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Anthracosis particulate matter causes changes in macrophages inflammatory response and in expression of genes related to xenobiotics metabolismo in co-culture of macrophages with BEAS-2B airway cells

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Introduction

Anthracosis is a term used to describe the black pigmentation of the lungs and tracheobronchial tree caused by the deposition of inhaled carbon particles [1-3]. Recent evidence indicates that air pollution play an important role in the establishment of anthracosis and possibly in the development of cancer in the respiratory system, specially due to polycyclic aromatic hydrocarbons (PAH) content [1,4]. The objective of this work is to study whether the composition of anthracosis particulate matter (APM) is still capable of causing changes in the expression of certain genes in the cells of the bronchial epithelium and interfere in the modulation of macrophages M1 and M2.

Methods

The environmental particulate material was collected from engine using a particulate retainer emitted from diesel exhaust and the anthracotic material was collected from corpse lungs of the Autopsy Verification Service in the city of São Paulo. The lung fragments were digested in an enzymatic process [5], the particulate was separated by centrifugation and lyophilized to obtain the black powder. The composition of PHA was analyzed by gas chromatography coupled with mass spectrometry. Cultures of BEAS-2B were exposed to APM and DEP (Diesel Exhaust Particles - as positive control) at concentrations of 50 or 100 µg/mL for 2, 4, 8, 12 and 24 hours and a citotoxicity test (LDH) was performed. Co-cultures of BEAS-2B and macrophages previously induced to M1 or M2 profile were exposed to APM and DEP for 24h, at 50 µg/mL and flow citometry was performed in order to imunophenotyping macrophages using HLADR and CD163 as M1 and M2 markes, respectively. Also, same samples were analyzed for RNAm expression, using TaqMan probes for genes CYP1A1 and CYP1B1 (P450 family) and AGR2 and TP53 (cell cycle control and repair).

Results

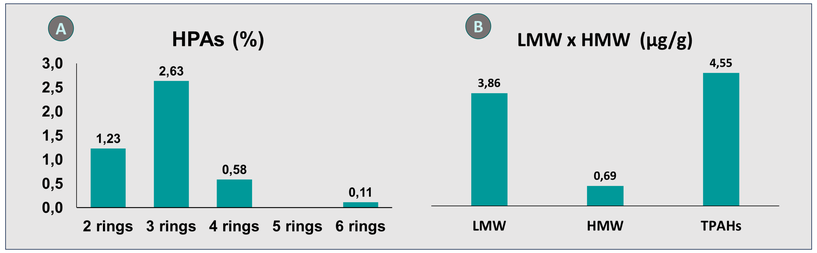

Fig 1. PHA content and characteristics in APM. LMW = Low molecular weight; HMW= high molecular weight

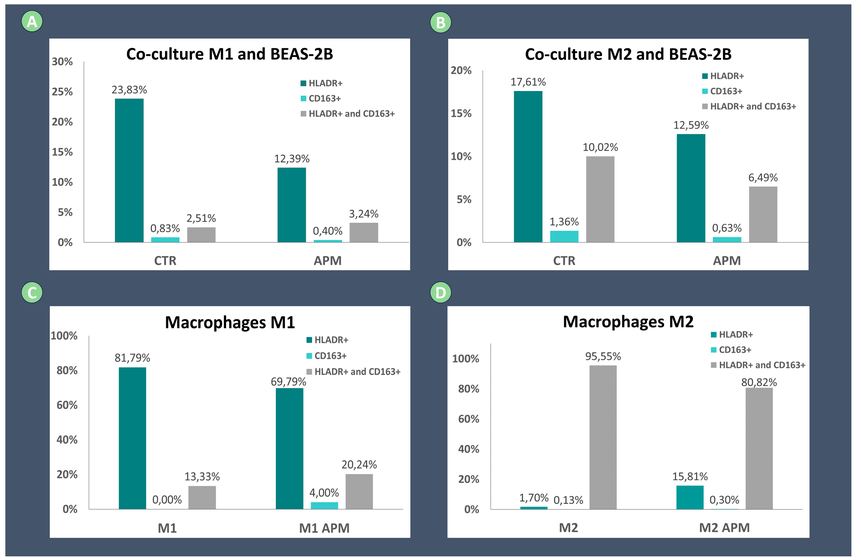

Fig 2. Co-culture macrophage (A-B) and Macrophages alone (C-D) profiles with or without 50 µg/mL APM exposure for 24h.

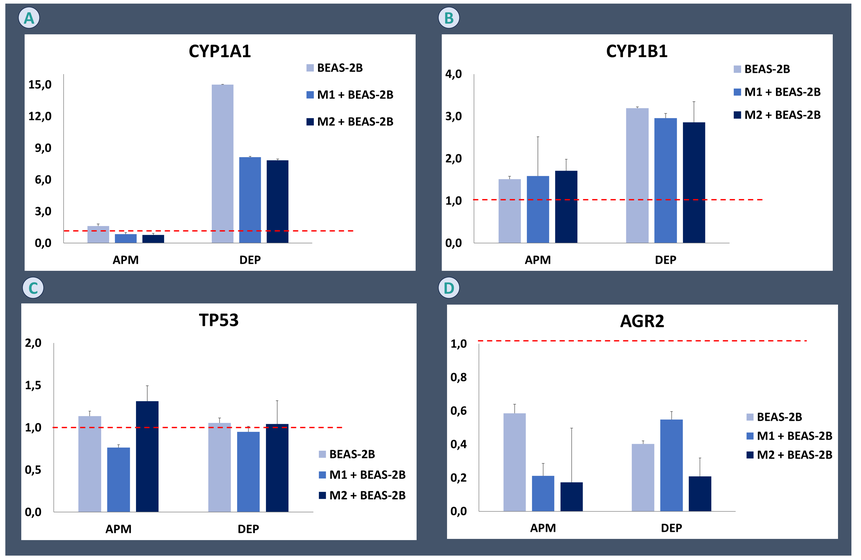

Fig 3 Expression of CYP1A1 (A), CYP1B1 (B) TP53 (C) and AGR2 (D) after 50 µg/mL APM or DEP exposure for 24h. The line represents control group (=1).

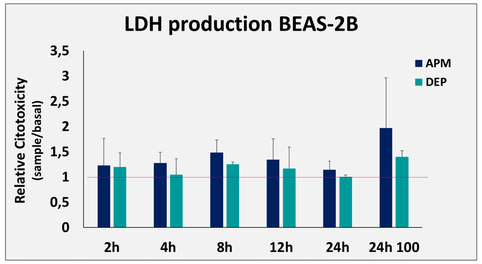

Fig 4. LDH production over time in BEAS 2B exposed to APM and DEP at 50 and 100 concentration µg/mL. The line represents control group (=1).

Conclusion

Anthracosis is not an inert particulate, probably because its composition including PAH, and is capable of causing similar LDH production to the environmental particulate in BEAS-2B cells. Previously induced M2 macrophages, showed a discreet change in its profile to M1, presenting a tendency towards a pro-inflammatory profile. APM also induced an increase in CYP1B1 expression and disrupted TP53 and AGR2 expression.

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Anthracosis is the term used to describe the black pigmentation of the lungs after long time exposure to biomass smoke and/or air pollution. But it is unknown whether this particulate presence is still capable of causing changes in tissues. Lung and lymph node fragments were collected from autopsies (36) for anthracosis particulate matter (APM) extraction. For detection of polycyclic aromatic hydrocarbons (PAH), gas chromatography coupled with mass spectrometry was performed; Diesel exhaust particles (DEP) was used for comparison. To assess biological effects, BEAS-2B airway cells were cultured with macrophages induced to M1 inflammatory or M2 anti-inflammatory profile and exposed to 50 µg/mL of APM or DEP for 24h. Flow cytometry was performed for macrophages immunophenotyping, culture supernatant was collected for LDH production analysis and RNA for real-time PCR. Studied genes were CYP1A1 and CYP1B1 (P450 family) and AGR2 and TP53 (cell cycle control and repair). Chemical composition showed PAH of low molecular weight (92%), with 2 to 3 rings (91.12%), most naphthalene (3.80±0.02 µg/g) and acenaphthylene (5.26±0.08 µg/g) species. APM or DEP caused no significant change in BEAS-2B LDH production (p=0.101); APM promoted inflammatory profile (M1), both in isolated or co-cultured macrophages; exposure induced the expression of CYP1A1 (DEP) and CYP1B1 (APM and DEP) genes, while a tendency to decrease AGR2 and increase TP53 expression (APM and DEP), both in BEAS-2B and in co-culture. The results suggest that APM has potential to cause immune and inflammatory response.