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The effect of acute carbon monoxide poisoning on micronuclei frequency and proliferation in human peripheral blood lymphocytes (case-control study)

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Objective: Carbon monoxide (CO) exposure is still one of the leading causes of unintentional poisonings. Although its neurological sequels have been extensively studied, the knowledge about cytogenetic consequences still remains very limited. The aim of this study was to estimate the genotoxic potential of carbon monoxide in the course of acute poisoning. Methods: The examined group consisted of 73 patients treated because of accidental acute CO poisoning, and 22 healthy control individuals. Poisoning severity was estimated on the basis of neurological symptoms at admission, age, duration of exposure, carboxyhemoglobin (COHb) level and blood lactate concentration. The cytochalasine-B (cytokinesis blocker) micronucleus assay (CBMN) was used to analyze the cytogenetic alterations in lymphocytes from peripheral blood of the patients. Results: Intoxicated patients displayed higher numbers of micronuclei (MN) than controls. The frequency of MN depended on the age of patients, loss of consciousness, neurological symptoms at admission, and the level of carboxyhemoglobin, but did not correlate with lactate level. We also observed differences in cell responses depending on the gender. Conclusion: Our results confirm the presence of cytogenetic changes after carbon monoxide poisoning. Based on these data we conclude, that CO might have genotoxic potential.

Ostre zatrucia tlenkiem węgla w zdecydowanej większości mają charakter przypadkowy, stanowiąc jedną z najczęstszych przyczyn tych zatruć. Wpływ CO na organizm pacjenta, oprócz bezpośredniego zagrożenia życia, powoduje trudne do oszacowania szkody na poziomie komórkowym. Mimo rozwoju wielu specjalistycznych technik diagnozowania wciąż pozostaje nierozstrzygnięta kwestia powstawania późnych powikłań u pacjentów z różnymi objawami klinicznymi po zatruciu CO oraz ich związku ze zmianami na poziomie komórkowym/ cytogenetycznym.

Głównym celem pracy jest ocena genotoksycznego działania tlenku węgla jak również ustalenie częstości uszkodzeń ludzkiego genomu w wyniku zatrucia na podstawie limfocytów z hodowli krwi obwodowych pacjentów zatrutych CO. Materiał i metoda; Badaniami objęto 73 dorosłe osoby w wieku 18-93 lat, hospitalizowane z powodu przypadkowego ostrego zatrucia tlenkiem węgla, u których stopień ciężkości zatrucia oceniono na podstawie wieku, czasu ekspozycji na CO, poziomu karboksyhemoglobiny (COHb) i mleczanów we krwi oraz objawów neurologicznych obecnych przy przyjęciu na oddział. Grupę kontrolną stanowiło 22 zdrowe osoby. Do oceny zmian cytogenetycznych w limfocytach krwi obwodowej wykorzystano cytogenetyczny test mikrojądrowy z użyciem cytochalazyny B (CBMN). Wyniki: U pacjentów ostro zatrutych CO wykazano podwyższoną częstość występowania mikrojąder w porównaniu do grupy kontrolnej, zależną od wieku i poziomu karboksyhemoglobiny. Nie stwierdzono jednak zmian skorelowanych z poziomem mleczanów czy objawami neurologicznymi prezentowanymi przez pacjentów podczas przyjęcia na oddział. Interesujące odkrycie stanowiła zdywersyfikowana odpowiedź komórkowa zależna od

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płci oraz związana z utratą przytomności podczas zatrucia CO. Wnioski: W niniejszej pracy wskazano zmiany cytogenetyczne zachodzące w limfocytach krwi pacjentów narażonych na toksyczne działanie tlenku węgla, mogące prowadzić do powstania klonów komórek o zmienionym genotypie, wskazując tym samym możliwe działanie genotoksyczne czystego tlenku węgla, jak również procesów towarzyszących samemu zatruciu.

Introduction

Observations of acute mass CO poisoning in previously healthy subjects reveal different clinical pictures, even in case of equal CO exposure [1-3]. Although symptoms of acute poisoning can develop in almost every organ, they primarily occur in those which are most sensitive to hypoxia, i.e. the cardiovascular system and the central nervous system (CNS).

Thus, consequences of hypoxia and ischemia due to CO toxicity include neuronal death, and result in delayed neurological effects after 1 to 6 weeks of the latent phase [4,5]. It is difficult to determine the real frequency of those late sequels, but it is being estimated at a level of 3-40% [6-9].

Despite multiple studies on CO toxicity and the availability of advanced imaging techniques, the relationship between changes in cardiovascular system and acute CNS symptoms with late neurological deficits is still not clear [10]. Better understanding of the pathophysiology on a systemic, as well as histological or cytological level after acute CO-exposure can be instrumental in establishing new methods of prognosis of late neurological changes.

Exposure to toxic substances, including CO, may result in serious consequences for a cell's genetic material. Mutagenic or genotoxic activity causes damages in the genome, leading to changes in the amount or structure of DNA, which interrupts the cell's or tissue's normal functioning. The genotoxic effect of mixed substances containing CO (cigarette smoke, fire smoke, coal stoves smoke) has been demonstrated in many studies, but only few concerned the genotoxic effect of CO alone [11,12]. In an *in vivo* study performed on pregnant mice and murine fetuses *Kwak* and co-authors [13] indicated genotoxic properties of CO and suggested the same effect in other mammals, including human. The pilot studies performed by *Guratowska* et al. [14], *Kubik* et al. [15], and *Tarik* et al. [16] on carbon monoxide-poisoned patients revealed disturbances in cell division and impaired DNA repair ability, indicating the need of more comprehensive studies on that issue.

The aim of the present study was to directly assess the genotoxic potential of carbon monoxide on the blood cells of patients exposed to CO.

Material and methods

Patients

The study was approved by the local ethics committee. All participants gave their written informed consent for genetics studies in order to become included in the research. The examined group consisted of 73 patients aged 18-93 years (median = 30.0), treated at the Clinical Toxicology Ward in Krakow because of accidental acute CO poisoning due to incomplete combustion of natural gas. In the group of 41 females, 16 declared to be smokers, and the group 32 males contained 13 smokers. Material was collected in winter time, when accidental acute CO poisonings are most frequent. Only patients without prior head injury, CNS inflammatory changes, alcoholic diseases, epilepsy, migraine or systemic injuries were qualified for the examination. All patients received treatment with 100% oxygen. Peripheral blood samples were collected during routine clinical examination.

Poisoning severity (Tab. I) was estimated on admission, on the basis of neurological symptoms (according to *Pach* scale, age, duration of exposure, COHb level and blood lactate concentration [17]).

The reference group consisted of 22 healthy volunteers (aged 18-78; median = 45.0), inhabitants of the Malopolska region, with similar sex and age distribution to the control group.

CBMN test

In order to assess the genotoxic effect of carbon monoxide, the cytochalasine B micronucleus (CBMN) assay was performed in the Karyology Laboratory of the Department of Anthropology, Jagiellonian University, using a modified procedure of *Fenech* [18]. This test allows to estimate early genotoxic effects. It is based on the analysis of micronuclei (MN) - structures formed in the course of inappropriate cell division. Because of the simplicity of its performance on human lymphocytes, this assay may be successfully used in population screenings.

Whole blood cell cultures were established to isolate material for the micronucleus test. At the day of blood receiving cells were plated in RPMI medium supplemented with 10% bovine serum, and phytohemmaglutinine (PHA). Cytokinesis-blocker cytochalasine B (Sigma) was added at a final concentration of 6 µg/ml after 44 hours. The cultures were harvested by centrifugation at 700 rpm after 72 h of culturing. The supernatant was discarded. Cells were treated with a hypotonic solution (0.075 M KCl) for 1 min, fixed several times with a 1:3 mix of glacial acetic acid and methanol, and centrifuged. Three slides were obtained for each patient. The slides were coded and left to air-dry. Afterwards, slides were stained with a 20% Giemsa solution in distilled water for 8 minutes, rinsed twice in deionized water and left to air-dry.

The micronuclei fraction was determined in 1000 mono- and binuclear lymphocytes (fractions MN1 and MN2, respectively), as well as nucleoplasmic bridges and buds in binuclear cells, meeting the criteria of *Fenech* et al. [19,20] - collectively called "2N damages". All analyses were performed on a light microscope (Nikon) at a magnification of 1600-2000x.

Next, to assess the degree of cell stimulation and proliferation, the Nuclear Division Index (NDI) was counted using the formula:

NDI = (1M1 + 2M2 + 3M3 + ... + kMk)/N,

where M1, M2, M3, ..., Mk are the numbers of cells with k nuclei, and N is the total scored cell number.

One-dimensional and multidimensional statistical analyses of the data were performed. Due to the lack of normal distribution of the majority of parameters, non-parametrical tests were used (*Mann-Whitney U* test and *Spearman's* rank correlation). Forward stepwise logistic regression analysis has allowed for verification of the statistical significance of age, sex, cigarette smoking, CNS damage, blood lactate concentration and level of carboxyhemoglobin on DNA damages.

Results

The first step of our analysis was estimating the poisoning severity on admission, on the basis of neurological symptoms according to *Pach* scale, age, duration of exposure, COHb level and blood lactate concentration (table I). Considering criteria listed in table

Table I
The point scale of carbon monoxide poisoning severity.
Skala punktowa ciężkości zatrucia tlenkiem węgla.

Parameter	Point scale			
	0	1	2	3
Age [years]	< 29	30 – 39	40 - 49	>50
Duration of exposure [min]	< 30	31 – 60	61 - 120	>120
Pach scale (table 2)	I°	II°	III°	IV°
COHb level [%]	0	< 15	15 - 30	>30
Lactate concentration [mmol/L]	1.0 - 1.78	1.8 - 3.6	3.7 - 5.4	<5.5

Severity grading :I° minor 1-4 , II° moderate 5-8, III° >9 points

I, a minor poisoning was diagnosed in 18 (25%) of the patients, moderate in 47 (64%), and severe poisoning was diagnosed in 8 (11%) of the study patients. Indices of CO poisoning severity in the examined group are presented in table II.

To analyze the cytogenetic changes in blood cells of examined patients, blood sample were collected for in vitro studies. The CBMN assay was performed for individual patients and data were pooled for the examined groups.

In the initial analysis the total frequency of micronuclei in mononuclear and binuclear cells (MN1 and MN2, respectively), damages in binuclear cells ("2N damages"- nucleoplasmic bridges and buds), and the cell division index were estimated. Basic descriptive statistics (median, range and standard deviation) for compared groups is summarized in table III.

The frequency of micronuclei in binuclear cells was significantly higher in the poisoned group than in the control group (CO: 5,0 ± 4‰, n = 73 vs. control: 2,0 ± 1,41‰, n = 21; p <0,001; figure 1).

To examine the influence of age on this difference *Spearman's* correlation test between this parameter and the number of MN was calculated. Linear regression (Fig. 2) revealed a positive correlation of both MN1 and MN2 frequency with age in the CO poisoned group which was not observed in control group. The frequences of micronuclei in mononuclear as well as binuclear cells were significantly dependent on the patients' age: p = 0.045 and 0.009 for MN1 and MN2, respectively. The correlation between MN2 and age in the patient group is shown in figure 2. In the control group, only MN1 correlated with the age of the patients (p = 0.034), whereas MN2 did not (p = 0.972) – data not shown. This is in accordance with the fact that the frequency of MN in mononuclear cells represents the amount of mutations accumulated in the organism during life.

Since smoking may influence the results of the micronuclei assay, we examined the influence of that factor on the CBMN parameters. We detected statistically significant differences in NDI between smokers and non-smokers in the examined and the control group (Tab. IV).

To verify the relationship between the cytogenetic changes in blood cells and neurological alterations, We analyzed the correlation between CNS damage diagnosed using the *Pach* scale, and MN frequency, by *Spearman's* correlation. This parameter did not affect genomes significantly (p>0.05).

Two-way Anova revealed a statistically significant interaction between poisoning severity and loss of consciousness in CO-poisoned patients on the number of micronuclei in mononuclear cells (Fig. 3). In patients with no loss of consciousness, a dose-effect correlation was observed - the amount of genetic damages was elevated in more severe cases of poisoning. Such an effect was not observed in patients with a history of loss of consciousness.

A similar analysis indicated the interaction between gender and loss of consciousness. We observed that in intoxicated males, but not females, loss of consciousness decreased the frequency of micronuclei in mononuclear cells (Fig. 4).

The amount of 2N damages (buds and bridges between nuclei of binuclear cells) were additionally correlated negatively with the time of admission to hospital in CO-poisoned patients (Fig. 5). Increased values were observed in patients tested immediately after admission, whereas decreased in those tested at a later time point. No such

Table II

Indices of CO poisoning severity.

Wskaźniki ciężkości zatrucia CO.

Parameter	mean	SD	min	max
Age [years]	35	16.2	18	93
COHb [%]	24.4	8.6	1.7	47.9
Lactate concentration [mmol/l]	2.998	2.21	0.64	12.4
Period of unconsciousness [min]	6.98	13.7	0	60
Duration of exposure [min]	67	97	5	420
Duration to exposure cessation – time of waiting for medical help [min]	93.93	106	15	600
Time since admission to blood harvesting for cytogenetic examination [hours]	18	6.27	3.5	28.3

Table III

Descriptive statistics of micronuclei test parameters for patients after carbon monoxide poisoning and for the control group.

Statystyka opisowa parametrów testu mikrojąder dla pacjentów po zatruciu tlenkiem węgla i grupy kontrolnej.

	CO poisoned patients				Control group			
	median	minimum	maximum	std deviation	median	minimum	maximum	std deviation
NDI	1.11	1.02	1.42	0.09	1.10	1.05	1.68	0.14
MN1 [‰]	1.00	0.00	8.00	1.81	1.00	0.00	3.00	1.17
MN2 [‰]	5.00	0.00	25.00	4.00	2.00	0.00	4.00	1.41
2N damages	10.00	0.00	43.00	6.65	4.00	0.00	12.00	3.02

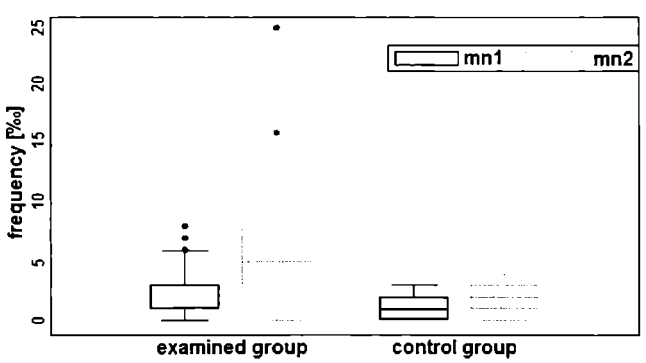

Figure 1

Frequencies [‰] of micronuclei in the examined and the control group, micronuclei in mononuclear cells (MN1) p=0.034, micronuclei in binuclear cells MN2 p<0.001 (Mann-Whitney test).

Częstość [‰] mikrojąder w grupie badanej i kontrolnej, mikrojądra w komórkach jednojądrzastych (MN1) p=0,034, mikrojądra w komórkach dwujądrzastych MN2 p<0,001 (test U Manna-Whitneya).

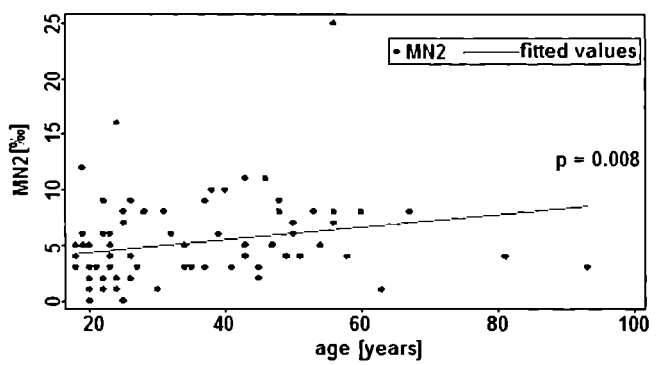

Figure 2

Frequency [‰] of micronuclei in binuclear cells of CO-poisoned patients in relation to their age (Spearman's Rho=0.3044; p=0.0088).

Częstość [‰] mikrojąder w dwujądrzastych komórkach pacjentów po zatruciu CO w odniesieniu do ich wieku (Spearman Rho=0,3044; p=0,0088).

Table IV

Comparison of micronuclei test results among smokers and non-smokers in the patient and the control group. Mann-Whitney test. α = 0.05

Porównanie wyników tesu mikrojąder dla palaczy i osób niepalących w grupie pacjentów i kontroli. Test U Manna-Whitneya. α = 0,05.

	CO poisoned patients							Control group						
	smoking		non-smoking		U	Z	p	smoking		non-smoking		U	Z	p
	N	∑ rank	N	∑ rank				N	∑ rank	N	∑ rank			
NDI	29	1268.0	43	1360.0	414.0	-2.400	0.016	6	73.5	14	136.5	31.5	-0.825	0.409
MN1 [‰]	29	1043.5	44	1657.5	608.5	0.334	0.738	7	91.0	14	140.0	35.0	-1.073	0.282
MN2 [‰]	29	1130.5	44	1570.5	580.5	-0.646	0.518	7	76.0	14	155.0	48.0	0.038	0.969
2N damages	29	1217.0	45	1558.0	523.0	-1.432	0.152	4	35.5	11	84.5	18.5	-0.395	0.693

Table V

Results of forward stepwise logistic regression for the control and the patient group – p values/beta. α = 0.05.

Wyniki regresji krokowej postępującej dla grupy kontrolej i pacjentów – wartości p/beta. α = 0,05.

	CO poisoned patients							Control group		
	age	sex	cigarettes	CNS damage	loss of consc	lactate	HbCO	age	sex	cigarettes
MN1	—	—	---	---	0.0519/-0.2415	—	0.0882/0.2110	0.1319/-0.3161	0.0258/0.4891*	—
MN2	0.0222/0.2624	—	—	0.0017/0.3668	—	—	---	—	0.0564/-0.4448	—
2N	0.0442/0.2411	—	---	0.0281/0.2744	0.2439/0.1452	—	---	—	0.1332/-0.4392	---
NDI	—	—	0.0580/0.2383		0.3194/-0.1238	—	---	—	—	0.2657/0.2770

--- not included in the logistic regression result

*** Positive correlation with female sex**

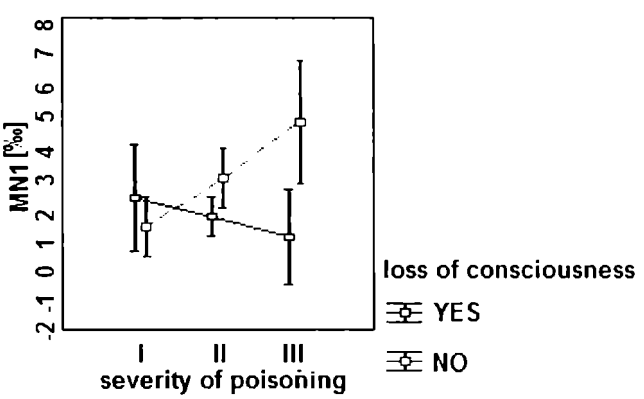

Figure 3

Comparison of micronuclei frequencies [‰] in mononuclear cells in patients with different severity of poisoning, considering loss of consciousness (current effect: F(2, 63)=4.3921, p=0.01638).

Porównanie częstości mikrojąder [‰] w komórkach jednojądrzastych u pacjentów o zróżnicowanej ciężkości zatrucia, uwzględniające utratę przytomności (efekt bierzący: F(2, 63)=4,3921, p=0,01638).

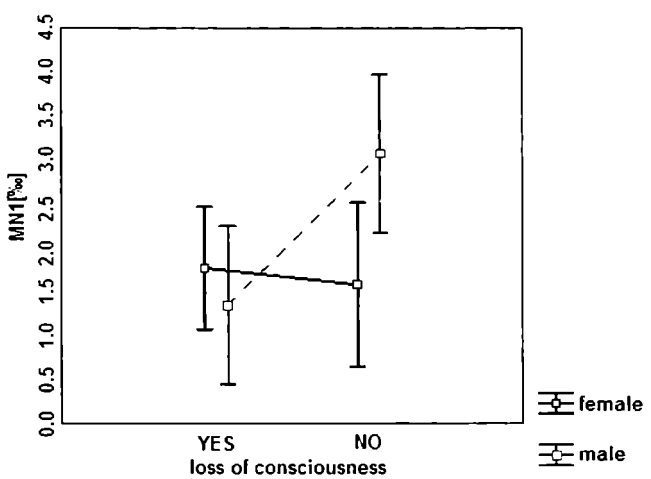

Figure 4

Comparison of micronuclei frequencies [‰] in mononuclear cells in CO-poisoned patients, considering loss of consciousness and gender (current effect: F(1, 65)=4.8983, p=0.03040).

Porównanie częstości mikrojąder [‰] w komórkach jednojądrzastych u pacjentów o zróżnicowanej ciężkości zatrucia, uwzględniające utratę przytomności i płeć (efekt bieżący: F(1, 65)=4,8983, p=0,03040).

correlations were observed for micronuclei frequency in mono- or binuclear cells.

In case of 2N damages we found a difference between men and women in the reaction to poisoning. Although there was no effect of gender on the level of severity parameters (COHb and lactates), there was an almost twice increase in the frequency of 2N damages in men with confirmed medical history of a loss of consciousness during CO intoxication (*Mann-Whitney* test: z=-2.610; p=0.0091).

Forward stepwise logistic regression analysis was performed including age, sex, and smoking status in all participants, and additionally serum lactate and HbCO concentrations, CNS damage, and loss of consciousness in the examined group. (table V) Although the model for micronuclei in mononuclear cells of CO-poisoned patients did not contain statistically significant parameters, the amount of MN1 in this group showed a trend of correlation with loss of consciousness, together with HbCO concentration.

Discussion

Here we present a comprehensive study on genotoxic effects of carbon monoxide in acutely poisoned patients in relation to poisoning severity and medical intervention.

Patients after acute CO poisoning were characterized by an elevated frequency of DNA damages in mono- and binucleated lymphocytes, when compared to the control group. Those data clearly indicate that a genotoxic agent had influenced acute carbon monoxide-poisoned people.

The increased amount of DNA damages in blood cells of intoxicated patients might be the result of a significant genotoxic effect of CO. The frequency of damages is difficult to correlate with the concentration of CO, to which patients have been exposed, because of the lack of information from the place of the event. Nonetheless, obtained results cannot be attributed to culture conditions, because they were in accordance with accepted methods for assessing the exposure to genotoxic agents in vitro. The observed alterations in DNA are visible even for concentrations which do not cause significant decrease in cell survival. Increased frequency of micronuclei in intoxicated patients'

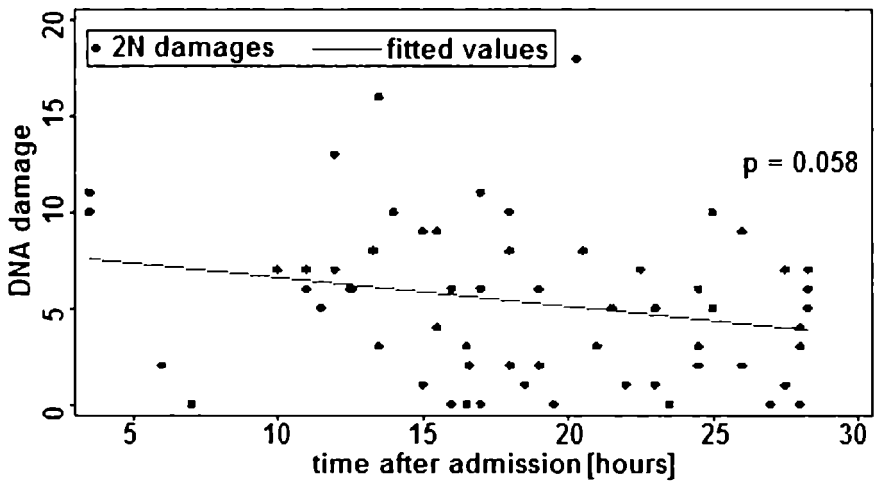

Figure 5

Frequency of 2N damages in binuclear cells of CO-poisoned patients in relation to time after admission.
Częstość uszkodzeń 2N w dwujądrzastych komórkach u pacjentów po zatruciu CO w odniesieniu do czasu od przyjęcia.

cells points to genotoxic action of CO. An increase in the number of micronuclei indicates that the tested population of cells has been exposed to a clastogen (which causes structural aberrations of chromosomes) and/or an aneuploidogen (which causes numerical chromosome abnormalities). The micronucleus test is informative not only about structural changes in the genetic material, but also allows to assess the dynamism of cell growth, by determining the nuclear division index (NDI) [21,22]. The frequency of MN in mononuclear cells allows to assess the amount of genome/chromosomal mutations accumulated in the organism, whereas the frequency in binuclear cells is informative about DNA damages arisen in vivo during phase G0 as well as the first cell cycle in vitro in the cell culture [19,21,23].

The genotoxic effect of CO on may be exerted by inducing changes in the genetic material in an indirect way, most probably by initiating oxidative processes. The latter may lead to an increase in the amount of DNA damages. Other mechanisms of CO genotoxicity include impaired oxygen assimilation impairment or interfering with cellular respiration by binding to mitochondrial cytochrome oxidase. These events eventually lead to energetic deficit and cellular structure disintegration as described by others [5,7,9,21]. Moreover, besides the genotoxic activity of CO, epigenetic alterations caused by carbon monoxide should also be considered CO can induce local modifications of chromatin structure, and thus disturb the proper transcriptional activity of many genes, while not causing changes in nucleotide sequences.

Alternatively, the increased number of micronuclei can be explained by the indirect effect of the therapy. The discontinuation of exposure to CO and oxygen therapy increase the speed of carbon monoxide dissimilation from hemoglobin, and restoration of normal oxygen transport into tissues. In parallel, it decreases the level of COHb in the blood without the restoration of enzymatic pathways in the cell. Free radicals arisen during re-oxygenation may not be effectively suppressed and removed, eventually leading to lipid peroxidation. CO bound to hemeproteins during re-oxygenation extends the time of oxygen and energy deficit, enhances oxidative stress in the cell, and enlarges the probability of the cell's death [24].

The determination of lactate levels reveals important information about the extent of hypoxia in a cell, because its increase is the effect of hypoxia and disturbed oxygen exploitation in a tissue [25]. However, this indicator did not reach statistical significance in relation to the micronucleus test ($p>>0.05$).

We observed a strong correlation between age and micronuclei frequency and damages in binuclear cells in people exposed to carbon monoxide but not in those of the control group. Age is usually the only endogenous factor taken into account in the assessment of poisoning severity and of damages in the genetic material. Positive correlation between age and the frequency of micronuclei has been reported previously [20,25,26]. The susceptibility of a cell to form micronuclei, which increases with age in patients after CO poisoning, might explain the worse outcome of older patients and the more frequently observed health complications after hospitalization [27,28].

Within the group of patients that lost consciousness after CO poisoning, men were more susceptible to the toxic activity of carbon monoxide, as indicated by increased frequency of micronuclei formation in mononuclear cells. In women, there is typically a higher frequency of micronuclei formation involving the inactive X chromosome, which is characterized by a faster age-dependent telomere shortening [29]. For these reasons, under normal conditions women demonstrate a 1.2 to 1.6-fold higher amount of micronuclei than men [30].

In studies [28,31] employing brain perfusion scintigraphy using ^{99m}Tc-HMPAO it was found that the probability of pathological changes in women was significantly lower than in men (OR = 0.32, p=0.02). Our results are in line with these observations.

Our data indicate that loss of consciousness influences the frequency of micronuclei formation in mononuclear cells in CO-poisoned patients. In those without loss of consciousness a "dose-effect" correlation was observed, i.e. in more severe cases of poisoning the number of damages in the genetic material increased. In available reports, the intensity of changes in brain perfusion scintigraphy using ^{99m}Tc-HMPAO in acute CO poisoning does not depend significantly on the duration of loss of consciousness [10,32,33], however intensity of scintigraphic changes increased with the severity of poisoning [28].

Fatal consequences for CO-poisoned patients may be also due to its negative impact on mechanisms controlling the cell cycle, especially mutations in proto-oncogenes and suppressor genes. These alterations might lead to uncontrolled proliferation of the damaged cell, and eventually induce tumorigenesis [34,35]. Multi-centre prospective studies within the HUMN project revealed a significantly higher risk of developing a tumor in patients with high or medium amounts of micronuclei in blood lymphocytes than in those with low amounts, independently of their ethnic origin or the locus of tumor development [36]. Exposition to CO, besides causing micronuclei formation, probably also induces the division of cells resistant to apoptosis, which might initiate tumor formation in intoxicated patients. The lack of correlation between the degree of damages in *in vitro* cultured lymphocytes and NDI may be a consequence of the elimination of cells with higher amounts of DNA damages in the patient's body [14], or the stimulating action of phytohemagglutinin added to the in vitro culture. The limitations of the CBMN test make it impossible to determine the fate of cells in with DNA damages have occurred.

The cytotoxic activity of CO may be an indirect result of hypoxia caused by COHb formation, or else a direct effect of CO action on the cell and its enzymes. The binding of CO with compounds of the cell [37], cytochrome P450 monooxidase, or cytochrome c oxidase [38], the formation of free oxygen [39,40] or nitrogen [41,42] radicals, and the induction of the mitochondrial apoptosis pathway [43] are just some examples of CO activities, that have been reported so far, which can be an explanation for the obtained data.

Conclusions

- Here we present a pilot study on the genotoxic effect of carbon monoxide poisoning, based on the CBMN assay.
- Our results require urgent verification on cultured lymphocytes exposed to controlled concentrations of CO.
- Further research is also needed in order to address whether the combination of laboratory tests used in this study, together with imaging methods (SPECT, MRI) might help in fast and effective diagnostics, and prediction of late sequels of CO intoxication of patients after acute CO poisoning.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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