INTESTINAL BARRIER INTEGRITY UNDER EFFECT OF PARTICULATE MATTER FROM COTTONWOOD COMBUSTION

The epidemiological data pointed out that exposure to ambient air pollution particulate matter (PM) may increase the risk of irritable bowel syndrome and ulcerative colitis. Air pollutants might affect the intestine through blood, mucociliary clearance of PM from the lungs, by polluted food or water intake. In the present study, we tested the hypothesis that water-suspended smoke preparations (SP) derived from the combustion of cottonwood might affect the integrity of the intestinal mucosa, induce an inflammatory response, and increase the susceptibility to chemically-induced colonic inflammation. Smoke from cottonwood was collected under laboratory conditions during the entire flaming, smouldering, and mixed combustion phases. Male Wistar rats (180–200 g) were injected with SP in two ways: 1) into the tail vein at a dose of 500 µg/100 g; 2) by gavage at a dose of 180 µg/100 g. The experimental colitis was induced 1 hour after SP injection by a single rectal injection of 0.1 ml 6% iodoacetamide (7 cm from the anus). Rats were euthanized in 2 h after colitis induction. We examined colonic vascular permeability by Evans blue extravasation (mg/g wet colon), myeloperoxidase level, total glycoprotein level at surface mucus by PAS-staining, and intestinal permeability by the translocation of bacteria into the blood of the portal vein. This study demonstrates that intravenous administration of the SP decreases the amount of surface mucus in the large intestine and increases myeloperoxidase activity in the rat colon. It did not increase the negative effect of iodoacetamide-induced colitis on changes in the state of the endothelial barrier. But, intravenous administration of SP led to the appearance of blood in the urine, which may be due to the damage to the fenestrated capillaries of the glomerulus. At the same time, oral administration of SP increased the translocation of bacteria into the blood of the portal vein which represents the increased gut permeability accompanied by a reduced level of total glycoprotein of colonic surface mucus. However, oral administration of SP did not have a significant effect on colonic vascular permeability and the activity of myeloperoxidase vs. the placebo group with colitis. The airborne PM obtained from the combustion of natural components such as cottonwood can cause primary pro-inflammatory changes in the intestines in a very short time after penetration into the organism through the blood and oral ingestion. Moreover, they can contribute to the violation of the integrity of the intestinal barrier.

Keywords: particulate matter, air pollution, mucosa integrity, intestinal barrier permeability, experimental colitis.

Introduction. According to the World Health Organization (WHO), air pollution is responsible for nearly seven million deaths worldwide annually and ranks the 13th among leading causes of mortality [5]. Nine out of ten people breathe air that exceeds the WHO’s guidelines for pollutants, with those living in developing countries most affected [54]. Air pollution can be caused by any chemical, physical or biological agents that change the natural characteristics of the atmosphere. More and more attention is being paid specifically to air pollution by particulate matter (PM).

PM is a general term used to describe the air pollution components comprising carbon-containing particles with associated adsorbed organic chemicals and reactive metals [42]. Typical components of PM include nitrates, sulfates, polycyclic aromatic hydrocarbons, endotoxins, and metals such as iron, copper, nickel, zinc, and vanadium [13, 14].

Among all environmental problems, air pollution with PM has the most significant negative impact on human health. PM2.5 and PM2.5 (or less) are the main components of urban air pollution and are admitted as risk factors for mortality [15, 29]. In addition, there is a growing body of evidence showing a link between air pollution with PM and the risk of cardiovascular disease [14], respiratory diseases such as asthma [31] and chronic obstructive pulmonary disease [2], lung and urinary tract cancer [9, 32, 54]. Air pollution by PM2.5 was associated with neurological diseases and disorders, including Alzheimer’s disease, Parkinson’s disease, neurodevelopmental disorders, lowered cognitive function, autism, neurodegenerative disease, dementia in adults, and stroke [8, 17, 25]. Recent researchers also claim a link between PM and gastrointestinal diseases such as irritable bowel syndrome [20], appendicitis [7, 24], and colorectal cancer [33]. Studies on IL-10−/− mice have shown that once PM10 enters the body, they increase proinflammatory cytokines in the small and large intestine, bacterial translocation into mesenteric lymph nodes, and change the composition of short-chain fatty acids and microbiota, which confirms their ability to cause disturbances in the intestinal tract [26, 43]. Ananthakrishnan et al. associated air pollution exposure with an increased risk of incident inflammatory bowel disease (IBD) [6].

IBD is a global health problem. The incidence of IBD is increasing rapidly in European and Eastern countries, particularly in developing countries [34, 35]. IBD includes two main types: Crohn’s disease, which affects the entire gastrointestinal tract [53], and ulcerative colitis [28], which affects the colon and rectum. The aetiology of IBD often involves genetic predisposition, immune responses, and changes in the gut microbiome [22, 27, 35]. Moreover, it has recently been suggested that environmental factors, particularly air pollution with PM, play an equally important role in IBD pathogenesis [1]. Also, Duan et al. revealed a connection between short-term PM2.5 pollution and an increase in the number of patients with ulcerative colitis [21]. The connection of PM with the worsening of the negative symptoms of colitis like intestinal oedema, enhanced production of proinflammatory cyto- and chemokines, and weight loss was also shown in an experiment with mice that drank ash-water dilution containing PM [39].

However, for the time being, the action mechanisms of solid air particles on the integrity of the intestinal barrier after their entry into the gastrointestinal tract remain unknown. We hypothesized that water-suspension PM-containing smoke preparations (SP) obtained from the combustion of a natural component on the state of the intestinal barrier and susceptibility to the development of inflammatory processes in the intestine. Previously, we tested SPs from different types of wood and wood-related components, such as pine wood and needles, birch wood and bark, as well as cottonwood for their toxicity on the functioning of nerve terminals and gut preparations/cells. Among all studied SPs, the preparation from cottonwood had the most prominent effect increasing the extracellular level of GABA in nerve terminal preparations, the paracellular permeability of the intestinal barrier of mucosal-submucosal preparations, and causing the death of COLO 205 cell culture [40].

Therefore, this study aimed to investigate the effects of SP from cottonwood on the integrity of the intestinal mucosa,
induction of inflammatory response, and an increase in the susceptibility to chemically-induced colonic inflammation after the introduction of SP intravenously or through a gastric tube.

**Materials and Methods.** Sampling and monitoring of smoke preparation. Smoke from cottonwood was collected during the entire flaming, smoldering, and mixed combustion (when the flaming and smoldering phases are present simultaneously) phases under laboratory conditions, then suspended in water and purified as described in [12, 48]. The emission of PM was monitored as described in [12]. Optical absorption of SP was registered in the UV region within the range of 250–300 nm using a spectrophotometer (Lambda Bio, Perkin Elmer). The fluorescence emission spectra of SP were monitored using a spectrofluorimeter (QuantaMaster, Photon Technology International).

**Ethical approval of animal experiments protocol.** Male Wistar rats (180-200 g, n = 28) were kept in an accredited vivarium of the Educational and Scientific Center "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv following the standards established by the law of Ukraine "On the protection of cruelty to animals dated 02/21/2006 No. 3447-IV", European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, and ARRIVE guidelines 2.0 [41]. All animals were kept under standard conditions and provided with dry food and water *ad libitum*. Experiments were carried out following the Institutional Regulations of the procedures for conducting scientific experiments using animals and the bioethical requirements of the Commission of the Institute of Biology and Medicine.

**Scheme of the experiment.** The experiment was carried out in two ways as shown in Fig. 1. For the first method, SP was injected into the tail vein of rats (n = 14) at a dose of 500 µg/100 g of body weight. Control group (n = 14) was injected with 0.1 ml of 0.9 % sodium chloride solution. The second method involved oral administration of SP to rats (n = 10) at a dose of 180 µg/100 g of body weight using a gastric tube. Control group (n = 10) received 0.3 ml of saline. Regardless of the administration route, animals were euthanized 3 hours after SP administration.

**Modelling of experimental colitis.** One hour after the administration of SP, experimental colitis was induced in rats (n = 8) by a single rectal injection of 0.1 ml of a 6 % iodoacetamide (IA), dissolved in a 1 % methylcellulose, at a distance of 7 cm from the anus using a rubber catheter S8 (Rüsch, Germany) [46]. Control group (n = 8) was injected with 0.1 ml of 1 % methylcellulose. Rats were euthanized with CO2 inhalation followed by cervical dislocation 2 hours after rectal administration. During the autopsy, the mass of the intestine was weighed in terms of 100 g of rat weight and oedema was assessed.

**Determination of colon endothelium permeability.** Colon endothelium permeability was determined using Evans blue (EB) dye [3, 30]. Rats were anaesthetized with urethane (1.1 g/kg, intraperitoneally, "Sigma-Aldrich", USA), after which 0.4 % EB was injected into the jugular vein at a dose of 0.4 mg per 100 g of rat weight 15 min before autopsies. Fragment of the colon (7 cm, which corresponded to the area for endothelial permeability) was removed, and the mucosa was scraped off, immersed in formamide, and incubated for 24 h on a shaker at T=50 °C. The optical density of the supernatant was measured on a spectrophotometer at a wavelength of 612 nm against formamide. Endothelial permeability was determined by the amount of dye in the blood (µg/g intestine).

**Glycoproteins determination by Schiff reaction on PVDF-membrane.** 1 cm of the colon was excised and immersed in tubes containing 3 ml of 6 N N-acetyl-1-cysteine (Sandoz, Switzerland). Each tube was vortexed for 4 min, then the samples were centrifuged at 3000 g for 15 min. The supernatant was taken with sediment and a supernatant liquid of 1 ml volume remained. The content of the tubes was resuspended and homogenized on a T10 basic ULTRA-TURRAX® (IKA, Germany) disperser at speed 5 (20 500 rpm) for 20 s. The level of surface mucus glycoproteins was determined using the Schiff reaction on a polyvinylidene fluoride (PVDF) membrane as described in [4, 23]. Image analysis was performed by measuring the density of positively stained dots using Image Studio™ Lite Ver 5.0 image processing and quantitative analysis software (LI-COR Biosciences, USA).

**Myeloperoxidase activity level in the colonic mucosa.** 20–30 mg of the colon mucosa was homogenized by grinding in a mortar with liquid nitrogen to a powder state. Hexadecyltrimethylammonium bromide (HTAB) buffer was added to the homogenized sample at the rate of 1 ml per 50 mg of the sample and transferred to a microtube, which was carried out after 3 cycles: 1 min in liquid nitrogen; 10 min in a water bath (T=37°C). The sample was sonicated.
on ice for 10 seconds on an ultrasonicator and centrifuged for 15 min (14,000 rpm, T = +4°C).

14 µl of the supernatant and 200 µl of the reaction buffer (5 * 10^{-4} % solution H_2O_2 – 6.1 ml, ODHC solution – 4.1 ml, phosphate buffer – 4.4 ml) were added to the microplate. After 5-10 min, the optical density was measured at a wavelength of 450 nm. Measurements were made on a Synergy HT spectrophotometer (BioTek Instruments, USA).

Detection of bacterial translocation. Rats were intraperitoneally anaesthetized with urethane at the rate of 1.1 g/kg, laparotomy was performed in aseptic conditions. 1 ml of blood was collected from the portal vein and tenfold dilutions were prepared in a sterile physiological solution. The total number of microorganisms in the blood was detected by sowing working dilutions on 5 % blood agar. The number of microorganisms was expressed as lg CFU in 1 ml of blood (lg CFU/ml).

Statistical analysis. The statistical analysis of the obtained research results and the construction of graphs were performed using the Prism software v.9 (GraphPad Software, USA). The mean value (M) and standard deviation (SD) were calculated to assess the quantitative indicators. The results are presented as M±SD. The Shapiro-Wilk test was used to test the hypothesis of normal distribution. Comparative data analysis was conducted using the Kruskal-Wallis test or one-way ANOVA with the post hoc Fisher LSD test. The critical level of probability for accepting the null hypothesis (p) was set at less than 0.05.

Results and discussion. PMs used for the study contained carbon with lignin decomposition products, particularly guaiacol and syringol. The average size of PM was ~30 nm. Absorption spectra were similar to those of carbon nanoparticles described in [52].

Intravenous administration of SP from cottonwood increased the permeability of the endothelial barrier of the colon of rats (Fig. 2A). This was indicated by the higher amount of Evans blue dye present in the SP group compared to the control group (p < 0.05). Specifically, after 3 hours, the amount of Evans blue dye in the SP group was 3.8±0.86 µg/g wet colon, while it was 2.30±0.89 µg/g wet colon in the control group. In the excised colon fragmentsof the SP group, we observed local redness that was absent in the control (Fig. 2B). This further supported the disruption of endothelial integrity in the colon due to the administration of SP.

We demonstrated changes in MPO activity level in the colon of rats after intravenous administration of SP (Fig. 2C). MPO is an enzyme associated with inflammatory processes. We found that this indicator significantly increased from 12.18±3.07 to 17.44±2.60 U/g wet colon compared to the control (p < 0.05). At the same time, there were no significant differences in the weight of the intestines between the SP group and the control group. However, there was a tendency for an increase in the SP group, suggesting a potential effect of SP on intestinal weight (Fig. 2D).

Next, Schiff reaction on the PVDF membrane was conducted to assess colonic surface mucus glycoproteins. This revealed a significant 2.5-fold decrease in the amount of colonic surface mucus glycoproteins between the intravenous SP group and the control (Fig. 2E).

As anticipated, the development of experimental colitis had a significant impact on endothelial permeability, as shown in Figures 2A and 2B. The control group with induced colitis exhibited a substantial increase in the amount of Evans blue stain, measuring 29.00±3.60 µg/g wet colon, which was approximately ten times higher than the control group without colitis (p < 0.0001). This difference between the groups was also evident in the levels of MPO activity and the total quantity of surface mucus glycoproteins, as depicted in Fig 2C and 2D, respectively. Moreover, the control group with simulated colitis displayed an increase in intestinal mass, confirming the presence of significant inflammation in the colon. Collectively, these findings provide strong evidence of considerable inflammation in the colon of the control group with induced colitis.
However, the intravenous administration of SP did not exacerbate the negative effects of IA-induced colitis on any of the parameters studied. This suggests that intravenous administration of SP may not have an additional detrimental effect in the context of colitis.

Since one of the indicators of the increased permeability of the colonic epithelium is the translocation of bacteria from the intestinal lumen into the systemic bloodstream, we determined the total number of microorganisms in the blood sample from the portal vein of rats after oral administration of SP from poplar wood. We found that after 3 hours of SP exposure, the number of microorganisms in the blood of rats increased from $10^3.6±0.18$ to $4.4±0.58$ CFU/ml of blood, which indicates a violation of intestinal integrity (Fig. 3).

![Fig. 3. The effect of oral administration of SP from poplar wood on bacterial translocation into portal vein.](image)

Rats received SP using a gastric tube (180 µg/100 g of body weight).

(A) Total bacteria number in the blood of the portal vein (M±SD, *p < 0.05.
(B) Representative photos of Petri dish with Blood agar

These disruptions were accompanied by a sharp decrease in the level of glycoproteins in the surface mucus of the colon. As shown in Figure 4A, the number of glycoproteins in the group which received SP orally decreased by half compared to the control. However, oral administration of SP from poplar wood had no significant effect on the level of MPO activity (Fig. 4B) and the integrity of the intestinal barrier (Fig. 4C,D). There were also no changes in the weight of the colons (Fig. 4E).

![Fig. 4. The effect of oral administration of SP from poplar wood.](image)

Rats received SP using a gastric tube (180 µg/100 g of body weight).

(A) Level of surface mucus glycoproteins (B) MPO activity level (C) Concentration of Evans dye in the mucous membrane of the colon.
(D) Representative images of the colon. (E) Colon weight changes. M±SD, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 vs. Control. #p < 0.05 vs. Control+colitis.
Oral administration of SP did not increase inflammatory processes in the colon in the group with experimental colitis. In contrast, SP reduced the level of endothelial barrier permeability from 28.00±2.50 to 23.00±2.0 µg/g wet colon compared to the control group with modeled colitis. This suggests that SP from poplar wood may have an absorbent effect, which prevented the development of colitis to the control values.

In this study, we demonstrated the ability of nano-sized PM from natural component to promote intestinal proinflammatory processes in vitro. The obtained results correlate with previous studies on epithelial cells of the gastrointestinal tract [37] and confirm the hypothesis that PM of natural origin can lead to an increase in intestinal permeability.

One of the key aspects of this study is the application of two administration models: intravenous and oral. Such a decision was made since there are several options for moving PM after their entrance into the body. Most particles settle in the upper respiratory tract or trachea or bronchi [43], but ultrafine particles can be deposited in the alveoli and can penetrate the interstitial and blood [38]. Alternatively, they can be removed from the bronchios and alveolar spaces through mucociliary clearance, subsequently transported to the oropharynx, and then swallowed [36] or consumed directly by food and water contaminated with PM [18, 47].

Having reached the large intestine through the blood, SP from cottonwood manifested its aggressive effect, primarily due to the destruction of the endothelium of the vessels of the mucous membrane of the large intestine. This process was accompanied by significant intestinal inflammation, the marker of which was an increase in the level of MPO – the abundant heme peroxidase enzyme, present in neutrophils and monocytes. MPO catalyzes the production of powerful ROS, which, on the one hand, provide the enzyme’s antimicrobial function, and on the other, provoke oxidative damage, mucosal damage, and inflammation [16]. Previously, increased amounts of MPO were found in faecal samples of patients with ulcerative colitis and Crohn’s disease [44].

Upon reaching the PM of the large intestine after swallowing, the mucin-type glycoproteins, which are the main high-molecular component of mucus, were the first to take the hit. Their ability to produce a protective gel and high content of oligosaccharides form their major function – to protect epithelial surfaces from mechanical damage and penetration of chemicals (for example, toxins), heavy metals, and pathogenic microorganisms [19, 50]. The decrease in the number of glycoproteins, which we observed, indicates the degradation of the mucus of the large intestine and, accordingly, possible damage to its epithelium. This assumption was confirmed by the fact that 3 hours after the introduction of SP, the number of bacteria in the portal vein increased significantly.

Speaking about the ability of SP from cottonwood to deepen the negative effect of chemically-induced intestinal inflammation, unexpectedly, it showed the opposite effect both with intravenous and oral administration. We assume that PM from a natural component could exert an absorbing property, neutralizing the effect of IA.

The results of PM’s effect on glutamate receptors are a considerable connecting link in the study of the relationship between the intestines and the brain. In our previous studies, we have already demonstrated that PM and carbon-containing nanoparticles are neurotoxic and capable of disrupting synaptic transmission by affecting excitotoxicity in nerve terminals [10, 11]. In addition, PMs reduce synaptosomal transporter-mediated uptake of glutamate and GABA, thereby causing an imbalance in excitatory-inhibitory signalling [12, 52]. However, there is currently no literature data that would fully reveal how regulating the gut-brain axis interacts with inflammatory processes in the colon.

This study had several shortcomings. In particular, it was not taken into account that the time required to reach the SC of the colon after oral administration is different from the time taken for the SC to reach it with the bloodstream. Therefore, the 3 hours set for both models of the experiment cannot be considered equivalent. To overcome this difference, a prolongation of the experiment with oral administration of SP is necessary. And although the conducted research deepens the knowledge about the connection between PM and IBD, it still does not sufficiently reveal the mechanism of action of PM on the development of inflammatory changes in the intestine and requires further research. We also consider it promising to continue the study of this problem under the conditions of chronic exposure to SP of various origins.

Conclusions. This study describes the pro-inflammatory features of the airborne particulate matter obtained from the combustion of natural components such as cottonwood. Getting into the intestines through the blood or oropharynx PM can cause primary pro-inflammatory changes in a concise period of time. Moreover, they can cause disruption of the integrity of the intestinal barrier. However, further studies are required to elucidate the long-term effects of PM on the intestine.

References


ЦІЛІСНІСТЬ КИШКОВОГО БАР'ЄРА ПІД ВПЛИВОМ ТВЕРДИХ ЧАСТИНОК, ОТРИМАНИХ ВІД ЗГОРЯННЯ ДЕРЕВИНИ ТОПОЛІ

Епідеміологічні дани вказують, що під впливом атмосферного забруднення поширюється широкий спектр хвороб. Найбільш часто вони слідкуємо за розвитком синдрому подразненого кишечника та ентерогенного коліту. Збільшення частоти появ таких хвороб може бути вплинуто на цілісність кишкового бар'єра, котрий може дати підставу для створення взаємозв'язку поміж атмосферним забрудненням та інфікційною патологією.

Однак зарубіжні дослідники вважають, що такі зміни можуть бути ініціювані впливом інших факторів. Наприклад, у досліді про вплив забруднення на цілісність кишок тварин, було виявлено, що збільшення частоти появ інфікційної патології є результатом не тільки непосереднього впливу, але й ендокрінних та імунологічних змін.

У даний статті в Україні здійснено дослідження, що включає вивчення впливу забруднення на цілісність кишкового бар'єра у тварин. Основна мета дослідження - зіставити зміни цілісності кишкового бар'єра та ефективність протибактеріальних препаратів.

Цілісність кишкового бар'єра визначалась за допомогою відбірки кишкових клітин та вивчення їх функціонального стану. В результаті досліджень було виявлено, що збільшення забруднення приводить до зменшення цілісності кишкового бар'єра.

Цей дослід може бути використаний в рамках профілактики та лікування хвороб, що пов'язані з забрудненням відходами. Зокрема, дані дослідження можуть бути використані в розробці нових методів захисту організму від негативних впливів забруднення.