamins. In the submandibular glands, the radiation dose was of 25 Gy and 23 Gy in the two groups, respectively. The xerostomia scores were high one month after the end of radiotherapy in both groups; however, in the patients that used the vitamins there was significant reduction of these scores after five months. The study showed that the use of vitamins C and E had a beneficial effect in patients irradiated, preserving part of the salivary function.

Resveratrol

Resveratrol is a polyphenolic substance mainly found in the red wine, in the red grape skin and also in peanuts. It is known for its antiinflammatory, antioxidant, vasodilating, antitumoral, anti-aging, among other properties. Moreover, it is related to the modulation of transcription factors, inhibition of kinase proteins, suppression of antiapoptotic genes and inflammation mediators.[61,62] Some studies suggest that resveratrol can present radioprotective potential.[28,63] In irradiated rats, resveratrol diminished apoptosis and enhanced cell tolerance to oxidative stress in the hippocampus. In the presence of this substance, there was an increase in the activity of SIRT-1 enzyme.[63]

Şimşek et al.[34] observed, in Wistar rats, that resveratrol in high dosages protected the salivary glands from the harmful effects of ionizing radiation. The animals underwent total body irradiation, in a single dose of 7.2 Gy, 24h after the infusion of intraperitoneal resveratrol in doses of 10 mg/kg or 100 mg/kg. The group that received 100 mg/kg of resveratrol showed a higher preservation of acinar structure and diminished necrosis in submandibular glands when compared to the group of 10 mg/kg or the control group ($p<0.05$). This group had results similar to the *sham*-irradiated group ($p>0.05$) in histological analysis. It was also shown an increase in glutathione and a reduction in malondialdehyde activity when a dose of 100 mg/kg of resveratrol was used.

Another *in vivo* study evaluated salivary flow and morphologic and biochemical alterations in submandibular glands of mice who underwent radiotherapy and treatment with resveratrol. An intraperitoneal infusion of resveratrol was administered in doses of 20 mg/kg a day, in the three days before radiotherapy (15 Gy) and 30 minutes before the procedure. Resveratrol prevented the radiotherapy-induced hypofunction in submandibular glands. Salivary flow in the animals that received resveratrol treatment was significantly higher in the 24 h ($p<0.01$) and 30 days ($p<0.05$) periods after radiotherapy. Moreover, there was no significant drop in superoxide dismutase enzyme in the experimental group. In the histological evaluation of submandibular glands, eight hours after the irradiation, the control group presented modifications such as acinar vacuolization, nuclear alterations and inflammatory infiltrate. These were even more evident throughout time and, after 30 days, fibrosis was observed. Furthermore, the group that received resveratrol presented milder alterations in relation to vacuolization, inflammatory infiltrate, as well as the absence of fibrosis in all of the periods.[28]

OTHER RADIOPROTECTIVE METHODS

Other non-pharmacological methods for radioprotection of salivary glands have also been researched, such as acupuncture, low level laser therapy and submandibular gland transfer.

Acupuncture

Acupuncture is a possible method for prevention and treatment of xerostomia and hyposalivation associated to radiotherapy.[64] Braga et al.[40], in a study with irradiated patients (n=24) in head and neck regions (≥50 Gy), observed that acupuncture diminished the grade of xerostomia in comparison to the control group. Between 16 and 20 acupuncture sessions were performed along the whole anti-tumoral treatment, reaching local (ST-3, ST-4, ST-5, ST-6, ST-7, GB-2, SI-19, TB-21), distal (LI-4, LI-11, LR-3, ST-36, KI-3, KI-5, GV-20) and auricular points (Shen-Men, central nervous system, neurovegetative system, kidney, spleen, pancreas and mouth), totalizing 29 points in each session. Salivary flow, both in resting as well as under stimulation, was significantly higher in the acupuncture group ($p<0.001$). Moreover, the patients also complained less about oral mucosa burning sensation, pain and dysgeusia.

In a controlled study involving 86 patients irradiated on head and neck regions, Meng et al.[65] used acupuncture three times a week, concomitantly to radiotherapy (média de 71 Gy). In the first 11 weeks after the end of treatment, the group that underwent acupuncture sessions presented salivary flow in resting and under stimulation significantly higher than the control group. Six months after the treatment there was no significant difference between the groups for salivary flow in resting. However, salivary flow under stimulation remained significantly superior in the acupuncture group. Furthermore, patients who underwent acupuncture sessions presented less xerostomia symptoms in comparison to the control group.

In a systematic review, Zhuang et al.[64] concluded that the acupuncture used to prevent xerostomia in patients with head and neck cancer has little or no side effects. Although studies suggest efficacy in combating xerostomia, there are still no clear evidences for the acupuncture to be considered a standard treatment in oncologic treatment centers.

Low Level Laser Therapy (LLLT)

The effect of LLLT on salivary glands is a source of discussions in literature. There are few studies that support the effect of this therapeutic alternative on saliva's production and the modification of its composition. Studies suggest that low level laser, through photophysical and photochemical reactions, acts on cellular metabolism, inducing cellular proliferation and protein synthesis. According to the authors, LLLT can enhance local vascularization of irradiated salivary glands.[41,66,67]

Acauan et al.[41] investigated the effect of LLLT in the prevention of radiotherapy-induced alterations in the parotid glands of mice. The LLLT (830 nm, 100 mW, 0.028 cm^2, 3.57 W/cm^2) was applied punctually in the region of the parotid glands with the energy of 2 J (20 s, 71 J/cm^2) or 4 J (40 s, 135 J/cm^2) immediately before the radiotherapy session (10 Gy) and 24h after it. The method preserved acinar structure, reducing the incidence of vacuolization and apoptosis, mainly when the 4J protocol was applied.

Lopes et al.[68], in a controlled study, assessed the LLLT effect on the prevention of salivary glands hypofunction in 60 patients with head and neck carcinoma who underwent chemo and radiotherapeutic treatment. The laser of InGaAlP (Aluminium Gallium Indium Phosphide, 685 nm, 35mW) was employed in doses of 2 J/cm^2 per point over the jugal mucosa, floor of mouth, tongue, tonsillar pillar, uvula, submandibular and parotid glands, during 58 seconds. Patients who underwent laser therapy presented stimulated and non-stimulated salivary flow close to normal, with significant difference ($p<0.001$) when compared to the control group.

Gonnelli et al.[66] performed 21 sessions of laser therapy (InGaAIP) intra and extraoral, three times a week, in alternate days, during radiotherapy (66-70 Gy) and chemotherapy (cisplatin 40 mg/m^2 weekly) in head and neck cancer patients (n=23). For the intraoral application, a 660 nm wavelenght, 40 mW potency and 10 J/cm^2 dose for 10 seconds per point were employed. For the extraoral application, a 780 nm wavelenght, 15 mW potency and a 3,8 J/cm^2 dose for 10 seconds per point was used. Saliva production with or without stimulation was assessed previously to the first radiochemotherapy session and 30 days after the last session. Salivary flow, both in resting (p=0.014) as well as under stimulation (p=0.013) was significantly higher in the laser group.

In another study, Gonnelli et al.[67], using the same described parameters, evaluated the effect of LLLT in non-stimulated saliva production in patients with head and neck cancer (n=27) treated with radiotherapy (66-70 Gy) and chemotherapy. The patients were evaluated in different periods: before radiotherapy, after 15 sessions, after the last session, 30 and 90 days after the end of treatment. Patients of the laser group showed salivary flow significantly higher than the control group after the 15^{th} session of radiotherapy (p=0.015), at the end of the radiochemoterapy treatment (p=0.014) and 30 days (p=0.023) after the conclusion of the oncological treatment. Nevertheless, after 90 days from the end of treatment, there was no significant difference between the groups regarding salivary flow.

Submandibular Gland Transfer

Submandibular gland transfer (SGT) is also a procedure used to prevent salivary glands hypofunction, improving the quality of life of the patients that will undergo radiotherapy on head and neck regions.[69] It is performed through a surgical procedure of transferring the submandibular gland to the submental region. The SGT is indicated for patients with primary tumors of the posterior region of the oral cavity, oropharynx, nasopharynx and larynx who do not have impaired lymph nodes.

In the Table 3 are described clinical trials assessing the effect of submandibular gland transfer in the preservation of salivary flow in patients irradiated in the head and neck region.

Table 3. Clinical trials assessing the effect of submandibular gland transfer (SGT) in the prevention of radiotherapy-induced xerostomia and hyposalivation in patients with head and neck cancer.

Authors	**Groups and Radiation Doses**	**SGT**	**Results**
SEIKALY et al. [70]	CR (n=12) CR + SGT (n=26) 50-70 Gy	Surgery performed 4-6 weeks before the CR.	After 2 years of radiotherapy, the SGT group still presented salivary flow significantly superior to the control group (p<0.005).
JHA et al.[71]	CR + SGT (n=60) CR + pilocarpine (n=60) 54-70 Gy	Surgery performed 4-6 weeks before the CR. Pilocarpine: 4X/day, from 3 days prior to the CR until 3 months after it.	At 3 and 6 months after the CR, the SGT group produced higher amount of non-stimulated (p=0.036 and p=0.001) stimulated saliva (p=0.002 and p=0.003). The incidence of xerostomia was lower in the SGT group after 3 and 6 months from the radiotherapy (p=0.038 e p=0.017). After 6 months, there was no significant difference between the groups regarding palate alterations (p=0.548), swallowing (p=0.305) and phonation (p=0.092).
LIU et al.[72]	CR (n=34) CR + SGT (n=36) 60–70 Gy	Surgeries performed 2 weeks before the CR.	At 3 and 60 months after the CR, the incidence of mild and severe xerostomia in the control group was of 76.5% and 78.6% and in the SGT group of 13.9% and 12.9%, respectively (p=0.000). The volume of saliva was higher in the SGT group after 3 and 60 months from the CR, with significant difference in comparison to the control group (p<.0001).
RIEGER et al.[73]	CR + SGT (n=36) CR + pilocarpine (n=33) 54-70 Gy 80% of the parotids were included in the radiation field with ≥50 Gy	Surgery performed previously to the beginning of the CR.	In the first 6 months, the patients who underwent SGT presented better results, with significant difference in comparison to pilocarpine group (dysphagia, p <0.001; xerostomia, p=0.001; viscous saliva, p=0.03). After one year, the SGT group sustained better results in comparison to the pilocarpine group.
ZHANG et al.[74]	CR (n=14) (40-60 Gy) CR + SGT (n=24) (50-70 Gy)	Surgery performed 1-2 months before the CR.	Salivary flow in the control group drastically dropped shortly after the radiotherapy. There was significant difference between the groups in salivary flow in resting and under stimulation 1, 3, 6 and 12 months after the CR (p≤0.006). SGT did not prevent dysphagia.
ZHANG et al. [75]	CR (n=33) CR + SGT (n=32) 65–75 Gy	Surgery performed previously to the beginning of the CR.	Three, 6 and 12 months after radiotherapy, the SGT group showed lower incidence of mild and severe xerostomia, significantly differing from the control group (p<0.001). The symptoms continued to diminish along the time. Five years after the CR, patients of the SGT group presented salivary flow superior to the ones of the control group (p<0.001).

CR (convetional radiotherapy); SGT (submandibular gland transfer).

Sood et al.[76], through a meta-analysis, investigated the incidence of xerostomia, maintenance of salivary flow (during and after radiotherapy) and perception regarding the maintenance of glandular function in patients who underwent submandibular gland transfer. In 82.7% of 177 cases, SGT prevented radiotherapy-induced xerostomia. Nevertheless, ionizing radiation decrease both stimulated and non-stimulated salivary flow 2-3 months after the end of radiotherapy. However, after 10-12 months there was a salivary recovery, reaching 76% of stimulated and 88% of non-stimulated flow in comparison to the *baseline*.

Wu et al.[77] also investigated through meta-analysis the effect of SGT on radiotherapy-induced xerostomia. Twelve studies were analyzed, in which 369 patients were treated. The SGT was capable of reducing in 69% the incidence of acute xerostomia and in 81% of chronic xerostomia.

The SGT does not interfere in the efficacy of the oncological treatment. After five years of follow-up, there is no significant difference in survival rate of patients who underwent or did not underwent SGT.[72,75,77] Yet, this kind of procedure also have disadvantages. The patient must undergo surgery, which can lead to complications, and needs pre and postoperative care. Moreover, the described method is only applied to submandibular glands.

DISCUSSION

Radiotherapy frequently causes irreversible damages to salivary glands, and xerostomia is one of the most frequent complaints of patients irradiated on head and neck region. In the present review, resources capable of preventing radiotherapy-induced xerostomia were considered, because a prophylactic approach can save recovery time and medical care, spare the patients from physical and emotional exhaustion, as well as being less burdensome to patients and health care services. Preventive pharmacological methods were approached, such as the use of cholinergic agonists (pilocarpine and bethanechol), antioxidant substances, acupuncture, LLLT and submandibular gland transfer.

Bethanechol and pilocarpine are muscarinic agonist drugs whose effect on salivary hypofunctions treatment is well established.[37,42,43] Pilocarpine, although widely studied in the prevention of radiotherapy-induced xerostomia, presents controversial results. While Zimmermann et al.[45] and Haddad and Karimi[46] evidenced positive results of this drug in the prevention of salivary hypofunctions, Burlage et al.[38] and Warde et al.[48] demonstrated no preservation of glandular function in irradiated patients. As to bethanechol, even though the results are positive, only two clinical trials assessed its effect in the prevention of radiotherapy-induced xerostomia.[37,43]

Amifostine is the most widely used drug for the prevention of xerostomia in patients irradiated on head and neck regions due to its antioxidant properties.[18,23,33,52,53] However, the results of these studies are also controversial. For Buntzel et al.[51] and Brizel et al.[23], amifostine was effective in the control of xerostomia, but Jellema et al.[52] showed preservation of glandular function only in the first 6 months of evaluation. On the other hand, Rudat et al.[18] and Gu et al.[53] believe in the late effect of amifostine against radiotherapy-induced xerostomia.

Regarding vitamin E, two clinical trials demonstrated satisfactory results in the prevention of radiotherapy-induced salivary hypofunctions.[35,61] As to resveratrol, there are no clinical results, once the studies were conducted only in animal models.[28,34] The SGT has presented good results regarding the prevention of xerostomia in patients who underwent radiotherapy.[72,75] Nonetheless, not all of the patients are eligible for this kind of procedure, such as patients with level I neck and ganglia involvement in both sides.

Clinical trials also corroborate the efficiency of acupuncture to prevent radiotherapy-induced xerostomia. However, besides being scarce and heterogeneous, it is impossible to make double-blind studies with this technique. The low level laser therapy, even if still little studied in prevention of radiotherapy-induced xerostomia, has presented positive results in animals and in humans, [41,66-68] suggesting it might be used as a preventive method for xerostomia in patients who undergo radiotherapy.

At this point in time, however, there is no procedure or drug capable of completely preventing the development of radiotherapy-induced xerostomia and hyposalivation. Controlled clinical trials are yet necessary in order to find therapeutic modalities that preserve the structure of the salivary glands exposed to ionizing radiation, preventing the occurrence of hyposalivation and xerostomia.

REFERENCES

1. Kaufman E, Lamster I. The diagnostic applications of saliva— A Review. Crit Rev Oral Biol Med. 2002;13(2):197-212. doi:10.1177/154411130201300209

2. Souza R, Lehn C, Denardin O. Níveis sérico e salivar de imunoglobulina A em portadores de câncer da boca e orofaringe. Rev Ass Med Bras. 2003;49(1):40-44. doi:10.1590/s0104-42302003000100032

3. Abiko Y, Saitoh M. Salivary defensins and their Importance in oral health and disease. Curr Pharm Des. 2007;13(30):3065-3072. doi:10.2174/138161207782110417

4. Tibor, KF, Fejérdy P, Csermely P. Saliva in health and disease (Chemical Biology of). In: Begley T, Wiley Encyclopedia Of Chemical Biology. 1st ed. NJ, USA: John Wiley & Sons, Inc; Hoboken; 2007:1–9. doi:10.1002/9780470048672.wecb643

5. Wiesner J, Vilcinskas A. Antimicrobial peptides: The ancient arm of the human immune system. Virulence. 2010;1(5):440-464. doi:10.4161/viru.1.5.12983

6. Saleh J, Figueiredo MAZ, Cherubini K, Salum FG. Salivary hypofunction: an update on aetiology, diagnosis and therapeutics. Arch Oral Biol. 2015;60(2):242-255. doi:10.1016/j.archoralbio.2014.10.004

7. Eisbruch A, Rhodus N, Rosenthal D, et al. How should we measure and report radiotherapy-induced xerostomia? Semin Radiat Oncol. 2003;13(3):226-234. doi:10.1016/s1053-4296(03)00033-x

8. Epstein JB, Thariat J, Bensadoun R-J, et al. Oral complications of cancer and cancer therapy: from Cancer Treatment to Survivorship. CA Cancer J Clin. 2012;62(6):400-422. doi:10.3322/caac.21157

9. Mortensen H, Jensen K, Aksglæde K, Behrens M, Grau C. Late dysphagia after IMRT for head and neck cancer and correlation with dose–volume parameters. Radiother Oncol. 2013;107(3):288-294. doi:10.1016/j.radonc.2013.06.001

10. Wong HM. Oral complications and management strategies for patients undergoing cancer therapy. Sci World J. 2014;2014:1-14. doi:10.1155/2014/581795

11. Vissink A, Burlage FR, Spijkervet FKL, Jansma J, Coppes RP. Prevention and treatment of the consequences of head and neck radiotherapy. Crit Rev Oral Biol Med. 2003;14(3):213-225. doi:10.1177/154411130301400306

12. Grundmann O, Mitchell GC, Limesand KH. Sensitivity of salivary glands to radiation: from animal models to therapies. J Dent Res. 2009;88(10):894-903. doi:10.1177/0022034509343143

13. Stone HB, Coleman CN, Anscher MS, McBride WH. Effects of radiation on normal tissue: consequences and mechanisms. Lancet Oncol. 2003;4(9):529-536. doi:10.1016/s1470-2045(03)01191-4

14. Visvanathan V, Nix P. Managing the patient presenting with xerostomia: a review. Int J Clin Pract. 2010;64(3):404-407. doi:10.1111/j.1742-1241.2009.02132.x.

15. Mercadante S, Aielli F, Adile C, et al. Prevalence of oral mucositis, dry mouth, and dysphagia in advanced cancer patients. Supp Care Cancer. 2015;23(11):3249-3255. doi:10.1007/s00520-015-2720-y

16. Nutting CM, Rowbottom CG, Cosgrove VP, et al. Optimisation of radiotherapy for carcinoma of the parotid gland: a comparison of conventional, three-dimensional conformal, and intensity-modulated techniques. Radiother Oncol. 2001;60(2):163-172. doi:10.1016/s0167-8140(01)00339-5

17. van Rij CM, Oughlane-Heemsbergen WD, Ackerstaff AH, Lamers EA, Balm AJM, Rasch CR. Parotid gland sparing IMRT for head and neck cancer improves xerostomia related quality of life. Radiat Oncol. 2008;3(1):41. doi: 10.1186/1748-717X-3-41

18. Rudat V, Münter M, Rades D, et al. The effect of amifostine or IMRT to preserve the parotid function after radiotherapy of the head and neck region measured by

quantitative salivary gland scintigraphy. Radiot Oncol. 2008;89(1):71-80. doi:10.1016/j.radonc.2008.07.016

19. Duarte VM, Liu YF, Rafizadeh S, Tajima T, Nabili V, Wang MB. Comparison of dental health of patients with head and neck cancer receiving IMRT vs conventional radiation. Otolaryngol - Head Neck Surg. 2013;150(1):81-86. doi:10.1177/0194599813509586

20. Marta GN, Silva V, Carvalho HD, et al. Intensity-modulated radiation therapy for head and neck cancer: systematic review and meta-analysis. Radiother Oncol. 2014;110(1):9-15. doi:10.1016/j.radonc.2013.11.010

21. Li J, Shan Z, Ou G, Liu X, Zhang C, Baum BJ, et al. Structural and functional characteristics of irradiation damage to parotid glands in the miniature pig. Int J Radiat Oncol Biol Phys. 2005;62(5):1510-1516. doi:10.1016/j.ijrobp.2005.04.029

22. Murdoch-Kinch CA, Kim HM, Vineberg KA, Ship JA, Eisbruch A. Dose-effect relationships for the submandibular salivary glands and implications for their sparing by intensity modulated radiotherapy. Int J Radiat Oncol. 2008;72(2):373-382. doi:10.1016/j.ijrobp.2007.12.033

23. Brizel DM, Wasserman TH, Henke M, et al. Phase III randomized trial of amifostine as a radioprotector in head and neck cancer. J Clin Oncol. 2000;18(19):3339-3345. doi:10.1200/jco.2000.18.19.3339

24. Roesink JM, Moerland MA, Battermann JJ, Hordijk GJ, Terhaard CHJ. Quantitative dose-volume response analysis of changes in parotid gland function after radiotheraphy in the head-and-neck region. Int J Radiat Oncol. 2001;51(4):938-946. doi:10.1016/s0360-3016(01)01717-5

25. Coppes RP, Vissink A, Konings AWT. Comparison of radiosensitivity of rat parotid and submandibular glands after different radiation schedules. Radiother Oncol. 2002;63(3):321-328. doi:10.1016/s0167-8140(02)00129-9

26. Boraks G, Tampelini FS, Pereira KF, Chopard RP. Effect of ionizing radiation on rat parotid gland. Braz Dent J. 2008;19(1):73-76. doi:10.1590/s0103-64402008000100013

27. Tuji FM, Pontual ML, Barros SP, Almeida SM, Bóscolo FN. Ultrastructural assessment of the radioprotective effects of sodium selenite on parotid glands in rats. J Oral Sci. 2010;52(3):369-375. doi:10.2334/josnusd.52.369

28. Xu L, Yang X, Cai J et al. Resveratrol attenuates radiation-induced salivary gland dysfunction in mice. Laryngoscope. 2013;123(11):E23-E29. doi:10.1002/lary.24276.

29. Motallebnejad M, Abedi SM, Seyedmajidi M, et al. Evaluation of protective effect of propolis on parotid salivary glands in gamma-irradiated rats. J Contemp Dent Pract. 2014;15:8-11. doi:10.5005/jp-journals-10024-1479

30. Abedi SM, Yarmand F, Motallebnejad M, et al. Vitamin E protects salivary glands dysfunction induced by ionizing radiation in rats. Arch Oral Biol. 2015;60(9):1403-1409. doi:10.1016/j.archoralbio.2015.06.003

31. Eisbruch A, Ten Haken R, Kim H, Marsh L, Ship J. Dose, volume, and function relationships in parotid salivary glands following conformal and intensity-modulated irradiation of head and neck cancer. Int J Radiat Oncol Biol Phys. 1999;45(3):577-587. doi:10.1016/s0360-3016(99)00247-3

32. Konings A, Cotteleer F, Faber H, van Luijk P, Meertens H, Coppes R. Volume effects and region-dependent radiosensitivity of the parotid gland. Int J Radiat Oncol Biol Phys. 2005;62(4):1090-1095. doi:10.1016/j.ijrobp.2004.12.035

33. Buentzel J, Micke O, Adamietz I, Monnier A, Glatzel M, de Vries A. Intravenous amifostine during chemoradiotherapy for head-and-neck cancer: a randomized placebo-controlled phase III study. Int J Radiat Oncol Biol Phys. 2006;64(3):684-691. doi:10.1016/j.ijrobp.2005.08.005

34. Şimşek G, Gurocak S, Karadag N, et al. Protective effects of resveratrol on salivary gland damage induced by total body irradiation in rats. Laryngoscope. 2012;122(12):2743-2748. doi:10.1002/lary.23609

35. Chung MK, Kim DH, Ahn YC, Choi JY, Kim EH, Son Y-I. Randomized trial of vitamin C/E complex for prevention of radiation-Induced xerostomia in patients with head and neck cancer. Otolaryngol - Head Neck Surg. 2016;155(3):423-430. doi:10.1177/0194599816642418

36. Ramos FM, Pontual ML, Almeida SM, Bóscolo F, Tabchoury C, Novaes P. Evaluation of radioprotective effect of vitamin E in salivary dysfunction in irradiated rats. Arch Oral Biol. 2006;51(2):96-101. doi:10.1016/j.archoralbio.2005.06.008

37. Jham BC, Teixeira IV, Aboud CG, Carvalho AL, Coelho MM, Freire ARS. A randomized phase III prospective trial of bethanechol to prevent radiotherapy-induced salivary gland damage in patients with head and neck cancer. Oral Oncol. 2007;43(2):137-142. doi:10.1016/j.oraloncology.2006.01.013

38. Burlage F, Roesink J, Kampinga H, et al. Protection of salivary function by concomitant pilocarpine during radiotherapy: a double-blind, randomized, placebo-controlled study. Int J Radiat Oncol Biol Phys. 2008;70(1):14-22. doi:10.1016/j.ijrobp.2007.06.016

39. Jha N, Seikaly H, Harris J, et al. Prevention of radiation induced xerostomia by surgical transfer of submandibular salivary gland into the submental space. Radiother Oncol. 2003;66(3):283-289. doi:10.1016/s0167-8140(03)00023-9

40. Braga F, Lemos Junior CA, Alves FA, Migliari DA. Acupuncture for the prevention of radiation-induced xerostomia in patients with head and neck cancer. Braz Oral Res. 2011;25(2):180-185. doi:10.1590/s1806-83242011000200014

41. Acauan MD, Gomes A, Braga-Filho A, de Figueiredo MAZ, Cherubini K, Salum FG. Effect of low-level laser therapy on irradiated parotid glands - study in mice. J Biomed Opt. 2015;20(10):108002. doi:10.1117/1.jbo.20.10.108002

42. Gorsky M, Epstein JB, Parry J, Epstein MS, Le ND, Silverman S. The efficacy of pilocarpine and bethanechol upon saliva production in cancer patients with hyposalivation following radiation therapy. Oral Surg, Oral Med Oral Pathol. 2004;97(2):190-195. doi:10.1016/j.tripleo.2003.08.031

43. Jaguar GC, Lima ENP, Kowalski LP, et al. Double blind randomized prospective trial of bethanechol in the prevention of radiation-induced salivary gland dysfunction in head and neck cancer patients. Radiother Oncol. 2015;115(2):253-256. doi:10.1016/j.radonc.2015.03.017

44. Cooper N, Gilbert JC, Gristwood RW, Wyllie MG. Use of bethanechol for treatment of xerostomia. 2015. https://www.google.com/patents/US9149454. Accessed Jan 25, 2017.

45. Zimmerman RP, Mark RJ, Tran LM, Juillard GF. Concomitant pilocarpine during head and neck irradiation is associated with decreased posttreatment xerostomia. Int J Radiat Oncol. 1997;37(3):571-575. doi:10.1016/s0360-3016(96)00557-3

46. Haddad P, Karimi M. A randomized, double-blind, placebo-controlled trial of concomitant pilocarpine with head and neck irradiation for prevention of radiation-induced xerostomia. Radiother Oncol. 2002;64(1):29-32. doi:10.1016/s0167-8140(02)00104-4

47. Yang W, Liao G, Hakim SG, Ouyang D, Ringash J, Su Y. Is pilocarpine effective in preventing radiation-induced xerostomia? A systematic review and meta-analysis. Int J Radiat Oncol. 2016;94(3):503-511. doi:10.1016/j.ijrobp.2015.11.012

48. Warde P, O'Sullivan B, Aslanidis J, et al. A Phase III placebo-controlled trial of oral pilocarpine in patients undergoing radiotherapy for head-and-neck cancer. Int J Radiat Oncol. 2002;54(1):9-13. doi:10.1016/s0360-3016(02)02890-0

49. Wiseman LR, Faulds D. Oral pilocarpine. Drugs. 1995;49(1):143-155. doi:10.2165/00003495-199549010-00010

50. http://www.fda.gov/drugs/developmentapprovalprocess/howdrugsaredevelopedandapproved/drugandbiologicapprovalreports/ucm081869.htm. Accessed Nov 16, 2016.

51. Buntzel J, Kuttner K, Frohlich D, Glatzel M. Selective cytoprotection with amifostine in concurrent radiochemotherapy for head and neck cancer. Ann Oncol. 1998;9(5):505-509. http://annonc.oxfordjournals.org/. Accessed April 29, 2016.

52. Jellema AP, Slotman BJ, Muller MJ, et al. Radiotherapy alone, versus radiotherapy with amifostine 3 times weekly, versus radiotherapy with amifostine 5 times weekly. Cancer. 2006;107(3):544-553. doi:10.1002/cncr.22020

53. Gu J, Zhu S, Li X, Wu H, Li Y, Hua F. Effect of amifostine in head and neck cancer patients treated with radiotherapy: a systematic review and meta-analysis based on randomized controlled trials. PLoS One. 2014;9(5):e95968. doi:10.1371/journal.pone.0095968

54. Rades D, Fehlauer F, Bajrovic A, Mahlmann B, Richter E, Alberti W. Serious adverse effects of amifostine during radiotherapy in head and neck cancer patients. Radiother Oncol. 2004;70(3):261-264. doi:10.1016/j.radonc.2003.10.005

55. Bukowski R. Amifostine (Ethyol®): dosing, administration and patient management guidelines. Eur J Cancer. 1996;32:S46-S49. doi:10.1016/s0959-8049(96)00328-0

56. Gosselin TK, Mautner B, Wilkinson K. Amifostine as a radioprotectant. Clin J Oncol Nurs. 2002;6(3):175-176. doi:10.1188/02.cjon.175-176

57. Koukourakis MI. Amifostine: is there evidence of tumor protection? Semin Oncol. 2003;30:18-30. doi:10.1053/j.seminoncol.2003.11.014

58. Sert C, Celik MS, Akdag Z, Ketani MA, Nergiz Y. The radioprotective effect of vitamins C, E and vitamin E + glutathione on the small intestine and the thyroid gland in rats irradiated with X-rays. Turkish J Med Sci. 2000;30(5):417-425. http://journals.tubitak.gov.tr/medical/issues/sag-00-30-5/sag-30-5-3-9901-8.pdf. Accessed Apr 19, 2016.

59. Gomes CC, Ramos-Perez FM, Perez DE, Novaes PD, Boscolo FN, Almeida SM. Radioprotective effect of vitamin e in parotid glands: a morphometric analysis in rats. Braz Dent J. 2013;24(3):183-187. doi:10.1590/0103-6440201301781

60. Singh VK, Singh PK, Wise SY, Posarac A, Fatanmi OO. Radioprotective properties of tocopherol succinate against ionizing radiation in mice. J Radiat Res. 2012;54(2):210-220. doi:10.1093/jrr/rrs088

61. Chitra S, Shyamala Devi C. Effects of radiation and α-tocopherol on saliva flow rate, amylase activity, total protein and electrolyte levels in oral cavity cancer. Indian J Dent Res. 2008;19(3):213. doi:10.4103/0970-9290.42953

62. Marques FZ, Markus MA, Morris BJ. Resveratrol: cellular actions of a potent natural chemical that confers a diversity of health benefits. Int J Biochem Cell Biol. 2009;41(11):2125-2128. doi:10.1016/j.biocel.2009.06.003

63. Li J, Feng L, Xing Y, et al. Radioprotective and antioxidant effect of resveratrol in hippocampus by activating Sirt1. Int J Mol Scis. 2014;15(4):5928-5939. doi:10.3390/ijms15045928

64. Zhuang L, Yang Z, Zeng X, et al. The preventive and therapeutic effect of acupuncture for radiation-induced xerostomia in patients with head and neck cancer: A systematic review. Integr Cancer Ther. 2012;12(3):197-205. doi:10.1177/1534735412451321

65. Meng Z, Garcia MK, Hu C, et al. Randomized controlled trial of acupuncture for prevention of radiation-induced xerostomia among patients with nasopharyngeal carcinoma. Cancer. 2012;118(13):3337-3344. doi:10.1002/cncr.26550

66. Gonnelli FAS, Palma LF, Giordani AJ, et al. Low-level laser therapy for the prevention of low salivary flow rate after radiotherapy and chemotherapy in patients with head and neck cancer. Radiol Bras. 2016;49(2):86-91. doi:10.1590/0100-3984.2014.0144

67. Gonnelli FAS, Palma LF, Giordani AJ, et al. Low-level laser for mitigation of low salivary flow rate in head and neck cancer patients undergoing radiochemotherapy: a prospective longitudinal study. Photomed Laser Surg. 2016;34(8):326-330. doi:10.1089/pho.2016.4104

68. Lopes CO, Mas JRI, Zângaro RA. Prevenção da xerostomia e da mucosite oral induzidas por radioterapia com uso do laser de baixa potência. Radiol Bras. 2006;39(2):131-136. doi:10.1590/s0100-39842006000200012

69. Jha N, Seikaly H, McGaw T, Coulter L. Submandibular salivary gland transfer prevents radiation-induced xerostomia. Int J Radiat Oncol. 2000;46(1):7-11. doi:10.1016/s0360-3016(99)00460-5

70. Seikaly H, Jha N, Harris J, et al. Long-term outcomes of submandibular gland transfer for prevention of postradiation xerostomia. Arch Otolaryngol Head Neck Surg. 2004;130(8):956. doi:10.1001/archotol.130.8.956

71. Jha N, Seikaly H, Harris J, et al. Phase III randomized study: oral pilocarpine versus submandibular salivary gland transfer protocol for the management of radiation-induced xerostomia. Head Neck. 2009;31(2):234-243. doi:10.1002/hed.20961

72. Liu XK, Su Y, Jha N, et al. Submandibular salivary gland transfer for the prevention of radiation-induced xerostomia in patients with nasopharyngeal carcinoma: 5-Year outcomes. Head Neck. 2011;33(3):389-395. doi:10.1002/hed.21461

73. Rieger J, Jha N, Lam Tang J, Harris J, Seikaly H. Functional outcomes related to the prevention of radiation-induced xerostomia: oral pilocarpine versus submandibular salivary gland transfer. Head Neck. 2011;34(2):168-174. doi:10.1002/hed.21682.

74. Zhang Y, Guo CB, Zhang L, et al. Prevention of radiation-induced xerostomia by submandibular gland transfer. Head Neck. 2012;34(7):937-942. doi:10.1002/hed.21859

75. Zhang X, Liu F, Lan X, et al. Clinical observation of submandibular gland transfer for the prevention of xerostomia after radiotherapy for nasopharyngeal carcinoma: a prospective randomized controlled study of 32 cases. Radiat Oncol. 2014;9:62. doi:10.1186/1748-717x-9-62

76. Sood AJ, Fox NF, O'Connell BP, et al. Salivary gland transfer to prevent radiation-induced xerostomia: a systematic review and meta-analysis. Oral Oncol. 2014;50(2):77-83. doi:10.1016/j.oraloncology.2013.10.010

77. Wu F, Weng S, Li C, Sun J, Li L, Gao Q. Submandibular gland transfer for the prevention of postradiation xerostomia in patients with head and neck cancer: a systematic review and meta-analysis. ORL J Otorhinolaryngol Relat Spec. 2015;77(2):70-86. doi:10.1159/000371854

ARTIGO DE PESQUISA

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EFFECT OF RESVERATROL AND VITAMINS C AND E ON PAROTID GLANDS: A STUDY IN RATS IRRADIATED IN HEAD AND NECK REGION

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EFFECT OF RESVERATROL AND VITAMINS C AND E IN PAROTID GLANDS: A STUDY IN RATS IRRADIATED IN HEAD AND NECK REGION

RESVERATROL, VITAMINS C AND E IN PAROTID

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ABSTRACT

Objective: To assess the radioprotective effect of vitamin E, of the association of vitamins C and E, and of resveratrol on the parotid glands of irradiated rats.

Design: Seventy Wistar rats were distributed in five groups: Control group (CG, 1 mL/kg of saline); Irradiated group (IrradG, 1 mL/kg of saline); Vitamin E group (VitEG, 360 mg/kg, 200 UI/mL of vitamin E); Vitamins C and E group (VitCEG, 360 mg/kg, 200 UI/mL of vitamin E and 100 mg/kg, 200 mg/mL of vitamin C); Resveratrol group (ResG, 100 mg/kg of resveratrol). Substances were administered by gavage 24, 48 and 72 h before irradiation. The animals were submitted to a single dose of 20 Gy. After euthanasia both parotid glands of each animal were dissected for histological analysis.

Results: Seven days after radiotherapy, no differences were observed between the groups regarding the presence of acinar disorganization, cytoplasmic vacuolation, apoptosis suggestive changes and nuclear pleomorphism on parotids. Thirty days after radiotherapy, VitEG and ResG presented less cytoplasmic vacuolation and larger nuclear area in comparison to IrradG group. Furthermore, at 30 days, the ResG showed less pleomorphism when compared to IrradG group.

Conclusions: Vitamin E and resveratrol attenuated the harmful effects of ionizing radiation on parotid glands. Resveratrol seems to have been more efficient in comparison to vitamin E.

Keywords: Radiotherapy, Rats, Salivary gland, Prevention xerostomia, Vitamin E, Resveratrol.

INTRODUCTION

Though it is effective for the treatment of head and neck malignancies, radiotherapy frequently causes acute and chronic collateral effects to the healthy tissues adjacent to the tumor. Salivary glands are frequently inserted in the radiation fields and even with their low mitotic level they are extremely radiosensitive. Consequently, there is dose-dependent glandular hypofunction, with hyposalivation and xerostomia (Nagler, Baum, Miller, & Fox, 1998; Eisbruch et al., 2001; Coppes, Vissink, & Konings, 2002; Grundmann, Mitchell, & Limesand, 2009). Such salivary alterations affect the quality of life of patients, and can cause dysgeusia, dysphagia, dysphonia, oral burning, besides increasing the risk of caries and oral opportunistic infections (Lin et al., 2003; Saleh, Figueiredo, Cherubini, & Salum, 2015).

Several therapeutic modalities such as the use of antioxidant substances (vitamins and resveratrol), muscarinic receptor agonist drugs, submandibular gland transfer, acupuncture, low level laser therapy (Jha et al., 2003; Ramos et al., 2006; Jham et al., 2007; Burlage et al., 2008; Teymoortash et al., 2009; Braga, Lemos Junior, Alves, & Migliari, 2011; Şimşek et al., 2012; Acauan et al., 2015) among others have been investigated in clinical and laboratory studies in the prevention of radiotherapy-induced xerostomia.

Antioxidants act against free radicals, impeding their action on lipids, amino acids, polyunsaturated fatty acids and DNA bases, which prevent cellular lesions. They also act in a repairing way, rebuilding the cellular membrane that had already been damaged by the free radicals (Bianchi & Antunes, 1999). Vitamin E (α-Tocopherol Acetate), the main liposoluble vitamin in blood plasma (Catania, Barros, & Ferreira, 2009), acts by interrupting the chain of lipidic peroxidation (Catania et al., 2009; Pocernich & Butterfield, 2012; Almeida et al., 2013). The protective potential of vitamin E over glandular alterations triggered by radiotherapy has been researched (Ramos et al., 2006; Chitra & Shyamala Devi, 2008; Abedi et al., 2015). Ramos et al. (2006) observed that rats treated with vitamin E presented a higher salivary flow in comparison to control 30 days after radiotherapy. Additionally, radiotherapy-induced morphologic alterations (15 Gy) in the parotid glands were milder in the animals treated with vitamin E (Abedi et al., 2015).

The vitamin C, in turn, reacts with oxygen, also reducing oxidative stress biomarkers. Besides, it acts on vitamin E regeneration process, although it is not synthesized in the human organism (Enwonwu, 1992; Manela-Azulay, Mandarim-de-Lacerda, Perez, Filgueira, & Cuzzi, 2003; Verrax & Calderon, 2008; Catania et al., 2009). Chung et al. (2016) evaluated 45 patients with head and neck cancer who used vitamins C and E during radiotherapy. The patients presented lower scores of xerostomia after six months from radiotherapy.

Resveratrol is a natural polyphenolic non-flavonoid compound found mainly in peanuts, red wine and grape skins. This compound is a natural activator of Sirtuin-1 (SIRT1) and acts as antioxidant (Marques, Markus, & Morris, 2009; Chong, Shang, Wang, & Maiese, 2012). In high doses it seems to have radioprotective effect against hyposalivation. After 24 hours of radiotherapy, resveratrol preserved the acinar structure of submandibular glands in rats (Şimşek et al., 2012). Xu et al. (2013) researched the effect of resveratrol in a dose of 20 mg/kg, administered during three days previously to radiotherapy in mice. Despite the hyposalivation, the superoxide dismutase enzyme did not have its function altered in the submandibular glands of the animals which received resveratrol.

Considering the antioxidant properties of these substances, the aim of the present study was to evaluate the effect of resveratrol, of vitamin E and of the association of vitamins C and E in the prevention of radiation-induced morphologic alterations in the parotid glands of rats.

MATERIALS AND METHODS

The study was approved by the Ethics Committee on Animal Use (CEUA) of the Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil, under protocol number 15/00454. The sample consisted of 70 male Wistar rats weighing between 300 and 450 grams. They were kept in the Center for Experimental Biological Models of PUCRS in temperature-controlled (23±1°C) chambers equipped with input and output air filters and with a 12-h light-dark cycle. They were housed in cages appropriate for rodents with free access to water and food.

The animals were randomly divided into five groups, each one containing 14 rats (Figure 1): Control group (CG, 1 mL/kg of saline); Irradiated group (IrradG, 1 mL/kg of saline); Vitamin E group (VitEG, 360 mg/kg, 200 UI/mL of vitamin E); Vitamins E and C group (VitCEG, 360 mg/kg, 200 UI/mL of vitamin E and 100 mg/kg, 200 mg/mL of vitamin C); Resveratrol group (ResG, 100 mg/kg of resveratrol). The dosages were based on previous studies (El-Nahas, Mattar, & Mohamed, 1993; Ramos et al. (2006), Kanter & Akpolat, 2008; Şimşek et al., 2012; Gomes et al. 2013).

The substances were administered by gavage 24, 48 and 72 h before irradiation. The animals of CG and IrradG received saline solution in the same periods when the drugs were administered in the other groups. In order to be administered by gavage, resveratrol was diluted in saline solution and Tween 80 at 0.05% (Ramos et al., 2006; Şimşek et al., 2012; Aguiar, 2014).

Radiotherapy

All the animals, except for the control group, were subjected to ionizing radiation. Radiotherapy was performed in a single session in the Radiotherapy Department of São Lucas Hospital. The animals were placed in prone position and irradiated using a ^{60}Co teletherapy unit (Theratron Phoenix 760, Theratronics, Ottawa, Canada). The radiation dose used was 20 grays (Gy) (Konings et al., 2005; Grundmann et al., 2009). The yield of the radiation source was 56.84 cGy/min, and the distance between the emission point of the radioactive beam and the animals was 77 cm. The irradiation field corresponded to 30 cm x 30 cm, and only the head and neck region were exposed.

Euthanasia and Preparation of Tissues

The animals underwent euthanasia through the inhaling of a superdose of isoflurane after the anesthesia with 100mg/kg of Ketamine (Dopalen) and 10 mg/kg of Xylasine (Anasedan 2%), according to the Guidelines for the Practice of Euthanasia of the National Council of Animal Experimentation. Seven animals of each group underwent euthanasia seven days after radiotherapy. The remaining animals were sacrificed 30 days after irradiation. Due to the adverse effects of radiotherapy, one IrradG animal was lost, in addition to two animals from each VitEG, VitCEG and ResG, in this period.

The right and left parotid glands of each animal were dissected and immersed for 24 h in 10% buffered formalin. They were then dehydrated in increasing concentrations of alcohol, cleared and embedded in paraffin. Four-micrometer thick sections were obtained and stained with hematoxylin and eosin (HE).

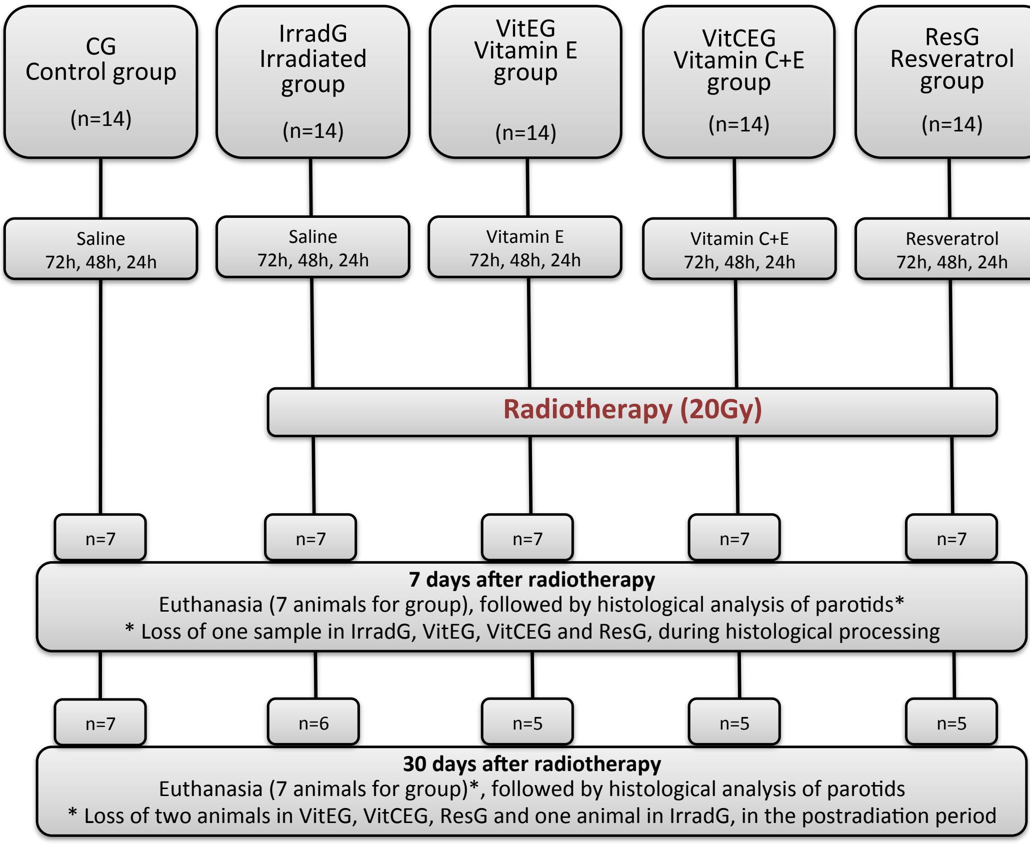

Figure 1. Flowchart representing the stages of the study.

Morphological Analysis

HE-stained slides were examined under a light microscope (Zeiss Axiolab; Zeiss, Berlin, Germany). Histological evaluation of the glandular tissue was performed by a single blinded and calibrated examiner. Calibration was performed applying two analysis of 20 samples with one-week interval between them.

Initially, the histological cuts of both parotid glands, right and left, were assessed in all their extension. We carried out an analysis observing the presence or absence of acinar disorganization, cytoplasmic vacuolation, apoptosis suggestive changes, nuclear pleomorphism and inflammatory infiltrate. We considered as apoptosis suggestive alterations the presence of nuclear fragmentation and pyknosis, with chromatin condensation, forming aggregates close to the nuclear membrane.

After this analysis, we measured the nuclear area of acinar cells. From each lamina, we captured five equidistant fields in 400X magnification, using the Olympus BX50 microscope (Olympus Corporation, Japan) attached to the Moticam 5 capture system (System ShiftCapture, China). Through the ImageJ 1.50 software (National Institutes of Health, USA), the area of 20 nuclei was measured in each histological field. Briefly, after the application of a black and white filter in the images, the nuclei were evidenced by brightness adjust and circumscribed with the *wand tracing* tool (Figure 2). The five largest and the five smallest measurements were excluded. The mean nuclear area of each lamina was calculated through the remaining nuclei.

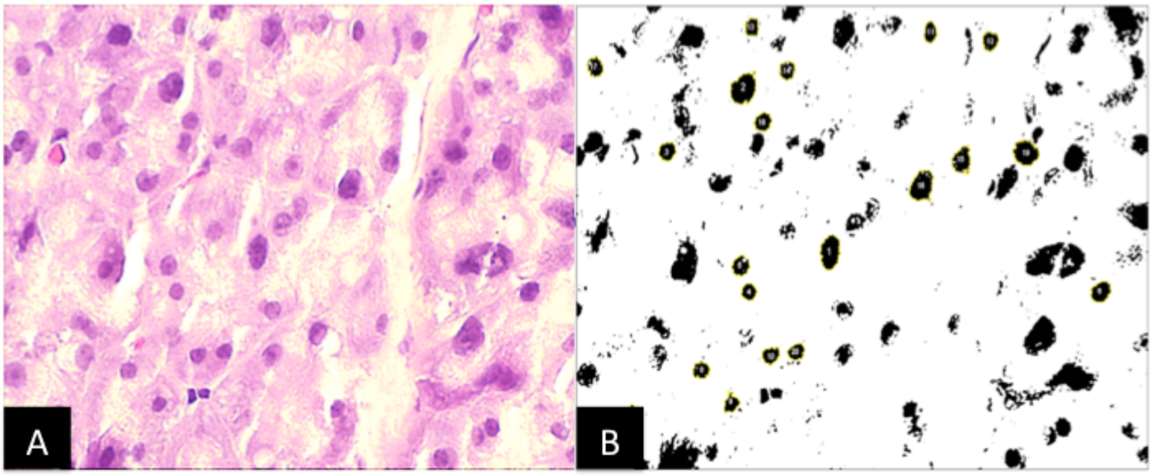

Figure 2. Histologic examination of parotid gland structure (400X). Irradiated group: (A) Original image; (B) Evidenced nuclei filtered image.

Data Analysis

Initially the data of histological evaluation were registered and analyzed through descriptive statistics. In order to compare the presence of acinar disorganization, cytoplasmic vacuolation, nuclear pleomorphism and apoptosis suggestive alterations among the groups, Fischer's exact test was employed. Anova followed by Tukey test was used to compare the nuclear area among the groups. The value established to reject the null hypothesis was of p≤0.05. The *software* used to perform statistical analysis was SPSS version 18.0.

RESULTS

The nuclear area measurements are presented on table 1. The animals of the irradiated group presented inferior values seven days after radiotherapy, however, without a significant difference to the other groups (p=0.078). Thirty days after radiotherapy the VitEG and ResG presented a significantly superior nuclear area in comparison to the others (p=.000).

Table 1. Mean (±DP) of nuclear area (μm²) of control (CG), vitamin E (VitEG), vitamins C and E (VitCEG), resveratrol (ResG) and irradiated (IrradG) groups in the periods of seven and 30 days.

	CG	VitEG	VitCEG	ResG	IrradG	P
7 days	128.25 (±30.76)	127.34 (±33.46)	129.86 (±22.78)	124.98 (±37.82)	90.08 (±12.83)	.078
30 days	103.42[b] (±7.71)	175.09[a] (±17.61)	97.13[b] (±6.75)	158.11[a] (±28.8)	98.34[b] (±13.95)	**.000***

ANOVA and Tukey´s tests, significant at P≤0.05
Distinct letters in the line indicate significant difference among the groups.

Seven days after radiotherapy, morphological alterations in the parotid glands such as acinar disorganization, cytoplasmic vacuolation and nuclear pleomorphism were found in all of the irradiated groups, without significant differences. Although the VitEG has not presented apoptosis suggestive alterations, it did not differ significantly from the other irradiated groups regarding this variable at seven days of experiment (Figure 3).

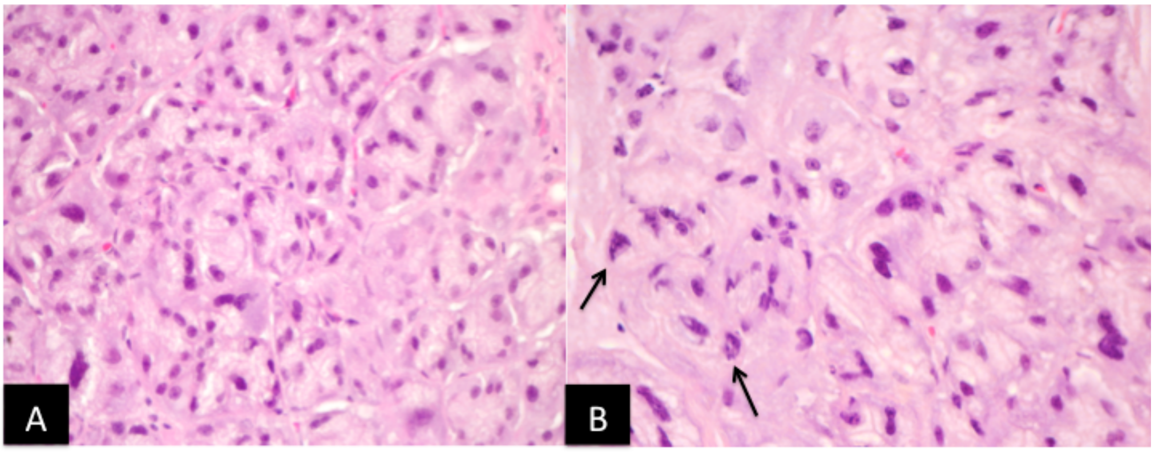

Figure 3. (A) Presence of disorganization acinar, glandular parenchyma showing changes in size, shape, and organization of acinar cells (VitEG, 7-day, HE 400X). (B) Nuclear pleomorphism in acinar cells (arrows), changes in nuclei shape and size (VitCEG, 7-day, HE 400X).

Thirty days after the irradiation, the morphological alterations in the parotid glands were further expressive than at seven days. The IrradG presented acinar disorganization and apoptosis suggestive alterations in 100% of cases, nonetheless with no significant difference to the other irradiated groups. Cytoplasmic vacuolation was significantly superior in IrradG (p=0.015) in comparison to ResG and VitEG. The IrradG showed cytoplasmic vacuolation in 100% of cases, while the ResG and VitEG had this alteration in 20% of the analyzed glands. There also was a significant difference concerning nuclear pleomorphism, which was superior in the IrradG when compared to the ResG (p=0.015), which did not exhibit this alteration 30 days after radiotherapy (Figure 4). No inflammatory infiltrate was observed in the evaluated glands.

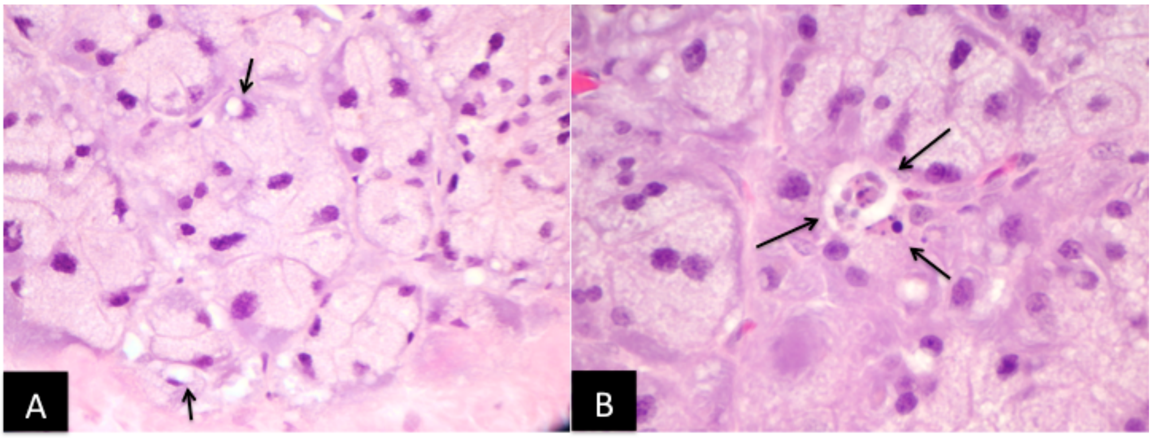

Figure 4. (A) Cytoplasmic vacuoles (arrows), (IrradG, 30-day, HE 400X). (B) Presence of nuclear fragmentation, suggestive of apoptosis (arrows), (ResG, 30-day, HE 400x).

DISCUSSION

Salivary hypofunction is one of the main complications in patients irradiated on head and neck region. Many studies have been performed with the aim of investigating methods capable of preventing the sequelae caused by ionizing radiation on salivary glands. Until the moment, however, there is no procedure or drug capable of completely preventing the onset of radiation-induced salivary hypofunctions.

In the present study we evaluated the effect of the antioxidants resveratrol, vitamin E and the association of vitamins C and E in the prevention of morphologic alterations induced by radiotherapy in the parotid glands. The vitamin E acts on the cellular membrane, interrupting the lipidic peroxidation chain and forming the α-tocopheroxyl radical when reacting with the peroxyl radical (Kayden & Traber, 1993). Studies have demonstrated its potential in protecting salivary glands from damages caused by ionizing radiation (Ramos et al., 2006; Abedi et al., 2015). The vitamin C acts as reduction agent, diminishing the concentration of oxygen inside the cell. Moreover, it promotes the regeneration of vitamin E (Verrax & Calderon, 2008;

Catania et al., 2009). Because of that, in one of the groups of this study, the association of both vitamins was investigated. The resveratrol acts in the activation of sirtuins. SIRT1 is related to several cellular processes such as DNA repair, lipid metabolism, cellular survival, among others, beyond inducing antioxidant enzymes (Marques et al., 2009). In irradiated salivary glands there is an elevation of the MDA (methane dicarboxylic aldehyde) levels and reduction of SOD (superoxide dismutase) levels, one of the most important enzymes against the ROS (reactive oxygen species). Resveratrol is capable of enhancing SOD activity, as well as decreasing MDA (Xu et al., 2013).

Irradiated glandular tissue presents morphological alterations such as acinar disorganization, characterized by cytoplasmic degeneration, with alteration in acinar form and size, presence of cytoplasmic vacuoli, nuclear pleomorphism, pyknotic nuclei with chromatin condensation and fragmentation, which characterizes apoptosis, as well as other alterations (Pontual et al., 2007; Choi, Wu, Jung, Lee, & Kwon, 2009; Domingos, Pontual, Pasetto, Devito, & Almeida, 2009; Tuji, Pontual, Barros, Almeida, & Bóscolo, 2010; Xu et al., 2013; Abedi et al., 2015). Thus, in the present study these glandular morphologic alterations produced by ionizing radiation were analyzed in parotid glands of rats. Abedi et al. (2015), Xu et al. (2013), and Şimşek et al. (2012) corroborate the concept that the modifications in the structure of the irradiated tissue, observed in the histological evaluation, represent evidences of damages related to the decrease in salivary glands function. In our study, seven days after radiotherapy there was no significant difference among the irradiated groups, treated or not with vitamins E, C or resveratrol, in relation to the acinar disorganization, cytoplasmic vacuolation, pleomorphism or presence of apoptosis suggestive alterations. In the second analysis, after 30 days, there was still no difference among those groups regarding acinar disorganization and apoptosis suggestive alterations. On the other hand, also after 30 days, the animals treated with resveratrol presented less cytoplasmic vacuolation and nuclear pleomorphism in the parotid glands when compared to the irradiated group. Xu et al. (2013) demonstrated that mice irradiated and treated with resveratrol presented more subtle morphologic alterations in the submandibular glands in comparison to the irradiated group. Şimşek et al. (2012) also observed, 24 hours after radiotherapy, preservation of glandular morphologic pattern in animals treated with 100 mg/kg of resveratrol. In our study, after 30 days, a milder effect of radiotherapy on the glandular tissue of rats treated with resveratrol was also observed. Such results suggest a possible effect of resveratrol in the preservation of irradiated glands morphology.

As previously reported, in 30 days, the animals treated with vitamin E presented less cytoplasmic vacuolation in comparison to the irradiated group. The presence of vacuoli can indicate an active process of autophagy (Krysko, Vanden Berghe, D'Herde, & Vandenabeele, 2008; Sridhar, Botbol, Macian, & Cuervo, 2012; Ghavami et al., 2014). Some studies in irradiated animals as well as in humans have demonstrated the preservation of salivary flow with the use of vitamin E, associated or not to vitamin C (Ramos et al., 2006; Chitra & Shyamala Devi, 2008; Chung et al., 2016). In our study we did not perform scintigraphy exams, nor measurement of the animals' salivary flow, limiting the comparison to other studies which demonstrate preservation of glandular function through these parameters (Xu et al., 2013; Abedi et al., 2015). On the other hand, in a morphometric analysis, Gomes et al. (2013) did not observe an effect of vitamin E in preventing the occurrence of damages in the parotid glands of rats eight hours or 30 days after radiotherapy. The number of acinar cells did not vary among the groups. When measuring the proportion of the tissues present in the salivary glands three and 70 days after the end of radiotherapy, Abedi et al. (2015) did not observe any difference between the animals treated with vitamin E and the ones of the control group. However, morphological changes were less marked when vitamin E was used before radiotherapy.

Together with the previously described morphologic evaluation, in the present study the nuclear area of acinar cells was measured since the ionizing radiation can lead to a reduction of the nuclear size. Seven days after radiotherapy, although the irradiated group had presented the lowest average nuclear area, there was no significant difference among the groups. On the other hand, in 30 days, the groups treated with vitamin E and resveratrol showed a significantly superior nuclear area when compared to the irradiated group. The increased nuclear area in Vitamin E and resveratrol groups can indicate a higher ability to repair the damages caused by ionizing radiation to the DNA of acinar cells (Ježková et al., 2014). An unusual finding was that the nuclear area of the control group, not irradiated, was also significantly inferior to the ones of animals treated with resveratrol and vitamin E. The small sample may have contributed to this finding.

In the literature, morphologic alterations resulting from ionizing radiation in salivary glands have been evaluated since one hour up to several months after radiotherapy (Konings et al., 2006; Choi et al., 2009, Abedi et al., 2015). In our study, we chose to perform histological evaluation seven and thirty days after radiotherapy with the objective to observe early and late alterations caused by the ionizing radiation. Radiotherapy was applied in a single session at a dose of 20 Gy, since this dose was employed in previous studies on rats, being considered enough to cause the previously discussed morphological alterations (Vissink, Gravenmade, Ligeon, & Konings, 1990; Grundmann et al., 2009; Xiang et al., 2013). On the contrary to what was reported by Ramos et al. (2006), after seven days, collateral effects of ionizing radiation were clearly observed such as hair loss, weight loss and fatigue in irradiated animals. After seven days, four animals which had undergone ionizing radiation were lost and, at 30 days, seven animals.

Based on the morphological analysis performed in this study, it was observed that the salivary glands treated with resveratrol and vitamin E presented larger nuclear areas and less cytoplasmic vacuolation 30 days after radiotherapy. Furthermore, in this period less nuclear pleomorphism was observed in the parotid glands treated with resveratrol. Hence, according to the employed methodology, it is possible to suggest that vitamin E and resveratrol attenuated the harmful effects of ionizing radiation on the acinar cells of the parotid glands. Resveratrol seems to have been more efficient in comparison to vitamin E, since the animals treated with this substance presented less apoptosis suggestive alterations.

REFERENCES

Abedi, S.M., Yarmand, F., Motallebnejad, M., Seyedmajidi, M., Moslemi, D., Ashrafpour, M., et al. (2015). Vitamin E protects salivary glands dysfunction induced by ionizing radiation in rats. *Archives of Oral Biology, 60*(9), 1403-1409. doi:10.1016/j.archoralbio.2015.06.003

Acauan, M.D., Gomes, A.P.N., Braga-Filho, A., Figueiredo, M.A.Z., Cherubini, K., & Salum, F.G. (2015). Effect of low-level laser therapy on irradiated parotid glands—study in mice. *Journal of Biomedical Optics, 20*(10), 108002. doi:10.1117/1.jbo.20.10.108002

Aguiar, T.M.D. (2014). *Antinociceptive effect of Resveratrol in an experimental model of osteoarthritis* (Master's dissertation). Porto, Portugal. Retrived from: http://hdl.handle.net/10216/73042

Almeida, I.V., Düsman, E., Heck, M.C., Pamphile, J.A., Lopes, N.B., Tonin, L.T.D., et al. (2013). Cytotoxic and mutagenic effects of iodine-131 and radioprotection of acerola (Malpighia glabra L.) and beta-carotene in vitro. *Genetics and Molecular Research, 12*(4), 6402-6413. doi:10.4238/2013.december.10.1

Bianchi, M.L.P., & Antunes, L.M.G. Free radicals and the main dietary antioxidants. (1999). *Revista de Nutrição, 12*(2), 123-130. doi:10.1590/s1415-52731999000200001

Braga, F.P.F, Lemos Junior, C.A., Alves, F.A., & Migliari, D.A. (2011). Acupuncture for the prevention of radiation-induced xerostomia in patients with head and neck cancer. *Brazilian Oral Research, 25*(2), 180-185. doi:10.1590/s1806-83242011000200014

Buentzel, J., Micke, O., Adamietz, I.A., Monnier, A., Glatzel, M., & de Vries, A. (2006) Intravenous amifostine during chemoradiotherapy for head-and-neck cancer: a randomized placebo-controlled phase III study. *International Journal of Radiation Oncology, Biology, Physics, 64*(3), 684-691. doi:10.1016/j.ijrobp.2005.08.005

Burlage, F.R., Roesink, J.M., Kampinga, H.H., Coppes, R.P., Terhaard, C.T., Langendijk, J.A., et al. (2008). Protection of salivary function by concomitant pilocarpine during radiotherapy: a double-blind, randomized, placebo-controlled study. *International Journal of Radiation Oncology, Biology, Physics, 70*(1), 14-22. doi:10.1016/j.ijrobp.2007.06.016

Catania, A.S., Barros, C.R., & Ferreira, S.R.G. (2009). Vitamins and minerals with antioxidant properties and cardiometabolic risk: controversies and perspectives. *Arquivos Brasileiros de Endocrinologia & Metabologia, 53*(5), 550-559. doi:10.1590/s0004-27302009000500008

Chitra, S., & Shyamala Devi, C.S. (2008). Effects of radiation and α-tocopherol on saliva flow rate, amylase activity, total protein and electrolyte levels in oral cavity cancer. *Indian Journal of Dental Research, 19*(3), 213-218. doi:10.4103/0970-9290.42953

Choi, J.H., Wu, H., Jung, K.C., Lee, S.H., & Kwon, E.K. (2009). Apoptosis and expression of AQP5 and TGF-β in the irradiated rat submandibular gland. *Cancer Research Treatment, 41*(3), 145-154. doi: 10.4143/crt.2009.41.3.145

Chong, Z.Z., Shang, Y.C., Wang, S., & Maiese, K. (2012). SIRT1: new avenues of discovery for disorders of oxidative stress. *Expert Opinion on Therapeutic Targets, 16*(2), 167-178. doi:10.1517/14728222.2012.648926

Chung, M.K., Kim, D.H., Ahn, Y.C., Choi J.Y., Kim, E.H., & Son, Y. (2016). Randomized trial of vitamin C/E complex for prevention of radiation-induced xerostomia in patients with head and neck cancer. *Otolaryngology - Head and Neck Surgery, 155*(3), 423-430. doi:10.1177/0194599816642418

Coppes, R.P., Vissink, A., & Konings, A.W.T. (2002). Comparison of radiosensitivity of rat parotid and submandibular glands after different radiation schedules. *Radiotherapy and Oncology, 63*(3), 321-328. doi:10.1016/s0167-8140(02)00129-9

Domingos A.C., Pontual M.L.A., Pasetto S., Devito K., & Almeida S.M. (2009). Avaliação morfológica dos efeitos da radiação em glândulas parótidas de ratos. *Saúde Coletiva, 6*(32), 181-183. Retrived from: http://www.redalyc.org/pdf/842/84212205005.pdf

Eisbruch, A., Ten Haken, R.K., Kim, H.M., Marsh, L.H., & Ship, J.A. (1999). Dose, volume, and function relationships in parotid salivary glands following conformal and intensity-modulated irradiation of head and neck cancer. *International Journal of Radiation Oncology, Biology, Physics, 45*(3), 577-587. doi:10.1016/s0360-3016(99)00247-3

Eisbruch, A., Kim, H.M., Terrell, J.E., Marsh, L.H., Dawson, L.A., & Ship, J.A. (2001). Xerostomia and its predictors following parotid-sparing irradiation of head-and-neck cancer. *International Journal of Radiation Oncology, Biology, Physics, 50*(3), 695-704. doi:10.1016/s0360-3016(01)01512-7

El-Nahas, S.; Mattar, F.; Mohamed, A. (1993). Radioprotective effect of vitamins C and E. *Mutat Res*, 301 (2), 143-147. Retrived from: http://www.sciencedirect.com/science/article/pii/016579929390037V?via%3Dihub

Enwonwu, C.O. (1992). Ascorbate status and xerostomia. *Medical Hypotheses, 39*(1), 53-57. doi:10.1016/0306-9877(92)90140-8

Ghavami, S., Shojaei, S., Yeganeh, B., Ande, S.R., Jangamreddy, J.R., Mehrpour, M., et al. (2014). Autophagy and apoptosis dysfunction in neurodegenerative disorders. *Progress in Neurobiology, 112*, 24–49. doi: 10.1016/j.pneurobio.2013.10.004

Gomes, C.C., Ramos-Perez, F.M., Perez, D.E., Novaes, P.D., Bóscolo, F.N., & Almeida, S.M. (2013). Radioprotective effect of vitamin E in parotid glands: a morphometric analysis in rats. *Brazilian Dental Journal, 24*(3), 183-187. doi:10.1590/0103-6440201301781

Grundmann, O., Mitchell, G.C., & Limesand, K.H. (2009). Sensitivity of salivary glands to radiation: from animal models to therapies. *Journal of Dental Research, 88*(10), 894-903. doi:10.1177/0022034509343143

Ježková, L., Falk, M., Falková, I., Davídková, M., Bačíková, A., Štefančíková, L., et al. (2014). Function of chromatin structure and dynamics in DNA damage, repair and misrepair: γ-rays and protons in action. *Applied Radiation and Isotopes, 83*, 128–136. doi: 10.1016/j.apradiso.2013.01.022

Jha, N., Seikaly, H., Harris, J., Williams, D., Liu, R., McGaw, T., et al. (2003). Prevention of radiation induced xerostomia by surgical transfer of submandibular salivary gland into the submental space. *Radiotherapy and Oncology, 66*(3), 283-289. doi:10.1016/s0167-8140(03)00023-9

Jham, B.C., Teixeira, I.V., Aboud, C.G., Carvalho, A.L., Coelho, M.M., & Freire, A.R.S. (2007). A randomized phase III prospective trial of bethanechol to prevent radiotherapy-induced salivary gland damage in patients with head and neck cancer. *Oral Oncology, 43*(2), 137-142. doi:10.1016/j.oraloncology.2006.01.013

Kanter, M.; Akpolat, M. (2008). Vitamin C protects against ionizing radiation damage to goblet cells of the ileum in rats. *Acta Histochem*, 110 (6), 481-490. doi: 10.1016/j.acthis.2008.07.006

Kayden, H.J., & Traber, M.G. (1993). Absorption, lipoprotein transport, and regulation of plasma concentrations of vitamin E in humans. *J Lipid Res, 34*(3), 343-358. Retrived from: http://www.jlr.org/content/34/3/343.full.pdf

Konings, A.W.T., Cotteleer, F., Faber, H., van Luijk, P., Meertens, H., & Coppes, R.P. (2005). Volume effects and region-dependent radiosensitivity of the parotid gland. *International Journal of Radiation Oncology, Biology, Physics, 62*(4), 1090-1095. doi:10.1016/j.ijrobp.2004.12.035

Konings, A.W.T., Faber, H., Cotteleer, F., Vissink, A., & Coppes, R.P. (2006). Secondary radiation damage as the main cause for unexpected volume effects: a histopathologic study of the parotid gland. *International Journal of Radiation Oncology, Biology, Physics, 64*(1), 98-105. doi:10.1016/j.ijrobp.2005.06.042

Krysko, D.V., Vanden Berghe, T.V., D'Herde, K., & Vandenabeele, P. (2008). Apoptosis and necrosis: detection, discrimination and phagocytosis. *Methods 44*(3), 205–221. doi: 10.1016/j.ymeth.2007.12.001

Lin, A., Kim, H.M., Terrell, J.E., Dawson, L.A., Ship, J.A., & Eisbruch, A. (2003). Quality of life after parotid-sparing IMRT for head-and-neck cancer: a prospective longitudinal study. *International Journal of Radiation Oncology, Biology, Physics, 57*(1), 61-70. doi:10.1016/s0360-3016(03)00361-4

Manela-Azulay, M., Mandarim-de-Lacerda, C.A., Perez, M.A., Filgueira, A.L., & Cuzzi, T. Vitamin C. (2003). *Anais Brasileiros de Dermatologia, 78*(3), 265-274. doi:10.1590/s0365-05962003000300002

Marques, F.Z., Markus, M.A., & Morris, B.J. (2009). Resveratrol: cellular actions of a potent natural chemical that confers a diversity of health benefits. *The International Journal of Biochemistry & Cell Biology, 41*(11), 2125-2128. doi:10.1016/j.biocel.2009.06.003

Nagler, R.M., Baum, B.J., Miller, G., & Fox, P.C. (1998). Long-term salivary effects of single-dose head and neck irradiation in the rat. *Archives of Oral Biology, 43*(4), 297-303. doi:10.1016/s0003-9969(97)00120-9

Pocernich, C.B., & Butterfield, D.A. (2012). Elevation of glutathione as a therapeutic strategy in Alzheimer disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1822*(5), 625-630. doi:10.1016/j.bbadis.2011.10.003

Pontual, M.L.A., Tuji, F.M., Barros, S.P., Bóscolo, F.N., Novaes, P.D., & Almeida, S.M. (2007). Ultrastructural evaluation of the radioprotective effect of sodium selenite on submandibular glands in rats. *Journal of applied oral Science, 15*(3), 162-168. Retrived from: http://www.scielo.br/pdf/jaos/v15n3/a03v15n3.pdf

Ramos, F.M., Pontual, M.L.A., Almeida, S.M., Bóscolo, F.N., Tabchoury, C.P., & Novaes, P.D. (2006). Evaluation of radioprotective effect of vitamin E in salivary dysfunction in irradiated rats. *Archives of Oral Biology, 51*(2), 96-101. doi:10.1016/j.archoralbio.2005.06.008

Saleh, J., Figueiredo, M.A.Z., Cherubini, K., & Salum, F.G. (2015). Salivary hypofunction: an update on aetiology, diagnosis and therapeutics. *Archives of Oral Biology, 60*(2), 242-255. doi:10.1016/j.archoralbio.2014.10.004

Şimşek, G., Gürocak, Ş., Karadağ, N., Karabulut, A.B., Demirtas, E., Karatas, E., et al. (2012). Protective effects of resveratrol on salivary gland damage induced by total body irradiation in rats. *The Laryngoscope, 122*(12), 2743-2748. doi:10.1002/lary.23609

Sridhar, S., Botbol, Y., Macian, F., & Cuervo, A.M. (2012). Autophagy and Disease: always two sides to a problem. *The Journal of Pathology, 226*(2), 255-273. doi:10.1002/path.3025

Teymoortash, A., Müller, F., Juricko, J., Bieker, M., Mandic, R., Librizzi, D., et al. (2009). Botulinum toxin prevents radiotherapy-induced salivary gland damage. *Oral Oncology, 45*(8), 737-739. doi:10.1016/j.oraloncology.2008.11.014

Tuji, F.M., Pontual, M.L.A., Barros, S.P., Almeida, S.M., & Bóscolo, F.N. (2010). Ultrastructural assessment of the radioprotective effects of sodium selenite on parotid glands in rats. *Journal of Oral Science, 52*(3), 369-375. Retrived from: https://www.jstage.jst.go.jp/article/josnusd/52/3/52_3_369/_pdf

Urek, M.M., Bralic, M., Tomac, J., Borcic, J., Uhac, I., Glazar, I., et al. (2005). Early and late effects of x-irradiation on submandibular gland: a morphological study in mice. *Archives of Medical Research, 36*(4), 339-343. doi:10.1016/j.arcmed.2005.03.005

Verrax, J. & Calderon, P.B. (2008). The controversial place of vitamin C in cancer treatment. *Biochemical Pharmacology 76*(12), 1644–1652. doi: 10.1016/j.bcp.2008.09.024

Vissink, A., Gravenmade, E.J., Ligeon, E.E., & Konings, W.T. (1990). A functional and chemical study of radiation effects on rat parotid and submandibular/sublingual glands. *Radiation Research, 124*(3), 259-265

Xiang, B., Li, Y., Zhao, X., Zou, Y., Yu, Z., Zhao, Y., et al. (2013). Mechanism of the protective effect of phenylephrine pretreatment against irradiation-induced damage in the submandibular gland, *Experimental and Therapeutic Medicine, 5*(3), 875-879. doi: 10.3892/etm.2012.867

Xu, L., Yang, X., Cai, J., Ma, J., Cheng, H., Zhao, K., et al. (2013). Resveratrol attenuates radiation-induced salivary gland dysfunction in mice. *The Laryngoscope, 123*(11), E23-E29. doi:10.1002/lary.24276.

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DISCUSSÃO COMPLEMENTAR

5 DISCUSSÃO COMPLEMENTAR

A radioterapia frequentemente causa danos irreversíveis às glândulas salivares e a xerostomia é uma das queixas mais frequentes de pacientes irradiados na região de cabeça e pescoço (VISSINK et al., 2003; GRUNDMANN; MITCHELL; LIMESAND, 2009). No primeiro artigo desta dissertação foi desenvolvida uma revisão de literatura com o objetivo de abordar métodos preventivos de hipofunção salivar radioinduzida. Foram abordados métodos farmacológicos preventivos tais como o uso de agonistas colinérgicos (pilocarpina e betanecol), substâncias antioxidantes, acupuntura, TLBP e transferência da glândula submandibular. Estudos com betanecol, pilocarpina e amifostina corroboram o efeito profilático dessas drogas sobre as glândulas salivares. Entretanto, as mesmas possuem efeitos colaterais que contra-indicam seu uso em diversos pacientes. Por esta razão, estudos com métodos mais toleráveis e com menos efeitos colaterais têm sido realizados. A acupuntura e a terapia laser de baixa potência, apesar de ainda pouco estudadas parecem apresentar resultados benéficos quanto à preservação da função salivar e redução da intensidade de xerostomia. Estudos pré-clínicos e ensaios clínicos controlados são ainda necessários a fim de encontrar modalidades terapêuticas que preservem a estrutura glandular submetida à radiação ionizante, prevenindo a ocorrência de hipossalivação e xerostomia.

As substâncias antioxidantes estão entre os métodos pesquisados para preservar a estrutura glandular de alterações radioinduzidas (CHITRA; SHYAMALA DEVI, 2008; XU et al., 2013; GU et al., 2014; CHUNG et al. 2016). Portanto, no segundo estudo desta dissertação foi investigado o efeito do resveratrol, da vitamina E, bem como da associação das vitaminas C e E na prevenção de alterações morfológicas em glândulas parótidas de ratos. Estas substâncias possuem reconhecida ação contra radicais livres e prevenção de lesões celulares (BIANCHI; ANTUNES, 1999). Os resultados obtidos neste estudo indicaram que as glândulas salivares tratadas com resveratrol e vitamina E apresentaram maior área nuclear e menos vacuolação citoplasmática 30 dias após a radioterapia. Além disso, neste período foi observado menos pleomorfismo nuclear nas parótidas tratadas com resveratrol. Portanto, de acordo com a metodologia empregada é possível sugerir que a vitamina E e o resveratrol atenuaram os efeitos nocivos da radiação ionizante nas glândulas parótidas de ratos. O resveratrol parece ter sido mais eficiente em comparação à vitamina E, pois além de terem apresentado menos vacuolação citoplasmática, as glândulas dos animais tratados com essa subtância apresentaram menos pleomorfismo nuclear.

Corroborando os resultados do presente estudo, Şimşek et al. (2012) observaram que o resveratrol apresentou efeito em prevenir a ocorrência de vacuolação e morte celular em parótidas de ratos irradiados. Xu et al. (2013) também observaram indícios do potencial efeito protetor do resveratrol sobre tecidos irradiados, agindo contra espécies reativas de oxigênio (ROS). O resveratrol foi capaz de aumentar a atividade da SOD (superóxido dismutase), e diminuir a MDA (methane dicarboxylic aldehydee), mantendo o nível do fluxo salivar.

Abedi et al. (2015) também encontraram resultados positivos da vitamina E na proteção de glândulas salivares de ratos irradiados. As alterações morfológicas foram menos evidentes quando a vitamina E foi utilizada previamente à radioterapia. Na análise por meio de cintilografia, os resultados do grupo vitamina E foram semelhantes aos do grupo-controle não irradiado. Ao avaliarem o fluxo salivar em ratos irradiados, Ramos et al. (2006) observaram que a vitamina E preservou a função glandular. Em nosso estudo, não foi realizada avaliação do fluxo salivar dada a dificuldade técnica em obter as amostras salivares.

Apesar de a vitamina E ter demonstrado efeito em reduzir a presença de vacuolação citoplasmática nas células acinares, no grupo em que essa foi associada à vitamina C não se observaram diferenças na morfologia acinar em relação ao grupo irradiado. Não há estudos que tenham testado a associação de ambas, vitaminas C e E e que tenham realizado análise morfológica semelhante no tecido glândular irradiado. Chung et al. (2016) em estudo clinico prospectivo, duplo-cego, randomizado, placebo-controlado, demonstraram eficiência da combinação das duas vitaminas na prevenção da xerostomia radioinduzida. Os pacientes utilizaram altas doses dessas substâcias (200 UI de vitamina E e 1000 mg de vitamina C) durante cerca de três meses. Após o primeiro mês da radioterapia todos os pacientes queixaram-se de xerostomia. A diminuição dos sintomas de xerostomia foi demonstrada apenas após seis meses do término da radioterapia. Os resultados podem ter divergido ao do presente estudo devido às diferentes metodologias aplicadas. Ao utilizarmos um modelo animal, não foi possível analisar os mesmos parâmetros que Chung et al. (2016), além disso, a avaliação morfológica foi realizada em sete e em 30 dias após a radioterapia e as substâncias foram administradas somente durante três dias. Nossos resultados em relação à associação das vitaminas C e E assemelham-se aos encontrados por e Banu (2004), onde não foi observada maior eficácia dessa associação contra a formação de radicais livres, em ratos submetidos à atividade de estresse. Ratos do grupo que ingeriu apenas vitamina E apresentaram menos sinais de estresse oxidativo do que aqueles que utilizaram as vitaminas C e E em conjunto.

Ainda não há um método capaz de prevenir totalmente a ocorrência de disfunções salivares radioinduzidas. Existem métodos que atenuam essas alterações. Nossos resultados sugerem uma possível eficácia da vitamina E e, em maior grau, do resveratrol na preservação da morfologia de glândulas parótidas de ratos submetidos à radiação ionizante. Deste modo pesquisas clínicas também devem ser realizadas com o objetivo de averiguar o potencial efeito radioprotetor destas substâncias em pacientes submetidos à radioterapia de cabeça e pescoço.

CONCLUSÕES

6 CONCLUSÕES

Com base na metodologia empregada e nos resultados encontrados, pode-se concluir que:

• Fármacos agonistas colinérgicos, substâncias antioxidantes, terapia laser de baixa potência, acupuntura e transferência da glândula submandibular têm sido investigados na prevenção de alterações radioinduzidas em glândulas salivares. Entretanto, a falta de padronização dos estudos dificulta a comparação entre os mesmos. Até o momento, não há um método capaz de prevenir totalmente a ocorrência de hipossalivação induzida pela radioterapia de cabeça e pescoço.

• Animais tratados com vitamina E e resveratrol previamente à radioterapia apresentaram alterações morfológicas mais sutis em comparação aos animais não tratados, sugerindo que estas substâncias tenham potencial de atenuar os efeitos da radiação ionizante nas células acinares.

• Das substâncias investigadas, o resveratrol parece ter sido mais eficiente na prevenção de alterações morfológicas nas glândulas parótidas de ratos irradiados.

REFÊRENCIAS COMPLEMENTARES

7 REFERÊNCIAS COMPLEMENTARES

ABEDI, S. et al. Vitamin E protects salivar glands dysfunction induced by ionizing radiation in rats. **Arch Oral Biol***,* v. 60, n. 9, p. 1403-1409, Sept. 2015.

ACAUAN, M. et al. Effect of low-level laser therapy on irradiated parotid glands—study in mice. **J Biomed Opt***,* v. 20, n. 10, p. 108002, Oct. 2015.

AHMED, M. et al. Reducing the risk of xerostomia and mandibular osteoradionecrosis: the potential benefits of intensity modulated radiotherapy in advanced oral cavity carcinoma. **Med Dosim**, v. 34, n. 3, p. 217-224, Fall 2009.

BIANCHI, M.; ANTUNES, L. Radicais livres e os principais antioxidantes da dieta. **Rev Nutr**, v. 12, n. 2, p. 123-130, maio/ago. 1999.

BLANCO, A. et al. Dose-volume modeling of salivary function in patients with head-and-neck cancer receiving radiotherapy. **Int J Radiat Oncol Biol Phys**, v. 62, n. 4, p. 1055-1069, July 2005.

BORAKS, G. et al. Effect of ionizing radiation on rat parotid gland. **Braz Dent J***,* v. 19, n. 1, p. 73-76, 2008.

BURLAGE, F. et al. Protection of salivary function by concomitant pilocarpine during radiotherapy: a double-blind, randomized, placebo-controlled study. **Int J Radiat Oncol Biol Phys***,* v. 70, n. 1, p. 14-22, Sept. 2008**.**

CATANIA, A.; BARROS, C.; FERREIRA, S. Vitaminas e minerais com propriedades antioxidantes e risco cardiometabólico: controvérsias e perspectivas. **Arq Bras Endocrinol Metab**, v. 53, n. 5, p. 550-559, July 2009.

CHITRA, S.; SHYAMALA DEVI, C. Effects of radiation and α-tocopherol on saliva flow rate, amylase activity, total protein and electrolyte levels in oral cavity cancer**. Indian J Dent Res**, v. 19, n. 3, p. 213-218, July/Sept. 2008.

CHONG, Z. et al. SIRT1: new avenues of discovery for disorders of oxidative stress. **Expert Opin Ther Targets**, v. 16, n. 2, p. 167–178, Feb. 2012.

CHUNG, M. et al. Randomized Trial of Vitamin C/E Complex for Prevention of Radiation-Induced Xerostomia in Patients with Head and Neck Cancer. **Otolaryngol Head Neck Surg**, n. 155, v. 3, p. 423-430, Sept. 2016.

CONKLIN, K. Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects**. Nutr Cancer***,* v. 37, n. 1, p. 1-18, 2000.

COPPES, R.; VISSINK, A.; KONINGS, A. Comparison of radiosensitivity of rat parotid and submandibular glands after different radiation schedules. **Radiother Oncol**, v. 63, n. 3, p. 321–328, June 2002.

DIAS, J. **Determinação dos conteúdos de resveratrol em vinhos tintos de duas regiões brasileiras**. 100 f. Dissertação (Mestrado em Ciência de Alimentos) - Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2009.

DU, J.; CULLEN, J.; BUETTNER, G. Ascorbic acid: chemistry, biology and the treatment of cancer. **Biochim Biophys Acta**, v. 1826, n. 2, p. 443-457, Dec. 2012.

DUARTE, V. et al. Comparison of Dental Health of Patients with Head and Neck Cancer Receiving IMRT vs Conventional Radiation. **Otolaryngol Head Neck Surg**. v.150, n.1, p. 81-86, Jan. 2014.

EISBRUCH, A. et al. Partial irradiation of the parotid gland. **Semin Radiat Oncol**, v.11, n. 3, p. 234-239, July 2001.

ENWONWU, C. Ascorbate status and xerostomia. **Med Hypotheses**, v. 39, n.1 p. 53-57, Sept.1992.

EPSTEIN, J. et al. Oral complications of cancer and cancer therapy: from treatment to survivorship. **CA Cancer J Clin**, v.62, n.6, p.400-422, Nov./Dec. 2012.

GRUNDMANN, O.; MITCHELL, G.; LIMESAND, K. Sensitivity of salivary glands to radiation: from animal models to therapies. **J Dent Res**, v. 88, n. 10, p. 894-903, Oct. 2009.

GU, J. et al. Effect of Amifostine in Head and Neck Cancer Patients Treated with Radiotherapy: A systematic review and meta-analysis based on randomized controlled trials. **PLoS ONE**, v. 9 n. 5. May 2014.

HADDAD, P.; KARIMI, M. A randomized, double-blind, placebo-controlled trial of concomitant pilocarpine with head and neck irradiation for prevention of radiation-induced xerostomia. **Radiother Oncol**, v. 64, n. 1, p. 29-32, July 2002.

JHA, N. et al. Submandibular salivary gland transfer prevents radiation-induced xerostomia. **Int J Radiat Oncol Biol Phys**. v.1, n.46, p. 7-11, Jan. 2000

JHAM, B. et al. A randomized phase III prospective trial of bethanechol to prevent radiotherapyinduced salivary gland damage in patients with head and neck cancer. **Oral Oncol**, v. 43, n. 2, p. 137–142, Feb. 2007.

KLEIN, J.; LIVERGANT, J.; RINGASH, J. Health related quality of life in head and neck cancer treated with radiation therapy with or without chemotherapy: a systematic review. **Oral Oncol**, v. 50, n. 4, p. 254-262, Apr. 2014.

KONINGS, A. et al. Volume effects and region-dependent radiosensivity of the parotid gland. **Int J Radiation Oncol Biol Phys**, v. 62, n. 4, p. 1090-1095, July 2005.

LI, J. et al. Structural and functional characteristics of irradiation damage to parotid glands in the miniature pig. **Int J Radiat Oncol Biol Phys**, V.62, n. 5, 1510-1526, Aug. 2005.

MANELA-AZULAY, M. et al. Vitamina C. **An Bras Dermatol**, v. 78, n. 3, p. 265-274, maio/jun. 2003.

MARINHO, T. et al. Complicações de radioterapia de cabeça e pescoço: revisão de literatura. **CGBS**, v. 13, n.13, p. 81-87, jan./jun. 2012.

MARQUES, F.; MARKUS, A.; MORRIS, B. Resveratrol: Cellular actions of a potent natural chemical that confers a diversity of health benefits**. Int J Biochem Cell B**, v. 41, n.11, p. 2125–2128, Nov. 2009.

MARTA, G. et al. Intensity-modulated radiation therapy for head and neck cancer: Systematic review and meta-analysis. **Radiother Oncol***,* v.110, n.1, p. 9-15, Jan. 2014.

MENG, Z. et al. Randomized controlled trial of acupuncture for prevention of radiation-induced xerostomia among patients with nasopharyngeal carcinoma. **Cancer***.* v. 118, n. 13, p. 3337–3344, July 2012.

MERCADANTE, S. et al. Prevalence of oral mucositis, dry mouth, and dysphagia in advanced cancer patients. **Support Care Cancer**, v. 23, n. 11, p. 3249-3255, Nov. 2015.

MONTERO, S.; REYES, M.; CIFUENTES, J. Complicaciones orales del tratamiento no quirúrgico del cancér de cabeza y cuello. **Rev Dent Chile**, v.93, n.3, p.12-6, nov. 2002.

MOSS, R. Do antioxidants interfere with radiation therapy for cancer*?* **Integr Cancer Ther**, v. 6, n. 3, p. 281-292, Sept. 2007.

MOTALLEBNEJAD, M. et al. Evaluation of protective effect of propolis on parotid salivay glands in gamma-irradiated rats. **J Contemp Dent Pract**, v. 15, n. 1, p. 8-11, Jan. 2014.

MURDOCH-KINCH, C. et al. Recovery of salivary epidermal growth factor in parotid saliva following parotid sparing radiation therapy: a proof-of-principle study. **Oral Surg Oral Med Oral Pathol**, v. 111, n. 1, p. 64-70, Jan. 2011.

NAGLER, R. et al. Long-term salivary efects of single dose head and neck irradiation in the rat. **Arch Oral Biol**, v. 43, n. 4, p. 297-303, Apr. 1998.

NUTTING, C. et al. Optimisation of radiotherapy for Carcinoma of the Parotid gland: a comparison of conventional, three-dimensional conformal, and intensity-modulated techniques. **Radiother Oncol**, v. 60, n. 2, p. 163-172, Aug. 2001.

POCERNICH, C.; BUTTERFIELD, A. Elevation of glutathione as a therapeutic strategy in Alzheimer disease. **Biochim Biophys Acta**, v.1822, n. 5, p. 625–630, May 2012.

RAMOS, F. et al. Evaluation of radioprotective effect of vitamin E in salivary dysfunction in irradiated rats. **Arch Oral Biol**, v. 51, n. 2, p. 96-101, Feb. 2006.

RIEGER, J. et al. Functional outcomes related to the prevention of Radiation-induced xerostomia: oral pilocarpine Versus submandibular salivary gland transfer. **Head Neck**, v. 34, n. 2, p. 168-174, Feb. 2012.

van RIJ, C. et al. Parotid gland sparing IMRT for head and neck cancer improves xerostomia related quality of life. **Radiother Oncol**, v. 9, n. 3, 41p, Dec. 2008.

RUDAT, V. et al. The effect of amifostine or imrt to preserve the paroti function after radiotherapy of the head and neck region measured by quantitative salivary gland scintigraphy. **Radiother Oncol**, v.89, n. 1, p. 71-80, Oct. 2008.

SEGRETO, H.; SEGRETO, R. Revisão e atualização em radiobiologia. Aspectos celulares, moleculares e clínicos. **Folha Med**, São Paulo, v.119, n. 4, p. 9-27, out./nov./dez. 2000.

ŞIMŞEK, G. et al. Protective effects of resveratrol on salivary gland damage induced by total body irradiation in rats. **Laryngoscope**, v. 122, n.12, p. 2743–2748, Dec. 2012.

STONE, H. et al. Effects of radiation on normal tissue: consequences and mechanisms. **Lancet Oncol**, n. 4 v.9. p. 529–36, Sept. 2003.

TROTTI, A. Toxicity in head and neck cancer: a review of trends and issues. **Int J Radiat Oncol Biol Phys**, v. 47, n. 1, p. 1-12, Apr. 2000.

TUJI, F. et al. Ultrastructural assessment of the radioprotective effects of sodium selenite on parotid glands in rats. **J Oral Sci**, v. 52, n.3, p.369-375, Sept. 2010.

VALKO, M. et al. Free radicals, metals and antioxidants in oxidative stress-induced cancer. **Chem Biol Interact**, v. 160, n. 1, p. 1-40, Mar. 2006.

VISSINK, A. et al. Prevention and treatment of the consequences of head and neck radiotherapy. **Crit Rev Oral Biol Med**, n. 14, v. 3, p.213-225, 2003.

VISSINK, A. et al. Clinical management of salivary gland hypofunction and xerostomia in head-and-neck cancer patients: successes and barriers. **Int J Radiat Oncol Biol Phys**, v. 78, n. 4, p. 983-991, Nov. 2010.

VISVANATHAN, V.; NIX, P. Managing the patient presenting with xerostomia: a review. **Int J Clin Pract**, v. 64, n. 3, p. 404-407, Feb. 2010.

XU, J. et al. Resveratrol attenuates radiation-induced salivary gland dysfunction in mice. **Laryngoscope**, v. 123, n. 11, p. E23-E29, Nov. 2013.

ZAIDI, S.M.; BANU, N. Antioxidant potential of vitamins A, E and C in modulating oxidative stress in rat brain. **Clin Chim Acta**, v. 340, n 1-2, p. 229-233, Feb. 2004.

ANEXO A

APROVAÇÃO DA COMISSÃO CIENTÍFICA E DE ÉTICA DA FACULDADE DE ODONTOLOGIA DA PUCRS

PUCRS

S I P E S Q

Sistema de Pesquisas da PUCRS

PROPESQ

Código SIPESQ: 6387

Porto Alegre, 28 de maio de 2015.

Prezado(a) Pesquisador(a),

A Comissão Científica da FACULDADE DE ODONTOLOGIA da PUCRS apreciou e aprovou o Projeto de Pesquisa "EFEITO DO RESVERATROL E DAS VITAMINAS C E E EM GLÂNDULAS PARÓTIDAS DE RATOS SUBMETIDOS À RADIOTERAPIA" coordenado por FERNANDA GONCALVES SALUM. Caso este projeto necessite apreciação do Comitê de Ética em Pesquisa (CEP) e/ou da Comissão de Ética no Uso de Animais (CEUA), toda a documentação anexa deve ser idêntica à documentação enviada ao CEP/CEUA, juntamente com o Documento Unificado gerado pelo SIPESQ.

Atenciosamente,

Comissão Científica da FACULDADE DE ODONTOLOGIA

ANEXO B

APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA PUCRS

PUCRS CEUA Comissão de Ética no Uso de Animais

Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA, INOVAÇÃO E DESENVOLVIMENTO
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Ofício 50/2015 - CEUA

Porto Alegre, 14 de agosto de 2015.

Prezado Sr(a) Pesquisador(a),

A Comissão de Ética no Uso de Animais da PUCRS apreciou e aprovou seu Protocolo de Pesquisa, registro CEUA 15/00454, intitulado **"Efeito do resveratrol e das vitaminas C e E em glândulas parótidas de ratos submetidos à radioterapia".**

Sua investigação, respeitando com detalhe as descrições contidas no projeto e formulários avaliados pela CEUA, está **autorizada** a partir da presente data.

Informamos que é necessário o encaminhamento de relatório final quando finalizar esta investigação. Adicionalmente, ressaltamos que conforme previsto na Lei no. 11.794, de 08 de outubro de 2008 (Lei Arouca), que regulamenta os procedimentos para o uso científico de animais, é função da CEUA zelar pelo cumprimento dos procedimentos informados, realizando inspeções periódicas nos locais de pesquisa.

Nº de Animais	Espécie	Duração do Projeto
56	Rattus novergicus	05/2015 – 01/2016

Atenciosamente,

Prof. Dr. João Batista Blessmann Weber
Coordenador da CEUA/PUCRS

Ilma. Sra.
Profa. Dra. Fernanda Gonçalves Salum
FO
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