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Análise do Perfil Oxidativo de Usuárias de Crack em Processo de Desintoxicação

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Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Biologia Celular e Molecular da Pontifícia Universidade Católica do Rio Grande do Sul como requisito para a obtenção do grau de Mestre.

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Dedico essa conquista à minha mãe que nunca mediu esforços para que eu chegasse onde estou.

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LISTA DE ABREVIATURAS

DA – dopamina

DAT – transportadores de dopamina

EO – estresse oxidativo

EROS – espécies reativas de oxigênio

GABA – ácido gama-aminobutírico

GPx – glutationa peroxidase

GR – glutationa redutase

GSH – glutationa reduzida

GSSG – glutationa oxidada

GST – glutationa transferase

NADPH – nicotinamida adenina dinucleotídeo fosfato

SNC – sistema nervoso central

SOD – superóxido dismutase

TRAP – potencial antioxidante reativo total

TRx – tiorredoxina

VTA – área ventral tegmental

“O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis”.

José de Alencar

RESUMO

O crack é uma substância derivada da cocaína que age como estimulante do sistema nervoso central. É caracterizado pelo baixo custo, rápida ação e alto poder de dependência. Pouco se sabe sobre os efeitos a nível celular causados pelo crack e sobre a capacidade de recuperação do organismo no período de abstinência. Estudos tem demonstrado uma elevação da produção de espécies reativas de oxigênio (EROS) após a administração da cocaína. O estresse oxidativo ocorre quando há um desequilíbrio entre a produção de EROS e a capacidade do sistema de defesa antioxidante em combater ou prevenir sua ação. Essa condição pode ser nociva para as células, causando danos a biomoléculas como DNA, lipídeos e proteínas. Com o objetivo de melhor compreender a ação do crack e da abstinência no sistema de defesa antioxidante e no dano oxidativo, esse estudo recrutou trinta voluntárias pacientes de um programa para desintoxicação e trinta voluntárias que não faziam uso de drogas tidas como grupo controle. Amostras de sangue foram coletadas após o 4º e o 18º dia de tratamento e o plasma foi utilizado para as análises bioquímicas. Foram realizadas quantificações de marcadores oxidantes, como proteínas carboniladas e tióis proteicos, ambos demonstram modificações proteicas feitas por EROS e marcadores antioxidantes, tanto enzimáticos como glutationa peroxidase e superóxido dismutase quanto não enzimático como glutationa reduzida (GSH) e o potencial antioxidante reativo total (TRAP). As variáveis psicológicas foram avaliadas através dos escores obtidos nos questionários: Cocaine Selective Severity Assessment, Questionário de Tolerância de Fagerstrom, Inventário de Depressão de Beck versão II (BDI-II) e o Addiction Severity Index. Após o 4º dia de abstinência observamos um aumento dos marcadores oxidantes em comparação ao fim do tratamento. Após dezoito dias de abstinência há uma recuperação das defesas antioxidantes. Também evidenciamos uma correlação positiva entre as proteínas carboniladas e variáveis psicológicas e uma correlação negativa entre os níveis de TRAP e as variáveis psicológicas. Dessa forma, nossos resultados sugerem que o período de abstinência pode propiciar uma recuperação das defesas antioxidantes, diminuindo assim, a propensão ao dano oxidativo.

Palavras-chave: Estresse oxidativo; Cocaína, Abstinência; Antioxidantes.

ABSTRACT

Crack is a cocaine-derived substance that acts as a stimulant of the central nervous system. It is characterized by low cost, quick action and high power dependency. Little is known about effects at the cellular level caused by the crack and body resilience of body during period of abstinence. Studies have shown an increased production of reactive oxygen species (ROS) after administration of cocaine. Oxidative stress occurs when there is an imbalance between the production of ROS and the ability of the antioxidant defense in combat or prevent its action. This condition can be harmful to cells, causing damage to biomolecules such as DNA, lipids and proteins. Aiming to better understand the action of crack and abstinence in redox state, this study enrolled thirty patients of program for detoxification and thirty volunteers who did not use drugs taken as control group. Blood samples were collected after 4th and the 18th day of treatment and plasma was used for biochemical analysis. Quantifications were performed oxidants markers such as protein carbonyl and protein thiols, both demonstrate protein modifications by ROS, and antioxidant markers such as glutathione peroxidase and superoxide dismutase and non-enzymatic markers such reduced glutathione (GSH) and total reactive antioxidant potential (TRAP). Psychological variables were assessed through the scores obtained Cocaine Selective Severity Assessment, Fagerstrom Tolerance Questionnaire, Beck Depression Inventory version II (BDI-II) and Addiction Severity Index. After four days of abstinence we observe observed an increase in oxidative markers compared to end of the treatment. After eighteen days of abstinence there is a recovery of antioxidant defenses. Also, we observed e a positive correlation between protein carbonyls and psychological variables and a negative correlation between the levels of TRAP levels and psychological variables. Thus, our results suggest that abstinence period may provide a recovery of antioxidant defenses, thereby reducing propensity for oxidative damage.

Keywords: Oxidative stress; Cocaine; Abstinence; Antioxidants.

SUMÁRIO

1. CAPÍTULO 1

1. INTRODUÇÃO

1.1 Drogas de Abuso: crack

A cocaína é classificada como um estimulante do sistema nervoso central (SNC). Seus efeitos são centrais e periféricos incluindo: euforia, falta de apetite, autoconfiança, insônia, aumento da energia seguida por ansiedade, depressão e fadiga (1). O crack é uma forma potente da cocaína, que quando fumado gera pequenas partículas que são rapidamente absorvidas nos pulmões Essa droga tem alto potencial estimulante e seus efeitos psicoativos aparecem em poucos segundos após o consumo (2).

Drogas de abuso são conhecidas por afetar a estrutura neuronal e funções de regiões cerebrais específicas, o que resulta em mudanças estáveis e persistentes a níveis celular e comportamental (3, 4) . O mecanismo primário de ação da cocaína ocorre pela inibição da recaptação de monoaminas como a norepinefrina, serotonina e dopamina (DA), pelos seus transportadores na fenda pré-sináptica (5). Os efeitos físicos e psicológicos da cocaína estão relacionados com o rompimento dos mecanismos de recompensa, tolerância, abstinência, desejo e recaída no sistema límbico do sistema nervoso central (6).

A prolongação dos efeitos das monoaminas ocorre no sistema mesocorticolímbico, o qual envolve a área ventral tegmental (VTA), núcleo accumbens e córtex frontal, sendo responsável pelas vias de recompensa, reforço e funções cognitivas superiores. Embora a cocaína tenha afinidade por esses três neurotransmissores, acredita-se que as propriedades de reforço e recompensa da cocaína são causadas pela elevação indireta de DA no canal pré-sináptico da VTA (7).

A Figura 1 representa a inibição da recaptação de DA pelos seus transportadores (7). Ainda que o crack alcance o cérebro rapidamente, os seus efeitos tem curta duração, fazendo com que os usuários fumem com maior frequência, o que leva ao rápido desenvolvimento de dependência (8).

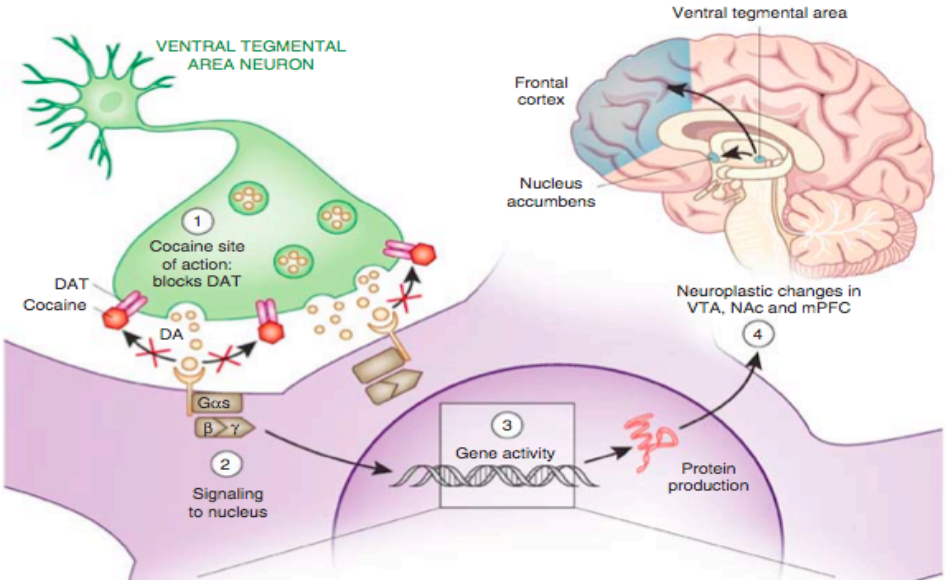

Figura 1: Bloqueio da recaptação de Dopamina na região mesocorticolímbica (figura adaptada) (7).

O uso de crack no Brasil vem sendo difundido desde o início de 1990, hoje estima-se que existam mais 1 milhão usuários ativos, o baixo custo e a facilidade do acesso são fatores facilitadores para o consumo, segundo a Secretaria Nacional de Políticas sobre Drogas (Senad) em parceria com a Fundação Oswaldo Cruz (FIOCRUZ) que realizaram levantamentos nacionais no ano de 2012 nas 27 capitais brasileiras, Distrito Federal e regiões metropolitanas. Esse levantamento recrutou 21 mil usuários das cenas de crack e demonstrou que a idade média desses indivíduos é de 30,28 anos, o sexo masculino é o predominante (78,68%) e 40% dos usuários eram moradores de rua (9). Atualmente é a dependência de crack é considerada um problema de saúde pública, sendo alvo de pesquisas e debates políticos.

A utilização da droga está mais concentrada entre indivíduos jovens e atinge todas as classes sociais. Algumas características são comuns entre os dependentes, tais como: morbidade mental, problemas de saúde e consumo de outras substâncias de abuso (10). Além dos danos físicos causados pela dependência ainda existe o impacto social, os usuários geralmente preferem o isolamento, abandonam emprego, suas casas e muitas vezes entram para a criminalidade, vivendo nas ruas e nas “cracolândias”.

Estudos recentes demonstram que a dependência de crack causa múltiplos danos cognitivos, prejudicando principalmente as habilidades executoras (11, 12). Usuários de drogas apresentam vários prejuízos neurológicos. Ao que diz respeito a cocaína, os

dependentes sofrem com convulsões, isquemia cerebral, hemorragia cerebral, infarto, neuropatias ópticas, atrofia cerebral, danos cognitivos, distúrbios de humor e equilíbrio(13).

Frequentemente o uso dessa droga encontra-se relacionado com o uso de outras substâncias como: álcool, tabaco, anfetamina, maconha e outros alucinógenos. Quando os usuários são submetidos a tratamentos de desintoxicação, há um aumento da ansiedade, depressão e do desejo pelo consumo da droga, que estão relacionados com mudanças de humor, emoção e cognição (14).

1.2 Estresse Oxidativo

Estresse oxidativo (EO) é definido como uma perturbação do equilíbrio entre componentes pró-oxidantes e antioxidantes em favor dos primeiros, gerando dano potencial. O EO é resultado de três fatores, que podem estar associados ou não: **(1)** aumento na geração de espécies reativas de oxigênio (EROS) e/ou de nitrogênio (ERN), através da acumulação de intermediários reativos; **(2)** prejuízo do sistema de defesa antioxidante (inibição de enzimas antioxidantes, depleção de antioxidantes não-enzimáticos); **(3)** incapacidade para reparar dano oxidativo (15).

As espécies reativas de oxigênio são assim classificadas, por possuírem elétrons desemparelhados na última camada eletrônica e spins antiparalelos, possuindo desta forma, alto potencial reativo. Elas em altas concentrações podem ser deletérias para as células, no entanto, quando estão em equilíbrio com o sistema antioxidante, participam da sinalização celular, fagocitose, inflamação, respiração celular e vasodilatação, no caso do Óxido Nítrico (espécie reativa de nitrogênio), suas funções benéficas estão representadas na Figura 2 (16).

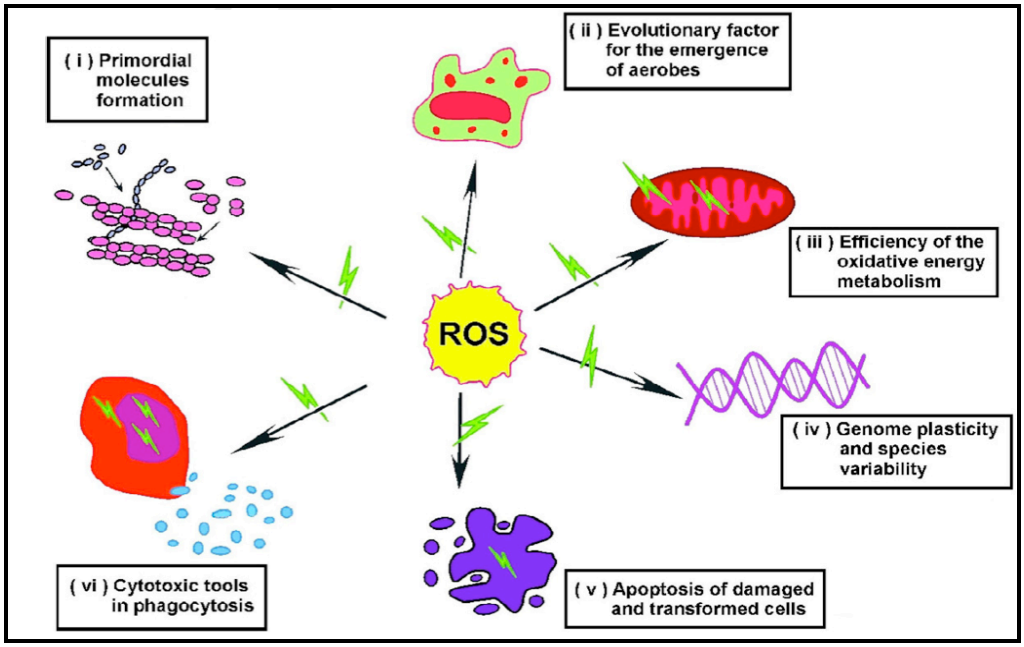

Figura 2: Funções benéficas das EROS (14).

Os sistemas biológicos oferecem condições favoráveis para ocorrência de reações de caráter oxidativo, devido à existência de lipídios insaturados, nas membranas celulares, e pela abundância de reações oxidativas que ocorrem durante o metabolismo basal (17). A suscetibilidade de uma célula ou de um tecido ao estresse oxidativo depende da disponibilidade de antioxidantes e a capacidade de inativação ou eliminação dos produtos oxidados formados.

1.3 Parâmetros Oxidantes

A formação endógena de EROS é uma característica do metabolismo de organismos aeróbicos. De 3 a 10% do oxigênio utilizado por tecidos são convertidos em intermediários reativos que podem ser prejudiciais para as células e tecidos (18). No entanto, a produção excessiva de EROS foi associado com injúrias teciduais e inúmeras patologias, tais como artrite reumatoide, diabetes, câncer, Alzheimer, Parkinson e obesidade, sendo também relacionado com o envelhecimento precoce (16). A condição de estresse oxidativo pode relacionar-se tanto com o desenvolvimento de patologias quanto como uma consequência das mesmas, refletindo em uma produção anormal e/ou uma deficiência na remoção de EROS, que tem sido relacionada como o mecanismo

chave que contribui para o dano tecidual e déficits funcionais em situações de hipóxia/isquemia cerebral e também esta intimamente associada com os danos causados pelas inflamações agudas e crônicas (19, 20).

Devido a cadeia de transporte de elétrons, a mitocôndria é considerada um dos principais sítios de geração de EROS via reação de Fenton, na qual é formado o radical hidroxil (HO•) (21). Sendo esse radical o mais nocivo para as células e o mais relacionado com os danos oxidativos referentes as espécies reativas de oxigênio (22).

O estresse oxidativo pode ocorrer no organismo a partir de um aumento na produção de EROS e uma resposta insuficiente ou ineficaz do sistema de defesa antioxidante, enzimático ou não, ocorrendo devido à carência de vitaminas e minerais, processos inflamatórios exacerbados, deficiências do sistema imune, situações de exercício intenso e condições exógenas (23-25). As principais fontes externas compreendem: exposição ao ozônio, radiação gama e ultravioleta, tabaco, medicamentos, consumo de substâncias tóxicas presentes em alimentos (aditivos químicos, hormônios, aflatoxinas) alto consumo de gorduras saturadas e consumo de bebidas alcoólicas (23, 26).

Existem evidências que o estresse oxidativo está relacionado com os processos patológicos e fisiológicos associados com a senescência (27). Havendo teorias que o estresse oxidativo decorrente do desacoplamento de reações de transporte de elétrons e do acúmulo dos níveis de metais, causam um aumento da produção de espécies reativas, oxidação de proteínas, elevação de proteínas inativas que associadas com a deficiência do sistema de degradação proteica, acumulam-se no organismo, levando a um desequilíbrio da homeostase (28). Além disso, ocorrem reações de auto-oxidação de substâncias endógenas, tais como catecolaminas e xenobióticos, e ainda há oxidação de metabólitos reduzidos, que também acabam por ser uma fonte de geração de EROS (29). As espécies reativas mais frequentes em metabolismos aeróbicos estão relacionadas na Tabela 1 (30).

Tabela 1: Caracterização das principais Espécies Reativas formadas *in vivo* (30).

Intermediário	Comentário	Meia-vida	Sítios de formação
Radical superóxido	Formado a partir da redução parcial do oxigênio molecular por 1 elétron.	Decomposição enzimática na velocidade aproximada de 5 x 10^5 $M^{-1}sec^{-1}$ em pH 7,0.	Reações de autoxidação envolvendo flavoproteínas e ciclos redox.
Peróxido de hidrogênio	Formado a partir da redução parcial do oxigênio molecular por 2 elétrons.	Decomposição enzimática.	Vias catalisadas por oxidases, e pela superóxido dismutase.
Radical hidroxil	Formado a partir da redução do oxigênio molecular, por 3 elétrons nas reações de Fenton e Haber-Weiss, catalisada por metais.	10^{-9} segundos	Locais adjacentes à formação de ânion superóxido/peróxido de hidrogênio na presença de metais, principalmente ferro; produto de reação do óxido nítrico com o radical superóxido.
Radical alcoxil	Radical orgânico centrado no oxigênio.	10^{-6} segundos	Intermediário na peroxidação de lipídios de membrana.
Radical peroxil	Formado a partir de hidroperóxidos orgânicos	7 segundos	Intermedário na peroxidação de lipídios de membrana.
Oxigênio molecular Simpleto. ($^1\Delta_g O_2$)	Primeiro estado excitado do Oxigênio Molecular com nível de energia de 22 kcal/moL acima do estado fundamental ou oxigênio tripleto ($^3O_2$).	10^{-5}segundos	Sem sítios metabólicos definidos.

1.4 Estresse Oxidativo, Cocaína e Crack

Estudos indicam que o alvo biológico principal da cocaína são os transportadores de dopamina (DAT), ao ligar-se a estes transportadores a cocaína interfere na recaptação deste neurotransmissor (31). O bloqueio da recaptação da dopamina na fenda sináptica faz com que seus níveis extracelulares aumentem de forma indireta, assim, a dopamina acaba por desempenhar um importante papel nos efeitos causados pela cocaína (32). Os efeitos físicos e psicológicos da cocaína ocorrem quando há o rompimento dos mecanismos de recompensa, abstinência, tolerância, desejo e recaída no sistema límbico do CNS (6).

A dopamina é removida da fenda via auto-oxidação ou por ação da enzima monoamina oxidase (33) que leva a produção de espécies reativas de oxigênio. Assim, a produção de EROS pode ter função relevante no metabolismo da dopamina quando a cocaína é administrada (34). Consistente com o mecanismo demonstrado através de um estudo da autoadministração de cocaína em ratos, onde foi verificado um aumento da

concentração de dopamina e também um aumento da oxidação deste neurotransmissor (35). Também existem estudos que relatam um aumento dos níveis de antioxidantes após a administração de cocaína, como uma tentativa de equilibrar a exacerbação da produção de EROS e evitar danos celulares (36). No entanto, no realizado por Muriach et al. (37) foi evidenciada uma depleção nas concentrações de glutationa reduzida (GSH) e uma diminuição da atividade enzimática da glutationa peroxidase (GPx) no hipocampo de camundongos expostos a cocaína. Tal redução nos níveis de GSH contribui para a perda da eficiência do sistema GABAérgico em atenuar os sinais neuronais excitatórios (38, 39).

O uso da cocaína também está relacionado com a supressão de células T citotóxicas, devido essa substância induzir o estresse oxidativo, uma vez que alterações de linfócitos T e citocinas vem acompanhadas da diminuição das defesas antioxidantes (40). Já foi demonstrado em modelos *in vitro* que a exposição aguda a cocaína de células neuronais progenitoras humanas eleva alguns biomarcadores de dano oxidativo (29, 32, 41). A intoxicação por cocaína leva ao aumento da peroxidação lipídica em várias estruturas cerebrais ligadas à síntese e liberação de dopamina (42).

As espécies reativas podem ter uma importante função no SNC durante situações patológicas nessa região, como elas produzem danos teciduais através de diferentes mecanismos, as EROS podem ser capazes de agravar doenças neurodegenerativas. Assim, o estudo acerca de terapias antioxidantes que possam atenuar o estresse oxidativo pode ser uma alternativa benéfica para controlar condições neurodegenerativas (43).

Recentemente, processos que envolvem transferência de elétrons, espécies reativas de oxigênio e estresse oxidativo estão sendo estudados para um melhor entendimento dos mecanismos que envolvem a toxicidade e a dependência de drogas de abuso (41). Esses estudos fundamentam o papel do estresse oxidativo como resultado da administração da cocaína, ficando evidente que o EO induz a morte celular no SNC (44-47) e a cocaína induz apoptose nos neurônios corticais (48). A lipoperoxidação, oxidação proteica, danos ao DNA e a inativação de enzimas podem ser responsáveis por alterar a função neuronal e causar injúrias que levam a diferentes patologias do sistema

nervoso (49). Tais evidências ressaltam a importância de mais estudos abrangendo o estado redox com o uso de drogas, principalmente entre dependentes do sexo feminino, visto que a maioria dos estudos são realizados com homens (50, 51).

1.5 Defesa Antioxidante

O balanço entre as espécies reativas e a prevenção do dano oxidativo é realizado pelo sistema de defesa antioxidante, que pode ser enzimático ou não enzimático e ainda através de respostas adaptativas (52). Antioxidantes são substâncias capazes de prevenir os efeitos deletérios da oxidação, inibindo o início da lipoperoxidação, sequestrando radicais livres e/ou quelando íons metálicos (53). Esse sistema age por meio de três mecanismos principais: prevenção da formação de ROS, eliminação de ROS já formadas e reparo de moléculas já modificadas pelas espécies reativas (52, 54).

Os mecanismos de defesa antioxidante enzimático compreendem as enzimas: (1) *Superóxido Dismutase (SOD)*: A qual é responsável pela dismutação do ânion superóxido (SO) em Peróxido de Hidrogênio (H_2O_2). Tal enzima existe sob a forma de três isoenzimas: SOD 1 (contém Cobre e Zinco como cofatores metálicos, localizada no citosol), SOD2 (contém Manganês presente nas mitocôndrias) e SOD 3 (contém Cobre e Zinco como cofatores, extracelular) (55). (2) *Glutationa:* família de enzimas que inclui Glutationa Peroxidase (GPx 1, 2, 3 e 4), Glutationa Transferase (GST) e Glutationa Redutase (GR). O sistema GSH tem função antioxidante essencial, sua depleção resulta em dano ao DNA e acúmulo de H_2O_2 . Durante a redução de H_2O_2 em H_2O e O_2 , a GSH é oxidada em GSSG pela GPx (56-58). (3) A enzima catalase tem como função principal eliminar peróxidos formados no peroxissoma, evitando assim a formação do radical hidroxil e ainda detoxificar diferentes substratos como fenóis e álcoois. (4) O sistema tioredoxina (Trx) compreende: Trx-1 (citosólica ou nuclear), Trx-2 (mitocondrial), Trx redutase (TrxR) e NADPH (59). Esse sistema está envolvido na proteção de diferentes tecidos contra danos oxidativos, manutenção da homeostase celular tiol-redox, controle do enovelamento de proteínas, regulação do crescimento celular e apoptose e ainda está relacionado com a regulação do sistema imune (60-62).

1.5.1 Superóxido Dismutase

A SOD 1 (Cu-Zn), assim chamada por ser a primeira descoberta, tem como função catalisar a reação do desproporcionamento do radical superóxido em oxigênio e peróxido de hidrogênio, aumentando em até 10^4 a velocidade da reação em comparação com a reação que acontece em pH fisiológico(25, 63).

Apesar da Mn-SOD ser uma isoforma mitocondrial, seu gene (SOD2) é codificado pelo DNA nuclear. O gene da SOD 2 possui um mecanismo regulatório, o qual pode ser induzido sob condições de inflamação e estresse oxidativo (64). A SOD 3 que codifica a forma extracelular (EC-SOD) é altamente expressa nos vasos sanguíneos, coração, pulmão, rins e placenta (65). A EC-SOD possui uma importante função biológica, ela é a única enzima responsável pela conversão do ânion superóxido no meio extracelular, protegendo os tecidos de possíveis injúrias causadas por essa espécie reativa. A atividade enzimática da EC-SOD foi detectada em linfócitos, plasma e líquido sinovial, o que a torna distinta das outas isoformas de superóxido dismutase (66).

1.5.2 Glutationa

A glutationa reduzida (GSH) é um tripeptídeo composto por glicina, ácido glutâmico e cisteína. É o tiol não proteico mais abundante em células animais, sendo responsável pela manutenção das reações de redução-oxidação (balanço redox) e por atuar nas vias de sinalização envolvidas com o processo de EO, tendo assim grande importância nas funções fisiológicas inter e intracelulares (67).(67). A GSH detoxifica peróxidos e compostos eletrofílicos através das ações catalíticas das isoformas S-transferases (GST) e Peroxidase(GPx) (68, 69). A GSH também atua nos processos de detoxificação de xenobióticos e metais pesados (70) regulação de proteínas e expressão gênica via tiol (reações de trocas dissulfídicas), participa do processo enzimático do sistema glioxalase e ainda da redução de ribonucleotídeos a deoxiribonucleotídeos (71).

Dentro das células a glutationa pode estar livre ou ligada a proteínas, a forma livre está, em sua maioria, sob forma reduzida. A glutationa redutase reverte a forma

livre em forma oxidada (GSSG) e a proporção entre a glutationa reduzida e oxidada frequentemente é utilizada como marcador da toxicidade celular (70). Alterações na expressão e na atividade da glutationa reduzida já foram relacionadas com envelhecimento e câncer (72).

Quando há uma diminuição da GSH e um aumento da GSSG pode-se dizer que existe uma propensão a condição de estresse oxidativo devido ao desequilíbrio redox (73, 74). Em condições normais, a razão GSH:GSSG pode ultrapassar 1:100, no entanto, em modelos para estresse oxidativo essa taxa pode diminuir para 1:10 ou até mesmo 1:1 (75). A manutenção do equilíbrio dessas taxas é essencial para a sobrevivência celular, sendo que uma deficiência de GSH deixa as células vulneráveis ao dano oxidativo. Estudos demonstram que a razão GSH/GSSG está diminuída em doenças neurodegenerativas como Parkinson e Alzheimer (57, 61) onde conforme a evolução da doença há um decréscimo dos níveis de GSH e um aumento dos níveis da razão GSH/GSSG.

A glutationa peroxidase (GPx) faz a redução do peróxido de hidrogênio (H_2O_2) a água, usando GSH como doador de elétron, logo a glutationa oxidada é reduzida a GSH novamente pela glutationa redutase usando NADPH (nicotinamide adenine dinucleotide phosphate-oxidase) como doador de elétron (76). Dessa forma, a GPx atua juntamente com a SOD e a catalase para evitar danos oxidativos nas células. Essa enzima pode existir sob quatro isoenzimas que atuam em diferentes tecidos. A cGPx que é a forma clássica está presente em todos os tecidos, GI-GPx presente apenas em células gastrointestinais, pGPx presente no plasma, a PHGPX é a fosfolipídeo hidroperóxido protege as membranas fosfolipídicas (56).

1.5.3 Defesa não enzimática

A defesa antioxidante não enzimática é composta principalmente pelas vitaminas C (ácido ascórbico) e E (α-tocoferol), diferentes compostos do selênio, ácido lipóico, albumina e ácido úrico (77, 78). Esses compostos podem ser tanto provenientes

da dieta quanto sintetizados pelo próprio organismo e atuam como *"scavengers"* de radicais livres e ainda protegendo as células dos danos causados por substâncias tóxicas, como o Cádmio (79, 80). A vitamina E é uma molécula lipossolúvel e tende a se concentrar no interior das membranas, agindo sinergicamente com ascorbato, estabilizando o processo de lipoperoxidação. Os carotenoides também se destacam como antioxidantes; estudos recentes indicam uma possível atividade antitumoral do β-caroteno, sendo usado como corante e antioxidante em alimentos (24).

1.6 Avaliações Clínicas

Além dos danos fisiológicos causados pela dependência do crack, existe uma relação com danos psicológicos e sociais, tendo em vista que os usuários dependentes apresentam características como depressão, déficit cognitivo, alterações na atenção, isolamento social, comportamentos transgressores e altos índices de desemprego. Assim, mensurar o grau desses impactos é tarefa fundamental na investigação dos usuários.

Através da aplicação do *Cocaine Selective Severity Assessment*, o qual avalia os sintomas de abstinência, é possível mensurar a severidade da dependência de cocaína. Tais sinais são considerados marcadores importantes para guiar o tratamento dos usuários bem como para avaliar a sua efetividade (81-83). Os sintomas comumente encontrados durante o cessar do uso são: distúrbios do sono e apetite, depressão, ansiedade, irritabilidade, diminuição da frequência cardíaca, fadiga e desejo pelo consumo da droga. Porém, esses sintomas podem variar de indivíduo para indivíduo (84) e podem estar relacionados com o início precoce da dependência e também com o uso concomitante de outras substâncias (85).

Usuários crônicos de crack apresentam grande dificuldade na adaptação social e déficits para tomar decisões, quando jovens apresentam problemas na escola e com a família enquanto que usuários adultos demonstram problemas com o emprego (86, 87). Além disso, existe a influência dos maus tratos na infância os quais estão associados com

uma maior propensão ao abuso de drogas, relacionadas com prejuízo nas funções executoras (12) e ainda uma elevação dos sinais de depressão e abstinência (88).

Desse modo, a aplicação do *Beck Depression Inventory (BDI-II)* (89), torna-se uma importante ferramenta para avaliar os sintomas depressivos, relacionados com a dependência de crack e outros tipos de drogas. Da mesma forma, através do *Addiction Severity Index (ASI-6)* (90, 91) pode-se abranger aspectos psiquiátricos, ocupacionais, sociais e familiares relacionados com a severidade da dependência. Ao conciliar essas abordagens é possível correlacionar variáveis psicológicas e biológicas, para facilitar o entendimento dos mecanismos que levam à dependência juntamente com as consequências do uso de drogas e os resultados obtidos com o tratamento.

2. JUSTIFICATIVA

Por ser uma droga com alto potencial estimulante e de menor custo, o crack tem sido cada vez mais difundido entre os usuários de drogas. O uso dessa droga leva à disfunções psicológicas e neuropsicológicas, alterando funções cognitivas e executoras, causando déficit de memória, atenção e aprendizado bem como pode afetar a fluência verbal. Além disso, dependência dessa droga é caracterizada como sério problema de saúde pública com consequências sociais, econômicas e políticas.

O estresse oxidativo é uma condição relacionada com inúmeras patologias, morte celular e danos teciduais e quando relacionado ao abuso de drogas, as respostas são contraditórias e as vias com que substâncias como a cocaína induzem o estresse oxidativo tanto no SNC quando em órgãos periféricos ainda não está bem definida. Os estudos anteriores sobre crack-cocaína avaliaram somente biomarcadores oxidantes, sem considerar os efeitos da droga sobre mecanismos antioxidantes.

No âmbito de ampliar os conhecimentos sobre os danos causados pelo uso de drogas ilícitas, o presente estudo visa avaliar de forma compreensiva os danos oxidativos, o potencial antioxidante e correlacioná-los com variáveis clínicas obtidas através de avaliações comportamentais das pacientes.

3. OBJETIVOS

3.1 Objetivo Geral

Avaliar parâmetros plasmáticos oxidantes e antioxidantes de usuárias de crack em processo de desintoxicação em diferentes períodos do tratamento, comparando-os com grupo controle e correlacionar as análises bioquímicas com as variáveis clínicas.

3.2 Objetivos Específicos

- ✓ Verificar a ocorrência de carbonilação proteica.
- ✓ Avaliar os níveis de tióis reduzidos totais.
- ✓ Verificar o potencial antioxidante não enzimático total.
- ✓ Analisar a atividade enzimática da Superóxido Dismutase.
- ✓ Analisar a atividade da enzima Glutationa Peroxidase.
- ✓ Avaliar os níveis de tióis não proteicos (GSH).
- ✓ Correlacionar variáveis bioquímicas com as variáveis clínicas relacionadas a Dependência Química durante o processo de desintoxicação e comparar com grupo controle sem dependência de drogas.

2. CAPÍTULO 2

2.1 ARTIGO CIENTÍFICO

EARLY ABSTINENCE OF CRACK-COCAINE IS EFFECTIVE TO ATTENUATE OXIDATIVE STRESS AND TO IMPROVE ANTIOXIDANT DEFENCES

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Conflicts of interest

The authors declare no conflict of interests.

Abstract

Rationale Preclinical studies have shown that cocaine exposure and withdrawal are associated with cellular oxidative stress damage. However, the impact of crack-cocaine dependence on oxidative stress biomarkers remains unclear. Here, we assessed peripheral oxidative stress and antioxidant defences during two periods of crack-cocaine detoxification treatment and associated these changes with psychological morbidity. *Methods* Thirty female inpatients were recruited and plasma samples were collected at the 4^{th} and 18^{th} days of abstinence; thirty healthy controls were also recruited. Plasma levels of protein carbonyl, protein thiol content, superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced reduced (GSH) and total reactive antioxidant potential (TRAP) were measured by standard methods; the questionnaires Cocaine Selective Severity Assessment, Beck Depressive Inventory and the Addiction Severity Index were applied. *Results* We report higher oxidative stress damage after four days of detoxification, as shown by increased total thiol content and protein carbonylation when compared with control group and after eighteen days of detoxification. After 18 days of treatment we observed a recovery of the oxidative stress damage and increase of the antioxidant defences, as shown by higher levels of SOD, GPx, GSH and TRAP. There was a positive correlation between protein carbonylation and psychological variables; in contrast, there was a negative correlation between TRAP levels and clinical assessments. *Conclusions* Taken together, these results suggest that drug rehabilitation treatment was effective in decreasing oxidative damage represented by the reduction in biological markers, which are closely related to the severity of withdrawal symptoms.

Keywords: Redox state; Cocaine; Drug withdrawal; Oxidative stress; Antioxidants.

Introduction

Chronic cocaine consumption is one of the most severe forms of addiction, especially regarding crack-cocaine use. In this case, the substance quickly reaches the brain and causes rapid dependence (92). This places individuals at high risk for criminal behavior, social isolation, early mortality and unemployment (93, 94), indicating that crack-cocaine dependence is an important social and economic problem.

Mounting evidence suggests the role of oxidative stress (OS) in the pathogenesis of psychiatric disorders, including drug addiction (41, 50, 95). The cocaine short-term effects leads to increased dopamine levels in the synaptic cleft in the brain reward system, but accumulation of not recaptured dopamine can cause cellular stress (96). In this context, dopamine clearance occurs via auto-oxidation or monoamine oxidase, which leads to increased production of reactive oxygen species (ROS) and cellular damage in various tissues (34). Preclinical studies have reported increased OS markers due to chronic drug exposure, including abuse of alcohol, nicotine, cocaine, opioid, and methamphetamine (36, 41, 97). Daily cocaine consumption leads to OS in the brain, as suggested by increased levels of superoxide dismutase and lipid peroxidation (42). Thus, OS responses can be an important mechanism for generating or even intensifying cocaine toxicity in the brain and peripheral organs, such as heart and liver (37, 98).

The imbalance between the production of ROS and antioxidant defences in favour of first is known by oxidative stress (OS). The OS responses can cause protein oxidation, DNA damage, and several enzyme impairments producing detrimental effects at both cellular and systemic levels (95). ROS is regularly produced during metabolic and physiological processes, and harmful oxidative reactions can occur in organisms if the antioxidant system is unable / insufficient to prevent or repair the damages caused by these molecules. Therefore to better estimate the balance of OS in blood samples both oxidative and antioxidative biomarkers (enzymatic and non-enzymatic) should be determined. Protein oxidation products measured by protein carbonylation and protein thiol modifications can estimate the oxidative damage. Carbonyl groups are formed when protein side chains are oxidised or by oxidative cleavage of proteins and this modifications are related with several diseases and psychological stress (99, 100), and protein thiol groups are formed through oxidative modifications in protein cysteines which may interfere with biological functions (101). Estimation of reduced

glutathione (GSH) and total reactive antioxidant potential (TRAP) are used to evaluate the non-enzymatic antioxidant capacity. Reduced glutathione is the most abundant thiol in mammalian cells and acts in the cellular protection against oxidative damage (67). Kovacic and colleagues (41) reported that decrease or depletion of glutathione levels occurs in different tissues after cocaine administration in the mouse. TRAP represents global potential antioxidant non-enzymatic and has already been linked with depression and nicotine dependence, showing decreased levels in smokers compared with never smokers (102). Enzymatic antioxidant capacity is represented here by the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx). SOD plays important biological role catalysing the dismutation of superoxide into oxygen and hydrogen peroxide. GPx converts peroxides and hydroxyl radicals into non-toxic forms by concomitant oxidation of GSH into the oxidised form, glutathione disulphide (GSSG)(22). During cocaine exposure, both enzymes show different results regarding their activities depending on the tissue analysed and treatment characteristics (36, 37, 40).

There is a scarce literature, however, regarding OS and crack-cocaine dependence, and discrepancies were reported by recent clinical studies focused on crack-related effects on oxidant biomarkers (50, 103). To extend previous studies and to provide a comprehensive assessment of OS response, we evaluated both enzymatic and non-enzymatic antioxidant markers and markers of oxidative damage in the peripheral blood of women with crack-cocaine dependence during early drug withdrawal stage, a critical period for cocaine treatment response (104). We also investigated the relationships between OS response and clinical variables during early abstinence.

Materials and Methods

Subjects

This follow-up study included a group of crack-cocaine dependent women during three weeks of early abstinence and a healthy control (HC) group. All parameters investigated were assessed in two moments, at the first (4^{th} day) and at third week (18^{th} day) after the beginning of detoxification treatment, and were compared with HC. The local institutional review board (IRB) approved the study, which is in accordance with the Helsinki Declaration of 1975. All volunteers provided their written consent following the explanation about the procedures involved in the research protocol.

The HC was recruited by convenience composed of thirty women aged 18 to 45 (mean age = 29.56±7.20 years), without medication use, acute or chronic diseases. Thirty female inpatients with crack-cocaine dependence were recruited from a public psychiatric hospital in Porto Alegre, Brazil. Participants had no access to drugs; alcohol or cigarettes and benzodiazepines were not prescribed during treatment. The inclusion criteria were as follows: (1) women aged 18 to 45; (2) primary diagnosis of cocaine dependence according to the Diagnostic and Statistical Manual of Mental Disorders IV-TR. The exclusion criteria were: (1) current infectious diseases or history of autoimmune, endocrine or coronary heart disease, rheumatoid arthritis, or neurological disorders; (2) pregnancy; (3) past or current psychotic disorders; and (4) the use of corticosteroids, antibiotics or anti-inflammatory drugs..

Clinical assessments

The Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders (SCID I) was applied to confirm the diagnosis of cocaine physiological drug dependence, as well as comorbid psychiatric diagnoses. Cocaine Selective Severity Assessment (CSSA) assessed withdrawal symptoms severity, which is an 18-item interviewer-administered questionnaire. Items are measured on a 7-point scale and the maximum score is 112. Beck Depressive Inventory (BDI) assessed depressive symptoms severity. Cocaine dependence severity was measured by Addiction Severity Index (ASI-6), which is a semi-structured interview and clinical/research instrument to assess several dimensions of the severity of substance use problems and patterns of substance use behavior (quantity, severity and frequency), resulting in a final score of cocaine drug dependence severity (90, 105). Expert psychologists and a senior psychiatrist administered all questionnaires.

Blood collection and plasma isolation

Two samples of five millilitres of peripheral blood were collected by venepuncture and stored in EDTA tubes prior to analyses. Since detoxification consisted in 21-day program, the first sample was collected on the 4th day of the detoxification period while the second one at the 18th day. Immediately after blood collection, the samples were centrifuged at 3000g during five minutes in order to obtain the plasma.

Biochemical analyses

To estimate the protein content was measured by the modified Lowry protein assay previously described by Lowry et al. (106). The amount of soluble protein in plasma sample was measured using the DC Protein Assay Kit according to the manufacturer's instruction (Bio-Rad Life Science), to allow expression of the biochemical results taken into account the protein content of each sample and was expressed in mg. All measures of colorimetric assays performed in this study were obtained with the SpectraMAX 190 (Molecular devices) spectrophotometer equipped with software Softmax.

Protein carbonylation

Quantification of carbonyl groups was measured as a parameter of protein oxidative damage. This technique is based on the reaction with dinitrophenylhidrazine (DNPH), as previously described (107). Proteins were precipitated by the addition of 20% trichloroacetic acid (TCA) and re-solubilized in DNPH. Then, the absorbance was read in a spectrophotometer at 370nm. Results are expressed as nmol carbonyl/mg protein.

Protein thiol content

The main objective of this assay is to analyse oxidative alterations in proteins, measured as the level of protein thiol content in plasma samples. Briefly, for total SH content measurement, an aliquot of sample was diluted in PBS 10 and 10 mM 5,5- dithionitrobis-2-nitrobenzoic acid in ethanol and the yellow color formed was read in a spectrophotometer at 412 nm after 60 min incubation at 25 ºC. Results are expressed as mmol SH/mg protein (108).

Estimation of reduced glutathione

Reduced glutathione is most abundant non-protein cellular antioxidant, which together glutathione disulfide (GSSG) modulates intracellular redox (Townsend et al., 2008). For GSH content, TCA solution (10% v/v) was added to an aliquot of plasma sample, centrifuged (10 000 g, 10 min), and the supernatants were used to measure the level of SH. The absorbance was analysed in a spectrophotometer at 412 nm after 60 min of addition of DTNB solution. The results are expressed as mmol GSH/protein (108).

Total reactive antioxidant potential

Total reactive antioxidant potential was used as an index of non-enzymatic antioxidant capacity and represents the total antioxidant capacity of the sample. This assay is based on the peroxyl radical (generated by AAPH solution, 2,2azobis[2-amidinopropane], with luminol) and it was used as a free radical source quenching by sample compounds. Luminescence detection of samples in microplate was counted by MicroBeta® JET. Sample addition decreases the luminescence proportionately to its antioxidant potential (109). As previously described by Dresch et al. (110), results were transformed in percentage and the area under the curve (AUC), which is inversely proportional to antioxidant capacity. Because of this was the last assay performed, we did not have enough samples for the assessment of control group subjects.

Antioxidant enzymes activities

We analysed two antioxidant enzymes activities, superoxide dismutase and glutathione peroxidase, both act as scavengers of ROS. Superoxide dismutase (SOD, EC 1.15.1.1) was assessed by quantifying the inhibition of superoxide-dependent adrenaline auto-oxidation in spectrophotometer at 480 nm and is expressed as Units SOD/mg protein (Misra and Fridovich, 1972). Activity of glutathione peroxidase (GPx, EC 1.11.1.9) was determined according to Wendel (111), using t-butyl hydroperoxide and GSH as substrates with further colorimetric reaction analysed in at spectrophotometer at 340 nm. Results are expressed as Units GPx/mg protein.

Statistical analyses

All variables presented normal distribution (tested by the Shapiro-Wilk test). Analyses of Variance (ANOVA) followed by Tukey's post-hoc test were performed to compare biochemical markers between crack-cocaine group at the first and at the third week of detoxification treatment, as well as control group reference values. Between groups comparisons for socio-demographic characteristics were performed using t-tests for independent samples. Paired t-test was also performed to compare TRAP measurements of clinical subjects between the first (4^{th} day) and the second assessment (18^{th} day). Specifically regarding TRAP measures; it was assumed an inverse relationship between raw data from the area under the curve and TRAP levels, which means that higher raw data represented lower antioxidant capacity. Exploratory correlation analyses between biochemical markers and

clinical parameters were performed using Pearson correlation coefficient. The significance level was set at $\alpha = 0.05$ (two-tailed). Statistical analyses were performed using SPSS 20.0 (IBM SPSS, Chicago, IL, USA). All values are presented as mean and SD.

Results

Sample characteristics

Socio-demographic characteristics of groups and clinical characteristics of crack-cocaine group are shown in Table 1. Regarding the clinical group, between-time-point comparisons of revealed a significant reduction in withdrawal ($t = 2.21$; $p = 0.05$) and depressive symptoms according to CSSA and BDI scores. In addition, there were no significant effects of use of medications and comorbid psychiatric diagnoses on plasma levels of biochemical markers (all $p > 0.05$, data shown in supplementary material table 1).

Effect of detoxification on protein damage

We found significant differences in protein carbonyl levels between groups, $F(2,57) = 11.87$; $p < 0.001$). Post hoc analyses revealed significant differences between HC and clinical group at the first week of treatment ($p < 0.001$) and among the first (4^{th} day) and second assessment (18^{th} day) within crack-cocaine group ($p = 0.007$). No differences were found between HC and clinical group at 18 days of detoxification ($p= 0.45$) (Figure 1a). Similarly, thiol content differed between groups, $F(2,87) = 152.11$; $p<0.001$, with significant differences between HC and crack-cocaine group at the first assessment ($p<0.001$) and at the second assessment ($p<0.001$), as well as within the first assessment and second assessment of crack-cocaine group ($p<0.001$) (Figure 1b). These results demonstrate that at the beginning of detoxification clinical participants exhibited higher levels of protein modifications in comparison to levels assessed in HC. After 14 days of detoxification treatment, carbonyl levels were similar among clinical and control participants, suggesting a decrease in protein carbonylation during crack-cocaine acute abstinence. Thiol content levels also significant reduced within clinical participants during detoxification, but not reached the lower levels observed in HC participants.

Effect of detoxification on antioxidant defences

Figure 2 shows the TRAP kinetics. At the first week of detoxification, crack-cocaine participants exhibited lower antioxidant capacity. Drug abstinence was associated with significant increase in the TRAP antioxidant capacity ($t = 4.61$; $p = 0.01$).

We observed significant differences in SOD levels ($F(2,87) = 91.13$; $p<0.001$) and post hoc analysis indicated significant differences between-time-point comparisons of clinical group and regarding HC group reference values (all $p = 0.01$) (Figure 3a). The GPx levels differed between groups ($F(2,73) = 73.96$; $p<0.001$) and post hoc analysis indicated differences between HC and first assessment of clinical group ($p<0.001$), as well as HC levels and second assessment of clinical group ($p<0.001$), while within clinical group comparisons no significant differences were found ($p=0.27$) (Figure 3b). The GSH levels also differed between groups ($F(2,87) = 12.29$; $p<0.001$) and post hoc tests revealed higher levels at the second assessment compared to first assessment within clinical group ($p=0.05$), and lower levels than HC ($p<0.001$) (Figure 3c). These results suggest an intensification of antioxidant defences at the end of detoxification treatment (day 18).

Correlations between plasma levels of biochemical markers and clinical assessment

Correlation analysis revealed associations between levels of oxidative stress markers and clinical variables. The SOD levels at the end of treatment period were negatively associated with depressive symptoms severity ($r = -0.46$; $p = 0.01$), withdrawal symptoms severity ($r = -0.39$; $p < 0.05$) and with the ASI score of severity of drug dependence ($r = -0.41$; $p < 0.05$). On the other hand, protein thiol content levels at the beginning ($r = 0.44$; $p = 0.01$) and at end of detoxification ($r = 0.49$; $p = 0.006$) were positively correlated with depressive symptoms severity. Similarly, protein carbonyl levels were positively associated with the ASI score of severity of drug dependence ($r = 0.56$; $p < 0.05$).

Discussion

This study investigated the redox state and associated it with clinical morbidity during early abstinence of crack-cocaine dependence. Briefly, women with crack-cocaine dependence showed higher levels of plasma biomarkers implicated with OS damage at first

week of abstinence, in comparison to higher levels of antioxidants biomarkers at the third week of abstinence. These data indicate an increased susceptibility to oxidative stress in the initial phase of detoxification treatment within women with crack-cocaine addiction. This is particularly important since recent epidemiological data support important sex differences regarding crack-cocaine dependence, showing that men are more likely to this drug use, while female users report higher craving severity and are more vulnerable to develop dependence (112).

Levels of protein carbonyls of clinical group decreased significantly over the treatment days, coming closer to levels of the healthy control group, suggesting crack-cocaine use can cause protein oxidation and over the days of detoxification treatment this condition is restored. In addition, protein thiol content also decreased significantly following the 3-week detoxification program when compared crack-cocaine users with HC and after four days of detoxification treatment. Thiol groups play an important role in stabilization, regulation, cell signalling and function of protein structures and ROS is already known to modify these groups (101, 113). Redox changes of several proteins and enzymes were identified in a variety of conditions, and correlated effects included cellular defence mechanisms for oxidative stress (58, 67). Taken together, these data suggest that many tissues could be affected by oxidative modifications in intracellular proteins and drug addiction may exacerbate these clinical conditions.

Furthermore, our data suggest that detoxification was capable in modifying both enzymatic and non-enzymatic antioxidant defences, as indicated by the increased GSH levels after treatment. GSH content could enhance the GSH/GSSG ratio and decrease lipid peroxidation. Lipton et al. (114) reported that chronic exposure to cocaine in rodents results in reductions in the level of glutathione and causes an imbalance between ROS and antioxidant concentrations. Interestingly, these data suggest a correlation between the periods of treatment with increased levels of this antioxidant. These findings are in accordance to previous preclinical studies using cell culture and animal models that have demonstrated cocaine-induced oxidative damage (42, 115). The primary enzymatic antioxidant protecting the body involves, among others, the enzymes superoxide dismutase and glutathione peroxidase. Significant increases were found in the SOD and GPx activity compared to the same patients after 18 days in absence of drugs. These enzymes act synergistically to control ROS actions: the SOD acts mainly hydrophilic regions while the GPx system protects hydrophobic regions

(116). Our study found positive association between increased levels of SOD and GPx with third week after the beginning of detoxification treatment.

At the same time, the measures of TRAP were found increased at the end of detoxification period, suggesting a recovery of non-enzymatic antioxidant defences when the drug is not consumed. This is a measure of global non-enzymatic antioxidant defences and covers uric acid, bilirubin, ascorbic acid, alpha-tocopherol and remaining antioxidants which are active free radical scavengers (25). Non-enzymatic antioxidants are considered first line of defence against lipid peroxides that attack cellular membranes when there is an exacerbation in ROS production (117). This is a first study that correlated total reactive antioxidant potential and drug addiction.

Pomierny-Chamiolo et al., (36) showed elevation of SOD activity in several areas in the brain after cocaine self-administration and the association between OS biomarkers in motivational process related to voluntary cocaine intake in rats. Recently a case control study conducted by Narvaez et al., (50) found no significant differences in TBARS when compared with users of crack-cocaine and healthy control group. However, Dietrich et al., (42) demonstrated in their study an increase in ROS production in frontal cortex and striatum after acute and chronic cocaine exposure. Many studies demonstrate that chronic use of different substances of abuse may increase biomarkers of oxidative stress, and this exacerbation of ROS may lead to apoptosis and cell damage in the brain (36, 41, 97). In addition, other researchers have shown a positive correlation between TBARS and severity of crack-cocaine use and a negative correlation between brain-derived neurotrophic factor (BDNF) and crack-cocaine use, suggesting these biomarkers may be indicative for severity of drug use (103). We already showed that crack-cocaine dependence is associated with higher BDNF plasma levels than healthy subjects during early drug abstinence, and such values remained stable over three weeks of detoxification treatment (118). High concentrations of ROS can affect processes closely linked with addiction, such as neuromodulation, transcription and transport of ions. Antioxidants can act eliminating (antioxidant enzymes) and protecting (antioxidant vitamins) the brain from the action of ROS (29).

Nevertheless, there are discrepancies between acute and chronic drug administrations that were reported for enzymatic activities with increased levels in acute administration, which may indicate a compensatory mechanism against the harm caused by drugs. In addition, previous findings in crack-cocaine and OS focused basically in oxidant biomarkers

individually and disregarded the effects of crack-cocaine upon antioxidant biomarkers (50, 103). However, exacerbation of ROS is associated with insufficient response of the antioxidant system, showing that it is important to evaluate both mechanisms to fully demonstrate OS (24). Furthermore, such studies drowned conclusions about OS based only on levels of lipid peroxidation, measured by the thiobarbituric acid reactive substances. Limitations of TBARS assay are widely known and this test is suggested to have poor specificity, often leading to overestimation of the levels of malondialdehyde in human fluids (e.g. plasma and biological tissues) (119, 120).

Here, correlation analyses indicated negative association between SOD activity with withdrawal symptoms severity and severity of drug dependence in the third week of treatment, highlighting the importance of this antioxidant enzyme and relating its activity with psychological factors. Moreover, protein carbonyl levels were positively associated with the severity of drug dependence. Although potential mechanism underlying this association remains unclear, these data suggest that crack-cocaine abstinence improves the enzymatic antioxidant defences and this use might cause oxidative damage in proteins.

One of the limitations of our study is the inclusion of participants who were also using tobacco and alcohol before detoxification treatment. However, it is known that crack-cocaine use is intimately linked with the use of other drugs (121). In addition, our data should be interpreted carefully due to large number of participants that used pharmacotherapeutic adjuvant treatment, even though no significant effects of medication were found on oxidative stress markers. Future studies should include both men and women subjects. The differences between females and males found regarding patterns of substance use, craving and abstinence symptoms severity, are probably due neurobiological specificities related to drug addiction (122, 123). For instance, female gonadal hormones seem to selectively influence regional dopamine neurotransmission, affecting the sensitivity to drug rewarding/aversive effects and certain aspects of drug-seeking behavior (123). Furthermore, regarding antioxidant defences, women had higher levels of reduced glutathione, total glutathione and vitamin E than men before antioxidant supplementation (124). Considering the exploratory nature of this study the authors acknowledge the relatively small sample size that could impact statistical power of the findings. Nevertheless, it is difficult to recruit participants to a follow-up study, particularly drug dependents. In this sense, even though previous investigations on cocaine and oxidative stress had larger samples, they do not completed repeated clinical and biological assessments

as herein performed, using only cross-sectional design. These data may help to understand the way the body recovers from oxidative damage caused by crack-cocaine. Future studies with longer follow-up and larger sample sizes are necessary to confirm these findings.

Conclusions

In summary, the present study demonstrates that crack-cocaine users are prone to the condition of oxidative stress, closely related to the severity of withdrawal symptoms. Treatment for detoxification may be effective for the partial recovery of oxidative stress, as well as both enzymatic and non-enzymatic antioxidant defences that despite not reaching the same levels of HC have a tendency to recovery of antioxidant defences.

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Figure Legends

Fig. 1 Effect of detoxification on protein damage. **a** Protein carbonyl and ***b*** Protein thiol modifications (SH). After early abstinence exists a decrease of damage on proteins and a tendency to reach the levels of the healthy control group. Data are shown as mean ± SE. Statistical significant group difference are indicated *p<0.05 and **p<0.01.

Fig. 2 Total reactive antioxidant potential (TRAP) kinetic graph **a**. A free radical source (AAPH) generating system produces peroxyl radical at a constant rate and the effect of antioxidants on free radical induced chemiluminescence that is measured as area under curve for 60 minutes. **b** The area under curve of total reactive antioxidant potential (arbitrary units). It was assumed an inverse relationship between raw data on the area under the curve and its concentration levels, which means that higher raw data represents a lower antioxidant capacity. Data are shown as mean ± SE. Statistical significant group differences are indicated **p<0.01.

Fig. 3 Effect of detoxification on antioxidants. ***a*** Enzymatic activity of Superoxide Dismutase (SOD). **b** Enzymatic activity of glutathione peroxidase (GPx) **c** Estimation of reduced glutathione (GSH). After early abstinence an increase of antioxidants biomarkers, suggesting that abstinence may restore the activity of free radical scavengers. Data are shown as meand ± SE. Statistical significant group differences are indicated *p<0.05, **p<0.01.

Table 1. Socio-demographic and clinical characteristics of sample.

Socio-demographic	**Crack Cocaine Group**	**Control Group**	**Statistics**
Age	29.17 (8.72)	29.56 (7.20)	(t = 0.19; p = 0.849)
Years of formal education	7.42 (2.69)	12.26 (2.08)	(t = 7.77; p = 0.001)
Pharmacotherapy – n (%)	19 (63.3)		
Mood stabilizers/Anticonvulsants	19 (63.3)		
Antipsychotics	20 (66.7)		
Antidepressants	1 (3.3)		
Psychiatric comorbidities – n (%)			
Mood disorders	2 (6.7)		
Anxiety disorders	6 (20)		
Age of first drug use			
Alcohol	16 (4.71)		
Cocaine	18.76 (6.26)		
Crack	22.72 (7.33)		
Cocaine withdrawal			
CSSA total score 4° day	53.68 (20.16)		
CSSA total score 18° day	45.63 (14.93)		
Depressive symptoms			
BDI total score 4° day	27.06 (17.51)		
BDI total score 18° day	17.40 (13.61)		
Nicotine dependence severity			
Fargestron total score	4.75 (3.99)		
ASI-6			
Drugs	62.74 (11.46)		
Days of abstinence prior to treatment enrollment	2.44 (5.06)		

Data shown as mean (SD) or n (%) when indicated. Abbreviations: ASI, The Addiction Severity Index; BDI, Beck Depression Inventory; CSSA, Cocaine Selective Severity Assessment

.

Fig. 1

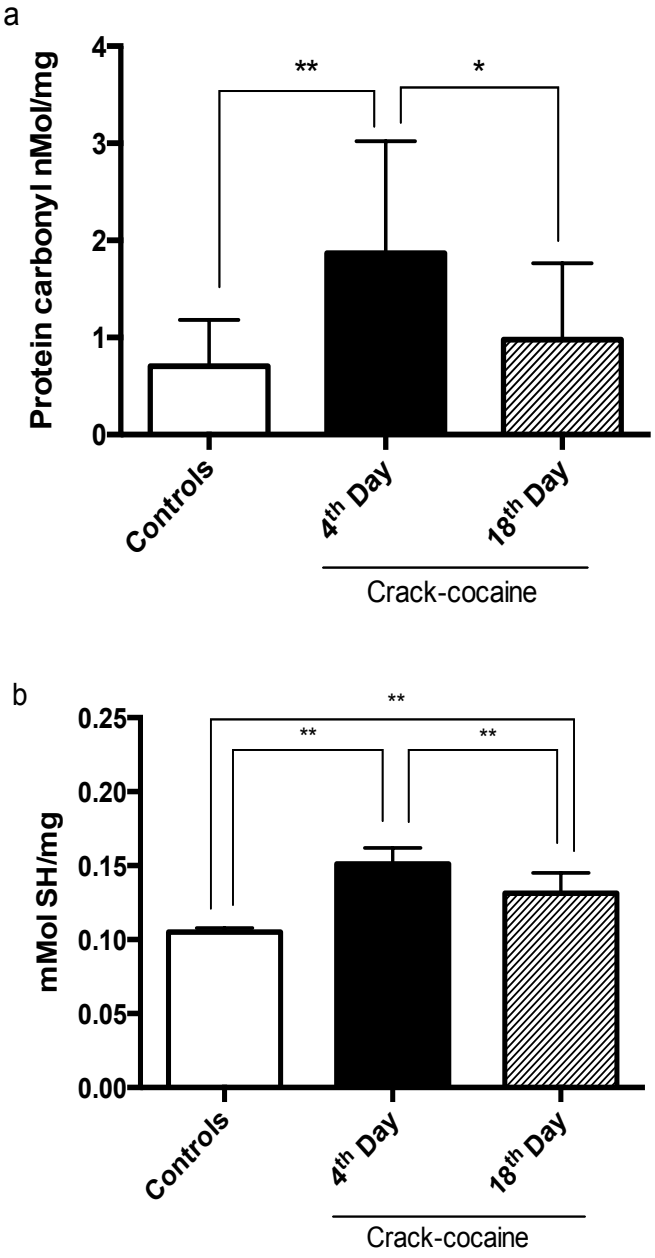
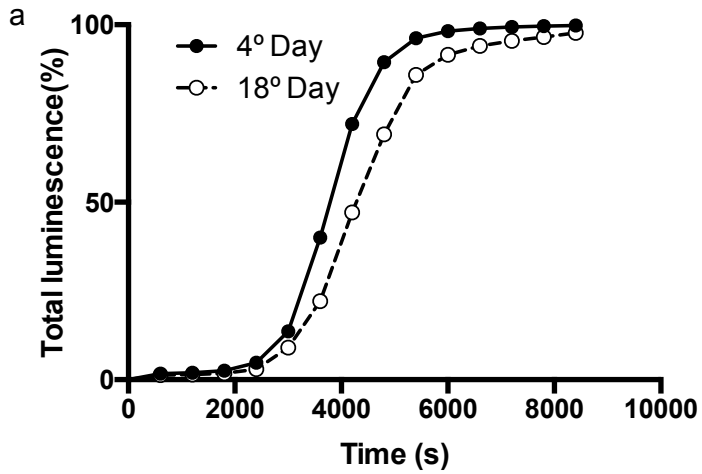

a
Protein carbonyl nMol/mg
4
3
2
1
0
**
*
Controls
4th Day
18th Day
Crack-cocaine
b
mMol SH/mg
0.25
0.20
0.15
0.10
0.05
0.00
**
**
**
Controls
4th Day
18th Day
Crack-cocaine

Fig. 2


a
100
50
0
Total luminescence(%)
4º Day
18º Day
0
2000
4000
6000
8000
10000
Time (s)

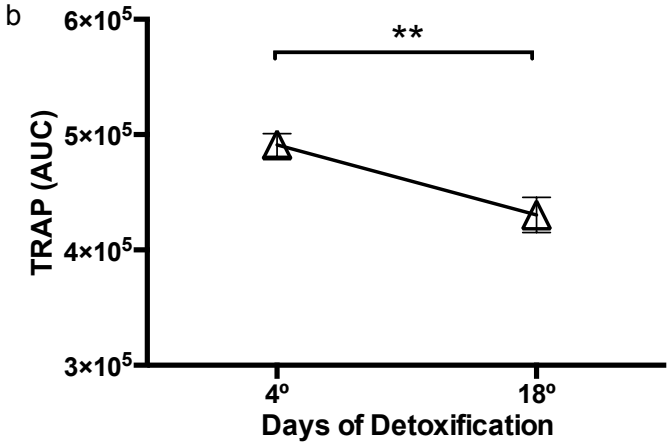
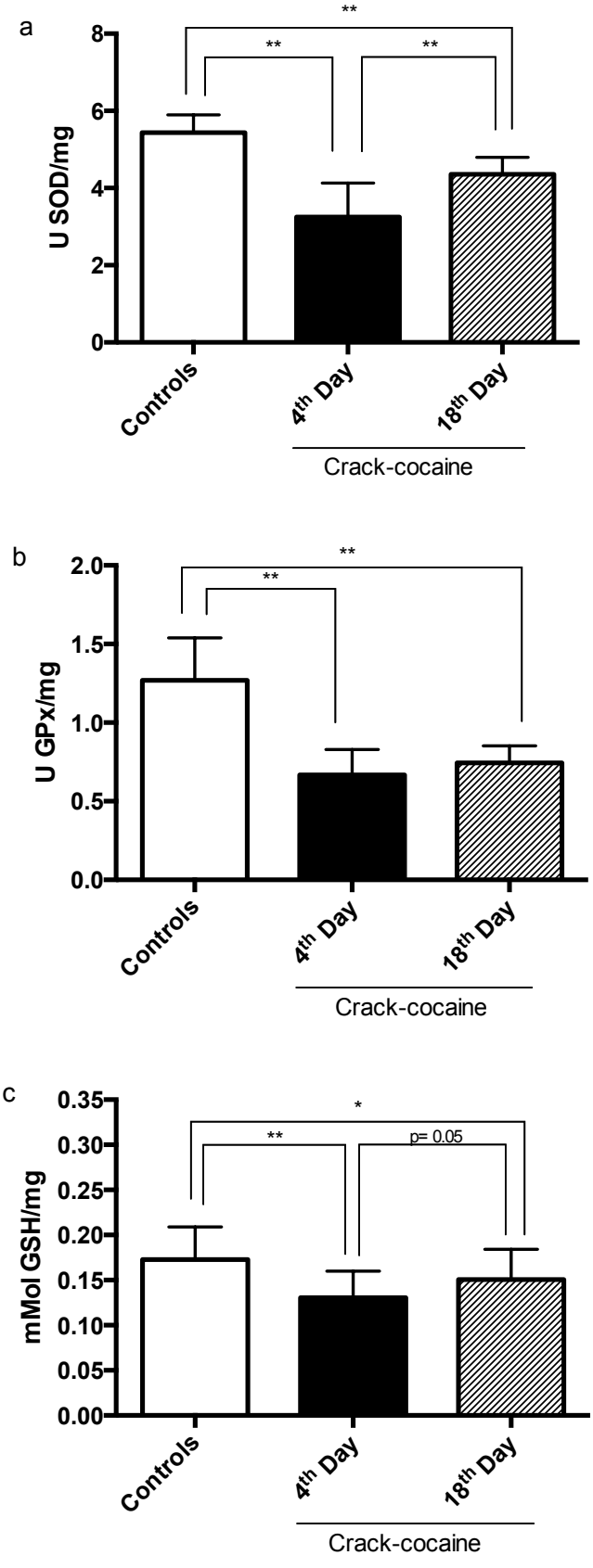

b
6×10^5
5×10^5
4×10^5
3×10^5
TRAP (AUC)
**
4º
18º
Days of Detoxification

Fig. 3


a
U SOD/mg
8
6
4
2
0
**
**
**
Controls
4th Day
18th Day
Crack-cocaine
b
U GPx/mg
2.0
1.5
1.0
0.5
0.0
**
**
Controls
4th Day
18th Day
Crack-cocaine
c
mMol GSH/mg
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
*
**
p= 0.05
Controls
4th Day
18th Day
Crack-cocaine

Supplementary material table 1. Effects of medications and comorbid psychiatric diagnosis on oxidative stress

	Neuroleptics	Anticonvulsivants	Anxiety Disorders Comorbidity
Carbonyl	*t*=0,013; *p*=0,99	*t*=0,016; *p*=0,98	*t*=0,064; *p*=0,95
Total thiol	*t*=-0,015; *p*=0,98	*t*=-0,155; *p*=0,87	*t*=1,354; *p*=0,18
TRAP	*t*=-0,994; *p*=0,32	*t*=-1,214; *p*=0,23	*t=-0,303; p=0,76*
SOD	*t*=1,665; *p*=0,10	*t*=0,875; *p*=0,38	*t*=-0,074; *p*=0,94
GPx	*t*=0,213; *p*=0,83	*t*=0,213; *p*=0,83	*t*=1,492; *p*=0,15
GSH	*t=-0,214; p=0,83*	*t*=-0,135; *p*=0,89	*t*=0,531; *p*=0,59

T-test for independent samples. Analyses performed with oxidative stress parameters at the end of detoxification (day 18th).

3. CAPÍTULO 3

3.1 CONSIDERAÇÕES FINAIS

Os resultados apresentados nesse estudo apontam um aumento dos parâmetros antioxidantes ao fim do tratamento para desintoxicação. Em nosso trabalho evidenciamos uma diminuição do potencial antioxidante total e atividade da SOD quando comparamos o 4º dia de abstinência com o 18º dia, resultados que corroboram com estudos anteriores que demonstram uma diminuição dos níveis de antioxidantes em animais expostos a cocaína.

Ao analisamos os marcadores de dano oxidativo como as proteínas carboniladas e as modificações nos grupamentos tióis, podemos perceber que ao término do tratamento, ambos os marcadores apresentam uma diminuição significativa de suas concentrações, destacando o fato de os níveis de proteínas carboniladas ao fim do tratamento estarem muito próximos dos níveis do grupo controle. Esse resultado pode estar associado com a melhora da efetividade do sistema antioxidante, que por sua vez age impedindo e prevenindo os danos oxidativos nas biomoléculas, em nosso caso, as modificações proteicas.

O fato que alguns estudos demonstram uma maior ação dos antioxidantes quando há uma exposição a cocaína e outros o contrário, nos faz perceber a necessidade de mais estudos que abordem a relação da produção de EROS e eficácia do sistema de defesa antioxidante frente ao consumo desta droga. Também existe a necessidade de maior compreensão sobre o mecanismo em que as EROS são formadas. No sistema nervoso central existem evidências que a autoxidação das MAO para "limpar" a fenda pré-sináptica da DA não recaptada é a responsável pelo aumento da produção de EROS. Já nos órgãos periféricos, a condição de estresse oxidativo pode ser justificada pela ação dos metabólitos da cocaína, visto que estudos de nosso grupo demonstraram que o uso de crack não está relacionado com o aumento da inflamação, a qual também poderia ser uma fonte de EROS.

As avaliações comportamentais como o BDI e CSSA apresentaram uma diminuição em seus escores quando comparados os períodos de início e término de tratamento. Esses resultados evidenciam uma diminuição dos sintomas de depressão e

da gravidade de sinais e sintomas de abstinência, respectivamente. Dessa forma, temos uma melhora tanto das variáveis bioquímicas quanto clínicas ao término do tratamento. Ao correlacionarmos esses dados, percebemos uma correlação positiva entre a quantificação de tióis totais ao início e ao fim do tratamento com a severidade dos sintomas de abstinência bem e os níveis de proteínas carboniladas. No entanto, houve uma correlação negativa entres os níveis de SOD com a severidade dos sintomas de abstinência e com os sintomas depressivos e entre os níveis de TRAP com o escore de dependência da droga.

Dessa forma, esse estudo demonstra que no primeiro momento (4ºdia) as pacientes estão mais propensas ao dano oxidativo, pois há uma depleção do sistema antioxidante e um aumento dos danos causados pelas EROS. Em contrapartida, ao término do tratamento para desintoxicação, percebemos uma melhora do sistema antioxidante enzimático e não-enzimático ao passo que os marcadores de dano oxidativo encontram-se diminuídos e aproximando-se do grupo controle, como no caso das proteínas carboniladas. Podemos concluir que com a abstinência é possível recuperar o balanço redox, evitando assim os danos causados às biomoléculas pelos oxidantes.

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