

COMPARATIVE STUDY BETWEEN HEART-TYPE FATTY ACID-BINDING PROTEIN AND ASYMMETRIC DIMETHYLARGININE AS A RISK MARKER OF CARDIOVASCULAR DISEASES IN RATS

Marwan A. Ibrahim

(Assistant Professor of Applied Physiology)
Majmaah University, Faculty of science, KSA

Abstract

Cardiovascular diseases (CVDs) are the leading cause of death worldwide. A number of comorbidities are associated with CVDs and prognosis is poor, with many patients experiencing disease progression. Recognizing the factors associated with CVDs progression enables high risk patients to be identified and given more intensive treatment if necessary. The identification of new predictive markers might improve the understanding of the pathogenesis and progression of CVDs. This study discusses a comparison between heart-type fatty acid-binding protein (H-FABP) and asymmetric dimethylarginine (ADMA) as a risk marker by induction cardiovascular problems in male adult albino rats by exposing to acute Carbon monoxide (CO) poisoning. CO intoxication causes cardiovascular problems as a result of diffuse tissue hypoxia. The rats were exposed to a mixture of either 3000 (group A) or 5000 (group B) parts per million (ppm) CO in air, or to ambient air (group C, control group). Blood samples were taken just before, immediately after and 6 hours after the exposure, and serum H-FABP and ADMA levels were measured. Serum H-FABP levels increased just after the CO exposure in both groups A and B. Additionally, H-FABP level was higher in group B than in group A, immediately after the exposure. However, plasma ADMA levels only increased at 6 hours after the CO exposure in groups A and B. The study results suggest that H-FABP might have potential to be an early and quantitative parameter of clinical severity and prognosis in CVDs.

Keywords: H-FABP-ADMA-risk marker-cardiovascular diseases-male rats

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide. People of all ages and backgrounds can get the condition. About 600,000 people die of heart disease in the United States every year—that's 1 in every 4 deaths (Kochanek et al., 2011). Every year about 715,000 Americans have a heart attack. Of these, 525,000 are a first heart attack and 190,000 happen in people who have already had a heart attack (Finegold et al., 2012 and Go et al., 2013). Knowing the warning signs and symptoms of a CVDs is key to preventing death, but many people don't know the signs, so there is an urgent need to develop a new risk marker for CVDs.

Heart-type fatty acid-binding protein (H-FABP) is a low-molecular-weight protein found in the cytoplasm of cardiac myocytes at high concentrations in comparison with other human cells (Valle et al., 2008). As myocardial cell membrane is damaged by ischemia, H-FABP leaks to the extracellular space and enters the blood circulation very easily and quickly due to its small size and water solubility. Owing to its early elevation, it is suggested that H-FABP maybe a useful tool in the early assessment of acute coronary syndrome (Okamoto et al., 2000 and Nakata et al., 2003).

Asymmetric dimethylarginine (ADMA) is a naturally occurring chemical found in blood plasma. It is a metabolic by-product of continual protein modification processes in the cytoplasm of all human cells. It is closely related to L-arginine, a conditionally essential amino acid. ADMA interferes with L-arginine in the production of nitric oxide, a key chemical to endothelial and hence cardiovascular health so it is conceder as an important marker of cardiovascular disease (Böger et al., 2000; Krzyzanowska et al., 2006; Meinitzer et al., 2007 and Edrees, 2010).

The clinical profile is the most important factor in making decisions about the treatment of CVDs patients (Fuster et al., 2010 and Mendis et al., 2011). Currently, myocardial injury is determined by elevation of cardiac enzymes and ischemic electrocardiography (ECG) changes in the clinical practice. However, due to their comparatively late elevation, the cardiac enzymes may cause the delay in the assignment of strategy for the treatment. Therefore, an early biochemical marker that would help to evaluate the severity of CVDs and to decide the management strategy is needed. The aim of this study was to compare between the serum H-FABP level and plasma ADMA in the early period of CVDs as a risk marker of cardiovascular diseases in rats.

Material and Methods

I-Material

This study was carried out on male albino rats *Rattus rattus* as an animal model for induction of CVDs. 30 young adult male albino rats were

employed in the current study. They were obtained from the Serum and Antigen Laboratories at Helwan with an average weight of 120 ± 10 g representing 10 ± 1 weeks of age. Animals were allowed ten days pre-experiment period to adapt to laboratory conditions in order to avoid any complications along the course of the experiment. They were housed in metallic cages at $28 \pm 20^\circ\text{C}$ and 50% relative humidity and received food and water ad-libitum with fresh supplies presented daily.

II-Methods

Rats were anesthetized (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine; intraperitoneally [i.p.]) and right jugular vein was catheterized for blood sampling. The venous catheter was tunneled subcutaneously and externalized at the nape of the neck for access during blood sampling. After surgery, rats were housed individually, with free access to food and water during the 48-hours recovery period before commencing the experiments.

The rats were exposed to a mixture of either 3000 (group A, n = 12) or 5000 (group B, n = 12) ppm CO in air or to ambient air (group C, control group, n = 6) at a rate of 4 L/min for 30 min in 16-L Plexiglas chamber. 14 Blood samples were taken just before, immediately after and 6 hours after the exposure to either CO or ambient air. Blood samples were separated into two batches of blood samples (with or without heparin as anticoagulant) were collected from each rat on each time interval. Serum and plasma were separated and divided into considerable aliquots to avoid the effects of repeated thawing and freezing. All specimens were stored at -20°C until use. The level of consciousness was evaluated by testing the response of rat to painful stimulus (pricking the rat's sole) at the end of the CO exposure, 14 and the rate was monitored for 7 days.

Biochemical methods:

The serum H-FABP levels were measured by enzyme-linked immunosorbent assay with commercially available kits (Life diagnostic, Inc., West Chester, Pennsylvania, USA); while Plasma ADMA was determined using commercial kits purchased from American Laboratory Products Company (Alpco Diagnostics), USA and according to the method of Schulze et al., 2004. In addition, blood samples taken just after the CO poisoning were also analyzed for carboxyhemoglobin (COHb) levels. Blood COHb was measured by using Roche OMNI S Blood Gas Analyzer (Roche Diagnostics Inc, Indianapolis, Indiana, USA).

Statistical analysis:

All data are expressed as means + S.E. Following the assurance of normal distribution of biochemical data (i.e. serum levels of H-FABP and plasma levels of ADMA), repeated measures analysis of variance (ANOVA) was used to evaluate the effect of time for each group. The comparisons between groups within a time point were evaluated with Student’s t test. The results of the consciousness and survival rates were analyzed using two-tailed Fisher’s exact tests. Differences were considered statistically significant if $p < 0.05$. All Statistical analyses were performed using the SPSS 15.0 software.

Results

Blood COHb levels just after the CO poisoning were higher in group A ($41\% \pm 2.5\%$, $p < 0.01$) and group B ($59 \pm 5.1\%$, $p < 0.01$), compared to group C ($0.4\% \pm 0.2\%$). Additionally, COHb level was higher in group B than in group A ($p < 0.01$).

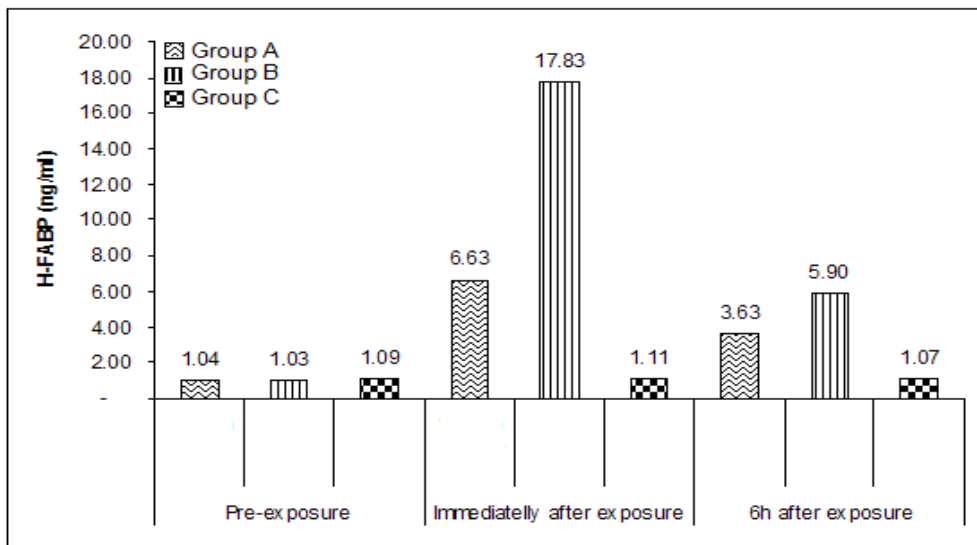


Figure 1. Serum H-FABP levels before, immediately after and 6 hours after the CO exposure.

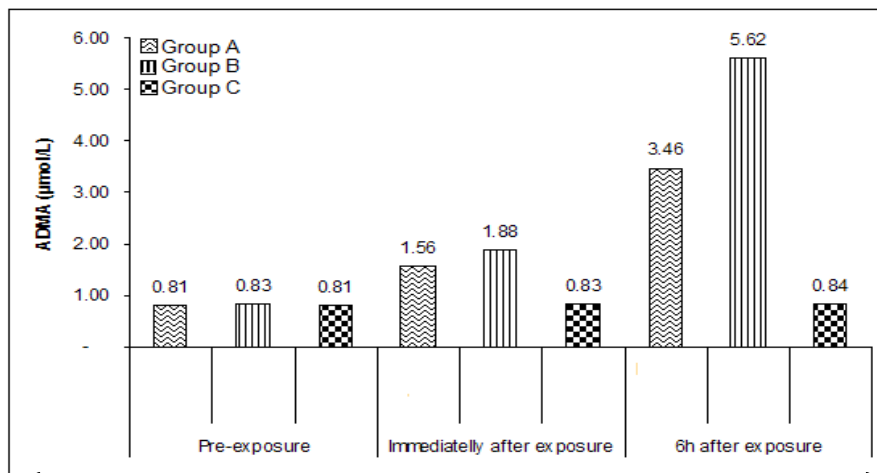


Figure 2. Plasma ADMA levels before, immediately after and 6 h after the CO exposure.

Figure 1 shows serum H-FABP levels in group A (3000 ppm CO exposure), group B (5000 ppm CO exposure) and group C (ambient air exposure) at different time points. In group C, serum H-FABP levels did not change along the study period (6 hours). Serum H-FABP levels acutely increased just after the CO exposure in both group A ($p < 0.001$) and B ($p < 0.001$), compared to pre-exposure. At 6 hours after the exposure, although there was a marked reduction, H-FABP levels were also higher in groups A ($p < 0.05$) and B ($p < 0.05$), compared to pre-exposure.

Figure 2 shows plasma ADMA levels in three groups at different time points. Plasma ADMA levels had slight changed just after CO exposure in either group A or group B, compared to pre-exposure but not significant statistically. At 6 h after the exposure, ADMA levels were high in group A ($p < 0.01$) and B ($p < 0.001$), compared to pre-exposure.

Discussion

Egyptians are most vulnerable to heart diseases at an early age. The possible causes of this increase are the progressive ageing of the population, urbanization, dietary changes, sedentary lifestyles, smoking and stress (Alexandria conference, 2013). This study discusses a comparison between H-FABP and ADMA as a risk marker by induction cardiovascular problems in male adult albino rats by exposing to acute carbon monoxide (CO) poisoning. CO intoxication causes cardiovascular problems as a result of diffuse tissue hypoxia. So, the rats were exposed to two different doses of CO (3000 and 5000 ppm) in air. In both groups, H-FABP levels significantly increased immediately after CO exposure and, although it was relatively decreased, remained high at 6 hours after the exposure. On the other hand,

ADMA levels only increased at 6 hours after poisoning in both groups. These results show that serum level of H-FABP increases in early phase of acute CO poisoning prior to ADMA in rats. When it was assessed with respect to different CO doses, H-FABP level was higher in the high-CO group than that in low-CO group immediately after the exposure. It appears that the increased H-FABP concentrations are related to severity of CO poisoning which indicate to CVDs. These results suggest that serum H-FABP level is related with the clinical profile and prognosis in CVDs in rats.

The pathological changes associated with CO poisoning are mainly related to CO-induced hypoxia. CO binds rapidly to hemoglobin, leading to the formation of COHb, and the oxygen carrying capacity of the blood decreases, causing tissue hypoxia (Prockop and Chichkova, 2007). Elevated COHb measurements are used to confirm a clinical diagnosis of exposure to CO and, in some instances, assess the severity of poisoning (Ernst and Zibrak, 1998). In current study, blood COHb levels increased after CO exposure in both groups, and COHb level was higher in high-CO group than in low-CO group.

It is suggested that patients vulnerable to CVDs should be screened for myocardial injury (Henry et al., 2006). Tissue hypoxia in CO poisoning is often responsible for cardiac damage that is not always registered in ECG recordings. Therefore, it maybe necessary to look for other indicators such as biochemical markers (Hampson and Hauff 2008). The molecule of H-FABP is smaller than that of myoglobin, ADMA and creatine kinase isoenzyme MB (CK-MB), permitting it leak from damaged cardiomyocyte earlier. At 15 min after acute myocardial infarction in rats, plasma concentration of H-FABP was found to be four times higher than that of the baseline level (Aslan et al., 2006). Moreover, a significant correlation was found between the amount of released H-FABP and the infarct size in humans and mice, which maybe helpful in anticipation of infarction prognosis clinically (Glatz et al., 1994 and Meng et al., 2006). These results showed that serum level of H-FABP increases in early phase of the acute CO poisoning prior to ADMA in rats (Lajer et al., 2008). Additionally, H-FABP, but not ADMA, level was higher in the high-CO group than in the low-CO group immediately after the exposure. These results suggest that H-FABP is an acute reactant and a more sensitive marker than ADMA in CVDs in rats.

Conclusion

In conclusion, our results suggest that increased serum H-FABP levels are associated with CVDs in rats. This result also indicates that H-FABP might have a potential to be an early and quantitative parameter of clinical severity and the prognosis in CVDs. This study now needs to be supported by further experimental and clinical studies.

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