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Therapeutic effect of magnesium sulphate on carbon monoxide toxicity-mediated brain lipid peroxidation

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Abstract. – BACKGROUND: Carbon monoxide (CO) toxicity primarily results from cellular hypoxia caused by impedance of oxygen delivery. Studies show that CO may cause brain lipid peroxidation and leukocyte-mediated inflammatory changes in the brain.

AIM: The aim of this study was to investigate whether magnesium sulphate could prevent or diminish brain lipid peroxidation caused by carbon monoxide toxicity in rats.

MATERIALS AND METHODS: Fourty rats were divided into five groups of 8 rats each. Group I was not received any agent during the experiment. Group 2 was inhaled CO gas followed by intraperitoneally normal saline 30 minutes (min) later. Group 3 was inhaled CO gas followed by 100 mg/kg magnesium sulphate intraperitoneally 30 min later. Group 2 and Group 3 rats was undergone laparotomy and craniotomy while still under anesthesia at 6 hour, and tissue sample was obtained from the cerebrum. Group 4 was inhaled CO gas followed by intraperitoneally normal saline 30 min later. Group 5 was inhaled CO gas followed by 100 mg/kg magnesium sulphate intraperitoneally 30 min later. Group 4 and Group 5 rats was undergone laparotomy and craniotomy while still under anesthesia at 24 hour, and tissue sample was obtained from the cerebrum.

RESULTS: Nitric oxide levels were no significantly different between all groups. Malonyldialdehyde levels increased in intoxication group (group 2) and decreased in treatment group (group 3). Activities of superoxide dismutase decreased in intoxication group (group 2) and increased in treatment group (group 3). Activities of catalase increased in intoxication group (group 2) and decreased in treatment group (group 3). Activities of glutathione peroxidase (GSH-Px) decreased in intoxication group (group 4) and increased in treatment group (group 5).

CONCLUSIONS: CO poisoning caused significant damage, detected within the first 6 hours. Due to antioxidant enzymes, especially GSH-Px activity reaching the top level within 24th hours, significant oxidative damage was not observed. The protective effect against oxidative damage of magnesium sulfate has been identified within the first 6 hours.

Key Words:
Carbon monoxide toxicity, Magnesium sulphate, Brain, Lipid peroxidation.

Introduction

Carbon monoxide (CO) is a colorless, odorless and non-irritant gas produced by the incomplete combustion of carbon-containing fuels[1]. CO poisoning is one of the commonest public health problems in our country and around the world (1.2). It is the most common cause of death resulting from poisoning in the United States[3]. Every year, about 40,000 people apply to Emergency Services for CO poisoning in the United States[1]. In Turkey, CO poisoning is reported the most frequent cause of death with a rate of approximately 31%[2]. CO poisoning is a toxic condition resulting from inhaling gases developing from any combustion reaction, car exhaust, improper chimney structure, industrial substances, or a fire in a non-aerated environment[2,4,5].

CO creates a reversible bind with respiratory enzymes and pigments such as hemoglobin, myoglobin, cytochrome P450 and cytochrome aa3. CO binds to hemoglobin 230-270 times more avidly than oxygen. Small concentrations such as 100 ppm create a COHb level of 16% approximately; this level is sufficient for the occurence of clinical symptoms[4,6]: primary tissue hypoxia occurs and accompanying damages develop. CO has been shown to cause brain lipid peroxidation and leukocyte-mediated inflammatory changes in the brain[4]. Increase

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of thiobarbituric acid reactive products (TBARS) in the rat brain tissue after oxidized proteins, reduces glutathione poisoning and lead to oxidative stress. Nitric Oxide (NO) may lead to elevation of glutathione after CO poisoning. Due to the changes associated with NO, extravasations of intravascular cells and the lipid peroxidation in brain cells occur[7]. Brain damage may develop if hypoxia accompanied with a period of loss of consciousness[7]. Additionally, glutamate level, an excitatory amino acid, increases after CO poisoning. Glutamate binds to N-methyl-D-aspartate receptors and leads to intracellular calcium release that cause delayed neuronal cell death[7]. Central nervous system is one of the sensitive organs to CO. Headache, dizziness, and ataxia may occur at COHb level of 15-20%. Long-term exposure to CO may cause syncope, convulsions, focal epileptic seizures and coma[5,8]. Patients may have acute stroke symptoms and leukoencephalopathy and brain edema may develop[5].

Treatment of CO poisoning is still controversial due to the confusing clinical trials[5]. Mankind benefits from the therapeutic effect of magnesium (Mg) for centuries. Today, Mg is known to have different effects on almost every system in our body[9]. A sudden increase in the serum Mg concentration in the cerebral circulation leads to a rapid and concentration dependent vasodilatation in cerebral arteries. Magnesium sulfate protects the spinal cord from ischemic injury in dogs with spinal cord ischemia. Another study[10] has revealed that magnesium sulfate have neuroprotective effects on axonal function and lipid in rats. It was also reported that magnesium sulfate reduce neuronal apoptosis in cerebral ischemia-reperfusion injury. It was suggested[11] that systemically administered magnesium have neuroprotective effects and shrink the area of stroke in animal models of stroke. An increase of lactate and MDA levels in brain tissue may cause ischemia reperfusion injury and the magnesium sulfate supress this increase[12].

To our knowledge, the effects of magnesium sulfate in CO poisoning has not been investigated yet. The purpose of this study is to investigate whether magnesium sulfate have therapeutic effect on oxidative damage due to CO poisoning in the brain.

Materials and Methods

Preparation of Animals

Male Sprague-Dawley rats weighing 160 to 250 g were housed under standard laboratory conditions and were allowed free access to food and water. These experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Afyon Kocatepe University and were in accordance with National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH publication, vol 25, no. 28, 1996). This research was supported by Afyon Kocatepe University Scientific Committee. The rats were divided into five groups as follows;

Group 1: No test procedure was performed. Brain tissue was removed immediately after sacrificing the animal (eight rats).

Group 2: The rats were exposed to CO gas and treated with saline. Evaluations were carried out at the 6th hour and the animals were sacrificed and then brain tissue was removed (eight rats).

Group 3: The rats were exposed to CO gas and treated with magnesium sulfate. Evaluations were carried out at the 6th hour and the animals were sacrificed and then brain tissue was removed (eight rats).

Group 4: The rats were exposed to CO gas and treated with saline. Evaluations were carried out at the 24th hour and the animals were sacrificed and then brain tissue was removed (eight rats).

Group 5: The rats were exposed to CO gas and treated with magnesium sulfate. Evaluations were carried out at the 24th hour and the animals were sacrificed and then brain tissue was removed (eight rats).

CO Gas Exposing Protocol

Rats to be poisoned with CO were placed in an anesthesia chamber made of transparent glass (dimensions 60 × 27 × 27 cm). Eight rats were placed into the cup at every session. Rats were randomized to a control group (N=4) and a magnesium group (N=4). In the beginning, CO gas exposure was set to be 1000 ppm and this process continued for 120 minutes. Then CO level was raised from 1000 to 2000 ppm and this process continued about 30 minutes. Rats were exposed to ambient air. Observations were made by the same observer throughout the experiment.

Magnesium Sulphate and Saline Administration

Magnesium sulfate was administered to both group 3 and group 5 in the same way. After 30-minute exposure to CO, rats were administered intraperitoneal injection of magnesium sulfate in a dose of 100 mg/kg. Intraperitoneal injection of saline was given to control groups

(group 2 and group 4) after 30-minute exposure to CO in the same amount.

Biochemical Analysis

At the sixth and twenty-fourth hour of the study, rats were sedated with intramuscular injection of 50 mg/kg ketamine. Abdomen was incised in the midline and rats were sacrificed by drawing blood from abdominal aorta. After incision of the skull, the brain tissue was removed as a whole for biochemical analysis without any damage. Following biochemical analyses were performed on the brain tissue.

Determination of SOD Activity

Tissue SOD (EC 1.15.1.1) activity was determined using the nitroblue tetrazolium (NBT) method described by Sun et al[13] and modified by Durak et al. In this method, NBT is reduced to blue formazan by superoxide (O_2^-), which has a strong absorbance at 560 nm. One unit (U) of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50%. SOD activity was expressed as units per mg tissue protein (U/mg protein).

Determination of Catalase (CAT) Activity

Tissue CAT (EC 1.11.1.6) activity was determined according to Aebi's method. The principle of the assay is based on the determination of the rate constant k (s^-1) or the H_2O_2 decomposition rate at 240 nm. Results were expressed as k (rate constant) per g of protein (k/g protein).

Determination of GSH-Px Activity

GSH-Px (EC 1.11.1.9) activity was determined according to Paglia et al[16].

Determination of MDA

Measurement of MDA levels was based on the coupling of MDA with thiobarbituric acid at +95°C. Results were expressed as nanomole per g protein (nmol/g protein).

Determination of Protein Content

The method of Lowry et al[18] with bovine serum albumin (IBSA) was used to measure the protein content of cerebellum homogenates. A Shimadzu spectrophotometer (Shimadzu Corp; Kyoto, Japan) was used for all assays.

Determination of NO

NO measurement is very difficult in biological specimens because it is easily oxidized to nitrite (NO^{2-}) and subsequently to nitrate (NO^{3-}), which serve as index parameters of NO production. The method for determination of nitrite and nitrate levels was based on the Griess reaction. Samples were initially deproteinized with Somogyi reagent. Total nitrite (NO^{2-} + NO^{3-}) was measured by spectrophotometry (Shimadzu, UV-Pharmaspec 1700, Kyoto, Japan) at 545 nm after the conversion of NO^{2-} to NO^{3-} by copperized cadmium (Cd) granules. Results were expressed as micromole per g tissue protein (mol/g protein).

Statistical Analysis

Statistical analyses were performed using SPSS for Windows, Version 13.0 (SPSS Inc., Chicago, IL, USA). All biochemical data were presented as means ± standard deviation (SD). They were analysed using a Kruskal-Wallis *H*-test. Differences between two groups were determined with Mann-Whitney *U*-test. $p < 0.05$ was considered statistically significant.

Results

Nitric oxide (NO) levels, malonyldialdehyde (MDA) levels and enzyme activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) are shown in Tables I and II.

Nitric Oxide (NO) Levels

No statistically significant difference was observed between groups in terms of NO levels ($p > 0.05$, Tables I and II).

MDA Levels

The increase in the MDA levels in group 2 (42.439 ± 2.285) was statistically significant than that in group 1 (35.162 ± 3.891) ($p < 0.05$). A statistically significant difference between group 1 and group 3 (35.200 ± 6.762) was observed ($p > 0.05$). Decrease in MDA levels was statistically significant in magnesium-treated group than that in group 2 ($p < 0.05$, Table I). No significant difference was observed between group 4 and group 5 (24^{th} hour groups) in terms of MDA levels (Table II).

GSH-Px Enzyme Activity

When compared with the group 1 (0.256 ± 0.033), a statistically significant increase was observed in group 2 (0.469 ± 0.184) ($p < 0.05$). There was no statistically significant difference

Table I. The levels of nitric oxide (NO), malonyldialdehyde (MDA), and activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in control (I), after 6 hour later carbon monoxide intoxication (II) and after 6 hour later carbonmonoxide intoxication plus magnesium (III) groups in rats' brain. Data represent means ± SD.

Group	NO (µmol/g protein)	MDA (nmol/g protein)	GSH-Px (U/g protein)	SOD (U/mg protein)	CAT (K/g protein)
I (n = 8)	2.898 ± 0.349	35.162 ± 3.891	0.256 ± 0.033	0.407 ± 0.031	0.097 ± 0.012
II (n = 8)	2.805 ± 0.261	42.439 ± 2.285	0.469 ± 0.184	0.282 ± 0.038	0.127 ± 0.015
III (n = 8)	2.755 ± 0.203	35.200 ± 6.762	0.339 ± 0.083	0.409 ± 0.046	0.097 ± 0.011
p values					
I-II	0.935	0.028	0.013	0.000	0.003
I-III	0.782	1.000	0.446	1.000	0.999
II-III	0.966	0.018	0.114	0.000	0.001

between group 1 and group 3 (0.339 ± 0.083) and group 2 and group 3 ($p > 0.05$, Table I). When experimental groups (study terminated at 24th hour) were analyzed, there were statistically significant differences between group 1 (0.256 ± 0.033) and group 4 (0.919 ± 0.201) and group 5 (1.288 ± 0.412) ($p < 0.05$). A significant difference was also observed between group 4 and group 5 ($p < 0.05$, Table II).

SOD Enzyme Activity

When compared with group 1(0.407 ± 0.031), a statistically significant decrease in SOD enzyme activity was observed in group 2 (0.282 ± 0.038) ($p < 0.05$). No statistically significant difference between group 1 and group 3 (0.409 ± 0.046) was observed ($p > 0.05$).

However, there was a statistically significant difference between group 2 and group 3 ($p < 0.05$, Table I). No significant differences were observed between group 4 and group 5 in terms of SOD activity ($p > 0.05$, Table II).

CAT Enzyme Activity

When compared with group 1 (0.097 ± 0.012), a statistically significant increase in CAT enzyme activity was observed in group 2 (0.127 ± 0.015) ($p < 0.05$). No statistically significant difference between group 1 and group 3 (0.097 ± 0.011) was observed ($p > 0.05$). When compared with group 2, group 3 showed a statistically significant reduction ($p < 0.05$, Table I). No significant difference was observed between experimental groups (group 4 and 5) in terms of CAT enzyme activity ($p > 0.05$, Table II).

Discussion

Carbon monoxide (CO) poisoning cases may occur by the utilization of charcoal barbecue and stoves especially in winter months and due to the suicide attempt. Clinical manifestations depend upon patients' physiologic reserve and the duration of CO exposure and the density of CO.

Table II. The levels of nitric oxide (NO), malonyldialdehyde (MDA), activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in control (I), after 24 hour later carbon monoxide intoxication (II) and after 24 hour later carbonmonoxide intoxication plus magnesium (III) groups in rats' brain. Data represent means ± SD.

Group	NO (µmol/g protein)	MDA (nmol/g protein)	GSH-Px (U/g protein)	SOD (U/mg protein)	CAT (K/g protein)
I (n = 8)	2.898 ± 0.349	35.162 ± 3.891	0.256 ± 0.033	0.407 ± 0.031	0.097 ± 0.011
IV (n = 8)	2.511 ± 0.258	39.918 ± 8.869	0.919 ± 0.201	0.434 ± 0.076	0.111 ± 0.013
V (n = 8)	2.753 ± 0.427	35.495 ± 8.284	1.288 ± 0.412	0.397 ± 0.037	0.111 ± 0.009
p values					
I-IV	0.138	0.499	0.001	0.626	0.154
I-V	0.875	1.000	0.000	0.935	0.085
IV-V	0.478	0.686	0.040	0.368	1.000

Headache, nausea, vomiting, muscle weakness, fatigue, malaise and changes in consciousness occur in mid-level poisoning cases. In cases of severe poisoning, more severe neurological symptoms may develop. CO poisoning affects the organs which have a high demand for oxygen such as brain and heart and impairs their functions. If the exact diagnosis is not established and appropriate treatment is not implemented, severe and permanent neurological and psychiatric symptoms may develop[5,6].

Carbon monoxide poisoning leads to a combination of extensive tissue damage and CO-mediated cell injury[20]. Although there have been many investigations on high mortality, long-term neurological dysfunction and possible mechanisms of toxicity and treatment methods of CO poisoning, they remain unclear[21,22]. Nevertheless, recent studies support CO-mediated toxicity. Another hypothesis about the reasons of central nervous system injury due to CO poisoning is the reoxygenation mechanism after tissue hypoxia[20]. Thom et al[23] suggested that CO-mediated brain injury is compatible with post-ischemic reperfusion phenomenon and xanthine oxidase-derived reactive oxygen species (ROS) are responsible for lipid peroxidation. In the organs which have a high need for oxygen such as brain and heart, cell injury due to hypoxia and lipid peroxidation may occur. Moreover, CO also binds to cytochrome aa3 enzyme system and stops cellular respiration[22].

Oxidative stress plays an important role in progression of CO-induced tissue damage during ischemic and reperfusion phases of CO-induced damage[24]. CO may cause oxidative damage and may affect leukocytes, platelets and endothelium[22]. When the tissue exposed to ischemia, a series of chemical reactions leading to cellular dysfunction begins. No reaction causing tissue damage alone has been identified. The data show that reduction of cellular energy sources and accumulation of toxic metabolites, leads to cell death. In this case, restoration of blood flow is inevitable. Perfusion which enables regeneration and excretion of toxic metabolites, paradoxically leads to a series of reactions causing tissue injury[23]. Toxic oxygen metabolites (superoxide radicals) are generated during normal aerobic metabolism in many cells and these superoxide radicals increase a lot under some pathological conditions such as ischemia-reperfusion and exceed the capacity of endogenous antioxidant mechanisms and lead to tissue injury[24].

ROS function as a general mediator in the programmed cell death in response to many toxic substances and pathological conditions[25]. MDA (or TBARS) is an indicator of membrane lipid peroxidation caused by interaction of ROS with cellular membranes. Membrane lipid peroxidation caused by ROS may lead to distortion of cellular homeostasis by changing the membrane characteristics[25,26]. The body develops several defense mechanisms in order to limit ROS levels. These are known as “anti-oxidant defense systems,” or simply “anti-oxidants”. Antioxidants inhibit lipid peroxidation by preventing peroxidation chain reaction and/or accumulating ROS. According to one approach, antioxidants are classified as enzymes and non-enzymes[26]. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) can be given as the main examples of enzymatic antioxidants[25]. In this study, in parallel with the literature, we observed an increase in MDA levels, an indicator of membrane lipid peroxidation, in the brain tissue resulting from CO poisoning.

Magnesium is a well-known neuroprotective agent in experimental brain injury and spinal cord ischemia[27-29]. The neuroprotective effects of magnesium therapy have shown in experimental studies after focal and global cerebral ischemia, traumatic brain injury, acute spinal cord injury and subarachnoid bleeding[27-31]. One possible mechanisms of this neuroprotective effect may be the prevention of glutamate toxicity by blocking the N-methyl-D-aspartate receptors. Second, it may prevent thrombosis of the vessels in critical segments by inhibiting the activation of platelets. Third, it may reduce endothelial and neuronal reperfusion injury by avoiding the use of Mg glutathione and inhibiting lipid peroxidation. Fourth, Mg may regulate ATP concentration after ischemia and reperfusion and re-form ATP[32]. In this study, we observed that Mg reduces the oxidative damage caused by CO poisoning, especially in the first six-hour (group 3).

Conclusions

CO poisoning causes significant damage in the first 6 hours. As antioxidant enzymes, especially GSH-Px activity, reach the highest level at 24th hour, no significant oxidative damage was observed. Magnesium sulfate has a protective effect against oxidative damage occurring in the first 6th hour.

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