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ARTICLE**

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Evaluation of Argyrophilic Nucleolar Organizing Region–Associated Protein Synthesis in Femoral Muscle Cells of Rats Exposed to 3000 ppm Carbon Monoxide Gas

ABSTRACT

Objective: Carbon monoxide (CO) is a colorless, tasteless, odorless, and nonirritant gas and it causes tissues hypoxia due to decreasing oxygen carrying capacity. Nucleolar-organizing regions (NORs) are genetic loci on chromosomes and they can be stained with silver when they are active. In this study, we aimed to investigate any possible effects 3000 ppm CO exposure on the NOR protein synthesis in femoral muscle cells of rats.

Method: The animals were divided into 2 groups as control (C) and 3000 ppm CO exposed group. One week after exposure to CO, the animals were anesthetized and femoral muscle tissues were obtained. Then mean argyrophilic NOR (AgNOR) number and total AgNOR area/nuclear area (TAA/NA) were detected in femoral muscle cells for each rat.

Results: There were significant differences between control group and 3000 ppm CO exposed group for mean AgNOR number ($Z=-6.407$ and $p=0.000$) and TAA/NA ratio ($Z=-7.718$ and $p=0.000$).

Conclusion: It was detected that there were a possible effects of CO exposure on the AgNOR proteins amounts in femoral muscle cells of rats.

Keywords: AgNORs, NOR, Carbon monoxide, muscle cells

Farelerin Femoral Kas Hücrelerinde 3000 ppm CO maruziyetinin Nükleolar Organize Edici Bölgeler (NOR) Protein Sentezi Üzerindeki Olası Etkilerinin İncelenmesi

ÖZ

Amaç: Karbon monoksit (CO), renksiz, tatsız, kokusuz ve irrtan olmayan bir gazdır ve oksijen taşıma kapasitesini düşürerek doku hipoksisine neden olur. Nükleolar organize edici bölgeler (NOR) kromozomlar üzerindeki bazı gen bölgeleridir ve aktif oldukları zaman gümüş ile boyanabilirler. Bu çalışmada, 3000 ppm'lik CO maruziyetinin farelerin femur kas hücrelerindeki NOR protein sentezi üzerindeki olası etkilerini incelemeyi amaçladık.

Metod: Hayvanlar, kontrol (C) ve 3000 ppm'e maruz kalmış grup şeklinde 2 gruba ayrıldı. CO maruziyetinden 1 hafta sonra hayvanlara anestezi verilip femoral kas dokusu alındı. Sonrasında, her farenin femoral kas dokusunda ortalama AgNOR sayısı ve toplam AgNOR alanı/çekirdek alanı (TAA/NA) tespit edildi.

Bulgular: Kontrol grubu ve 3000 ppm CO'e maruz kalan grup arasında ortalama AgNOR sayısı ($Z=-6.407$; $p=0.000$) ve TAA/NA oranı ($Z=-7.718$; $p=0.000$) açısından önemli farklar mevcuttu.

Sonuç: Farelerin femoral kas hücrelerinde CO maruziyetinin olası etkileri olduğu tespit edilmiştir.

Anahtar Kelimeler: AgNORs, NOR, Karbon Monoksit, Kas Hücreleri

INTRODUCTION

Carbon monoxide (CO) is a colorless, tasteless, odorless, and nonirritant gas, so it is also known as a 'silent killer', which leads to multiple human deaths each year, because of a home accident or disaster (1). CO is produced by an end product of incomplete combustion of hydrocarbons such as wood, charcoal, kerosene, or natural gas used for heating and cooking (2). CO causes tissues hypoxia due to decreasing oxygen carrying capacity and oxygen delivery of hemoglobin at tissue level after passing through the lungs without leading to any damage in lung tissue. Additionally Acute or chronic CO poisoning may cause death (3,4). Femoral muscle is rich for mitochondria. The mitochondrial biogenesis can be stimulated by CO (5) and this biogenesis is also obviously necessary for muscle conditioning (6,7).

The nucleolus is important structures within cell nuclei. The synthesis, process of ribosomal RNAs and its' assembling with ribosomal proteins are occurred in the nucleolus. The organization and size of nucleolus are directly associated with ribosome production and reflects the functional compartmentalization of the nucleolar machinery. Nucleolar-organizing regions (NORs) are genetic loci on chromosomes and composed of ribosomal DNA (rDNA) and proteins, some of that are argyrophilic. They are transcribed into ribosomal RNA, that is processed into preribosomes in the nucleolus and becoming part of mature ribosomes in the cytoplasm (8).

The active NORs are stained with silver by using Silver nitrate (AgNO_3) under suitable conditions in interphase via the precipitation of metallic silver granules and the quantity of these silver granules is closely associated with NORs activity (9,10). Silver binds with those transcriptionally active or transcribed and still retain residual rRNA nonhistone-associated proteins and silver staining methods is the most reliable to show nucleoli in interphase nuclei. Due to the silver affinity, those proteins are named as argyrophilic NOR (AgNOR)-associated proteins (11).

More studies have been performed about the importance of AgNOR proteins in evaluation and discrimination of thyroid lesions (12-16), in hair root cells of healthy individuals and person with alopecia (17, 18), in buccal epithelial cells of Down syndrome infants and healthy individuals (19, 20) and for the detection possible effects of CO exposure on the AgNOR proteins synthesis of lung and hearth cells (21-23).

In addition to this, to the best of our knowledge, there is not a research about the AgNOR proteins amount in the cells of CO exposed femoral muscle. Therefore, we decided to carry out the current study to detect the possible effects of

CO on the AgNOR protein synthesis in femoral muscle cells of the rats.

2. Materials and methods

2.1. Study design

Male albino Wistar rats weighing between 200 g and 230 g selected from the same breed were included in the study. The rats were cared for in accordance with the guide for the care and use of laboratory animals. They were divided into 2 groups as control (C) and 3000 ppm CO exposed group.

CO exposed group: A steel tubes containing 10 L of CO-air Mixture with a 3000 ppm concentrations were used for CO exposing. Rats were exposed to CO at a flow rate of 4 L/min for 30 min in an enclosed transparent jar with dimensions of 20 X40 X60 cm^3 . One week after exposure to CO, the animals were anesthetized by administering ketamine hydrochloride + xylazine hydrochloride, intraperitoneally. For the surgical procedure, the animals were placed in the supine position, and the anterior thoracic wall was shaved and disinfected using 10% povidone-iodine solution. Thoracotomy was performed through a midline incision. Perfusion fixation was achieved by intracardiac administration of 10% formaldehyde solution and femoral muscle of rats was taken. Samples were placed in 10% formaldehyde solution. Ethical approval was taken from the animal ethics committee according to accepted principles for laboratory animal use and care.

AgNOR detection

The femoral muscle tissue of the animals was dissected (approximately 1 x 1 x 1 cm^3 in size). After routine histological follow up, the femoral muscle tissue was cut to 5 mm thick sections for preparation and deparaffinized in xylene and then rehydrated in graded alcohol solutions. The slides were air-dried for 15 min at room temperature and fixed in fixative solution for 5 min. AgNOR staining method was performed according to the Benn and Perle protocol and the Lindner protocol, with a slight modification for all slides of groups (24,25). Further staining of nuclei after silver staining was not carried out, and the slides were rinsed with bidistilled water. The AgNOR staining cells of femoral muscle were transferred to the computer by means of a light microscope (Eclipse 80i, Nikon) and photographed via a digital camera (Digital Sight DS-fi1, Nikon). The captured images of femoral muscle cells were transferred to image processing software (Image J version 1.47t, National Institutes of Health, Bethesda, Maryland, USA). Fifty nuclei per slides were evaluated to detection of the total AgNOR area per nuclear area (TAA/NA) and mean AgNOR number via the "freehand selections" tool for each nucleus.

Pictures of the AgNOR staining cells of femoral muscle were seen in Figure 1.

Statistical analysis: Statistical analysis was performed using Statistical Package for Social Sciences (SPSS, Inc., Chicago, Illinois, USA) for Windows 15.0. The descriptive statistical methods (mean and standard deviation (SD)) and Mann–Whitney U tests were used for comparison of two groups. Results were given as mean ± SD, and p < 0.05 was accepted as statistically significant.

RESULTS

The differences between control group (1.38±0.635) and 3000 ppm CO exposed group (2.86±1.212) were significant for mean AgNOR number (Z=-6.407 and p=0.000). Additionally the differences between control group (0.017±0.01) and 3000 ppm CO exposed group (0.106±0.079) were significant for TAA/NA ratio (Z=-7.718 and p=0.000) (Table 1, Figure 2 and 3).

Table 1. Mean AgNOR number and TAA/NA of groups

Groups	Mean AgNOR Number (n=50)	Z value for AgNOR Number Comparison of Groups	P Value for AgNOR Number Comparison of Groups	Mean TAA/NA (n=50)	Z value for TAA/NA Comparison of Groups	P Value TAA/NA Comparison of Groups
C	1.38±0.635	-6.407*	0.000 *	0.017±0.01	-7.718*	0.000*
CO exposed (3000ppm)	2.86±1.212			0.106±0.079		

C: Control, CO: Carbon monoxide, TAA/NA: Total AgNOR Area / Nuclear Area, n: The numbers of evaluated muscle cells, &: For AgNOR number*: For TAA/NA AgNOR: argyrophilic NOR

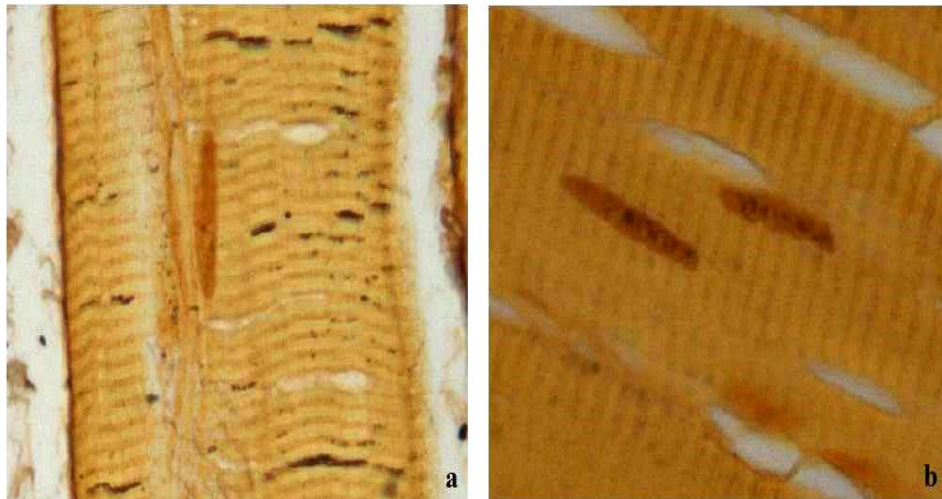


Figure 1. A demonstrative example of the (argyrophilic NOR (AgNOR) staining muscle cells (a: control; b: 3000ppm Carbon monoxide (CO) exposed group)

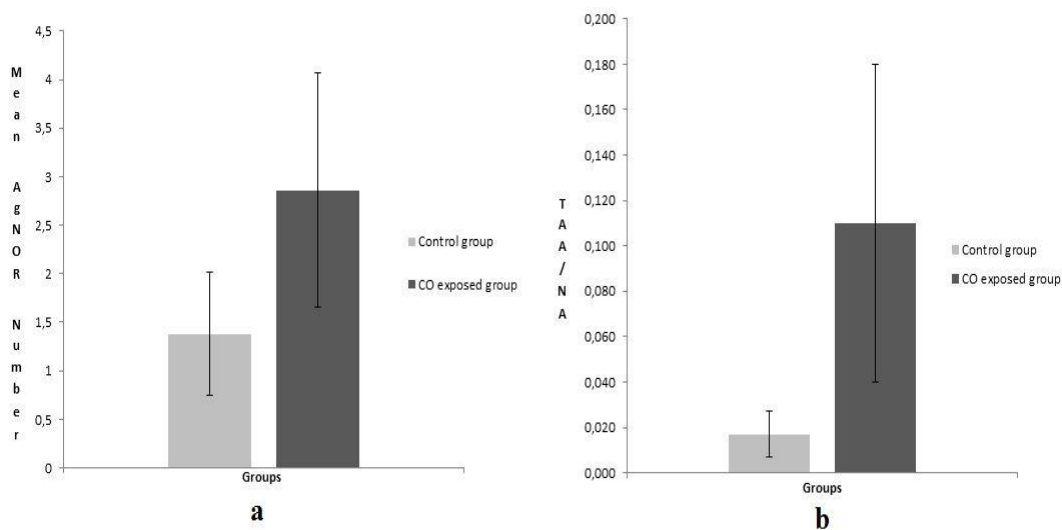


Figure 2. Comparison of control group and 3000ppm Carbon monoxide (CO) exposed group for mean argyrophilic NOR (AgNOR) number and TAA/NA ratio

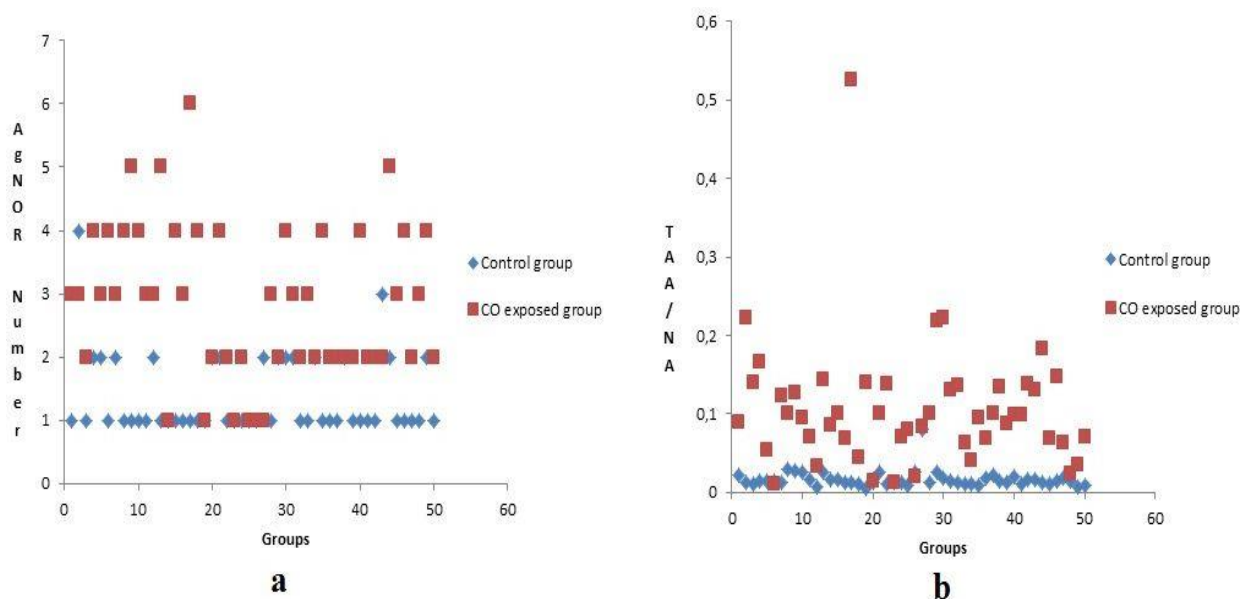


Figure 3. The distribution of mean argyrophilic NOR (AgNOR) number and TAA/NA ratio in control group and 3000 ppm Carbon monoxide (CO) exposed group

DISCUSSION

It was reported that CO stimulates mitochondrial biogenesis that activated by when CO binds to cytochrome c oxidase and increase mitochondrial hydrogen peroxide production. So, this disrupts oxidative phosphorylation, reduces cellular respiration and causes cellular hypoxia (5). Additionally, this biogenesis is also exactly necessary for muscle condition (6,7). CO binds myoglobin, also with a high affinity, up to 20-50 times. It leads to myocardial depression and hypotension by causing tissue hypoxia. CO has affinity to binds muscle myoglobin. Thus it reduces O_2 pressure and leads to rhabdomyolysis (26).

Mitosis, ribosome assembly, the generation of ribonucleoprotein complexes and stress response are important cellular processes and the maintenance and regulation of these important processes are associated with the nucleolus (27). NORs are surrounded by a great number of regulatory proteins in interphase and they are functional subunits of the nucleolus (10). We carried out different studies about the importance of AgNOR proteins in different disease and condition in different cell types (12-23).

In some of them we reported that the AgNOR protein amount increased depending on the increase of chronic and acute CO exposure (also that cause hypoxic condition) in the heart and lung cells in our previous studies (21-23). In this study, we also detected that the mean AgNOR number per total nuclear number and TAA/NA ratio were higher in the 3000 ppm CO exposed

cells than normal femoral cells of rats. To do best of our knowledge, this is the first studies about the possible effects of CO exposure on AgNOR proteins amounts in femoral muscle cells of rats.

All living cells tend to protect their structural and functional situation (metabolic process etc.) toward dangerous agents such CO. Our study showed that the expression capacity of rRNA gene that detectable with total TAA/NA and/or AgNOR number per total nuclear number, increased in CO exposed femoral cells. It may be said that the AgNOR proteins may have a protective role or may be trigger the synthesis of some other proteins that have protective roles in the signaling transduction pathways and gene expression regulation of hypoxic condition occurred by CO. Whether the duration (acute or chronic) and concentration of CO are changed, how changes may be occurred in the AgNOR proteins amounts. To obtain more certain knowledge about this topic, additional studies should be performed.

As a conclusion; it was detected that there were a possible effects of CO exposure on the AgNOR proteins amounts in femoral muscle cells of rats. Additional studies including different duration time and concentration of CO exposure in large sample series should be carried out to obtain more exact knowledge about the current topic.

Declaration of interest: There is no conflict of interest.

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