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Estudo do efeito da exposição à poluição do ar sobre o estresse oxidativo e o número células neuronais e microgliais no córtex e estriado de ratos

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Tese submetida ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Doutor.

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RESUMO

A exposição crônica à poluição atmosférica (PA) desencadeia mecanismos de neuroinflamação e de estresse oxidativo os quais estão envolvidos na fisiopatogênese de muitas doenças neurodegenerativas. O objetivo deste estudo foi investigar o efeito da exposição crônica à PA em período pré-natal, após o nascimento ou pré e pós-natal sobre biomarcadores de estresse oxidativo (EO), número de neurônios e de células microgliais do córtex e do estriado de ratos. O nosso trabalho também objetivou examinar se a troca de ambientes por ocasião do desmame alteraria parâmetros de EO ou histológicos. Setenta e dois ratos Wistar machos foram distribuídos em 4 grupos de exposição: 1) grupo F ou controle – exposto durante o período pré e pós-natal até a idade adulta ao ar filtrado;2) grupo NFF – exposto ao ar não filtrado no período pré-natal até o desmame e após, ao ar filtrado; 3) grupo FNF - exposto ao ar filtrado no período pré-natal até o desmame e após, ao ar não filtrado; 4) NF - exposto ao ar não filtrado no período pré-natal até a vida adulta. Após 150 dias de vida, córtex e estriado de 48 animais (n=12 por grupo de exposição) foram dissecados e submetidos às análises de EO: determinação da da atividade das enzimas superóxido dismutase (SOD) e catalase (CAT) e das concentrações de malondialdeido (MDA) e glutationa total (GSHt). Na sequência, córtex e estriado de outro lote de animais (24 - n= 6 por grupo de exposição) foram preparados para estudo histológico, por técnica estereologica, para a determinação do número de neurônios e de microglia. A concentração de MDA foi significativamente mais elevada no córtex e no estriado do grupo NF. O estriado apresentou um aumento e o córtex apresentou redução na concentração de GSHt no grupo FNF. A atividade da SOD foi reduzida no córtex de todos os grupos expostos em algum período da vida à PA e no estriado do grupo FNF. A exposição à PA não modificou a atividade da CAT nas regiões estudadas. Não houve diferença entre os grupos quanto ao número de neurônios ou de microglia no estriado. Por outro lado, o número de neurônios e de microglia foi significativamente mais elevado no córtex do grupo FNF. Nossos resultados sugerem que estriado e córtex têm limiares de reação e respostas adaptativas distintas ao EO gerado pela exposição a PA. A troca precoce de ambiente impediu e talvez reverteu o quadro de EO. Sugerimos que a PA seja capaz de induzir neurogênese no córtex, embora provavelmente de forma transitória e como mecanismo compensatório.

Palavras-chave: poluição do ar, estresse oxidativo, neurogênese

ABSTRACT:

Chronic exposure to air pollution (AP) triggers neuroinflammation and lipoperoxidation mechanisms involved in physiopathology of several neurodegenerative diseases. The aim of this study was to investigate the effect of AP on biomarkers of oxidative stress (OS) and number of neurons and microglial cells in cortex and striatum of rats chronically exposed to AP at ambient level. We also aim to examine if environment change at weaning age would modify OS or histological parameters. Seventy two male Wistar rats were distributed in four groups of exposure as follows: control group FA - exposed during pre and post-natal period until adulthood to filtered air; group NFA-FA - exposed to non-filtered air during pre-natal period until weaning and then to filtered air; group FA-NFA - exposed to filtered air during pre-natal period until weaning and then to non-filtered; group NFA - exposed during pre and post-natal period to non-filtered air. After 150 days, cortex and striatum of 48 rats (n= 12 of each group of exposure) were harvested and prepared for measuring the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), and concentrations of malondialdehyde (MDA) and total glutathione (GSHt). At the same time, the same brain structures of the remaining 24 animals (n= 6 of each group of exposure) were prepared for histological study through stereological method to determine the number of neurons and microglia. The cortex and striatum of NF group showed a significant increase in cortical MDA concentration. GSHt concentration was lower in cortex and higher in striatum of FNF group. There was a significant decrease of SOD activity in cortex of all groups exposed to AP. AP exposure did not alter CAT activity in cortex or striatum. The histological study showed no significant differences in neurons or microglial number of striatum between exposed or not to AP. On the other hand, neuron and microglial number were significantly high in FNF group. Our findings suggest that striatum and cortex have dissimilar thresholds to react to AP exposure and different adaptable responses to chronically AP-induced OS. At least for cortex, changing to a non-polluted environment early in life was able to avoid and perhaps reverse the OS. We also *hypothesize* that the exposure to AP can trigger cortical neurogenesis even though transiently maybe as a compensatory mechanism.

Key-words: neurogenesis, air pollution, oxidative stress.

LISTA DE ABREVIATURAS E SIGLAS

BHE	Barreira hematoencefálica
CAT	Catalase
CO	Monóxido de carbono
EO	Estresse oxidativo
ERN	Espécies reativas de nitrogênio
ERO	Espécies reativas de oxigênio
GSHt	Glutationa total
IQA	Índice de qualidade do ar
MDA	Malondialdeído
MP	Material particulado
MP_{10}	Material particulado com diâmetro aerodinâmico igual ou inferior a 10 micrometros ou grosso
$MP_{2,5}$	Material particulado com diâmetro aerodinâmico igual ou inferior a 2,5 micrometros ou fino
$MP_{0,1}$	Material particulado com diâmetro aerodinâmico igual ou inferior a 0,1 micrometros ou ultrafino
NO_2	Dióxido de nitrogênio
O_3	Ozônio
OMS	Organização Mundial de Saúde
PA	Poluição do ar
Pn	Pós-natal
ROFA	*Residual oil fly ash*
SNC	Sistema nervoso central
SO_2	Dióxido de enxofre
SOD	Superóxido dismutase
TNFα	Fator de necrose tumoral alfa

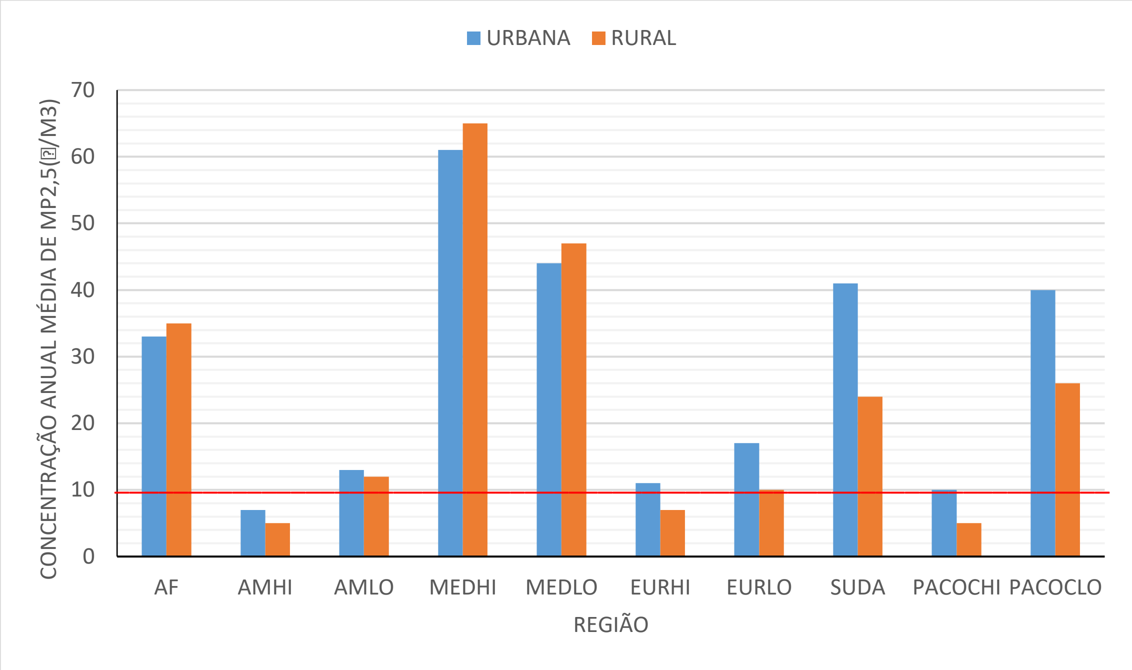
Sumário

1. INTRODUÇÃO

1.1 Composição da poluição do ar e controle da qualidade do ar.

A poluição do ar (PA) é um toxicante ambiental multifacetado composto de uma mistura dinâmica de substâncias cujos componentes podem variar na dependência de diversos fatores como condições climáticas, geografia e mecanismos geradores. Entram na constituição dos poluentes atmosféricos: material particulado (MP) que inclui componentes orgânicos, metais e todo o tipo de material sólido passível de se manter suspenso na atmosfera, gases (óxidos de nitrogênio, enxofre e ozônio) e líquidos. O MP é usualmente caracterizado de acordo com o seu diâmetro aerodinâmico em: MP_{10} ou grosso, composto de partículas com diâmetro igual ou inferior a 10μm, $MP_{2,5}$ ou fino que compreende partículas com diâmetro aerodinâmico igual ou inferior a 2,5μm e $MP_{0,1}$ ou ultrafino, composto de partículas ultrafinas com diâmetro aerodinâmico igual ou menor do que 0,1μm. Os veículos automotores são a principal fonte de PA, sendo que os motores a diesel movimentam uma ampla gama de veículos e máquinas usados no transporte terrestre e marítimo, construção civil, agricultura, mineração, etc. A combustão do diesel gera mais de 40 poluentes tóxicos e é o principal contribuinte na geração de MP, particularmente de $MP_{2,5}$ e $MP_{0,1}$. A figura 1 oferece uma visão dos dados obtidos pela Organização Mundial da Saúde (OMS) sobre os níveis de PA composta de $MP_{2,5}$ conforme a região do globo e, dentro de cada região, se rural ou urbana.

Figura 1 - Concentração anual média de $MP_{2,5}$ por região do planeta e suas áreas urbanas e rurais, 2016.



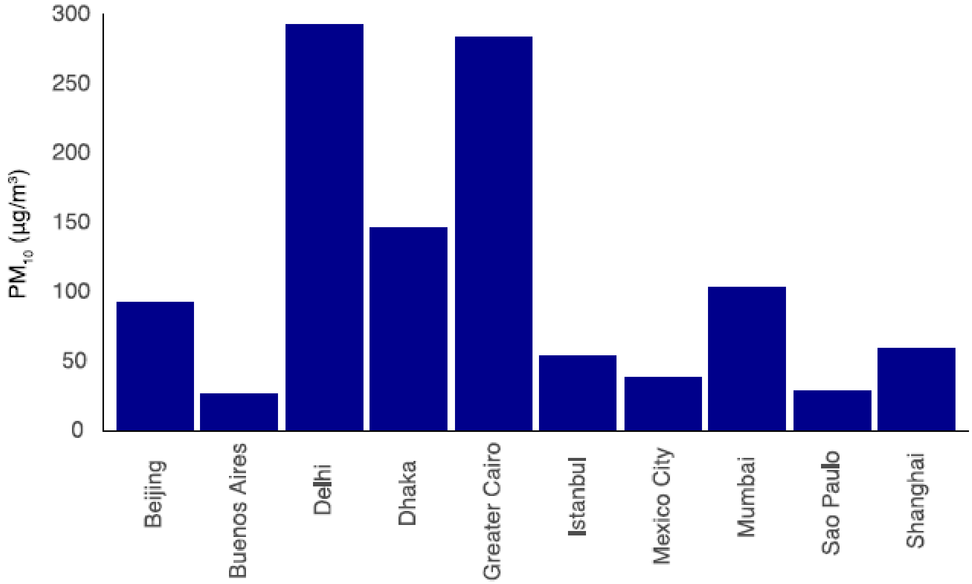
AF- AFRICA; AMHI - AMERICA *HIGH INCOME*; AMLO - AMERICA *LOW INCOME*; MEDHI - MEDITERRÂNEO ORIENTAL *HIGH INCOME*; MEDLO - MEDITERRÂNEO ORIENTAL *LOW INCOME*; EURHI - EUROPE *HIGH INCOME*; EURLO - EUROPE *LOW INCOME*; SUDA - SUDESTE ASIATICO; PACOCHI - PACÍFICO OCIDENTAL *HIGH INCOME*; PACOCLO - PACÍFICO OCIDENTAL *LOW INCOME*.

Fonte – OMS - *Exposure to ambient air pollution from particulate matter for 2016*.

Na maioria das regiões do mundo as médias anuais de $MP_{2,5}$ são mais elevadas do que os níveis estabelecidos pelas diretrizes de qualidade do ar da OMS que são de 10 µg/m^3 (linha vermelha na figura). As regiões com exposições particularmente mais intensas são o mediterrâneo oriental, o sudeste da Ásia e a região oriental do Pacífico. No mediterrâneo oriental as cidades mais comprometidas são Abu Dhabi, Doha, Riade e Ma'ameer. As cidades mais poluídas do sudeste da Ásia são Delhi e Daca, enquanto que Bagdá, Lahore e Cairo são as mais poluídas do pacífico oriental (OMS, 2018). A PA não se origina exclusivamente da atividade humana. Ela pode ser fortemente influenciada por fatores climáticos como tempestades de areia e poeira nas áreas rurais próximas a desertos como ocorre na África e no Mediterrâneo oriental, conforme demonstrado no gráfico acima. No entanto, nas demais regiões as concentrações nas áreas urbanas são maiores do que nas rurais.

A OMS (*https://www.who.int/airpollution/ambient/pollutants/en/*) considera o $MP_{2,5}$ um dos mais danosos poluentes à saúde humana. Desta forma, a mensuração deste poluente é particularmente importante porque pode ser utilizada diretamente para estimar o impacto da PA ambiental sobre a saúde. Quando as medidas de $MP_{2,5}$ não estão disponíveis, elas poderão ser obtidas por conversão a partir dos níveis de MP_{10}. Este método de obtenção indireta dos níveis de $MP_{2,5}$ pode comprometer a acurácia dos resultados em razão de dificuldades na determinação dos fatores de conversão adequados. Assim, incentiva-se internacionalmente a adoção de tecnologia que permita a determinação direta dos níveis de $MP_{2,5}$ (*https://www.who.int/airpollution/ambient/interventions/en/*). A figura 2 permite uma comparação visual entre os níveis de MP_{10} em algumas das principais megacidades, assim chamadas por possuírem mais de 10 milhões de habitantes (Organização das Nações Unidas - ONU).

Figura 2 - Níveis anuais de MP_{10} nas megacidades no período de 2010 a 2016.



Fonte: *OMS - ambient (outdoor) air quality database Summary results, 2018.*

A Agência Americana de Proteção Ambiental (*Environmental Protection Agency - EPA)* é o órgão responsável pelo cálculo do índice de qualidade do ar (IQA). O IQA é calculado a partir das concentrações dos cinco maiores poluentes atmosféricos a saber: monóxido de carbono (CO), ozônio (O_3) ao nível do solo, MP, dióxido de enxofre (SO_2) e dióxido de nitrogênio (NO_2). A partir do cálculo do IQA, é possível estabelecer os níveis de PA que determinam riscos para a saúde humana e quais as populações mais sensíveis. O quadro 1 ilustra estes dados e mostra também a convenção de cores utilizada para facilitar a identificação dos níveis de PA. O IQA é utilizado para monitorar permanentemente e em tempo real a qualidade do ar a nível global nas cidades que têm sistemas de detecção de poluentes atmosféricos. As informações em tempo real estão disponíveis no site http://aqicn.org/map/world.

Quadro 1 – Escala de indexação da qualidade do ar utilizada para avaliar a poluição do ar em tempo real em várias cidades do mundo.

IQA	Nivel de poluição do ar	Efeitos à saúde
0-50	Bom	Qualidade do ar é satisfatória com pequeno ou nenhum risco à saúde
51-100	moderado	Qualidade do ar é aceitável; porém dependendo dos poluentes e para um pequeno número de pessoas especialmente sensíveis a poluição atmosférica poderá causar problemas de saúde.
101-150	Insalubre para grupos sensíveis	Grupos sensíveis poderão apresentar problemas de saúde. A população geral não será afetada.
151-200	Insalubre	Qualquer pessoa poderá apresentar problemas de saúde que serão mais graves nos grupos sensíveis.
201-300	Muito insalubre	Alerta para situações de emergência. Possibilidade de afetar toda a população.
>300	Perigoso	Sério risco a saúde de qualquer pessoa.

Fonte: http://waqi.info/

No Brasil, o Conselho Nacional do Meio Ambiente (CONAMA) é o órgão que determina os padrões de medição de qualidade do ar que servem de parâmetro para o controle da PA no país. Em publicação oficial recente datada de novembro de 2018 o Conama estabelece novos padrões de medição da qualidade do ar que deverão ser implementados em etapas progressivas sendo que a primeira etapa já está em vigor desde a data da publicação da resolução. O quadro 2 descreve esses novos parâmetros de acordo com as etapas progressivas de implementação. O quadro 3 apresenta os parâmetros de poluentes atmosféricos que delimitam os estados de atenção, alerta e emergência para a saúde humana.

Quadro 2 - Padrões de concentração máxima de poluentes atmosféricos em 24 horas a serem implementados em etapas progressivas de controle da poluição do ar no Brasil.

Poluente atmosférico	**[&]PI - Concentração (µg/m³ em 24h)**	**P2 - Concentração (µg/m³ em 24h)**	**P3 - Concentração (µg/m³ em 24h)**	**PF**
Material Particulado - MP_{10}	120	100	75	50
Material Particulado - $MP_{2,5}$	60	50	37	25
SO_2	125	50	30	20
Fumaça	120	100	75	50
CO	-	-	-	9*
Partículas totais em suspensão - PTS	-	-	240	-

SO_2 = dióxido de enxofre; MP10 = material particulado com diâmetro aerodinâmico equivalente de corte de 10 µm; $MP_{2,5}$ = material particulado com diâmetro aerodinâmico equivalente de corte de 2,5 µm; CO = monóxido de carbono.
*Padrão de CO é medido em período de 8h com base na máxima móvel obtida no dia; [&]Padrão intermediário – primeira etapa da implementação dos novos parâmetros estabelecidos pelo CONAMA. PF – Padrão final.
Fonte: CONAMA/2018.

Quadro 3 – Níveis de atenção, alerta e emergência para poluentes atmosféricos e suas concentrações.

Nível	SO^2 (µg/m³ - média de 24h)	MP_{10} (µg/m³ em 24h)	$MP_{2,5}$ (µg/m³ em 24h)	O^3 (µg/m³ - média móvel 8h)	NO^2 (µg/m³ - média de 1h)	CO - ppm (média móvel de 8h)
Atenção	800	250	125	15	200	15
Alerta	1600	420	210	30	400	30
Emergência	2100	500	250	40	600	40

SO2 = dióxido de enxofre; MP10 = material particulado com diâmetro aerodinâmico equivalente de corte de 10 µm; MP2,5 = material particulado com diâmetro aerodinâmico equivalente de corte de 2,5 µm; CO = monóxido de carbono; O3 = ozônio; NO2 = dióxido de nitrogênio µg/m3; ppm = partes por milhão.
Fonte: CONAMA/2018

As concentrações médias diárias anuais de $MP_{2,5}$ de Porto Alegre no período do estudo foram de 28,1 µg/m^3. A composição química do material particulado ($MP_{2,5}$) incluiu elementos traço tóxicos – alumínio, chumbo, vanádio e níquel, bem como nitratos e sulfatos.

1.2 Poluição atmosférica e saúde humana

Estudos pioneiros datados do século passado já apontavam a PA como causadora de doenças respiratórias. O estudo de Pope (1989) cita na sua introdução a modificação dos parâmetros de poluição atmosférica máxima pela EPA em razão de evidências crescentes correlacionando o aumento das concentrações de MP com a redução da função pulmonar em crianças, o aumento do risco de bronquite e de outras doenças respiratórias. Outros estudos, em outros países, que avaliaram a saúde respiratória da população pediátrica na presença de diferentes graus de poluição atmosférica, chegaram a conclusões semelhantes. O estudo de Heinrich *et al.* (2002) demonstrou uma redução na prevalência de sintomas respiratórios não-alérgicos em crianças em idade escolar após a reunificação das Alemanhas em razão do declínio no MP total em suspensão e nas emissões de dióxido de enxofre secundários à combustão. Bayer-Oglesby (2005) encontrou redução na frequência de sintomas respiratórios de crianças em áreas da Suíça durante períodos de redução moderada da PA. O mesmo estudo demonstrou a correlação entre a presença de MP com diâmetro aerodinâmico igual ou inferior a 10 micrometros (MP_{10}), originário de uma fábrica de aço na região de Utah e um aumento na incidência de doenças respiratórias na população adulta e pediátrica, mesmo quando a concentração de MP_{10} permanecia igual ou inferior aos níveis máximos estipulados pela agência de proteção ambiental. Um achado interessante desse estudo foi a ausência de um limiar de concentração de MP_{10} para desencadeamento de efeitos tóxicos já que os autores observaram benefícios mesmo com reduções pequenas nessas concentrações e em ambientes moderadamente poluídos.

A partir destes dados pioneiros, as pesquisas têm se multiplicado no sentido de estabelecer uma relação de causa e efeito entre a PA, doenças respiratórias e mortalidade por diferentes causas. Recentemente, identificou-se uma associação entre a exposição crônica a ozônio e um aumento da mortalidade na população portadora de doença crônica das vias aéreas inferiores como asma e doença pulmonar obstrutiva crônica (Hao *et al.*, 2015). O grupo ESCAPE (*European Study of Cohorts for Air Pollution Effects*) concluiu que a exposição crônica a $MP_{2,5}$ associou-se a aumento do risco de morte por causas naturais mesmo em concentrações atmosféricas inferiores aos valores de limite médio anual europeu (Beelen *et al.,* 2014). Resultados semelhantes foram obtidos com o estudo de uma grande coorte americana seguida por 12 anos, envolvendo uma população de quase 70 milhões de pessoas (Di *et al*., 2017). Os achados destes autores revelaram que a população de condição socioeconômica inferior exposta durante longo período a $MP_{2.5}$ e ozônio apresentou risco aumentado de morte mesmo em concentrações de $MP_{2.5}$ inferiores aos níveis anuais propostos pelo órgão regulador da qualidade do ar. Os efeitos da exposição ao $MP_{2.5}$ também se revelaram mais acentuados no sexo masculino e nos afro-americanos.

As primeiras evidências do efeito da PA sobre o sistema nervoso central (SNC) foram aquelas fornecidas pelo estudo de Zhang *et al.* (1988) que estabeleceu um aumento no risco de acidente vascular encefálico em uma coorte exposta cronicamente a fumaça de carvão. Desde então, a correlação entre PA e doença cerebrovascular tem sido progressivamente identificada em vários trabalhos que atribuem um papel significativo e independente da PA no aumento da incidência de eventos cerebrovasculares isquêmicos (Hong *et al.,* 2002; Lisabeth *et al.,* 2008). A apoptose neuronal secundária ao estresse oxidativo (EO) gerado no tecido neural tem implicado a PA na fisiopatologia dos acidentes vasculares cerebrais e de diversas doenças neurodegenerativas crônicas incluindo doença de Parkinson e esclerose lateral amiotrófica (Becker & Bonni, 2004).

Dados epidemiológicos atuais demonstram o papel toxicológico dos constituintes da PA como as partículas ultrafinas na indução de respostas inflamatórias e pró-trombóticas no organismo humano resultando na promoção de aterosclerose, trombogênese e doença cérebrovascular e cardiovascular (Schulz *et al.,* 2005). Maheswaran *et al.* (2012) conduziram uma coorte retrospectiva de pacientes residentes em comunidades londrinas que sobreviveram ao primeiro episódio de acidente vascular hemorrágico ou isquêmico entre 1995 e 2005 com o objetivo de estudar se a exposição à PA afetaria a mortalidade a longo prazo. Estes autores encontraram que cada 10 $\mu g/m^3$ de incremento na exposição a NO_2 e MP_{10} correspondeu a um aumento na mortalidade de 28% e 52%, respectivamente.

As doenças neurodegenerativas como a doença de Alzheimer e a doença de Parkinson ocorrem predominantemente de forma esporádica sem um claro envolvimento de fatores genéticos. Fatores ambientais como a PA têm sido apontados como corresponsáveis na patogênese destas doenças e o envelhecimento é o principal fator de risco para ambas (Heusinkveld *et al.,* 2016). Do ponto de vista neuropatologico, ambas são caracterizadas pela presença de depósitos de proteína no tecido cerebral como as bandas neurofibrilares, agregados intracelulares contendo proteínas tau hiperfosforiladas, e placas senis que consistem principalmente de peptídeo amiloide β (peptídeo Aβ) na doença de Alzheimer (Selkoe, 2001). Na doença de Parkinson, inclusões proteicas de agregados de α-sinucleina, os chamados corpos de Lewy, predominam na substância negra. A presença destas proteínas anormais associa-se à perda de função, morte neuronal e degeneração do tecido nervoso (Selkoe, 2001). Uma revisão recente sobre fatores de risco ambientais para demência classifica como moderada a forte a força das evidências de participação de fatores relacionados à PA (Killin *et al.,* 2016).

Além das doenças neurodegenerativas citadas, a PA é considerada fator de risco para o desenvolvimento neurológico normal. Estudos epidemiológicos e experimentais sugerem que indivíduos jovens são particularmente sensíveis (Calderón-Garcidueñas *et al.,* 2008, 2011). Crianças e adolescentes expostos a diferentes níveis de PA apresentaram comprometimento cognitivo (Calderón-

Garcidueñas *et al.,* 2008, 2011; Sunyer *et al.,* 2008), hiperatividade e déficit atencional (Newman *et al.,* 2013, Kicinski *et al.,* 2015). Evidências recentes têm identificado uma associação entre os transtornos do espectro autista e a exposição gestacional e na infância precoce a PA derivada do trafego. Os resultados do seguimento dessas coortes demonstraram que a exposição a PA particularmente no terceiro trimestre da gestação apresenta uma forte correlação positiva com a incidência de transtornos do espectro autista (Raz *et al.,* 2015).

1.3 Mecanismos de neurotoxicidade da poluição atmosférica

O MP e as substâncias adsorvidas na sua superfície podem ter acesso ao cérebro através dos pulmões e da circulação sistêmica, determinando quebra da barreira hemato-encefálica (BHE), ou pela via olfatória através do bulbo olfatório.

Os pulmões têm a maior área exposta do corpo ao ambiente externo e são ricamente vascularizados servindo como ampla porta de entrada aos elementos que compõem a PA. Através deles, o MP desencadeará a geração de estressores metabólicos (biomoléculas oxidadas e seus produtos de degradação e citocinas pro-inflamatórias) (Gomez-Mejiba *et al.* 2009) que interagirão com diferentes receptores em diferentes tecidos incluindo o tecido neural. O $MP_{2,5}$ e o $MP_{0,1}$ estão predominantemente envolvidos na neuroinflamação e no estresse oxidativo neuronal (Calderón-Garcidueñas *et al.,* 2001, 2002, 2003, 2004, 2008; Gomez-Mejiba *et al.,* 2009; Oberdörster *et al.,* 2005a, 2005b; Oberdörster & Utell, 2002; Peters *et al.,* 2006). Estas partículas são inaladas e induzem toxicidade aguda nos pulmões e tecido cardiovascular e, penetrando a barreira alveolar, ganham a circulação sistêmica e o SNC. A inflamação pulmonar persistente em decorrência da deposição repetida das partículas finas e ultrafinas nos alvéolos pulmonares e a degeneração dos epitélios nasal e do trato respiratório acarretam a liberação de citocinas, como interleucinas 6 (IL-6), 1β (IL-1β) e fator de necrose tumoral alfa (TNFα) na circulação sistêmica. Estes mediadores inflamatórios ativam os receptores do endotélio vascular da BHE tornando-a mais permeável (Calderón-Garcidueñas *et al.*, 2008; Sunyer, 2008) o que por sua vez facilita a entrada de mediadores inflamatórios, espécies reativas de oxigênio (ERO) e de nitrogênio (ERN) e outras substâncias nocivas cujo acesso ao SNC é normalmente bloqueado pela BHE. Embora a BHE intacta seja capaz de impedir a entrada da grande maioria dos xenobióticos ao SNC, uma pequena porção tem acesso através de difusão ou transporte ativo mediado pela existência de proteínas e receptores na membrana celular. Nanopartículas podem danificar as células endoteliais, romper a BHE e reduzir a viabilidade das células endoteliais da microvasculatura cerebral através de modificações no potencial da membrana mitocondrial e redução na expressão das proteínas das *tight-junctions* endoteliais (Oppenheim *et al.,* 2013).

O bulbo olfatório constitui outra importante porta de entrada para o MP que é inicialmente capturado pelas terminações nervosas sensoriais do epitélio da cavidade nasal e, em seguida, por translocação axonal, atinge estruturas do SNC (Ajmani *et al.,* 2016). Estudos *post-mortem* mostraram aumento da expressão de marcadores inflamatórios no córtex pré-frontal, hipocampo e bulbo olfatório de crianças, adolescentes e adultos jovens expostos à PA urbana (Calderón-Garcidueñas *et al.,* 2004). Outras evidencias sugerem que partículas finas sejam capazes de se acumular nos bulbos olfatórios de pessoas expostas a elevados níveis de poluentes atmosféricos, incluindo MP e metais pesados desencadeando processos de inflamação local e dano neuronal (Calderón-Garcidueñas *et al.,* 2013).

A presença de MP no SNC induz EO e neuroinflamação *in loco* que são os principais mecanismos potencialmente geradores de lesão neural. A neuroinflamação, desencadeada através da ativação de vias como a c-Jun N-terminal kinase (JNK)t e a via do fator de transcrição nuclear kappa-b (NF-κB), produtora de citocinas, quimiocinas e ERO resulta em agregação proteica aberrante, comprometimento da neurotransmissão e da sinalização neurotrofica, remodelamento neuronal e neurodegeneração (Teeling *et al.*, 2009). O EO resulta de reações metabólicas que usam o oxigênio e representa um desequilíbrio entre as reações oxidativas e as defesas antioxidantes no organismo vivo (Valko *et al.,* 2007). As ERO embora sejam produtos normais do metabolismo celular e, em concentrações moderadas façam parte da resposta celular fisiológica ao dano, podem produzir EO em concentrações elevadas. O dano celular gerado pelo EO leva a apoptose por destruição dos componentes celulares incluindo lipídios, proteínas e ácidos nucleicos (Becker & Bonni, 2004). Sendo o encéfalo rico em ácidos graxos poli-insaturados e proteína e pobre em defesas antioxidantes, é fácil concluir pela elevada susceptibilidade do tecido neural ao efeito do EO. Um dos mecanismos pelo qual o EO determina dano celular acontece através de modificações no retículo endoplasmático. Esta organela intracelular é o principal local de síntese e enovelamento de proteínas, armazenamento e sinalização de cálcio e biossíntese de lipídeos. Consequentemente, os papeis fisiológicos do retículo endoplasmático incluem modulação de proteínas e homeostasia do cálcio intracelular. Entretanto, o retículo endoplasmático é sensível a dano oxidativo e a perturbações da homeostasia celular e, sob condições de estresse excessivo, pode iniciar o processo de apoptose celular de forma independente de outros mecanismos de sinalização (Rao *et al.,* 2004). As consequências desses desequilíbrios expressadas no aumento de marcadores de EO e de neuroinflamação, bem como deposição de proteína precursora amilóide secundários a ação da microglia ativada foram detectados em estudos *post-mortem* do tecido cerebral de indivíduos expostos (Block & Calderón-Garcidueñas, 2009; Calderón-Garcidueñas *et al.*, 2008, 2011).

1.4 Períodos críticos do desenvolvimento do sistema nervoso central e toxicologia ambiental

O processo de construção do SNC é determinado por diferentes estágios de desenvolvimento da expressão gênica, atividade neuronal intrínseca e de sinais moleculares que o orientam. Todos esses estágios estão sujeitos, por sua vez, à influência de fatores externos ao SNC como o ambiente materno durante o período embrionário. A limitação temporal dos estágios de desenvolvimento para migração neuronal, formação de circuitos neuronais e refinamento sináptico determina períodos críticos ou sensíveis que são janelas de tempo no desenvolvimento onde o processo é mais sujeito a mudanças. Em outras palavras, durante essas janelas, a plasticidade de fenótipos fisiológicos ou comportamentais está aumentada comparativamente aos outros estágios. O conhecimento desses períodos fornece uma rede de mapeamento das janelas de tempo para grandes mudanças e plasticidade cerebral durante o desenvolvimento normal e também sinaliza e particulariza os momentos de maior vulnerabilidade (Meredith, 2015).

A utilização do cérebro de roedores como modelo de sistema de desenvolvimento tem evidenciado a existência de períodos críticos e sensíveis pré e pós-natais e possibilitado a comparação com o que se observa no cérebro de primatas (Meredith, 2015). Entretanto, para possibilitar estudos translacionais de humanos a partir de modelos de roedores, é necessária a validação das informações para que se possa comparar períodos críticos similares entre as espécies. Esta comparação interespécies baseia-se em um grupo de indicadores que inclui: crescimento global do cérebro, momento da neurogênese e gliogênese, biomarcadores da migração neuronal e da formação sináptica (Semple *et al.,* 2013). O quadro 4 apresenta de forma resumida uma comparação entre o cérebro de roedores e humanos com relação aos períodos críticos de desenvolvimento macro e microscópico.

Quadro 4 - Resumo dos principais passos e processos de desenvolvimento cerebral com equivalência de idades em humanos e roedores (modificado de Semple et al., 2013).

PND – *pos-natal day* BHE – barreira hematoencefálica NT – Neurotransmissores.

Humano	Roedor	Marcos de desenvolvimento
23-32 semanas IG (pré-termo)	*PND 1-3	- Predomínio de oligodendrócitos mitoticamente ativos - Desenvolvimento do sistema imune - Estabelecimento da BHE
36-40 semana IG (termo)	PND 7-10	- Pico de desenvolvimento cerebral - Pico da gliogênese - Aumento da densidade axonal e dendrítica - Oligodendrócitos imaturos - Consolidação do sistema imune
2-3 anos de idade	PND 20-21	- Peso cerebral = 90-95% do adulto - Densidade sináptica > adulto - Pico da mielinização - Modificações nos NT e receptores.
4-11 anos de idade	PND 25-35	- Maturação do córtex pré-frontal - Espessura máxima cortical e da substância cinzenta
12-18 anos de idade	PND 35-49	- Redução da densidade sináptica com platô no adulto - Mielinização, aumento do volume da substância branca, refinamento da circuitaria cognitiva
20 anos	PND ≥ 60	- Níveis maduros de sinapses e neurotransmissores. - Mielinização em andamento, declínio da substância cinzenta.

O conceito de períodos sensíveis ou críticos de desenvolvimento cerebral tem sido usado na tentativa de elucidar os mecanismos fisiopatológicos envolvidos na gênese dos transtornos do desenvolvimento neuropsicomotor como os transtornos do espectro autista e da esquizofrenia (Meredith, 2015; Cattane *et al.,* 2018). A exposição pré-natal a condições que promovem EO e/ou neuroinflamação figura entre uma multiplicidade de fatores passíveis de determinar desequilíbrios na sequência normal de eventos desses períodos. Neste sentido, o envolvimento da PA na gênese dos transtornos do desenvolvimento do SNC tem sido apontado em alguns estudos clínicos. Calderon-Garcidueñas *et al.,* (2008, 2013) encontraram comprometimento cognitivo e um aumento importante de alguns mediadores inflamatórios no liquido cefalorraquidiano de crianças residentes em zona da cidade do México densamente poluída em comparação com crianças moradoras em região de baixo índice de PA. Outros estudos atribuíram atrasos no neurodesenvolvimento de crianças expostas à PA no período pré-natal (Yorifuji *et al.,* 2016).

Estudos *in vitro* com exposição de células tronco pluripotentes neuroepiteliais a altas concentrações de dexametasona promoveram aumento das concentrações intracelulares de ERO e *down-regulation* na expressão de enzimas antioxidantes em células nunca expostas diretamente ao corticoide. O desequilíbrio intracelular induzido pelas ERO associou-se com redução da diferenciação neuronal e de outros marcadores neuronais sugerindo um papel do EO no comprometimento dos processos precoces de diferenciação neuronal (Raciti *et al.,* 2016). Consequentemente, disfunção do sistema antioxidante e geração descontrolada de ERO podem prejudicar o desenvolvimento do SNC e resultar em predisposição para doenças neurológicas e psiquiátricas. Por outro lado, EO está fortemente ligado a inflamação em razão de que múltiplos mediadores inflamatórios são ativados pelas ERO enquanto que células ativadas imunes, como a microglia, geram ERO e ERN. A exposição pré-natal à PA constituída de partículas de escapamento de diesel induziu citocinas inflamatórias, modificação da morfologia microglial e alterações do volume cortical e número de neurônios de camundongos machos (Bolton *et al.,* 2017). A importância do EO no desencadeamento da ativação imune pré-natal é reforçada pelo fato de que a administração de N-acetilcisteina, um poderoso antioxidante, a camundongos prenhes protegeu a prole contra os efeitos nocivos como a hipomielinização, o comprometimento cognitivo e dos processos neuroplásticos induzidos pela administração pré-natal de lipopolissacarídeos (Lantè *et al.,* 2008).

O desenvolvimento da BHE é outro elemento importante a considerar quando se trata do acesso de xenobióticos ao SNC particularmente em fases precoces e sensíveis de desenvolvimento embrionário ou mesmo pós-natal. A BHE é um complexo de células altamente especializado responsável pela proteção do tecido nervoso, controle bidirecional do transporte de fluidos, macromoléculas exógenas e endógenas, tráfego de células imunes e progenitoras, primariamente, ao nível dos capilares cerebrais (Osipova *et al.,* 2018). A BHE está estabelecida e funcionante durante a embriogênese nos roedores e nos humanos e é regulada pelas interações das células endoteliais com pericitos (Semple *et al.,* 2013). Entretanto, ainda há um entendimento incompleto sobre as respostas idade-dependentes da BHE a injúrias como a isquemia neonatal. A integridade estrutural da BHE em ratos em geral é atingida na idade de 21 dias Pn, quando ocorre o desmame. Nos humanos, os dados mais definitivos estabelecem a idade de 6 meses após o nascimento para redução completa da permeabilidade a proteínas e fluoresceína (Mortuza *et al.,* 2019) o que provavelmente traduz um processo completo de maturação.

1.5. Modelos experimentais para estudo dos efeitos da exposição à poluição atmosférica

O desenvolvimento de modelos experimentais para o estudo dos efeitos da PA sobre a saúde dos seres vivos é relativamente recente e tem sofrido aprimoramentos ao longo do tempo. De uma forma geral os modelos podem ser classificados em modelos *in vitro* que utilizam células em cultivo e modelos *in vivo* que recorrem à exposição de animais à PA através de diferentes métodos como a instilação intratraqueal e intranasal, a exposição a concentrado de partículas, ao ar livre ou em câmaras de exposição.

1.5.1 Modelos *in vitro:*

O estudo dos efeitos da PA *in vitro* através de cultivo e exposição de células originadas de múltiplos tecidos como do epitélio brônquico humano, do sistema imunológico e do SNC tem sido empregado em diversos estudos (Becker *et al.,* 2005; Fagundes *et al.,* 2015; Cho *et al.,* 2018). Este tipo de estudo constitui ferramenta útil para fornecer informações de forma relativamente rápida, com vantagens adicionais em termos de custos e operabilidade. Entretanto, exige que as células sejam retiradas do seu meio biológico, em sua maioria, comprometendo a interação com células vizinhas. Essa característica priva os modelos *in vitro* de espelhar o contexto biológico da exposição do organismo por inteiro.

1.5.2 Modelos *in vivo:*

A aspiração ou instilação de substâncias para os pulmões pelas vias intratraqueal ou intranasal têm sido empregadas, experimentalmente, na investigação dos efeitos do MP sobre a saúde. A via intranasal requer a instilação com auxílio de micropipetas de um volume reduzido de uma suspensão de partículas em concentração previamente conhecida. A instilação provoca o reflexo de apneia seguido de inspiração profunda o que propulsiona o líquido para o interior dos pulmões e para o SNC através do bulbo olfatório (Zanchi *et al.,* 2008, 2010; Medeiros *et al.,* 2004; Antonini *et al.,* 2002). Por outro lado, a via intratraqueal requer sedação para evitar a tosse e as partículas em solução são instiladas através de canulação traqueal. Uma revisão das técnicas, indicações, vantagens e desvantagens do uso da via intratraqueal para administração de drogas é oferecida no trabalho de Driscoll *et al.* (2000). Entre as vantagens das técnicas estão o baixo custo e a fácil execução. Entre as limitações podemos citar: perda de parte da suspensão no momento da instilação intranasal ou por reflexo de tosse na via intratraqueal; a quantidade de solução instilada é limitada e a frequência deve ser monitorada pelo risco de edema pulmonar nos animais principalmente quando utilizada a via intratraqueal; comprometimento da precisão dos resultados porque não reproduzem com fidelidade a exposição aos níveis ambientais de PA, embora possam ser relevantes para estudos de exposição ocupacional ou mesmo ambiental. Os desfechos de estudos que utilizam instilação ou inalação podem diferir principalmente na dependência do tempo de exposição e eventualmente alguma variação temporal de distribuição dependendo do calibre da via respiratória, o que deverá ser considerado na análise dos resultados.

O estudo dos efeitos da PA sobre os organismos vivos também pode ser feito através da exposição dos mesmos ao ar livre ou através de câmaras de exposição que exigem suporte técnico e manutenção. Estes métodos reproduzem com maior precisão a fisiopatogenia das alterações induzidas pela PA nos diferentes tecidos. Entretanto, com a exposição ao ar livre, uma amostragem homogênea é mais difícil de se obter em razão de fatores como as diferenças de concentrações de MP determinadas pelo clima, altitude, latitude, entre outros. Entre os trabalhos descritos que utilizaram este método destacam-se os de Calderon-Garcidueñas *et al.* (2002) que estudaram cães expostos ao ar livre da cidade do México com altos níveis de PA comparativamente a cães moradores da cidade mexicana de Taxcala com baixos níveis de PA.

Câmaras de exposição à PA foram construídas com o objetivo de reduzir os custos experimentais e o tempo necessário para a obtenção de resultados comprometendo ao mínimo a validade dos mesmos. A técnica de exposição através dessas câmaras permite expor os animais aos níveis reais de PA, bem como a um ambiente com ar filtrado em função dos diferentes tipos de filtros que podem ser acoplados ao sistema. As câmaras de exposição permitem que os animais não fiquem isolados durante a exposição e permaneçam alojados em suas gaiolas de origem sem interferência com ciclo circadiano e alimentação. Outra vantagem do método é o fato de que os animais não necessitam de sedação ou treinamento. O tempo de exposição pode ser adaptado ao delineamento do estudo e pode se estender por meses (Shang & Sun, 2019).

O concentrador de partículas é outra forma de exposição à PA descrita por Sioutas *et al.* (1995). Atualmente, este método é empregado pelos laboratórios de pesquisa americanos e da cidade de São Paulo (Fonken *et al.,* 2011; de Brito *et al.,* 2014). O sistema de exposição mais representativo que pode gerar os desfechos mais produtivos é o que utiliza o princípio do crescimento por condensação de partículas ambientais submetidas ao impactador virtual de forma a concentrar o aerossol e remover as partículas com diâmetro aerodinâmico maior do que 2,5 μm e concentrar o aerossol restante através de técnicas de separação por inercia. A exposição ao aerossol final mimetiza a exposição ambiental real de todo o organismo a PA. A utilização de concentrador de partículas apresenta vantagens similares às descritas para as câmaras de exposição com a vantagem de redução do tempo de experimento sem comprometimento dos resultados. Por outro lado, a principal desvantagem é custo elevado dos equipamentos.

2. Justificativa

As evidências apontam para o envolvimento da PA, através de múltiplos mecanismos, na fisiopatologia de doenças neurodegenerativas que levam a perda de neurônios no SNC. Desconhecemos, até o momento, estudos que demonstrem objetivamente uma relação direta entre a exposição à PA em diferentes estágios de vida e possíveis alterações morfológicas e bioquímicas em diferentes regiões cerebrais. Tampouco é do nosso conhecimento que estudos prévios tenham determinado a influência da troca de ambientes (poluído e não poluído) sobre essas alterações. Assim, justifica-se a realização de um estudo com enfoque nos aspectos morfológicos e bioquímicos do tecido neural de animais aventando a hipótese de uma correlação entre a exposição à PA, troca de ambiente atmosférico em fase precoce de desenvolvimento pós-natal e os aspectos supracitados.

Ainda, como apontado por Calderon-Garcidueñas (2017) por ocasião de comentário de publicação previa (Chen *et al*., 2017), tem havido progresso modesto no entendimento da patogênese das doenças neurodegenerativas e é urgente que se desenvolvam paradigmas que integrem os níveis celular e molecular com o indivíduo e as alterações epigenéticas induzidas pelo ambiente.

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4. Objetivos

4.1 Objetivo geral:

Estudar o efeito da modulação da exposição pré e pós-natal à PA a nível ambiental sobre o número de neurônios e de microglia e sobre a bioquímica cerebral de ratos machos adultos.

4.2 Objetivos específicos:

Avaliar no córtex e estriado dos animais adultos (150 dias de idade) expostos a PA:

a) o efeito da troca de ambiente aos 21 dias de vida sobre a lipoperoxidação;

b) o efeito da troca de ambiente aos 21 dias de vida sobre a atividade das enzimas SOD e CAT e a concentração de GSH;

c) o efeito da troca de ambiente aos 21 dias de vida sobre o número de neurônios e micróglia utilizando-se análise histológica por técnica estereológica.

5. Artigo científico: The impact of chronic exposure to air pollution over oxidative stress parameters and brain histology.

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Abstract:

Chronic exposure to air pollution (AP) triggers neuroinflammation and lipoperoxidation also involved in physiopathology of several neurodegenerative diseases. The aim of this study was to investigate if cortex and striatum of rats chronically exposed to AP at ambient level display alterations of oxidative stress (OS) parameters and number of neurons and microglial cells modulated by environment change at weaning. Seventy two male Wistar rats were distributed in four groups of exposure: control group or FA, exposed during all life to filtered air; group PA-FA, exposed to polluted air during pre-natal period until weaning and then to filtered air; group FA-PA, exposed to filtered air during pre-natal period until weaning and then to polluted air and group PA, exposed during all life to polluted air. After 150 days, the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), and concentrations of malondialdehyde (MDA) and total glutathione (GSHt) were measured in cortex and striatum of 48 rats (n= 12 of each group of exposure). At the same time, the number of neurons and microglia of cortex and striatum were determined in the remaining 24 animals (n = six for each group of exposure). The cortex and striatum of the PA group showed significantly higher cortical MDA concentration. GSHt concentration was lower in cortex and higher in striatum of FNF group. SOD activity was significant decreased in cortex of all groups exposed to AP. AP exposure did not alter CAT activity in cortex or striatum. The number of neurons or microglia in the striatum did not differ between FA and PA. On the other hand, neurons and microglia cell numbers were significantly higher in the cortex of FA-PA group. Our findings suggest that striatum and cortex have dissimilar thresholds to react to AP exposure and different adaptable responses to chronically AP-induced OS. At least for the cortex, changing to a non-polluted ambient early in life was able to avoid and/or reverse the OS. We also hypothesize that the exposure to AP can trigger cortical neurogenesis at least transiently maybe as a compensatory mechanism.

Key words: neurogenesis, air pollution, oxidative stress.

INTRODUCTION

Recently, Di *et al*. (2017) demonstrated by overwhelming evidence the impact of air pollution (AP) in mortality of an open cohort with 460,310,521 person-years of follow-up. These authors showed that even low levels of $PM_{2.5}$ and ozone chronic exposure according National Ambient Air Quality Standard (NAAQS) were associated with an increased risk of death predominantly among black men and people with low income. The strength of the evidences justifies the permanent searching for methods to control and turn off the AP poisonous effects.

The first evidences of the toxic effects of AP to the central nervous system (CNS) were demonstrated by Zhang *et al*. (1988). These authors found an increased risk of stroke in a cohort of people exposed to indoor coal fumes. Afterward other authors (Hong *et al*., 2002; Lisabeth *et al*., 2008) confirmed the role of AP as an independent risk factor for stroke. Air pollution is also considered a risk factor for neurodegenerative diseases like Alzheimer's and Parkinson's (Kilian and Kitazawa, 2018). Several studies demonstrated an association between urban AP exposure and neuroinflammation, CNS oxidative stress (OS), and beta-amyloid deposition that are common mechanisms of different neurodegenerative diseases and result in the loss of neurons (Calderón-Garcidueñas *et al*., 2002, 2004, 2008a, 2010; Zanchi *et al*., 2008; Shulman *et al*., 2011).

The brain damage induced by AP exposure is mediated by fine and ultrafine particulate matter (PM) that reaches the CNS mainly by systemic circulation Muhlfeld *et al.*, 2008) and through olfactory epitelium (Gomez-Mejiba *et al*., 2009; Calderón-Garcidueñas *et al*., 2008a, 2010; Oberdörster *et al*., 2005a; Oberdörster *et al.,* 2005b; Oberdörster and Utell, 2002). The presence of PM in the brain promotes disruption of the blood-brain barrier (BBB), neuroinflammation, OS, endothelial activation and inflammatory cell trafficking (Calderón-Garcidueñas *et al*., 2008a). Zanchi *et al*. (2008) showed an increased level of lipoperoxidation in the striatum and cerebellum of rats exposed by intranasal instillation to residual oil fly ash (ROFA). The pre plus post-natal exposure of rats to environmental AP induced cortical OS and impairment of discriminative memory (Zanchi *et al*., 2010b). Fonken *et al*. (2011) demonstrated that mice chronically exposed to airbone fine PM displayed upregulation of inflammatory markers, reduced density and length of hippocampal dendritic spines, and was associated with depressive-like affective responses and cognitive impairment.

Calderón-Garcidueñas *et al*. (2004, 2008b) studied the effects of Mexico's city environmental AP in CNS of feral dogs and humans. These authors found upregulation of inflammatory markers with astrogliosis, OS, DNA damage in olfactory bulbs, frontal cortex and hippocampus, amyloid beta peptide ($A\beta_{42}$) deposition in neurons and astrocytes, white matter glial cells apoptosis and pre-frontal hyperintense lesions suggesting oligodendroglial cells impairment due to pollution. Besides the macro and micro alterations described above, OS, secondary to AP exposure or other mechanisms also induces cellular responses involving mitochondria and other organelles, notably the endoplasmic reticulum and eventually triggers the cell to enter a cell death pathway (Higgins *et al*., 2010; Xia *et al*., 2007).

Recently, Calderón-Garcidueñas *et al.* (2011) evaluated serum inflammatory mediators and brain volumetric alterations by magnetic resonance (MRI) of healthy children exposed or not to environmental AP of Mexico City and correlated the results with cognitive performance. The authors found that regardless of the presence of white matter hyperintensities, those children resident in a highly polluted atmosphere had a poorest performance across a variety of cognition tests. The children exposed to AP also exhibited significant systemic inflammation with the highest serum concentrations of inflammatory markers. Interestingly, there were no volumetric differences in subcortical structures suggesting that the endothelial dysfunctions and disruptions in BBB integrity, proposed as mechanisms of white matter damage in highly exposed subjects, occurred selectively just for certain higher cortical functions.

The major neuronal loss for Alzheimer's disease and Parkinson's and Huntington's chorea concentrates in cortex and in dopaminergic structures of midbrain respectively. Therefore, we aimed to investigate if there are morphological changes in the cortex and striatum of rats chronically exposed to the ambient AP of Porto Alegre city during distinct periods of life using a stereological technique. We also aimed to determine the OS in the same structures.

MATERIALS AND METHODS.

Air pollution exposure

The exposure chambers to polluted or filtered air were built in the garden of the Federal University of Health Sciences of Porto Alegre, the more southern capital city of Brazil. The university is located on a high traffic density avenue where the source of air pollution is predominantly automotive and follows. The exposure procedures were already described elsewhere (Damaceno-Rodrigues *et al*., 2009). The 11.8-m^3 chambers were sustained by a metallic structure and protected of ultraviolet radiation by a cover of plastic film. The air in the filtered air chamber (FAC) was supplied by a filtration air unit at a flow of 33 L/min. The filtration unit consisted of two pre-filters (PP-30 and pleat pre-filter of 18''), chemical (Puracarb® and PK-12; Borges–Katayama, Sao Paulo, Brazil) and high efficiency particulate arresting (HEPA) filters stages (Purafil® side access TAG B07-8338, Borges–Katayama, Sao Paulo, Brazil). The PP-30 had a 30% average efficiency and the pleat pre-filter of 18'', 60% of average efficiency. These first mechanical barriers removed the dust that could block the pores of pellets in the next stage of the unit. The next stage consisted of Puracarb® (activated coal) and PK-12 in 'V' which is made from acrylonitrile butadiene styrene plastic, that contains Purafil® CHEMI SELECT (alumen impregnated with potassium permanganate). Moreover, there were two stages of HEPA PH-97 which had a 99.97% highest efficiency (Borges–Katayama, Sao Paulo, Brazil). The percentages of H_2S, SO_2 and Cl_2 neutralized by Puracarb® are 20%, 5%, and 8%, respectively. The Purafil® CHEMI SELECT neutralizes H_2S, SO_2, nitric oxide (NO), and O_3 on percentages of 14%, 7%, 4.9%, and 90%, respectively (Borges–Katayama, Sao Paulo, Brazil). The HEPA filters retain 99.97% of dust and $PM_{2.5}$, besides the microorganisms (Borges–Katayama, Sao Paulo, Brazil). The only filter of air in polluted air chamber (PAC) was the mechanical one. Additionally, the pressure inside the chambers did not exceed the atmospheric pressure by more than 3 cm H2O. Approximately, 1960 l/h of air flow was provided by a fan assembled between the filtration unit outlet and the chamber inlet.

Animal care:

The study was approved by the review boards and ethical committee of the Federal University of Health Sciences of Porto Alegre and was carried out in accordance with international and national guidelines for animal welfare.

Experimental design

Seventy-two male Wistar puppies were used. Half of them were born after pre-natal exposure to FAC and the other half from prenatal exposure inside the PAC (n=36). All animals were maintained in polypropylene cages (47 cm × 34 cm × 18 cm), six rats per cage, in a temperature (22° + 2°C) and light-controlled 12-h-light/dark cycles (light from 7:00 a.m. to 7:00 p.m.) environment. Food (conventional laboratory diet Supra-lab, Alisul Alimentos S.A., São Leopoldo, RS, Brazil) and water were available ad libitum. On the 21st post-natal day (PND21) the animals were weaned and randomly allocated to compose the four experimental groups each one with six animals for histological studies and 12 animals for OS investigation as follows: control group FA - exposed during pre and post-natal period until adulthood to filtered air; group PA-FA - exposed to polluted air during pre-natal period until weaning and then to filtered air; group FA-PA - exposed to filtered air during pre-natal period until weaning and then to polluted air; group PA exposed during pre and post-natal period to polluted air.

At the PND150, six animals of each group of exposure were selected for histological studies. They were anesthetized intraperitoneally with ketamine (50mg/Kg) and e xylazine (20 mg/Kg) for cardiac perfusion with formaldehyde. The fixed brains were removed, and the cortex and striatum were dissected. Histological analyses are described below.

The other groups of 12 animals each were euthanized by decapitation in the 150 PND and cortex and striatum were immediately dissected and frozen in liquid nitrogen. Afterwards, these structures were homogenized in with KCl and phosphate buffer added with protein inhibitors and centrifuged to remove nuclei and cell debris. The pellets were discarded, and the supernatants were used as homogenates for OS analysis (described below).

Histological study

The wet weights of cortex and striatum were used to calculate the total volume of each structure using the formula: V=m/d (V= volume; m= mass and d=specific density of the tissue). The specific density of the tissue and the retraction factor were calculated using Cavalieri’s principle followed by shrinkage calculation (Dorph-Petersen *et al.* 2001). The sampling procedure for each of the brain structures was based on the fractionator random sampling and systematic random sampling (Mayhew, 2008). The fragments were embedded in paraffin wax and posteriorly were sliced by the disector method (Gundersen *et al.* 1988). The physical disector method was used to quantify the number of neuron and microglial cells. Two pair of 5 μm serial sections for cortex and striatum separated by a distance 150 μm were obtained. The sections were mounted on glass slides and stained using toluidine blue.

Nikon® ACT-2U Imaging software coupled to a light microscope (Nikon Opitphot®) was used for the microscopy. Digital images of two aligned consecutive sections stained by toluidine blue were obtained. Every pair of dissectors was composed by the reference plane on the superior section and the look-up plane on the inferior section and a squared counting frame of 2500 μm^2. For these frames, the upper and right sides of each frame are inclusion lines and the lower and left sides of the frame are exclusion lines. Only the cells inside the reference plane or touching the inclusion lines were counted (Sterio, 1984). The total number of neurons and microglial cells in cortex and striatum was obtained by the formula: Nt=DxVt (NT= total number of cells; D= numeric density of cells counted and Vt = total structure volume).

Analysis of Oxidative Stress Parameters

Lipid peroxidation was determined using the thiobarbiturc acid reactive substances (TBARS) method (Buege and Aust, 1978). TBARS concentration was expressed in nmol of malondialdehyde (MDA) per milligram of total proteins.

The methodology used to determine the SOD activity was the same described by Marklund (1985) and the results were expressed as units of SOD per milligram of total proteins. We used the method described by Aebi (1984) to quantify catalase's activity expressed in units per milligram of total proteins.

A gluthatione kit (Sigma CS0260, Saint Louis, MO, USA) was used to determine the total glutathione concentration and the results were expressed in nmol per milligram of total proteins.

All the above procedures are detailed elsewhere (Zanchi *et al.,* 2008, 2010a, 2010b, Fagundes *et al.,* 2015).

Statistical Procedures

Statistical analysis was performed through *Sigma Stt 3.11* software (Systat software Inc. 2004, Port Richmond, EUA). Results were expressed as mean ± standard error of the mean and submitted to one-way analysis of variance (ANOVA) test followed by the Student–Newman–Keuls post hoc multiple comparison test. The level of significance was set at 5%.

Results.

Analysis of OS:

OS level induced by chronic exposure to AP was quantified by the concentration of MDA (lipid peroxidation) and tGSH, as along with the activity of SOD and CAT in cortex and striatum, as showed in table 1. The cortex and striatum of rats exposed all life, both pre and postnatally to AP (PA) showed a significant almost 5-fold-increase in concentrations of MDA when compared to the other groups (P=0.007 and P=0.010 respectively). The cortex tGSH concentration was reduced in those group exposed only after weaning to AP (FA-PA) (P=0.003). On the other hand, these same animals showed an increase in tGSH concentration in striatum compared to the other groups (P=0.006).

A statistically significant decrease in SOD activity of cortex was found in all groups exposed to AP in some period of life in comparison to the FA group that was never exposed (P=0.012). The striatum showed a significant decrease in SOD activity only in the FA-PA group (P=0.004). CAT activity did not differ in cortex (P=0.440) or striatum (P=0.286) between the groups of exposure.

Table 1. Oxidative stress in córtex and striatum of the groups of rats chronically exposure to air pollution at ambient level.

Exposure group	MDA (nmol/mg total proteins)	SOD (U/mg total proteins)	CAT (pmol/mg total proteins)	GSHt (nmol/mg total proteins)
		CORTEX		
FA	1.73±0.628	6.509±0.74	0.02874±0.00805	776.79±61.707
PA-FA	1.101±0.217	**5.627±0.408****	0.04485±0.02832	653.81±69.352
FA-PA	1.014±0.3	**4.077±0.275****	0.06707±0.00304	**402.26±45.985***#
PA	**5.978±1.92***	**4.455±0.577****	0.04553±0.01325	669.11±63.683
		STRIATUM		
FA	0.975±0.347	6.075±0.493	0.06773±0.01237	148.627±8.689
PA-FA	0.516±0.139	5.384±0.16	0.03881±0.03703	155.897±9.603
FA-PA	0.903±0.277	**4.167±0.139**#	0.04969±0.00765	**195.207±12.193***#
PA	**2.415±0.656***	5.244±0.387	0.04737±0.00894	147.011±5.506

Data are expressed as means±SD; one-way ANOVA, SNK post-hoc.

*MDA concentration in córtex: P=0.007 and striatum P=0.010 compared with other groups.

**SOD activity in córtex: P=0.012 compared with FA; #SOD activity in striatum: P=0.004 compared with the other groups.

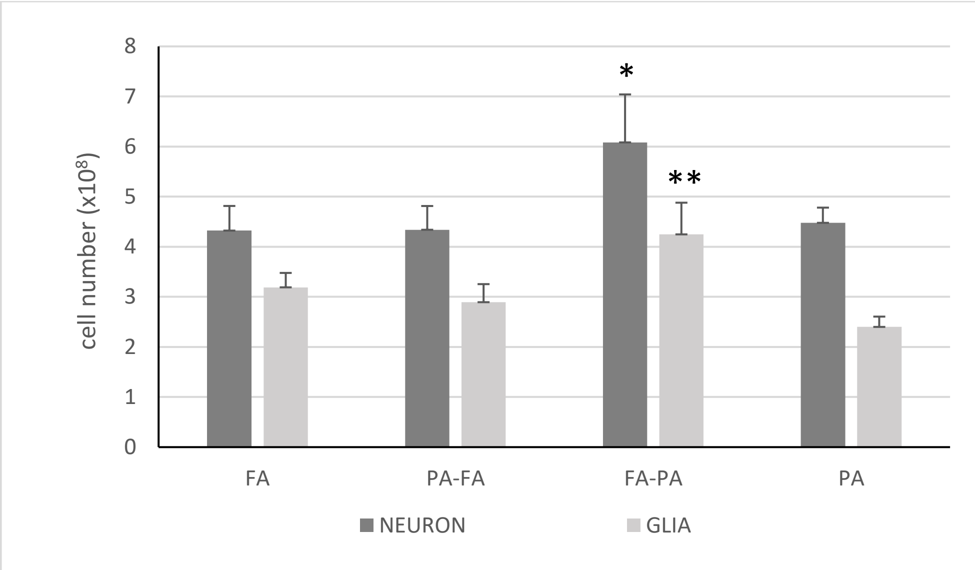
*#tGSH concentration in cortex P=0.003 and striatum P=0.004 compared with other groups.

FA – filtered air; PA-FA – non-filtered air-filtered air; FA-PA – filtered air-non-filtered air; PA – non-filtered air.

Histological analysis:

The figure 1 represents the total number of neuronal and microglia cells calculated by stereological technique. It can be observed in figure 1A that the cortex of FA-PA group showed a significant increase in the number of neurons (P=0.018) and microglial cells (P=0.02) when compared to the other groups. The striatum did not differ regarding neuron or microglial cells number between the four groups of exposure as showed in figure 1B. On the other hand, we can observe a tendency of increasing number of striatal neurons of animals exposed to AP in any period over their life time, as highlighted by dotted blue line. The dotted red line emphasizes that this tendency was not observed by microglial cells.

A



B

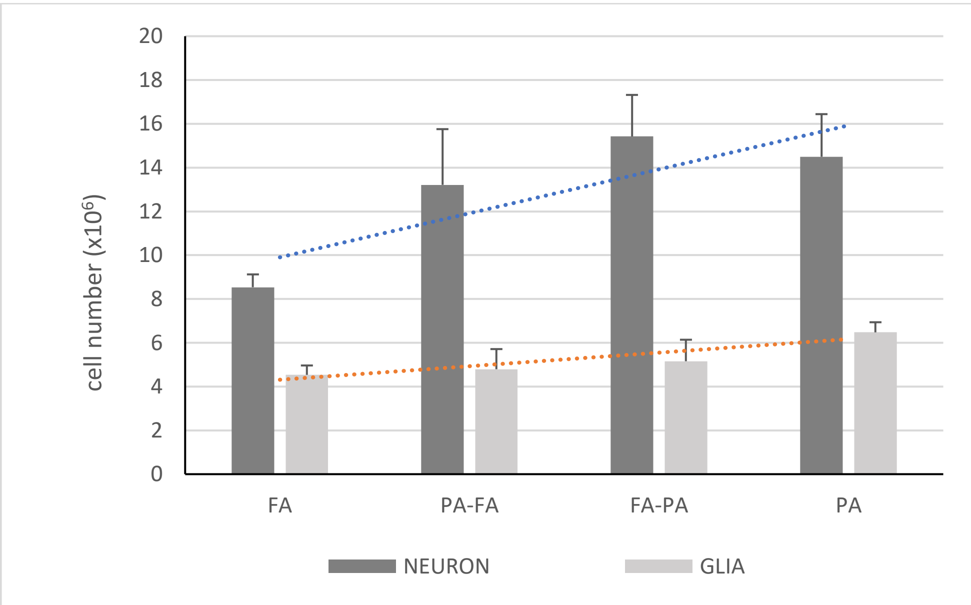


Figure 1. Mean number (±SEM) of neurons (grey bars) and microglial cells (white bars) of the groups of rats chronically exposure to air pollution at ambient level. FA (filtered air chamber); PA-FA (nonfiltered air – filtered air); FA-PA (filtered air-non-filtered air); PA (non-filtered air). (A) Cell number in cortex: significant increase in *neuron (*P*=0,018) and **microglial cells (*P*=0,02) in the FA-PA group in comparison with the other groups. (B) Cell number in striatum: the number of neurons or microglia of

striatum did not differ between groups. The dotted blue line highlights a tendency to an increase in striatal neurons in the groups exposed to AP some time over their lifetime.

Discussion

Our results showed an increase of lipid peroxidation in the cortex and in the striatum of rats after exposed during all life to automotive-derived AP. Likewise, the exposure to AP for at least one period of life induced an imbalance in the antioxidant system as revealed by a significant reduction of SOD activity in the cortex in all groups of rats exposed to AP at some point in life and a significant decrease in GSH concentrations in the FA-PA group. The striatum of rats of FA-PA group showed a decrease in SOD activity but an increase in GSH concentration. We also showed that changing from a polluted to a non-polluted ambient (PA-FA) reverted the lipid peroxidation in cortex and striatum and restored the SOD activity in striatum. Unexpectedly, the histological study of both cortex and striatum did not reveal a reduced number of neurons. Instead, we found that changing from a polluted to a non-polluted ambient resulted in a significant increase in neuron number in the cortex of rats. This same group of animals showed an increased number of microglial cells, which may be an evidence that AP promotes neuroinflammation.

The higher MDA concentrations found in cortex and striatum of the animals exposed during all life, from pre-natal to adults to polluted ambient reinforces the evidences that AP induces lipoperoxidation in brain regions. Our findings corroborate the results of several other studies. Marchini *et al.* (2014) demonstrated that female mice acutely exposed to residual oil fly ash (ROFA) by nasal instillation presented a plasmatic increase of TBARS up to the first 3 hours of exposure. In addition, chronic ROFA exposure of rats by nasal instillation induced lipoperoxidation in striatum, hippocampus and cerebellum with elevation of TBARS levels (Zanchi *et al.,* 2008, 2010b). Costa *et al*. (2017) have studied the effects of acute exposure of adult mice to $PM_{2.5}$ or ultrafine. These authors demonstrated high brain levels of lipoperoxidation and pro-inflammatory cytokines as well as microglia activation in exposed animals. There was also decrease of neurogenesis in hippocampal sub granular zone (SGZ) and subventricular zone (SVZ).

On the other hand, the activity of SOD in our study exhibited a more complex profile that probably reflects differences in susceptibility and/or thresholds of tissue reactions to OS. The striatum showed a significant decrease in SOD activity only in the group of animals exposed to AP after weaning (FA-PA) whereas in cortex the activity of SOD decreased significantly in rats exposed to AP in some period of post-natal life, independently of ambient change. This finding supposes that the exposure to AP early in life as demonstrated by PA-FA group triggers long lasting effects over the enzymatic antioxidant system of cortex even without an increase in MDA concentrations. Long lasting alterations of cellular enzymatic antioxidant systems can be a consequence of epigenetic mechanisms activated by OS during early stages of brain development. According to the free radical theory of development, the development of organisms is dependent of production and removal of reactive oxygen species (ROS). Therefore, the cells under development must increase their antioxidant defenses, for example, throughout the production of GSH (Hitchler & Domann, 2007). However, the increased GSH production interferes with epigenetic processes like DNA and histone methylation by reducing the availability of the cofactors required during epigenetic control of gene expression by DNA and histone methyltransferases (Hitchler & Domann, 2007). We hypothesize that the exposure to air pollution during pre-natal period could disrupt the redox balance in cortex favoring the production of ROS and an over expenditure of SOD. Consequently, the sustainable reduction in cortex SOD activity of the PA-FA group even after ambient change can be secondary to a long-lasting disturbance of antioxidant enzymatic defenses during pre-natal exposure to AP. Another consequence secondary to similar mechanism concerns to a model recently proposed by Lahiri *et al.,* (2007) and termed “Latent Early-Life Associated Regulation” model. This model postulates that exposure of developmental cells to environmental agents (heavy metals), cytokines, and dietary factors induces a long-lasting disruption in gene regulation that begins early in development but will be manifest later in life. Although longlasting chages in enzymatic antioxidative system, our results showed that ambient change during weaning period was able to bring MDA levels back to control values.

The literature gives evidence that the SOD extracellular component is reduced in a diversity of conditions that comprise OS as coronary arterial disease (Landmesser *et al.*, 2000). Delfino *et al.*, (2008) assessed biomarkers of OS and inflammation in a cohort of elderly with history of coronary arterial disease living in Los Angeles (US). These authors showed that PM induced systemic inflammation, platelet activation e reduction of erythrocyte Cu-Zn-SOD activity. Inactivation of antioxidant enzymatic system by electrophilic ambient environment pollutants is an additional mechanism for induction of OS by AP. *In vitro* studies to determine the sensibility of antioxidant enzymes to their own substrates demonstrate that hydrogen peroxide inactivates SOD (Pigeolet *et al.*, 1990). This mechanism could work together to reduce SOD activity in the cortex of the group exposed to AP during all life although does not explain the similar findings in the other groups where the concentrations of MDA were not elevated. While PA-FA group showed a sustained reduction in cortex SOD activity probably secondary to early exposure to AP, the FA-PA preserved the antioxidant enzymatic defenses that successfully neutralized the ROS generated by OS. These findings corroborate the hypothesis that ambient conditions during brain development impacts SNC ability to deal with OS.

The activity of SOD in striatum depicts a different profile in comparison to that of cortex where only the FA-PA showed a significant decrease in this enzyme activity. This group of animals also showed elevated concentration of GSH. Concurrently we did not identify differences in microglial cell number between the striatal groups. We consider that this finding may be secondary to discrepancies in the recruitment of inflammatory cells between cortex and striatum. Fagundes *et al*., (2015) using an *in vitro* model of acute exposure to increasing concentrations of PM, demonstrated a decrease of catalase activity in hippocampus, cerebellum, striatum and olfactory bulb when exposed to the highest PM concentrations. Nevertheless, SOD activity was higher in hippocampus and olfactory bulb, but cortex did not show differences in enzymatic activity. Guerra *et al*., (2013) used an inertial particle separator system as a method of exposure rats to inhaled PM and to study the profile of SOD2 mRNA and Heme-oxigenase-1 (HO-1) mRNA besides other biomarkers of inflammation and cell death in olfactory bulb, frontal cortex, striatum and hippocampus of animals exposed to PM_{10}, $PM_{2,5}$ and ultrafine PM. The olfactory bulb and striatum showed elevated levels of HO-1 mRNA unconcerned to MP diameter while in cortex and hippocampus only $PM_{2,5}$ and ultrafine PM determined significant increase in HO-1 mRNA. SOD mRNA was elevated in striatum independently of the diameter of PM and in hippocampus only in the group of animals exposed to ultrafine PM.

The comparison between different categories of brain cells demonstrated that non-neuronal cells have the highest concentrations of GSH (Dringen, 2005; Rae & Williams, 2017). Microglial cells are regularly exposed to high ROS levels what dictates their need for highly effective antioxidant defense systems to protect against oxidative damage (Chatterjee *et al.*, 2000; Vilhardt *et al.,* 2017). Besides the production of proinflammatory cytokines, superoxide, NO, ROS and proteases (Tang & Li, 2015), activated microglia, also termed “M1 microglia” have a high expression of GSH (Vilhardt *et al.,* 2017). After intrastriatal administration of quinolinic acid, activated microglia showed the highest activity of glutathione peroxidase compared to astrocytes (Lindenau *et al.*, 1998). Human studies demonstrated that glutathione peroxidase was higher in brain microglia of Parkinson's disease and dementia with Lewy bodies possibly involved in neuroprotection (Power and Blumbergs, 2009). According to these evidences, we hypothesize that the increased number of microglial cells of the FA-PA group contributed with the decrease of GSH levels in cortex and possibly had a protective role avoiding OS since there was not elevation in MDA levels in this group of AP exposure.

We need to consider the regulation of antioxidant response by Nuclear factor erythroid-2-related factor 2 (Nrf2). The Nrf2 and its specific repressor, the Kelch-like ECH-associated protein 1 (Keap1), responsible for Nrf2 sequestration in the cytoplasm, mediate cellular response to oxidative stress and to electrophilic xenobiotics (Osburn & Kensler, 2008). Included among the target genes regulated and induced by Nrf2 are: HO-1, SOD, CAT, NADPH-quinone oxidoreductase 1, glutathione S-transferase, glutathione reductase (GR), glutathione peroxidase (GPx), thioredoxin and glutamate cysteine ligase (Ahmed *et al.*, 2017; Vilhardt *et al.,* 2017). Nrf2 is the main regulator of antioxidant responses, mediates negative regulation on the transcription of pro-inflammatory cytokines and chemokines, adhesion molecules, metalloproteinases, cyclooxygenases and inducible nitric oxide synthase (iNOs) (Osburn & Kensler, 2008; Ahmed *et al.*, 2017; Vilhardt *et al.,* 2017). It also blocks the activation of nuclear transcriptional factor kappa B (NFκB) that is essential in many inflammatory responses signaling, immune function, apoptosis and cell growth (Ahmed *et al.*, 2017). The activation of NFκB predominates over Nrf2 in conditions of chronic inflammation with OS and cell death as consequences (Ahmed *et al.*, 2017; Lodovici & Bigagli, 2011). The Nrf2 controls the γ-glutamyl cysteine synthetase the rate-limiting enzyme in GSH biosynthesis (Ghosh *et al*., 2014). Even though we did not measure inflammatory biomarkers, the presence of lipoperoxidation and an elevated number of microglial cells in cortex of FA-PA group are indicative of inflammation induced by chronic exposure to AP. This chronic condition could compromise the Nrf2 activity and decrease SOD expression. Mixed cultures of glial cells treated with lipopolysaccharides and interferon-γ showed activation of iNOs and a drop in GSH concentration in microglial cells (Chatterjee *et al.,* 2000). On the other hand, microglial cultures treated with TNF-α increased GSH, MnSOD levels and MnSOD activity (Dopp *et al*., 2002). The diversity of microglia reactions to inflammatory stimuli in comparison with other glial cells is owing to the cytokines receptor expression (Dopp *et al*., 2002). Accordingly, the source of the stimuli will determine an increase in GSH concentrations secondary to enhanced synthesis or a decrease following enhanced demand (Dringen, 2005).

The dissimilar profile of cortex and striatum responses to chronic AP exposure reflects the well described phenomena of selective neuronal vulnerability from which the neurodegenerative diseases compose the best models (Morigaki & Goto, 2017; Johri & Beal, 2012). On the other hand, cortical neurons in culture showed high survival rate after exposure to different conditions involving OS (Wang & Michaellis, 2010) possibly because of lower levels of genes related to OS suggesting a limited intrinsic OS status with a low level of ROS/reactive nitrogen species formation (Wang & Michaellis, 2010). We cannot conclude for an established difference between cortex and striatum neurons vulnerability to chronic AP exposure based only in our findings of SOD activity and MDA concentrations. Although, we suppose that differences in adaptive responses determine a stepwise progression of neuron damage that is not identical between different brain regions. The interpretation of enzymatic antioxidant functions secondary to AP exposure must consider several variables that are difficult to standardize even using experimental models. Among these variables, we can cite the composition of the aerial and the solid phase of the AP, genetic background, gender and age of individuals involved in the studies, as well as the model and period of exposure. All these issues can modulate the OS and neuroinflammation circumstances and the responses of exposed individuals or tissues.

Another potentially harmful effect of the exposure to AP affects blood-brain-barrier (BBB) integrity. Human exposure to AP induces endothelial cellular damage by the formation of adhesion factors, cytokines and ROS that modify the permeability and the function and expression of transporter proteins (Hartz *et al.,* 2008). Chronic exposure to AP enhanced BBB permeability and expression of endothelial adhesion factors (Calderon-Garcidueñas *et al*., 2008a). An investigation of AP effects with brain magnetic resonance of children and dogs in Mexico City revealed abnormalities secondary to disruption of BBB (Calderon-Garcidueñas et al., 2008b). Experimental studies demonstrated an increase in BBB permeability after exposure to a mixed vehicular emission, a combination of gasoline and diesel engine exhausts, originated by the protein deterioration in tight junctions (Oppenheim *et al*., 2013). BBB permeability is also modified by metalloproteinases which genic expression is enhanced in the presence cytokines (IL-1,4,6 e TNF-α), ROS and NO stimuli (Rojas *et al.*, 2011). Even though the BBB is established and functional during embryogenesis in both humans and rodents, its complete maturation occurs between PND 14 and PND21 in rats (Semple *et al.*, 2013). In this regard, BBB rupture during chronic early AP exposure favors the infiltration of toxicants in brain tissues resulting in neuroinflammation and OS. The immature brain has a reduced capacity to deal with OS due to reduced antioxidant reserve and function of some antioxidant enzymes (Potts *et al.,* 2006; Mavelli *et al.,* 1982). These mechanisms and peculiarities of immature brain could potentiate cellular damage induced by lipoperoxidation and imbalance of epigenetic mechanisms and determine the higher MDA concentrations in cortex and striatum of our group of rats exposed during all lifetime to pollution.

Unexpectedly our histological study revealed a significant increase of neuron number in cortex of FA-PA group. This group also revealed a higher number of microglial cells. On the other hand, the number of microglial or neuron of striatum did not differ between groups. Nonetheless, we recognize a non-significant tendency of increasing neuron number in the groups exposed to AP in some period of life or during all lifetime. The microglial elevated cell number in cortex of FA-PA group suggests inflammation. Whereas the absence of this finding in the PA-FA group demonstrates the suppressant effect of changing to a non-polluted ambient over the cortical inflammatory process. Nevertheless, we cannot sustain the same explanation for the finding of non-elevated number of microglial cells in cortex of PA group. Undoubtedly, chronic exposition to AP during pre and post-natal periods triggers adaptive mechanisms to chronic inflammation which latency, intensity and extent are still unknown. The current knowledge about microglia structure reveals multifaceted and very dynamic phenotypes from proliferative, synaptic pruning, neuromodulation, phagocytosis and inflammation ones (Gomez-Nicola & Perry, 2015). Therefore, microglia can assume a protective role secondary to disease or a neurotoxic phenotype and cellular damage. Pharmacological or genetic ablation of microglia during brain development produced negative and long-lasting impact on synaptic maturation and establishment of CNS circuits (Nelson & Lenz, 2017). A recent review of Condello *et al*., (2017) propose a diverse role to neuroglia in Alzheimer's disease. According to these authors, microglia promotes encapsulation of amyloid deposits and creates a barrier that isolates and protects adjacent axons against neurotoxicity. Microglia also microglial regulates cortical neurogenesis in normal conditions by controlling the size of neural precursor cells reservoir of CNS. Neurogenesis, the process by which new neurons are formed, is present in the adult brain (Ehninger & Kempermann, 2008; Inta *et al.*, 2015). Early manipulation of fetal microglia through maternal immune activation or suppression modifies neural precursor cells in embryo and during pos-natal period (Cunningham *et al.,* 2013). Bolton *et al.,* (2017) studied the impact of pre-natal exposure of male and female mice to diesel exhaust particles over microglia development and function and found long-lasting alterations in microglial morphology and in neuron-glia interactions in exposed male mice. These findings were accompanied by an embryo increase of cortical volume with regression in the post-natal period and a non-significant elevated neuron number. The authors propose that these results could be secondary to excessive neurogenesis or anomalous phagocytosis and apoptosis function of neuroglia or other related cells. Ischemic striatal lesions induced experimentally revealed that microglia in the lesion zone takes different phenotypes compared to the subventricular zone. In this neurogenic niche, microglia adopt an intermediate profile of low activation grade capable to stimulate neurogenesis and promote migration and integration of the new neurons toward the lesion zone (Thored *et al.,* 2009). Striatal neuronal loss induced by middle cerebral artery occlusion promotes migration of neuroblasts to the lesioned zone where they will adopt the phenotype of dead neurons (Yamashita *et al.*, 2006). On the other hand, a growing body of evidences has supported a role of neurons in regulation of microglia state and this cross talk is mediated by chemokines like fractalkine (CX3CL1) highly expressed in neuron an its receptor CX3CR1 highly expressed in microglia. This chemokine is a neuroimmune regulatory molecule that maintains microglia in their resting phenotype (Kohman & Rhodes, 2013). The expression of this phenotype after inflammatory response suggests a compensatory and protector function to neurogenesis by producing factors that recover neuroblasts and induce their differentiation to neuronal cell as well as extend the neurogenic potential of stem neural cells. The neurogenic factors like IL-4, brain derived neurotrophic factor (BDNF), TGF-β e IGF-1 mediate microglial positive effects in adult neurogenesis in a context-dependent way (Walton *et al.,* 2006).

Finally, considering the importance of striatum for procedural or non-declarative memory (Rankin *et al.,* 2009; Squire and Dede, 2015), the studies that evaluate habituation memory can give information about the functional state of striatum. Zanchi *et al.,* (2010a) found a progressive decrease in objects exploration determined by chronic exposure to AP what could be representative of increased levels of habituation. Woodward *et al*. (2017) studied the impact of intermittent exposure of female mice to nPM from ambient urban traffic emissions in several parameters as neuritic atrophy, white matter degeneration, and microglial activation. They also tested the animals to recognition memory and spontaneous alternating behavior and found that nPM exposure decreased exploratory activity in both tests without impairing recognition memory. Human studies with brain magnetic resonance showed that the harmful effects of AP exposure occurred selectively without encompass subcortical structures although with impairment of cortical higher functions as demonstrated by cognitive challenges (Calderón-Garcidueñas et al., 2011). The tendency to an increase in neuron number in striatum of our rats exposed to AP could justify a habituation strengthening? Given the complexity of the effects of AP exposure in CNS homeostasis, there is a need to proceed the research about the impact in the different categories of memory.

Conclusions

The method used in our study simulates the quotidian exposure of population to AP and reveals that chronic and uninterruptedly pre and post-natal exposure to urban AP is necessary to induce higher levels of lipoperoxidation in cortex and striatum. Our results also point to an imbalance of CNS antioxidant enzymatic activity especially in cortex of the exposed animals probably reflecting dissimilar thresholds to react to OS. Even though we did not determine the levels of inflammatory biomarkers, the elevated number in microglial cells in cortex is an undoubted evidence of inflammation. Differently of striatum that shows no significant changes in neuron and microglial cells number, in cortex, the exposure to AP after weaning determined a significant elevation of both cells. This finding reflects differences in vulnerability between these regions. In conclusion, our findings support the idea that brain regions do not react identically to the exposure to AP. Further investigations are necessary to address the effects of AP in different brain regions with control of age, interval of exposure and gender.

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5. Conclusão

Os nossos resultados permitem concluir que a exposição crônica diária durante o período pré e pós-natal à PA mesmo em concentrações médias diárias anuais de $MP_{2,5}$ inferiores aos níveis de atenção para a qualidade do ar conforme estabelecido pelo CONAMA em 2008, induz estresse oxidativo no córtex e estriado. Constatamos também haver um desequilíbrio na atividade enzimática anti-oxidante manifestado principalmente no córtex dos animais expostos refletindo limiares diversos de reação ao EO entre as regiões cerebrais estudadas. Concomitantemente à presença de EO observamos uma elevação no número de células microgliais no córtex dos animais expostos à PA após o desmame reforçando a ideia de processo inflamatório associado. O cortex deste mesmo grupo de animais também apresentou número aumentado de neurônios. Contrariamente aos achados no cortex, o estriado não apresentou modificações significativas no número de células neuronais ou microgliais. Desconhecemos, até o presente momento, estudos que tenham demonstrado resultados semelhantes particularmente em termos de número de células neuronais. As respostas não homogêneas à exposição à PA por parte do córtex e do estriado quanto ao número de celulas microgliais e neuronais e atividade enzimática antioxidante pode ter origem no fenômeno da vulnerabilidade neuronal seletiva que envolve múltiplos mecanismos. Quanto ao aumento do número de neurônios, aventamos a hipótese de que se trate de neurogênese compensatória ao processo inflamatório desencadeado peal PA no córtex cerebral. Para comprovar esta hipótese há necessidade de prosseguirmos nos estudos com a utilização de técnicas específicas para identificação de neurônios jovens em diferentes etapas de desenvolvimento cerebral durante exposição à PA. É importante assinalarmos também que o fato de permitir ao SNC desenvolver-se e maturar-se em ambiente não poluído parece exercer efeito protetor contra o estresse oxidativo induzido por exposição à PA em fase mais tardia.

7 Anexos

7.1 Anexo I

Normas de publicação no periódico Inhalation Toxicology.

Inhalation Toxicology Instructions for Authors

About the Journal Aims and Scope Editor-in-Chief

Manuscript Submission

Manuscript Preparation File preparation and types Title Page Abstract Main Text Acknowledgments and Declaration of Interest statements References Tables Illustrations Notes on Style

Editorial Policies Authorship Redundant publications and plagiarism Peer Review Ethics and Consent Copyright and Permissions CrossCheck Declaration of Interest NIH and Public Access Policy

Additional Information Proofs Reprints Contact the Publisher

About the Journal

Aims and Scope

Inhalation Toxicology is a peer-reviewed monthly publication providing a key forum for the latest accomplishments and advancements in concepts, approaches, and procedures presently being used to evaluate the health risk associated with airborne chemicals.

The journal publishes original research, reviews, symposia, and workshop topics involving the respiratory system's functions in health and disease, the pathogenesis and mechanism of injury, the extrapolation of animal data to humans, the effects of inhaled substances on extra-pulmonary systems, as well as reliable and innovative models for predicting human disease.

Editor-in-Chief

Donald E. Gardner, PhD Savannah, GA USA

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