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EXPERIMENTAL STUDY

# The effect of antioxidants (N-acetylcysteine and melatonin) on hypoxia due to carbonmonoxide poisoning

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**Abstract:** We aimed to determine the effect NAC (N-acetylcysteine) and melatonin on the histopathological and biochemical paramethers in the rats poisoned with CO (Carbon monoxide) experimentally.
Winster albino female rats were placed in a plexiglass chamber and they were poisoned with CO. After the poisoning, rats were randomly divided into 3 groups. The group given only normal saline, was used as a control group (n=9). The second group was given 30 mg /kg intraperitonally NAC (n=10). And the third group was treated with 10 mg/kg of melatonin intramuscularly (n=9). It is determined that some biochemical values affected by NAC but not by melatonin.
CK, ALT, Lactate, MDA levels were significantly higher in NAC group than control and Melatonin group ($p<0.01$ for all comparisons). Thiol level was lower in NAC group than control group and Melatonin group ($p<0.01$ and $p<0.001$, respectively). There were no statistical significant differences between the melatonin and control group. There were statistically significant difference between control, NAC and Melatonin groups according to brain and lung tissue damage.
It is shown that both NAC and Melatonin are reducing the brain and lung tissue damage of CO poisoning but due to biochemical results worsened by NAC, Melatonin may recommend for CO poisoning *(Tab. 3, Ref. 21)*.
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Key words: CO poisoning, experimental study, NAC, melatonin.

Carbon monoxide (CO) poisoning is a frequently encountered problem in emergency departments, which can result in severe morbidity and death. The delivery of CO intracellularly and its binding to heme proteins other than hemoglobin may also account for its toxicity (1).

Ten to 15 % of the total body store of CO is extravascular (2). Some of this CO may be interfering with cellular respiration by binding to mithochondrial cytochrome oxidase (3, 4). Initial studies show that this binding is especially exaggerated in hypoxic and hypotensive NAC (N-Asetil sistein), an important antioxidant and cytoprotective agent is reported to replenish intracellular defense against oxidative stress (5). It acts as an antioxidant, both directly as a glutathione substitute and indirectly as a precursor for glutathione. It also causes vasodilatation by increasing cyclic guanosine monophosphate levels, inhibits platelet aggregation, acts as a sulphydryl donor to regenerate endothelial-derived relaxing factor and reduces IL-8 and TNF-alpha production. While there is evidence for its effectiveness as an antidote to paracetamol poisoning, its use in other disorders has only experimental or anecdotal support (5, 6).

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Melatonin reduces oxidative damage through its free radical eliminating and direct anti-oxidant effects (7). It has been shown to have an antioxidant effect and inhibitive effect on ADP ribose synthetase activation. A large number of experimental studies have documented that melatonin exerts important anti-inflammatory actions (3, 4, 8).

Treatment in CO poisoning in all other disorders it is still under evaluation. The common treatment modalities in use are supportive care and oxygen administration (9).

N-acetylcysteine and melatonin have antioxidant properties that may be useful in many clinical conditions. There is currently insufficient evidence to propose NAC for the treatment of carbon monoxide poisoning. Whilst there is experimental evidence for a variety of novel roles for NAC, further clinical studies are required before it can be recommended for the routine management of any disorders other than that of paracetamol poisoning (6, 7).

The aim of this study is to evaluate the NAC and melatonin treatment on histopathological, biochemical and behavioral parameters of experimentally CO poisoned rats.

## Methods

### *Animals*

This study was carried out with the prior approval of The Animal Experimental Ethics Committee of Erciyes University (Kayseri, Turkey)**.** Female Winster albino rats (weighing 190–

**Tab. 1. The classification of histopathological examination for lung and brain tissue damage.**

| | |
|---|---|
| Lung | |
| 0- | Only congestion |
| 1- | Mild damage; hemorrhage and edema. |
| 2- | Moderate damage; infiltration and aggregation of vessel wall. |
| 3- | Marked damage; the thickening of alveol wall and formation of hyaline membrane. |
| Brain | |
| 0- | No damage. |
| 1- | Mild damage; rare necrotic cells (‹10%) |
| 2- | Moderate damage; moderate frequency necrotic cells (10-50%) |
| 3- | Marked damage; marked frequency necrotic cells (›50%) |

200 g) were used in this study. Rats were kept under standardized conditions of light (14/10 h light/dark cycle) and temperature (23±2 °C), and fed standard rat chow (Aytekinler Yem Sanayi, Konya, Turkey). All procedures were performed in the Experimental Animals Breeding and Research Centre (Medical Faculty, Erciyes University).

*Experimantal groups*

Rats were randomly divided into 3 groups. The group given only physiologic saline solution (0.9% NaCl) was used as a control group (Control group, n=9). The second group was given 30 mg/kg intraperitonally NAC (Asistr) (NAC group, n=10). And the third group was melatonin group( Melatonin group, n=9).

Melatonin solution was applied intramusculer in this study group. The melatonin powder (Sigma; St.Louis, MO, USA) was thawed with 99 % ethanol and diluted with 2 cc physiologic saline in order to obtain 1 mg/cc melatonin. (10 mg/kg, approximately 2 mg/2 cc melatonin per rat).

The rats were placed in a plexiglass chamber, into which a small volume of pure CO was injected to achieve a concentration of 1000 ppm for 120 minutes. Following this procedure, additional CO with a concentration of 3000 ppm was administered for varying periods from 40 minutes. After the rats became unconscious and bradypneic, they were removed from the chamber. Immediately after their removal from the chamber, physiologic saline solution, melatonin or NAC were given to the rats.

Blood samples were obtained with aortic punction under anesthesia (Ketamine (20 mg/kg Ketalar$^{R}$, Eczacibasi, Istanbul, Turkey) and xylazine (5 mg/kg Rompum$^{R}$, Bayer, Leverkusen, Germany) was given intraperitoneally) and biochemical parameters were determined in plasma. Samples were stored for 2 month at -20 °C until measured.

Creatinine kinase (CK), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), pH, Lactate, COHb and Bicarbonate ($HC0_3$), Malonyldialdehyde (MDA) and Thiol levels were measured. The rats were sacrificed 6 hours later and brain, lung and heart were dissected out. Histopathological examination of lungs, brain and heart tissue were done by a pathologist.

*Pathological Examination*

The whole part of the lungs, brain and heart were resected and fixed in 10 % formalin. Tissues were processed routinely and embedded in parafin and preperad 5–8 μm thick sections. Sections were stained with hematoxylin-eosin. The hematoxylin-eosin stained sections were examined under light microscope. Examination was done by the same person. The damage score of the lung and brain was done according to the Table 1. Tissue damage classified into two groups for statistical analyses; No damage and damaged (mild + moderate + marked).

*MDA measurement*

MDA measurement was assessed using the Ohkawa method (10) and 1, 1, 3, 3 tetrametoksipropan was used as a standard.

The basic principle of this method is the measurement of the absorbency of the compound formed as a result of the reaction made by MDA with Thiobarbutyric acid at 532 nm. For this, 0.1 ml plasma sample was mixed with 0.2 ml sodium dodecyl sulphate 1.5 acetic acid and 1.5 TBA solution. This mixture was diluted to 4 ml with distilled water and 5 ml n-butanol/ /pyridine mixture was added to it. Following this, it was centrifuged at 40000 rpm for 10 minutes. The absorbency of the supernatant was read at 532 nm with a Shimadzu- UV 160A spectrophotometer.

Plasma Total Thiol was analysed by the method described by Sedlak et al (11).

*Statistical analyses*

Kruskal-Wallis test was used for comparing three study groups. Since analysis of variance was significant, comparisons were applied using the Mann-Whitney U test between two groups. Bonferroni's correction was applied ($p<0.05/n$; where n=number of comparisons) when multiple comparison were made. The incidence of tissue damage between the groups was analyzed by using the Chi square test. Results were presented as mean±SD, median (min-max) and n (%). A p value less than 0.01 considered as significant. Statistical analyses were performed using the statistical package SPSS v 12.0.

**Tab. 2. Biochemical features of the groups.**

| | Control (n=9) Mean±SD Med (min-max) | NAC (n=10) Mean±SD Med (min-max) | Melatonin (n=9) Mean±SD Med (min-max) | p value (Kruskall Vallis) |
|---|---|---|---|---|
| CK | 209,4±265,6 | 407,2±381,4 | 68,7±65,7 | |
| | 107(25-874) | 272(25-1209)** | 45(19-209)‡‡ | 0,01 |
| AST | 101,0±40,2 | 190,8±110,4 | 100,4±75,5 | |
| | 103(62-189) | 134,5(65-413)* | 84(39-291)‡ | 0,02 |
| ALT | 38,5±17,7 | 49,1±22,1 | 30,3±13,1 | |
| | 33(20-71) | 37,5(26-77) | 27(16-55) | 0,1 |
| PH | 0,2±0,1 | 0,3±0,1 | 0,2±0,1 | |
| | 0,3(1-0,4) | 0,3(0,1-0,4) | 0,3(0,1-0,3) | 0,4 |
| Lactate | 1,8±1,9 | 3,7±1,4 | 1,9±1,3 | |
| | 1,7(0,0-3,36) | 4,0(1,7-5,6)* | 2,0(0,0-3,6)‡ | 0,01 |
| COHb | 7,2±0,08 | 7,3±0,04 | 7,1±0,2 | |
| | 7,2(7,1-7,3) | 7,3(7,2-7,3) | 7,2(6,6-7,3) | 0,3 |
| $HCO_3$ | 17,8±3,8 | 19,5±2,9 | 16,8±8,3 | |
| | 18(11,5-23) | 20,6(14,7-22,5) | 21(2,6-24,2) | 0,7 |
| MDA | 3,4±1,2 | 6,9±2,7 | 3,6±1,35 | |
| | 3,4(1,8-5,64) | 6,2(3,8-12,8)** | 3,7(1,8-5,5)‡‡ | 0,004 |
| Thiol | 267,3±86,4 | 204,2±83,2 | 305,9±99,6 | |
| | 265,6 (136,7-390,6) | 169,9(120-390,6)** | 292,9(160-515,6)‡ | 0,04 |

*p<0.01, **p<0.001 between Control and NAC
‡ p<0.01, ‡‡ p<0.001 between NAC and Melatonin

## Results

Biochemical features of groups were shown in Table 2. CK, ALT, Lactate, MDA levels were significantly higher in NAC group than control and Melatonin group (p<0.001, p<0.01, p<0.01 and p<0.001, respectively for both comparisons). Thiol level was lower in NAC group than control group and Melatonin group (p<0.01 and p<0.001, respectively). There were no statistical significant differences between the melatonin and control group.

Microscopic examination of brain and lung tissue samples obtained from the all groups showed marked histopathological changes. All 9 rats (100.0 %) had brain damage in control group. While 3 rats had brain damage in NAC group, none of them had brain damage in Melatonin group. Both were significantly different than control group (p=0.003 and p=0.0001, respectively). While lung tissue damage was 77.0 % in control group, it was 40 % in NAC group and 22.2 % in Melatonin group (p=0.1 and p=0.05, respectively compared to control group). There were statistically significant difference between control, NAC and Melatonin groups according to tissue damage (Tab. 3)

## Discussion

We found some protective effects of NAC and melatonin in brain tissue, while the biochemical variables results were worsened by NAC and hardly influenced by melatonin.

There were no significant differences between the melatonin and control group according to biochemical features. But, as CK, ALT, AST, Lactate levels were significantly higher than control and Melatonin group in NAC group, this may suggest NAC is insufficient to reverse hypoxic injury in CO toxicity. These parameters were lower in melatonin group compared to control group and this may suggest melatonin had been more effective than NAC in CO toxicity. In NAC group, decrease in thiol levels has been detected.

CO toxicity appears to result from a combination of tissue hypoxia and direct damage at the cellular level (12). The most common manifestations of CO poisoning are neurological, cardiovascular, gastrointestinal, and pulmonary (13, 14).

In several studies, agents with antioxidant properties have been shown to be protective in hypoxic (15, 16). As NAC and melatonin have antioxidant effects, the potential beneficial

**Tab. 3. Frequency of tissue damage according to the groups.**

| Tissue Damage | Control n (%) | NAC n (%) | Melatonin n (%) |
|---|---|---|---|
| Heard | | | |
| No damage | 9 (100) | 9 (90.0) | 9 (100) |
| Damaged | 0 (0.0) | 1 (10.0) | 0 (0.0) |
| Brain | | | |
| No damage | 0 (0.0) | 7 (70.0) | 9 (100) |
| Damaged | 9 (100) | 3 (30.0)** | 0 (0.0)*** |
| Lung | | | |
| No damage | 2 (22.2) | 6 (60.0) | 7 (77.8) |
| Damaged | 7 (77.8) | 4 (40.0) | 2 (22.2)* |

*p<0.05, ** p<0.01 ***p<0.001 between Control and NAC; and between Control and Melatonin

affects of these agents might be alternative treatments in CO toxicity.

The therapeutic efficacy of melatonin or N-acetylcysteine (NAC) was studied in the present study. Possible mechanism for CO mediated cardiac dysfunction is oxidative stress, which could contribute to free radical overload in the heart, similar to ischemia reperfusion injury. Such a mechanism has been described in CO-mediated neurotoxicity.

In a Feline model, central nervous system damage comparable to that associated by one interval of ischemia, confirming the ischemic-reperfusion model for central nervous system insult after CO poisoning (17).

Although we have shown histopathological damage in all animals in control group, no rat in melatonin group had this damage. This suggests as melatonin may be an alternative treatment in brain damage in CO toxicity. Histopathological findings in NAC group were lower than melatonin group. NAC also has beneficial effects compared to control group. These findings suggest antioxidant properties of these two agents might have a role. Further studies are needed to show the exact mechanisms.

Brando et al found that Thiobarbituric acid-reactive substances (TBARS), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities and ascorbic acid levels were not modified after mercury exposure. Mercuric chloride poisoning caused an increase in hepatic and renal melatonin levels and antioxidant treatments did not modify this parameter (18).

Antioxidants have long been known to reduce the free radical-mediated oxidative stress while, thiol chelators have been used to treat carbon monoxide toxicity.

In the present study, there was no significant difference between control and NAC group or control and melatonin group in terms of heart tissue injury. It can be concluded that, combined therapy with an antioxidant moiety and a thiol-chelating agent may be a better choice for tissue damage**.** Nehru B et al reported that thiol-antioxidant supplementation following Pb exposure might enhance the reductive status of brain regions by arresting the lipid peroxidative damage in brain regions (19). And another study suggest that the involvement of reactive oxygen species in lead toxicity and a pronounced beneficial role of NAC in therapeutic implications of lead poisoning when co-administered with a thiol chelator (DMSA) supporting the hypothesis that cellular redox status may be significantly reversed by utilizing a thiol containing antioxidant compound (16).

CO poisoning may cause damage, both to the lungs and to other organ systems. It causes cellular damage via formation of reactive oxygen species. The lung is protected from oxidative stress by the glutathione (GSH) antioxidant system which can be augmented by the thiol drug, N-acetylcysteine (NAC).

There are studies showing lung protective effect of melatonin and NAC in lung injury. Akca T et al reported that NAC has protective effect on pulmonary lipid peroxidation and tissue damage before and after lipopolysaccharide administration (20) and al investigated the protective effect of NAC on peroxidative changes in rat lungs exposed to inhalation of thinners for 8 weeks and they found that thinners are agents that cause imbalance between oxidants and antioxidants produced by aerobic cellular systems (21). This imbalance between oxidant and antioxidant systems is decreased by the effect of NAC.

In our study, lung injury was decreased both in NAC and melatonin group, It was markedly better in melatonin group.

As a result;

However, it is shown that both NAC and Melatonin are reducing the brain and lung tissue damage of CO poisoning, it is determined that some biochemical values worse affected by NAC but not by melatonin. Melatonin may recommend for CO poisoning.

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