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Toxicological Responses of Wistar Rats Exposed to Controlled Emissions of Carbon Monoxide and Nitrogen Dioxide

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***Abstract*—Context: The inhalation of pollutants of automobile origin induces blood disturbances, especially noncommunicable diseases, in humans. However, there are no studies highlighting the effects induced by car pollutants. From this perspective, this study aims to evaluate the effects of at least two (02) pollutants, carbon monoxide (CO) and nitrogen dioxide (NO_2), on oxidative stress and inflammation after a single exposure for four hours (04 h). Materials and methods: Ten male Wistar strain rats were randomly divided into two groups of five (05): the control group (179.00 ± 5.916 g) unexposed and the group exposed to CO and NO_2 (188.00 ± 13.13 g). CO and NO_2 were produced by the combustion gas oil using a device contained in a sealed metal box supplied with ambient air by a pump. The ranges of CO and NO_2 concentrations to which the rats were exposed varied between 35 and 45 ppm and between 0.2 and 0.3 ppm, respectively. The blood samples were taken at 24 hours after the end of the manipulations. A malondialdehyde (MDA) assay was performed on supernatant using the GENESYS 10S UV Visible Spectrophotometer. Tumour Necrosis factor alpha (TNF-α) levels in the supernatant stored at -80 ° C were examined with an ELISA kit . Results: The mean values of MDA (17,771 ± 6.624 nM / ml) and TNF-α (83,050 ± 7.483 µg / ml) observed in the exposed group were significantly higher ($p < 0.01$) than those recorded in the control group (MDA (7.097 ± 1.882 nM / ml) and TNF-α (6.410 ± 3.160 µg / ml).**
Conclusion: Exposure to CO and NO_2 for at least 4 hours induces the overproduction of reactive oxygen species (ROS) and the exacerbation of inflammation.

***Index Terms*—carbon monoxide; nitrogen dioxide; oxidative stress; inflammation.**

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I. Introduction

Air pollution is a danger in terms of public health. According to the WHO in 2014, air pollution in urban and rural areas is responsible for 6.5 million premature deaths worldwide (11.6% of deaths worldwide) [1]. Air pollution is not only in the form of dust but also in the form of pollutants emitted by combustion engines, including diesel. In France, a diesel reference country, the number of deaths due to pollution ranges between 17,000 and 48,000, thus representing an economic cost of approximately 33.6 billion US dollars [2]. Diesel gas emissions in the gaseous phase comprise several toxic gases, including nitrogen dioxide (NO_2) and carbon monoxide (CO) [3]. In Benin, the inhalation of car pollutants due to the relatively long duration induces respiratory disorders [4] in motorcycle taxi drivers exposed to a CO concentration greater than nine (09) ppm [5]. Cachon et al. [6] showed that the exposure of human bronchial epithelial cells to particulate matter (PM 2.5 and PM > 2.5) collected in the city of Cotonou (Benin) induces the significant secretion of pro-inflammatory cytokine proteins (TNF-α) and an increase in the level of MDA, regardless of the duration of the exposure (24, 48 and 72 h). These studies generally take into account suspended particles that are nothing more than a complex cocktail of pollutants that can cause disease in all sedentary strata (children, adults) and even athletes [7]. This situation makes it difficult to evaluate the role and the effects of each pollutant on the appearance of communicable and non-communicable diseases.

Studies take into account that the impact of compounds present in the gaseous phase of diesel emissions are almost rare [8]. Peel et al. [9] showed that an increase in daily concentrations of 0.02 ppm (parts per million) NO_2 induces cases of respiratory disorders, chronic obstructive pulmonary disease (COPD) and asthma. Another study on inflammatory responses has shown that the cytokine response of serum interleukin-6 (II-6) is dose-dependent at NO_2 exposure concentrations [10]. Subchronic exposure to NO_2 leads to a decrease in the endogenous antioxidant (reduced glutathione) in the bronchoalveolar fluid of rats [11] as well as a decrease in the activities of antioxidant enzymes, which leads to the extracellular formation of reactive oxygen species (ROS) responsible for lung damage [12].

With respect to carbon monoxide (CO), several studies have found rates ranging from 20 to 40 ppm in urban areas,

with peaks of up to 170 ppm [13]–[15]. A cross-sectional study conducted by Fourn and Fayomi in 2006 [16] in BENIN showed after analysis of the ambient air taken at the junctions and taxi-motorcycle parks, a peak CO concentration of 38.6 ppm in the morning and 78.6 ppm in the afternoon was observed against a standard of 30 ppm [16]. In the body, CO binds to haemoglobin with an affinity 250 times greater than that of oxygen (O_2) [17]. CO can also, according to Brown and Piantadiosi [18], bind to mitochondrial cytochromes, altering the electron transport chain and inhibiting the aerobic synthesis of ATP (adenosine triphosphate), thus contributing to the alteration of the metabolism of cardiomyocytes. In healthy volunteers, controlled exposure to diesel emissions for 1 h [19]–[21] or 2 h [22],[23] induced a significant increase in the level of stress and the expression of interleukin-13 and IL-8 in bronchial epithelium. Inflammatory cells, i.e., the number of neutrophils and B cells, CD4 + and CD8 + T lymphocytes, mast cells and platelets increase considerably following exposure to diesel [19][23].

Despite these convincing results, studies reveal biases due to the presence of several uncontrolled pollutants (especially in the gaseous state) whose mixture is not controlled but induces damage, especially noncommunicable diseases, such as asthma, diabetes and cancer [24]. Moreover, for ethical reasons, the concentrations of pollutants to which human subjects are exposed as well as the duration of exposure (1 or 2 hours) could not reflect the real image of the values of the concentrations of the different pollutants observed at great crossroads of our urban cities.

Despite the lack of knowledge of particle concentrations of diesel emissions, the lack of control of the effect of a pollutant specifically in the occurrence of diseases, the variations in the methodology from one study to another, the various studies on exposure to diesel, CO and NO_2 emissions revealed two phenomena: oxidative stress and inflammation. However, there are not studies highlighting the effects induced by each pollutant. To our knowledge, this study is the first to evaluate the effects of at least two (02) pollutants, carbon monoxide (CO) and nitrogen dioxide (NO_2) on oxidative stress and inflammation after a single four-hour exposure (04 h).

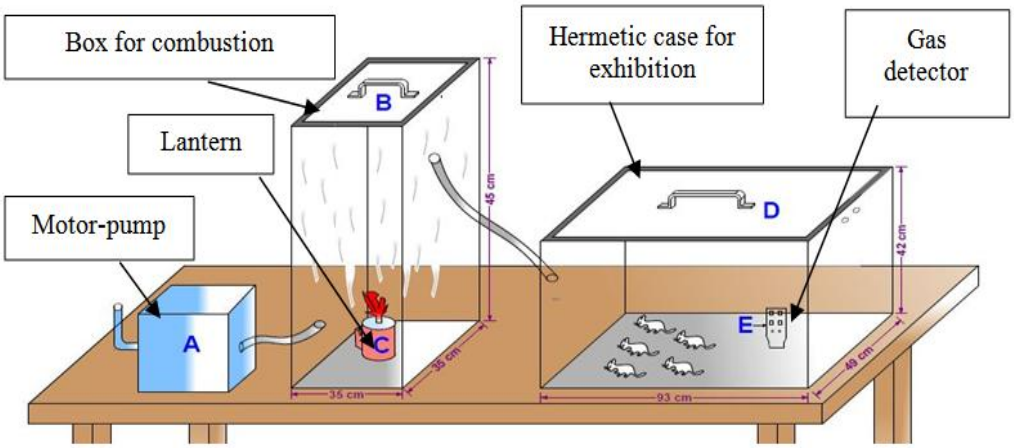
II. Materials and Methods

A. *Biological material*

We used ten (10) two-month-old male Wistar strain rats with body weights of 174 and 206 grams. The rats were randomly divided into two lots consisting of a control group and an experimental group in 2 conventional cages at the rate of 5 rats per cage. Access to water and food was ad libitum, and the animals were acclimatized to the conditions of the animal house for one week before the start of the experiments. The lighting followed a rhythm of 12 hours of day over 12 hours of night.

B. *Description of device used to produce (CO and NO_2) and oxygen (O_2)*

The device consisted of a KF, NEUBERGER 78 Freidburg (A) ambient air motor pump (temperature 21 °C and hygrometry rate 65 ± 5%) connected by a 7 mm diameter conductive pipe to a metal box (B) at 45 cm high rested on a base 35 cm long and 35 cm wide with a low entrance and a high exit. This metal box, hermetically closed, contained a gas-oil combustion device (C) that is operational from the ambient air propelled by the motor pump by the low inlet to maintain combustion. After the combustion of gas oil produced in the metal box, CO and NO_2 escaped through the top outlet and an 8.5 mm diameter conductor pipe to a hermetic enclosure (D) 42 cm in height rested on a base 93 cm long and 49 cm wide. This chamber houses rats and the VENTIS MX4 brand gas detector EXPLO-O2-CO-NO2 (E), which measures the concentrations of CO, NO_2 and O_2 from the metal box. Thus, the gas detector was programmed to tolerate ranges of the concentrations at which the animals were exposed: CO concentrations were between 35 and 45 ppm, NO_2 concentrations were between 0.2 and 0.3 ppm, and O_2 concentrations were between 20.4 and 20.6%. The gas detector emitted light beeps if the programmed concentrations were exceeded, which would preserve the physical integrity of the animals (fig 1).



Ambient air motor pump (A) ; Box for the combustion source (B) ; Lantern (C) ; Hermetic case for exhibition (D) ; Gas detector (E)

Fig 1: Device used to produce pollutants (CO and NO_2) and oxygen (O_2)

C. Modalities of acute exposure of animals to CO and NO_2 pollutants

Due to possible influences on the study-dependent variables, pollen exposures were carried out under the same conditions from 8 to 12 hours in both the experimental and control groups, resulting in an acute exhibition of 4 hours. In accordance with the exposure scheme explained above, the experimental group consisting of five rats were exposed to CO and NO_2 for 4 hours, i.e., 8 hours to 12 hours. Similarly, the next day, the control group of five rats was also placed in another enclosure of the same dimensions and hermetically closed with supply of ambient air provided by the motor pump. This group was not exposed to pollutants. At the end of the exposure of each study group, the animals were then placed in a standard pet shop environment, i.e., the air was filtered according to the standards of a pet shop (AFS).

D. Determination of biological parameters: Biochemical parameters

Blood sampling and determination of tumour necrosis factor alpha (TNF-α) and MDA (malondialdehyde)

Twenty-four hours (24 h) after each experiment, blood samples were taken from the retro-orbital vein of experimental and control rats. Blood was collected in tubes with EDTA anticoagulant and analysed. To avoid any deterioration and external contamination, the collected blood samples were conveyed as soon as possible to the laboratory and centrifuged at 3000 rpm for 15 minutes. The supernatant was stored at -80 °C until dosing. The TNF-α concentration was determined with an ELISA kit (Ray Bio® Rat TNF-alpha ELISA Kit Protocol, Aachen, Germany). The MDA assay was performed according to the method of Gbeassor and Agbonon [25] explained below.

E. Dosage of MDA

The lipid peroxidation was determined in the serum by measuring the concentration of malondialdehyde (MDA) according to the method, which consisted of successively adding 0.6 mL of phosphoric acid (H_4PO_3) at 1% and 1 mL of 1% thiobarbituric acid to a 200 µL aliquot of the supernatant. Then, the mixture was placed in a water bath at 100 °C for 50 minutes. The tubes containing the mixture were cooled in an ice bath for 10 minutes. Then, 2 mL of 1-butanol was added to the contents of each tube. The tubes were vigorously shaken and centrifuged at 3000 rpm for 10 minutes. The supernatant containing the thiobarbituric acid-malondialdehyde complex (TBA-MDA) was removed. The absorbance was read with the GENESYS 10S UV-visible spectrophotometer (Monaco, France, made in China, designed in the USA) at 535 nm against a blank. The control was made in the same manner as the tubes used to determine MDA, except that the organ homogenate supernatant is replaced with distilled water. The standard curve was determined by MDA at concentrations of 0, 15.75, 31.5 and 63 nM. The tests were repeated three times. 1, 1, 3, 3-tetramethoxypropane (MDA) was used as a standard to obtain the standard linear regression curve (0-60 nM, Y = 0.03860X + 0.04205). The correlation coefficient of the linear regression curve of the standard range is $r^2 = 0.9982$. The results were expressed in concentration (nM / mL).

F. Determination of TNF- α

The concentrations of TNF-α were determined by an ELISA (enzyme-linked immunosorbent assay) method (Rat TNF-α Picokine™ ELISA Kit). ELISA is an enzyme immunoassay conducted in the heterogeneous phase. The assay was performed according to the instructions of the supplier. The antibody adsorbed onto a 96-well microplate was contacted with a sample containing the antigen of interest for 1 hour. After 4 washes, a biotinylated anti-TNF-α antibody was added. After incubation for one hour, the excess antibody was removed by 4 washes, and peroxidase-conjugated streptavidin was added. After an additional incubation of 30 minutes, 4 washes are made, and then the substrate is added. After 1 hour of incubation, the addition of a hydrochloric acid solution stops the reaction. The optical density was read at 450 nm versus 630 nm using a microplate reader. A standard range was performed in parallel as a standard to obtain the standard linear regression curve of the form y = ax + b (7.8 µg / mL-500 µg / mL, Y = 0.0020 × 0.0979). The correlation coefficient of the linear regression curve of the standard range is $r^2 = 0.9990$. The results obtained were expressed as a concentration (µg / ml).

G. Statistical analysis

The recorded data were processed with StatView software version 5 AcabusConcept. The results of biochemical analyses were expressed as the mean ± ESM (standard deviation to mean). The Mann-Whitney test was performed to compare the control group (GT) and the group exposed for 4 hours (Grp_Expo4 h) to CO and NO_2. The level of significance of the tests was set at $p < 0.05$.

III. RESULTS

The analysis of the parameters related to oxidative stress and inflammation gave the results presented in the following table (table 1).

Table1 : Comparison of the effect of four (04) hours of exposure to CO and NO_2

	GT n = 5 m ± s	Grp_Expo$_{4\,h}$ n = 5 m ± s	P	Delta (%)
MC (g)	179.00 ± 5.916	188.00 ± 13.13	p > 0.05	-
MDA (nM/ml)	7.0970 ± 1.882	17.771 ± 3.624	p < 0.01	150.4
TNF-α (pg/ml)	6.4100 ± 3.160	83.050 ± 7.483	p < 0.01	1195.63

Grp_Expo$_{4h}$: Group exposed for 4 hours ; GT : a group of witnesses ; MC : body mass ; g : gramme ; MDA: Malondialdéhyde; TNF-α : Tumor Necrosis Factor- alpha, n : effective ; m ± s : average more or less standard deviation ; ** : significant difference to p < 0.01 ; % : percentage ; Delta = [(situation after-situation before)/situation after]×100.

Mean values for body mass in both groups showed no significant variation ($p > 0.05$).

The mean values of the MDA and TNF-α concentrations observed in rats exposed for 4 hours to diesel emissions (Grp_Expo4 h) were significantly higher than those observed in the control group (GT) ($p < 0.01$). of the concentrations were 17.771 ± 3.624 nM / ml and 83.05 ± 7.483 pg / ml in Grp_Expo4 h compared to 7.097 ± 1.882 nM / mL and 6.41 ± 3.16 pg / ml in GT.

IV. Discussion

The objective of this study was to evaluate the effects of carbon monoxide (CO) and nitrogen dioxide (NO_2) after a four-hour exposure (04 h) to oxidative stress and inflammation. Ten eight-week-old Wistar male rats with body masses ranging from 174 to 206 grams were used. The rats were divided into two groups: the group exposed for four hours to CO and NO_2 (Grp_Expo4 h) and the control group (GT). Access to water and food was ad libitum, and the animals were acclimatized to the conditions of the animal house for one week before the start of the experiments. The lighting followed a rhythm of 12 hours of day over 12 hours of night. To avoid the effects of the biorhythm, all the manipulations were started at the same time, i.e., eight hours (08:00). The markers used in this study were MDA (malondialdehyde) and TNF-α (tumour necrosis factor alpha), which represent the markers of oxidative stress and inflammation, respectively. Changes in body mass between the four-hour group exposed to CO and NO_2 and the control group showed no significant difference. The influence of body mass on oxidative stress and inflammation could not be invoked.

Analysis of the data revealed that exposure to CO and NO_2 for 4 hours significantly increased the mean concentrations of MDA and TNF-α. Most studies on air pollution reported the presence of several uncontrolled pollutants in isolation and whose mixture was not controlled. In addition, markers related to oxidative stress and inflammation vary from one study to another. For example, a recent study conducted in vivo by Niemann et al. [24] showed that exposure to pollutants from diesel emissions is partially responsible for cardiovascular diseases that would have resulted in impaired vasomotor response, endogenous fibrinolysis capabilities, and platelet activation. Similarly, myocardial ischaemic effects in stable coronary patients have been observed without attributing these effects to any gaseous or particulate compounds [26]. Several studies on the effects of particulate matter from diesel emissions have shown that the oxidative stress generated by these particles comes from the overproduction of reactive oxygen species (ROS), impaired mitochondrial function, or NADPH. Oxidase, activation of inflammatory cells capable of generating ROS, N reactive species and oxidative DNA [27], [28]. Other in vitro studies [27]–[30] have shown that exposure to particles increases the expression of related genes, including NF-ÎºB, and the activation of oxidant-dependent NF-ÎºB factors, such as TNF-α, IL6 and IL8.

In this quasi-experimental and controlled study, the lipid peroxidation test was performed using MDA because this compound is recognized as a potential biomarker of oxidative stress according to Atasayar et al. [31]. Indeed, following an aggression of the body, oxidative stress represents an imbalance between the production of reactive oxygen species (ROS) and intracellular antioxidant capacity, leading to good regulation of the redox state of the cells [32]. The mean MDA concentration observed after 4 hours of exposure in this study was 150.4 times higher in rats exposed to CO and NO_2 after 4 hours compared to the control group. However, according to Favier [33], the increase in oxidative stress marked by the increase in the concentration of MDA leads in the long term to an exacerbation of communicable or non-communicable diseases, cancer, cardiovascular diseases and an acceleration of ageing. It is therefore obvious that if an acute exposure of 4 hours to these pollutants induces a significant increase in oxidative stress, then repeated exposure, as in users of major cities, African capitals or pollutant controls, gives rise to major problems in public health.

To our knowledge, previous studies did not show the role played by these two pollutants simultaneously as in the context of our study, but they did show that in isolation, these pollutants are sources of oxidative stress and inflammation. Indeed, as for the particles, in the gaseous phase, exposure to NO_2 generates oxidative stress. This finding was confirmed by Yan et al. [34], who showed that the oxidative stress generated by exposure to NO_2 in the gas phase could be of mitochondrial origin. Indeed, these authors have shown that exposure to NO_2 induces overproduction of ROS from 2.5 ppm NO_2, which represents a concentration well above that used in this study (0.2 and 0.3 ppm). At this concentration, these authors found a decrease in the activity of the IV and V complexes and a decrease in ATP production. At 5 ppm, there is a loss of membrane permeability and a modification of the protein expression of NRF1 and TFAM, an actor of mitochondrial biogenesis [34]. Morphological modification of mitochondria is also observed in the cerebral cortex during exposure to NO_2 according to Li et al. [35]. These results are suggest that NO_2 crosses the brain barrier.

The NO_2 concentrations (2.5 and 5 ppm) used in the literature were higher than the range used in this study (0.2 and 0.3 ppm), suggesting that the obtained results are related exclusively to the concentration of CO used at 35 and 45 ppm. This finding confirms the work of Mc Donald et al. [36], who observed no change in markers of oxidative stress in APO E - / - mice exposed to 0.2 or 2 ppm NO_2. Favory et al. [37] showed that CO causes oxidative stress by lipid peroxidation resulting in a modification of myelin basic protein (MBP), which in turn induces immunological abnormalities that contribute to brain damage. In fact, once physiopathologically inhaled OC passes easily through the alveolocapillary membrane, it binds to haemoglobin (Hb), forming carboxyhaemoglobin (HbCO), which has a binding affinity 250-fold higher than that of oxygen (O_2) [17]. CO binds to cytochromes (haem compounds) and induces mitochondrial dysfunction due to overproduction of ROS [38]. The excess ROS formed due to the blockage of the mitochondrial chain thus generates specific brain lesions by lipid peroxidation, which induces oxidative stress [39]. A few animal model studies have concluded that CO exposure in rats induces brain damage due to free radicals generated by mitochondria but not tissue hypoxia [40], [41]. Oxidative damage to cell membranes in relation to the inhibition of mitochondrial respiratory chain enzymes is therefore at the

root of the pathophysiology of CO poisoning [42], and Piantadosi et al. suggested that CO induces the release of nitric acid (NO), a potent oxidizing agent that may be able to cause DNA damage, cell death and the activation of the immune system. Cases of sudden death following the inhalation of CO can be explained by the fact that it acts directly on the ion channels that regulate many vital processes [43]. The lack of oxygen following the inhalation of a significant amount of CO inhibits the production of ATP and stimulates the synthesis of inflammatory cytokines (interleukins, TNF-α, which is used in the context of this study). CO is responsible for the induction of lipid peroxidation, which can lead to the destruction of the pulmonary epithelium [44].

The mean TNF-α concentration in this study was 12.95 times higher in rats exposed to CO and NO_2 after 4 hours compared to that in the control group. In the case of inflammatory reactions, most studies were performed in vitro with stem cells. Indeed, the results obtained after the exposure of the mice to diesel particles have revealed a severe inflammatory response with the production of allergen-specific Ig-E antibodies, a lymphocyte response with a cytokine profile type Th-2 (production of interleukins: Il-4, Il-5, Il-6, Il-10, Il-13 and TNF-α), and then a decrease in the Th-1 type response (Interferon gamma) (IFN-γ) in nasal wash fluids [45]–[50], showed that inflammation is accompanied by cell necrosis and the disruption of the interaction between cellular organelles leading to the energetic deprivation of tissue. In vitro studies on the exposure of epithelial cells to diesel particles have shown that these cells inhibit the proliferation of particles [8] by inhibiting the proto-oncogene c-fos, which is involved in the cell cycle [51].

These cells are therefore able to endocytose diesel particles and then release pro-inflammatory cytokines, such as Il-8 and GM-CSF (Granulocyte Macrophage-Colony Stimulating Factor)[52]. Our results corroborate those of Shukla et al. [30], who showed that exposure for 6 hours to 300 µg/m3 of $PM_{2.5}$ from diesel emissions induced 24 hours after the expression of inflammatory and cytokine (NF)-kappaB genes in bronchial epithelial cells. However, most studies have focused more on the PM. Studies on controlled exposures, as in this study, are a new direction that can lead researchers to focus on each pollutant selectively. At rest, 20 hours after an acute exposure to diesel emissions for 3 hours, Xu et al. [53] found that total monocyte and leukocyte counts in the peripheral blood were higher in the exposed volunteers, and a trend towards increased serum IL-6 concentrations was also observed. These results show the presence of inflammation at rest, although the parameters used for inflammation, the different pollutants as well as the concentrations to which the subjects were exposed (PM: 300 µg / m^3 and NO_2: 1.3 ppm) were not the same as those used in this study. Moreover, other studies have shown that the exposure of volunteers to diesel emissions for two hours induces a significant increase in the expression of interleukin-13 and IL-8 in the cells of the bronchial epithelium [19]–[23]. Indeed, Stenfors et al. [23] showed that acute exposure of healthy subjects to 108 µg/m3 PM10 for 2 hours resulted in neutrophilia, lymphocytosis, and airway inflammation at 6 hours after exposure to diesel. This inflammation is characterized by increased interleukin-8 (IL-8) protein in the lavage fluid, increased IL-8 messenger ribonucleic acid expression in the bronchial mucosa, and increased regulation of the endothelial adhesion molecule. These authors also showed that inflammatory cells, namely, the number of neutrophils, B cells in the airway washes, neutrophils and platelets in the peripheral blood, and neutrophils, mast cells and CD4 + T cells and CD8 + in bronchial tissues, increase significantly as a result of diesel exposure [23]. These results are consistent with those obtained in this study, even if the inflammatory parameters studied are not the same. This effect seems to be normal because according to Noak and Kolopp-Sarda, inflammation by definition is a set of protective mechanisms by which the body defends itself from the various aggressions (infection by a pathogen, inhalation of a pollutant, allergy) and repairs the damaged tissues [54].

Thus, during the initial phase, inflammatory cells, such as tissue macrophages and mast cells, are activated and synthesize pro-inflammatory chemokines and cytokines, such as the TNF-α used in our study, which is responsible for the recruitment of the innate immune system: neutrophils (PN). The release of cytokines and chemokines will then act on endothelial cells and allow the recruitment of monocytes, dendritic cells and lymphocytes [54]. Since TNF-α is a cytokine essentially produced by T cells, neutrophils, endothelial cells, monocytes and macrophages [55], it is reasonable to propose that the increase in these inflammatory cells is related to the significant increase in TNF-α concentration observed in our study. In addition, Sehlstedt et al. [21] examined inflammatory responses in exposed volunteers for a shorter duration than that in the previous study (1 hour), and the results showed that exposure to diesel emissions at a particulate matter concentration of 270 µg / m3 resulted in an increase in the expression of vascular endothelial adhesion molecules (P-selectin), vascular cell adhesion molecule (VCAM-1) in the bronchial mucosa, and an increased number of eosinophils at the level of bronchoalveolar washes. These results, similar to those previously mentioned, are difficult to compare with the results obtained in the context of this study because the samples were taken at 6 hours after exposure, on the one hand, and do not show the role played by each pollutant on the other hand.

Despite the multitude of methods used in these different studies, the variety of markers used, the lack of knowledge of the concentrations of the different pollutants, and the choice of the exposure method (in vitro and in vivo), make it difficult to compare different results; nevertheless, the conclusions are virtually the same.

With respect to the inhalation of NO_2 alone, to our knowledge, the results of previous show the effects of repeated exposure, which is not the case in this study, which highlights the combined effects of CO and NO_2 following a single exposure of 4 hours. However, toxicological studies conducted by Kastner et al. [56] on cell cultures exposed to NO_2 at 0.08 ppm for four (04) days revealed a decrease in cell metabolism. In addition, exposure to diesel engine exhausts induces not only a severe inflammatory response in bronchoalveolar lavages characterized by allergen-specific Ig-E and Ig-G1 antibody production [57] but also a lymphocyte response with a cytokine profile type Th-2 (overproduction of Il-4) to the profile of Th-1 (IFN-gamma) during repeated exposure at 5 h / day for 7 days with 2.7 ppm NO_2 [58].

On the other hand, our results are contrary to those of van Berlo et al. [59], who found that short-term inhalation of diesel exhaust does not induce a significant increase in TNF-α even 18 hours after exposure. This discrepancy could be explained from a methodological point of view, since in these rats were exposed for 2 hours to diesel diluted against 4 hours in our study. Thus, the CO, NOx (NOx), NO (NOx) and NO_2 concentrations to which the animals were exposed were lower than in this study. In fact, the concentrations were 14.23 ppm (CO), 2.38 ppm (NOx), 1.43 ppm (NO) and 0.94 ppm (NO_2) against 35-45 ppm (CO) and 0.2-0.3 ppm (NO_2) in this study. The concentration of CO was relatively lower than that observed by Fourn and Fayomi in 2006 in BENIN [16], which was between 38.6 ppm in the morning and 78.6 ppm in the afternoon against a standard of 30 ppm [16]. However, these authors showed that the exposure of taxi-motorcycle drivers, commonly known as 'zemidjan', to diesel engine exhaust caused changes in the expression of mRNA-bound genes due to oxidative stress and inflammation.

This effect would affect all social classes living in polluted environments, including children, adults, sedentary athletes, due to the air brewing important to exercise, which leads to the hyperventilation of air laden with pollutants and environmental. However, the energy used in the form of adenosine triphosphate (ATP) comes from the mitochondria, which is the main source of reactive oxygen species. At the mitochondrial level, physiological radical production is linked to an electron leak located at the level of ubiquinone, which transfers the electrons of complex I or II to complex III. According to Turrens [60], oxygen (O2) then undergoes a univalent reduction and is transformed into the superoxide anion itself at the origin of the formation of the other ROS (hydrogen peroxide (H_2O_2) and hydronium ion • OH. The diffusion of ROS (from the mitochondria to the cytoplasm) would depend in part on their chemical nature [61]. O2 • - within the mitochondria is converted to H_2O_2 by the action of Mn-SOD. H_2O_2 can easily cross the mitochondrial membrane, and studies have shown that most of the extracellular increase in hydrogen peroxide levels during muscle contractile activity is mainly derived from mitochondria and diffuses through subcellular and plasmic membranes. For • OH, as chemically neutral but very reactive compounds, these particles diffuse little and exert their effects locally. Therefore, following an increased inhalation of pollutants due to the practice of physical exercise, pollution would cause much more damage to athletes.

V. Conclusion

Our results are therefore a contribution to the literature because at 24 hours after acute exposure to CO and NO_2 for 4 hours, the answers remain the same.

Short-term exposure to these pollutants thus causes oxidative stress and the activation of the inflammatory process. However, the inflammatory response observed in this study could not be attributed to NO_2 given the concentrations to which the rats were subjected (between 0.2 and 0.3 ppm), which are well below the thresholds used in the literature. Nevertheless, the inflammatory responses observed and the stress induced by CO and NO_2 in the context of this study are not without consequences on the human organism because the elevation of the oxidative and inflammatory stress parameters in humans leads to long term effects, such as accelerated ageing process, visual disorders (cataracts and macular degeneration), neurodegenerative diseases (ataxia, lateral sclerosis, Alzheimer's disease), cancer, sleep apnoea, and respiratory diseases, including bronchospasm. Public awareness of the dangers to which it is exposed every day is imperative. Exercising in heavily polluted areas is therefore discouraged. Another study on these same pollutants would be necessary to determine their effects on the practice of physical exercise.

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