Fundació La Marató 3

23rd SOCIAL RETURN OF THE RESEARCH
Strokes and traumatic spinal cord and brain injury

INFLUENCE OF DIESEL MOTOR PARTICLES AS ATMOSPHERIC POLLUTANTS IN BRAIN LESIONS AND REPAIR AFTER EXPERIMENTAL STROKE

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1. Project Summary

Air pollution in cities is composed of particulate matter (PM), gases, organic compounds and metals. However, it is considered that the particulate matter fraction from the artificial combustion of fossil fuels, namely diesel exhaust particles (DEP), is the most harmful for human health. As the particle becomes smaller in size, the more it can penetrate the organs and the more damaging actions it can inflict. Additionally, air pollution is ranked as an important risk factor for mortality and recent data indicates that global mortality and morbidity of cardiovascular disease are strongly influenced by PM damaging actions. Among cardiovascular diseases, stroke is the second leading cause of death globally and 85% of strokes are caused by the occlusion of blood vessels supplying the brain. In this context, PM is thought to contribute to the ischemic stroke pathology through mechanisms of inflammation, pro-thrombosis and/or impaired recovery.

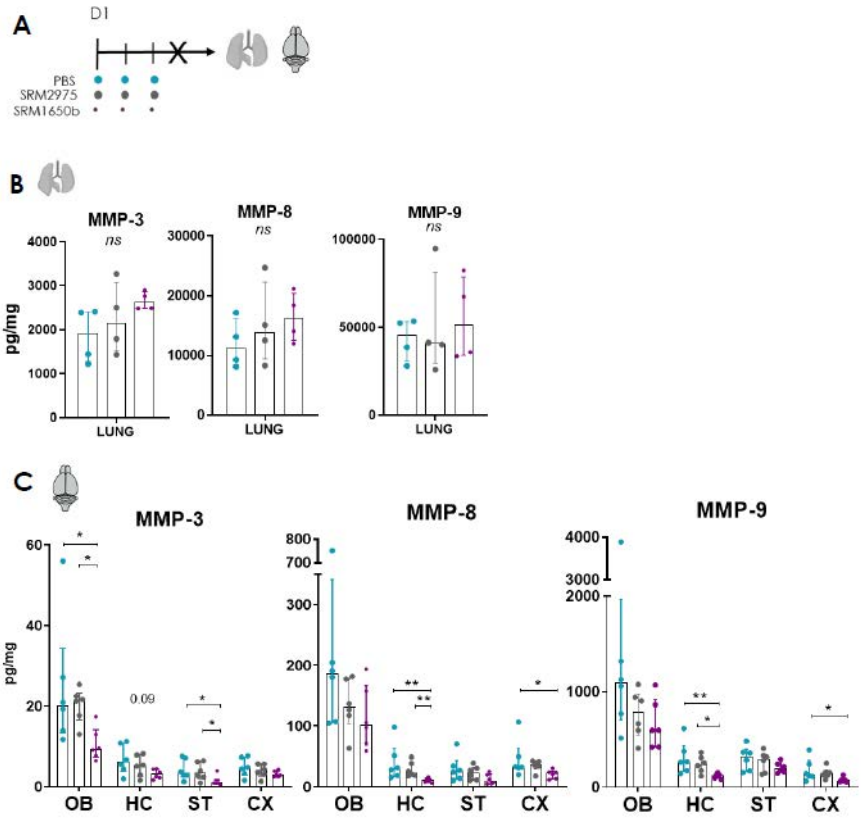
This project aims to investigate the possible interaction of DEP in the brain with the pathophysiological mechanisms of cerebral ischemia before and during the acute phase of the disease, by means of experimental models including an in vivo mouse model of cerebral ischemia, by in vitro neural stem cell (NSC) cultures and by an ex vivo turbidimetric assay of clot formation and tPA-induced lysis with plasma samples from humans and mice. Matrix metalloproteinases (MMPs) are key enzymes related to injury and inflammation in acute stroke but are also essential in post-stroke tissue remodeling. In the lungs of healthy mice exposed to DEP for 3 days (acute) or 3 weeks (chronic), MMP levels were not altered. However, in the brain they decreased after acute DEP exposure but raised after chronic exposure. Chronic exposure reduced the amounts of cortical neurons while both exposures showed fewer NSC in the rostral migratory stream matching with in vitro data where NSC viability was compromised. After acute or chronic DEP exposure, mice underwent permanent middle cerebral artery occlusion (MCAo, ischemia). In the lungs of the chronically exposed group, an altered MMP response was observed along with reduced alveolar macrophages and increased interstitial macrophages and monocytes. However, brain injury produced by brain ischemia was not exacerbated by the pre-exposure to DEP, and no changes in brain MMP levels or in the cell counts of different CNS cells were observed. The potential systemic effects of DEP exposure related to stroke were studied analyzing thrombogenic/thrombolytic plasmatic characteristics through turbidimetry. After

ischemia, mouse and human plasma presented pro-thrombogenic features in the presence of DEP, while thrombolysis slowed in the ischemic chronic group returning to basal levels after DEP exposure. Additionally, humans living in highly polluted areas presented accelerated thrombolysis compared to individuals living in less polluted areas.

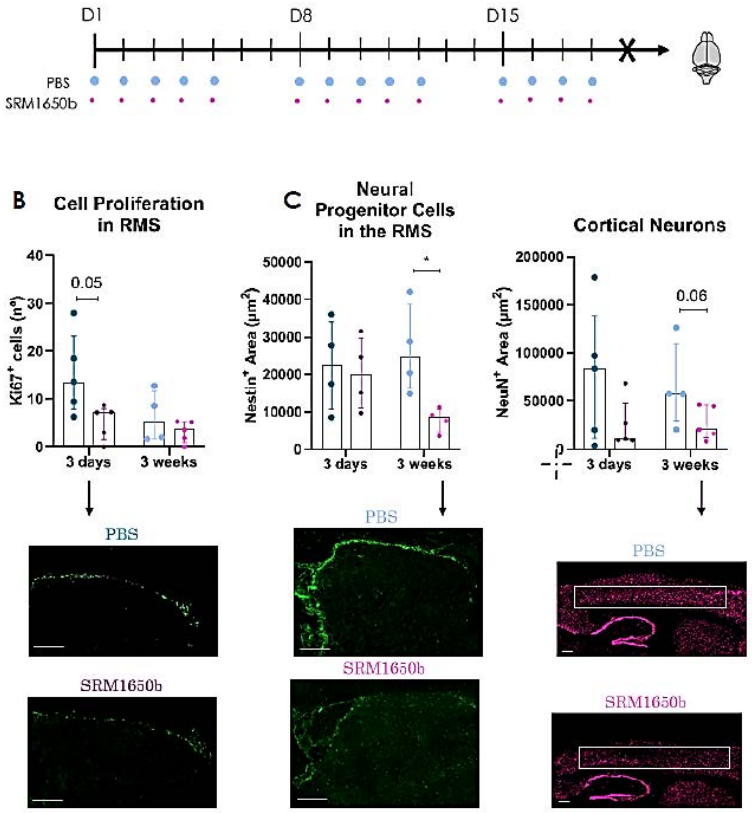
In conclusion, we have found that diesel exhaust particulate matter can regulate the expression of brain proteases in healthy animals while reducing the progenitor and mature neuronal pools, compromising the CNS physiological balance. The expected detrimental role of DEP exposure in the context of cerebral ischemia by exacerbating the infarct lesion has not been observed in the tested conditions although the influence in post-stroke repair needs to be elucidated. Systemically, DEP exposure influenced the recruitment of inflammatory leukocyte populations in the lungs after ischemia and induced pro-thrombotic characteristics in plasma, which could influence the brain clots in stroke disease and the outcome of thrombolytic therapies, which nowadays are the only treatment for ischemic stroke in the acute phase of the disease.

2. Results

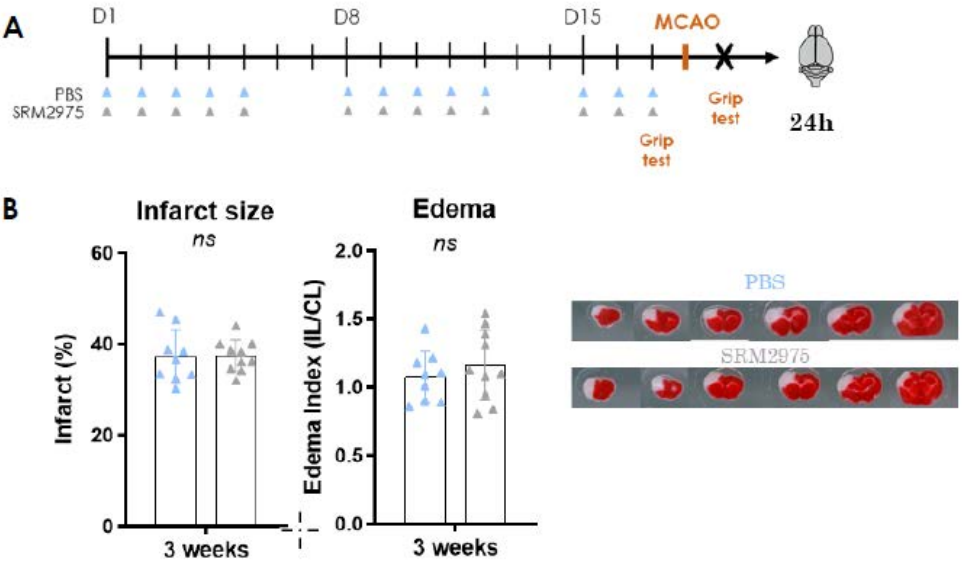
1-Acute exposure to SRM1650b DEP decreases MMP levels in the brain of naive mice. A: Experimental design: after 3 days exposure, brains and lungs were obtained for MMP analysis. B: Levels of MMP-3, -8 and -9 in the lungs. C: MMPs were analyzed in different brain regions showing decreased MMP levels in several of these areas. Data is represented as median (IQR). * p<0.05, ** p<0.01, PBS(n=6), SRM2975(n=6) or SRM1650b(n=6).



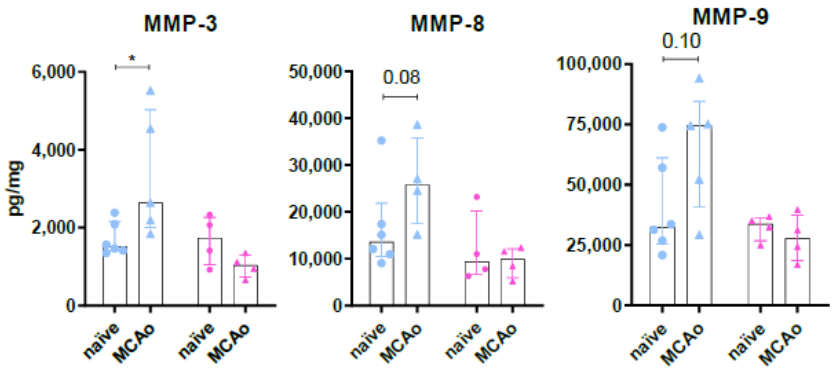
2- Brain neurogenesis markers were reduced after exposure to SRM1650b in naïve mice. A: Experimental design: 3 days and 3 weeks DEP exposure before the immunofluorescence study in brain. B: Bar graphs and representative images showing Ki-67+ reduction in acutely exposed mice. C: Bar graphs and representative immunofluorescence images of the Nestin+ and NeuN+ study showing reduced positive area in chronically exposed mice. Data is represented as median (IQR). Scale: 200 µm. Comparisons are made between DEP and PBS. * p<0.05. Acute-PBS(n=5), SRM1650b(n=5) and chronic-PBS(n=4), SRM1650b (n=5).



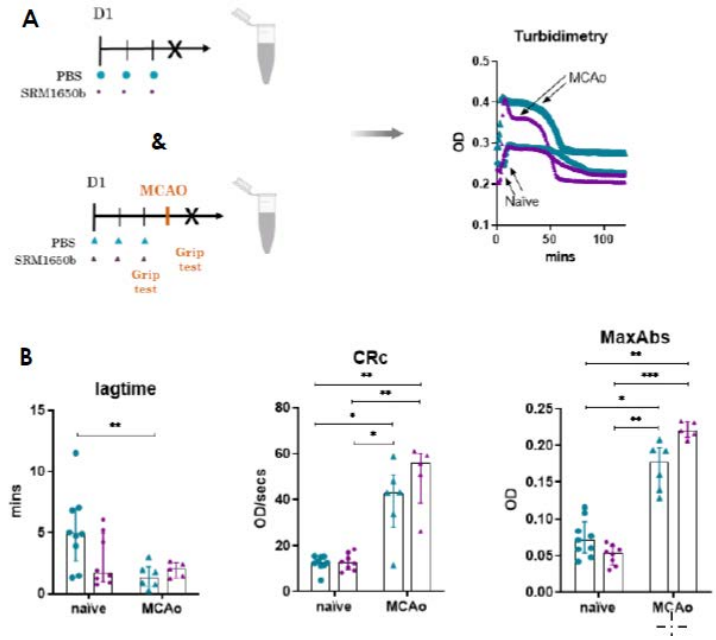
3- Infarct lesion measurements after chronic DEP exposure at 24 hours of permanent ischemia. A: Experimental design overview. B: Bar graphs showing the infarct and edema quantifications and TTC representative images. Data is represented as mean ±SD. *ns*: not significant. PBS (n=9), SRM2975 (n=10).



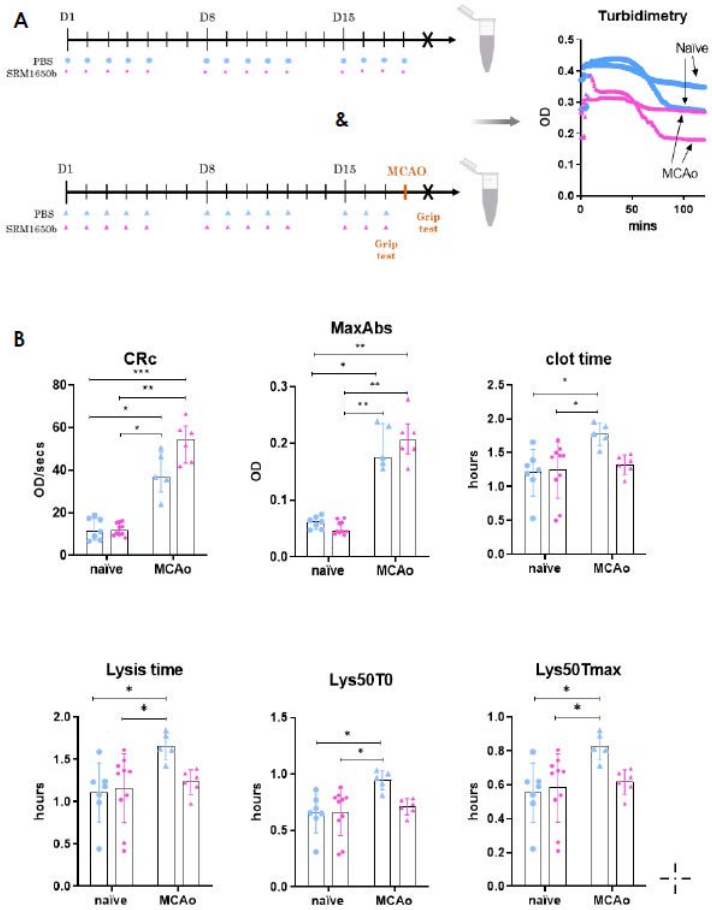
4- MMP alterations in the lungs after ischemia in chronically exposed mice. There is an inflammatory MMP response after ischemia in lungs of PBS-ischemic mice, which is not observed after the chronic exposure to SRM1650b. Data represented as median (IQR). * p<0.05. Naive: PBS (n=6), SRM1650b (n=4) and MCAo-PBS (n=5), SRM1650b (n=5).



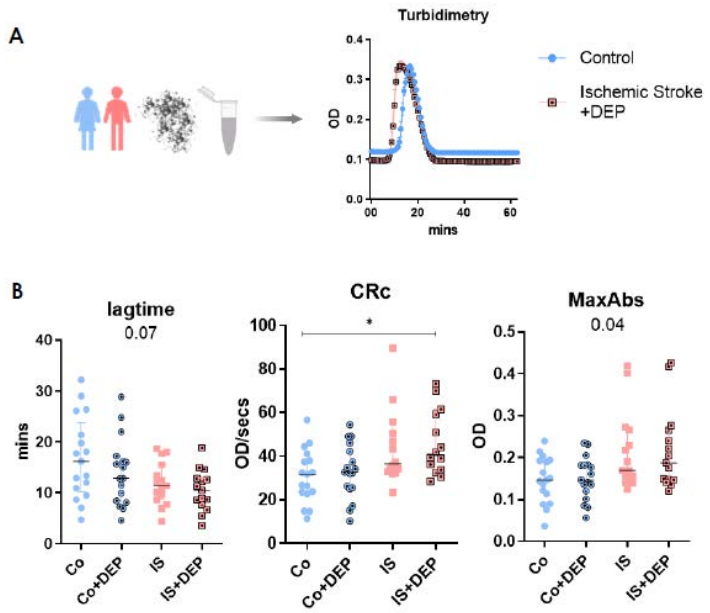
5- Turbidimetry study of clot formation and lysis in acutely exposed naïve and ischemic mice. A: Experimental design overview with representative turbidimetric curves. B:After ischemia the lag time shortens, the slope formation accelerates (CRc) and the density of the thrombus (MaxAbs) increases. Data represented as median (IQR). * p<0.05, ** p<0.01, *** p<0.001. naïve-PBS (n= 9), SRM1650b (n= 8) and MCAo-PBS(n= 6), SRM1650b (n= 5).



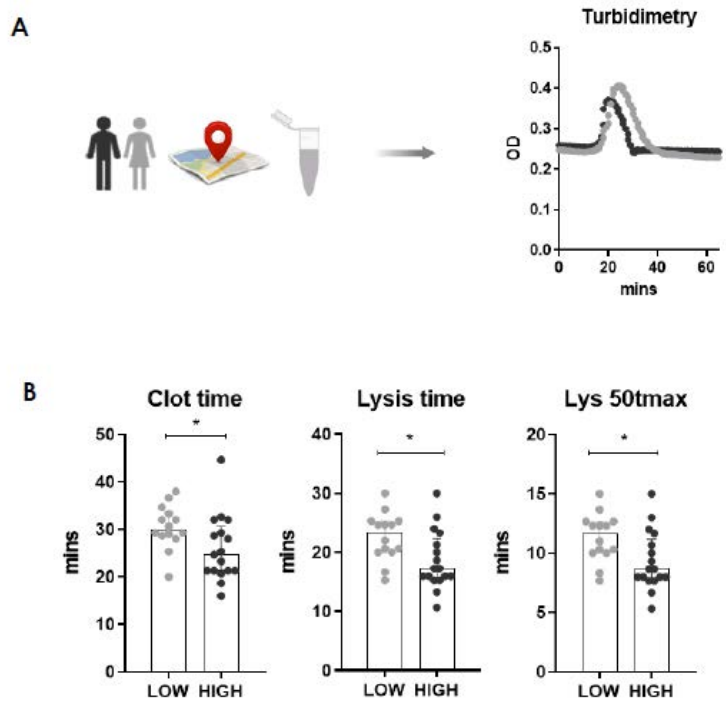
Thrombus formation and lysis in chronically exposed naïve and MCAo animals. A: Experimental design overview. B: CRc and MaxAbs, formation parameters, are altered in the ischemic group and boosted after DEP exposure, while lysis parameters showed differences only after ischemia in the DEP exposed mice Data represented as mean±SD or median (IQR). * p<0.05, ** p<0.01. *** p<0.001. Naïve-PBS (n= 7), SRM1650b (n= 9) and MCAo-PBS (n= 5), SRM1650b (n= 6).



7- Turbidimetric assay in plasmas of control and ischemic stroke patients. A: Experimental design overview and representative turbidimetric curves. B: Reduced latency times (lag time), increased clot formation rates (CRc) and maximum absorbances were observed in clots of IS patients whose plasma was exposed to DEP during the assay. This difference turned significant in clot formation rate when compared to non-exposed plasma from controls. Data represented as median (IQR). * p<0.05. Co: Control, IS: ischemic stroke.



8- Lysis of the clot is faster in the highly PM2.5 exposed group. A: Experimental design overview and representative turbidimetric curves. B: Graph bars displaying reduced clot time, lysis time and lysis 50 tmax in the highly exposed group compared to the lower-exposed one. Data represented as median (IQR).*p<0.05.



3. Relevance and potential impact

The results demonstrate the health risks of exposure to air pollutants such as diesel exhaust particles in urban or industrial environments, which could decrease the physiological neurogenic potential of the brain in healthy individuals and in specific diseases such as stroke where exposure to high levels of diesel particles could influence blood clotting and the response to thrombolytic drugs. Overall this experimental study positions air pollutants such as diesel exhaust particles as environmental risk factors for human health directly affecting the brain with potential impact in stroke patients.

4. Scientific Bibliography

Doctoral Thesis

"Brain, lung and plasma alterations induced by diesel exhaust particle exposure in the context of ischemic stroke". University and Doctorate Program: UAB, Neuroscience Thesis date: 1st March 2022. Author: Mercedes Arrúe Gonzalo.

Publications

"Plasma exposed to diesel exhaust particles exacerbates the pro-thrombotic characteristics after cerebral ischemia and accelerates tPA-induced thrombolysis" (*in preparation*)

"Neural progenitors are decreased after diesel exhaust particle (DEP) exposure in the mouse brain" (*in preparation*).

"Physical characterization of the diesel exhaust particle materials SRM2975 and SRM1650b representative of two different combustion engines to obtain smaller sized fractions" (*in preparation*).

Communications

Title: Acute exposure to diesel exhaust particles before cerebral ischemia induces pro-thrombogenic plasma changes while chronic exposure can impair the physiological response.

Authors: Arrúe-Gonzalo M, Penalba A, Pizarro J, Turner M, de Homdedeu M, Cruz MJ, Pilar

Delgado, Rosell A. Congress: 7th European Stroke Organisation Conference (ESOC). 1-3 September 2021. Communication type: Poster Presentation

Title: Traffic-related pollution can alter levels of matrix metalloproteinases in brain and lungs after cerebral ischemia in mice.
Authors: Arrúe-Gonzalo M, Rodríguez-Bodero A, Esquiva G, Penalba A, Simats A, de Homdedeu M, Cruz MJ, Montaner J, Rosell A. Congress: XIII Vall d'Hebrón Research Institute Scientific Meeting. Catalunya. December 2019. Communication type: Poster presentation.

Title: Traffic-related pollution can alter levels of matrix metalloproteinases in brain and lungs after cerebral ischemia in mice.
Authors: Arrúe-Gonzalo M, Rodríguez-Bodero A, Esquiva G, Penalba A, Simats A, de Homdedeu M, Cruz MJ, Montaner J, Rosell A. Congress: VII Neuroscience Institute Conferences. Catalunya. Oct 2019. Communication type: Oral presentation.

Title: Traffic related pollution reduces the levels of matrix metalloproteinases in hippocampus and striatum after cerebral ischemia in mice.
Authors: Arrúe-Gonzalo M, Rodríguez-Bodero A, Esquiva G, Penalba A, Simats A, de Homdedeu M, Cruz MJ, Montaner J, Rosell A. Congress: V European Stroke Organisation Conference. Milan. May 2019. Communication type: Poster Presentation.