Original Article

Risk Factors of Acute Myocardial Infarction in Rats Under Combined Exposure to Ethanol and Methylmercury

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ABSTRACT

The consumption of moderate doses of ethanol (EtOH) can be useful in the prevention of cardiovascular events. In contrast, methylmercury (MeHg) has been associated with myocardial infarction. However, the combined exposure to these neurotoxicants needs to be taken into consideration. Our objective, therefore, was to evaluate the biochemical alterations in animals that received EtOH and/or MeHg during their intrauterine life as risk factors for acute myocardial infarction. Pregnant rats received tap water or EtOH during pregnancy and breast-feeding. On the 15th day of pregnancy, half of the treatment groups received MeHg. The offspring groups were: Control, EtOH, MeHg, and EtOH+MeHg and were evaluated during an exhaustive swimming test. The results suggest the adult rat offspring that received only MeHg during their intra-uterine life were more susceptible to acute myocardial infarction after exhaustive activity than the groups that received EtOH or EtOH+MeHg. The MeHg group displayed an increase in almost all biochemical parameters, especially levels of the enzymes CPK and CKMB. Our data complement research related to the risk of acute infarction of the myocardium and the effect of EtOH and/or MeHg on that risk. However, the exact cytoprotective mechanism of EtOH against the effect of MeHg needs further investigation.

KEYWORDS: biochemistry; exhaustive swimming test; fetal alcohol syndrome; methylmercury; myocardial infarction.

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RESUMO

O consumo de doses moderadas de etanol (EtOH) pode ser útil na prevenção de eventos cardiovasculares. Ao contrário, o metilmercúrio (MeHg) tem sido associado ao infarto do miocárdio. A combinação desses dois neurotóxicos, entretanto, necessita ser levada em consideração. Nosso objetivo, portanto, foi avaliar as alterações bioquímicas de animais que receberam EtOH e/ou MeHg durante a vida intrauterina, como fatores de riscos para o infarto agudo do miocárdio. Ratas receberam água de torneira ou EtOH durante a gravidez e amamentação. No 15º dia de gravidez, metade dos grupos receberam MeHg. As proles foram divididas em: Controle, EtOH, MeHg e EtOH+MeHg, que foram avaliadas no teste do nado exaustivo. Os resultados sugerem que as proles adultas que receberam somente MeHg durante suas vidas intra-uterinas, foram mais susceptíveis ao infarto agudo do miocárdio após a atividade exaustiva do que os grupos que receberam EtOH ou EtOH+MeHg. O grupo MeHg exibiu um aumento em quase todos os parâmetros bioquímicos, especialmente nos níveis das enzimas CPK e CKMB. Nossos dados complementam pesquisas relacionadas ao risco do infarto agudo do miocárdio e os efeitos do EtOH e/ou MeHg. No entanto, o exato mecanismo citoprotetor do EtOH contra os efeitos do MeHg necessitam de investigações.
PALAVRAS-CHAVE*: bioquímica; teste do nado exaustivo; síndrome alcoólico-fetal; metilmercúrio; infarto do miocárdio.*

INTRODUCTION

Despite advanced medical and surgical treatments, heart failure (HF) remains a major public health issues with a high associated mortality rate[1]. Neurotoxicants such as ethanol (EtOH) and methylmercury (MeHg) appear to produce cardiovascular abnormalities, and play a role in the development of HF[2-5].

Chronic alcoholism is one of the risk factors for myocardial infarction and is associated with an increased mortality risk. Various studies have also reported that heavy alcohol consumption promotes the progression of atherosclerosis and that binge drinking might trigger an embolic stroke and acute myocardial infarction[6]. Another study has demonstrated that the heart is frequently affected in alcoholic men during alcohol withdrawal[7]. The excess of catecholamine and magnesium deficiency associated with this withdrawal[8] may induce myocardial ischemia by coronary vasoconstriction[9-11] and may possibly precipitate cardiac arrhythmias in alcoholics with coronary disease, even though alcohol consumption occurs in short periods of time[12]. Alcohol abuse during pregnancy in both human and animals can induce damage in the heart and must be considered an essential co-factor within the multifactorial etiology of congenital heart defects[13-15].

Among modifiable lifestyle factors, the regular consumption of fish is associated with a substantial reduction in the risk of death from heart attacks[16]. Consequently, the American Heart Association recommends the consumption of two fish meals per week[17]. However, there are populations that depend on fish as their main source of protein and consume more than two fish meals per week. Therefore, the most common route of MeHg exposure is through food, especially by eating fish from contaminated lakes. MeHg can be transferred to the fetus through the placenta and to the offspring through breast milk[18-20]. Therefore, one of the most serious issues is the postnatal effect of exposure to different levels of MeHg in the uterus. The vascular effects of MeHg exposure include oxidative stress, inflammation, thrombosis, vascular smooth muscle dysfunction, endothelial dysfunction, dyslipidemia, immune dysfunction, and mito-chondrial dysfunction, which have consequences such as hypertension and generalized atheros-clerosis, including biochemical alterations[21-22].

The main aim of the present study was to investigate the correlation between EtOH and MeHg intoxication and biochemical abnormalities that could impair the circulatory system.

SUBJECTS AND METHODS

Breeding and prenatal treatment

Three month old male and female Wistar rats were obtained from the Animal Facility, Faculty of Medicine, University of Brasília and housed in groups of 5 animals/sex in each polycarbonate cage, under controlled conditions of temperature (23±1 °C) and photoperiod (light:dark 12:12h), with free access to food and water. The entire set of experiments was carried out in the Pathology Laboratory. For mating, individual females were placed with a male overnight. The detection of a sperm plug the next morning denoted pregnancy at gestation day 1. Pregnant rats were housed individually in polycarbonate cages and randomly assigned to receive tap water or 6.5 g/kg per day of 22.5% w/v EtOH, by gavage[23] for 21 days of gestation and for a further 21 days during breast-feeding. On the 15th day of pregnancy, half of each group (EtOH or control) received 8 mg/kg of MeHg by gavage[24,25]. The control dams received only tap water. In total, all of the procedures involved four treatment groups of pregnant rats: control (*C*, n = 5); ethanol (*EtOH*, n = 5); methylmercury (*MeHg*, n = 5); and ethanol+methylmercury (*EtOH+MeHg*, n = 5).

Pregnancy outcome

The day of birth was designated as postnatal day 1. After birth, litters were culled to 10 pups per litter, and returned to their dams until weaning on postnatal day 21, after which they were grouped (n = 5) according to treatment regime. All behavioral experiments were carried out with 2.5 month-old male rats. Each group of 10 animals was divided into two subgroups (n = 5), for the following behavioral tests: 1) animals underwent intense exhaustive swimming activity, for at least 50 minutes, in a swimming pool with water at room temperature and 2) animals were not submitted to intense exhaustive swimming activity. On the day of the experiments, animals were acclimated to the laboratory for at least 1 h prior to the experimental procedures. All procedures were carried out between 8:00 and 12:00h in order to avoid the circadian influence. All experiments reported in this study were in accordance with our current guidelines for the care of laboratory animals, which is based on the Animal Ethics Committee of the Biology Institute of the University of Brasília.

Behavioral tests

Exhaustive swimming activity

Six hours before the beginning of the exhaustive swimming test, offspring from the Control, EtOH, MeHg and EtOH+MeHg groups were fasted, receiving only water *ad libitum.* Rodents were placed in a cylindrical tank (50 cm in diameter and 70 cm high) containing water at 23±1° C and left there in a position from which they could not escape. The water depth allowed the rats to swim or float without their hind limbs touching the bottom of the tank. The animals were forced to swim without any additional body weight until they were completely exhausted. Each test was run concurrently with two animals, one was a pretreated animal (EtOH, MeHg or EtOH+MeHg) and the other was a control. After an average of 30 minutes, all the animals started manifesting two kinds of behavior, either attempting to flee or stopping and sinking to the bottom of the swimming pool. When it was necessary to avoid drowning, the animals that presented the latter behavior were always rescued by the person conducting the experiment and brought to the surface of the water to continue the swimming activity. The criterion used to identify a possible drowning was the permanence of the animal under water, for more than 10 seconds, without showing any intention to return to the water surface[26]. Five additional offspring from the same groups were used as controls: these animals were not subjected to the swimming procedure.

Sacrifice of animals and blood sample collecting

Whether they underwent the exhaustive swimming test or not, the animals from all the experimental groups were anesthetized with a combination of ketamine (80 mg/kg) and xilazine (10 mg/kg), i.p, in order to have their abdominal and thoracic cavities cut open. The heart and aorta artery of the abdomen of each animal were located and exposed. The blood was then collected with washed needles and syringes containing 100 µL of heparin, which helps to prevent the collected blood from clotting, thus making it available for further biochemical measurements.

Mercury Measurements

The total mercury content in all hair samples was estimated using previously described techniques[27]. Hair samples from rats (dams and pups) were collected with scissors, by cutting 1 cm of hair from three different areas, then washed with acetone and heated in an oven at 800 °C. Mercury vapor was collected in gold plates and analyzed by atomic absorption spectrophotometry using a Mercury Analyzer (model SP3D/Nippon Instruments Corporation, Tokyo, Japan). The precision and accuracy of these measurements were verified using the IAEA 085 International Reference Standard (>95%).

Biochemistry analysis

Tubes containing the collected blood were centrifuged for separation and the plasma removed. Sample processing and measurement of lactic dehydrogenase (LDH)(DIASYS kit), creatinine phosphokinase (CPK) (DIASYS kit), creatine kinase MB (CKMB) (DIASYS kit), aspartate aminotransferase (AST) (DIASYS kit), Gamma-glutamyltransferase (γGT) (DIASYS kit), total cholesterol (TCL) (BIOSYSTEM kit), direct high-density lipoprotein (HDL)-cholesterol without precipitation (LABTEST kit), direct low-density lipoprotein (LDL)- cholesterol , without precipitation (BIOSYSTEM kit), triglycerides (TGD)(BIOSYSTEM kit), glucose (GLC) (LABTEST kit), urea (BIOSYSTEM kit) and creatinine (CRT)(LABTEST kit) were performed according to the criteria of the industries. Automatic measurements were taken by the SYNCHRON CX5 (BECKMAN).

Statistical analysis

Statistical comparisons were performed using one-way analysis of variance (ANOVA). Multiple post hoc comparisons were performed using the Newman-Keuls test. The data were expressed as the average ± SEM. P values less than 0.05 ($p<0.05$) were considered statistically significant.

RESULTS

Figure 1 shows the LDH levels in all treatment groups. In animals that were not subjected to the exhaustive swimming test (panel A), the concentration of LDL increased when compared to the control group [EtOH ($F_{(3,19)}= 71.75$, $p<0.001$); MeHg ($F_{(3,19)}= 27.12$, $p<0.001$); EtOH+MeHg ($F_{(3,19)}= 6.556$, $p<0.001$)]. The LDL levels in the EtOH+MeHg group were also altered when compared to the EtOH ($F_{(3,19)}= 65.20$, $p<0.001$) and MeHg groups ($F_{(3,19)}= 20.56$, $p<0.001$). When the groups that were subjected to the exhaustive swimming test (panel B) were analyzed, the EtOH+MeHg group displayed lower levels of LDH when compared with groups treated with each compound individually [EtOH ($F_{(3,19)}= 6.068$, $p<0.01$); MeHg ($F_{(3,19)}= 6.182$, $p<0.01$)]. The CPK levels were altered in some groups that performed (panel C) the exhaustive test [MeHg ($F_{(3,19)}= 9.174$, $p<0.001$)] and in some groups that did not (panel D) [EtOH ($F_{(3,19)}= 33.60$, $p<0.001$); MeHg ($F_{(3,19)}= 11.09$, $p<0.001$)]. The CKMB levels were also increased in all the treated groups (panel E) that did not perform the exhaustive swimming test [EtOH ($F_{(3,19)}= 31.04$, $p<0.001$); MeHg ($F_{(3,19)}= 20.96$, $p<0.001$); EtOH+MeHg ($F_{(3,19)}= 11.65$, $p<0.001$)], and this difference was maintained in the MeHg group (panel F) that was also submitted to the exhaustive swimming test ($F_{(3,19)}= 16.79$, $p<0.001$).

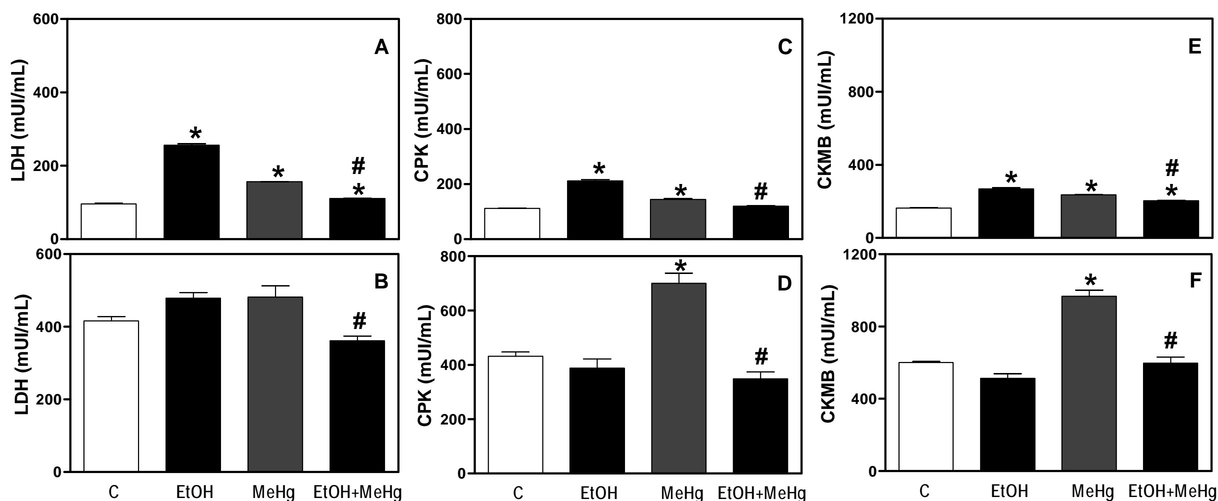

Figure 1 – Effects of EtOH and/or MeHg on LDH, CPK and CKMB levels. Panel A, C and E: Dosage of biochemical levels in the blood samples from rat offspring not subjected to exhaustive swimming activity. Panel B, D and F: Dosage of biochemical levels in the blood samples from rat offspring subjected to exhaustive swimming activity. Each value represents the mean±SEM of 5 rats. *$p<0.05$ when compared to the Control group, treated with tap water. #$p<0.05$ when compared to the EtOH and/or MeHg groups (Newman-Keuls test). LDH = lactic dehydrogenase, CPK = creatinine phosphokinase, CKMB = creatine kinase MB.

Among the hepatic enzymes (figure 2), the AST levels were altered in all of the treated animals , whether they were not subjected to the exhaustive swimming test (panel A) [EtOH ($F_{(3,19)}$= 5.065, $p<0.01$); MeHg ($F_{(3,19)}$= 5.789, $p<0.01$); EtOH+MeHg ($F_{(3,19)}$= 25.18, $p<0.001$)] or did perform the exhaustive swimming test (panel B) [EtOH ($F_{(3,19)}$= 5.998, $p<0.001$); MeHg ($F_{(3,19)}$= 7.949, $p<0.001$)]. Under these conditions, rats in the EtOH+MeHg group showed reduced AST values compared to those in the EtOH ($F_{(3,19)}$= 6.106, $p<0.01$) and MeHg ($F_{(3,19)}$= 8.057, $p<0.001$) groups. The γGT levels were altered only in groups that were exposed to exhaustive swimming test (panel D), while the EtOH ($F_{(3,19)}$= 31.36, $p<0.001$) and EtOH+MeHg ($F_{(3,19)}$= 30.43, $p<0.001$) groups displayed diminished levels of this enzyme. The TCL levels were altered only in groups that were not subjected (panel E) to the exhaustive swimming test [EtOH ($F_{(3,19)}$= 5.437, $p<0.01$); MeHg ($F_{(3,19)}$= 11.77, $p<0.001$); EtOH+MeHg ($F_{(3,19)}$= 6.328, $p<0.01$).

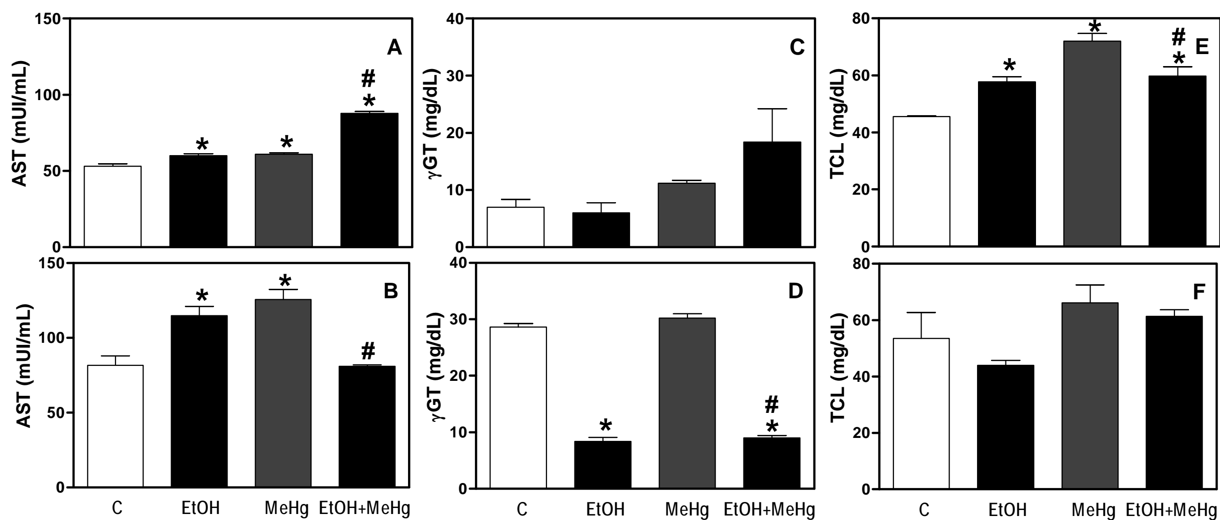

Figure 2 – Effects of EtOH and/or MeHg on AST, γGT and TCL levels. Panel A, C and E: Dosage of biochemical levels in the blood samples from rat offspring not subjected to exhaustive swimming activity. Panel B, D and F: Dosage of biochemical levels in the blood samples from rat offspring subjected to exhaustive swimming activity. Each value represents the mean±SEM of 5 rats. *$p<0.05$ when compared to the Control group, treated with tap water. #$p<0.05$ when compared to the EtOH and/or MeHg groups (Newman-Keuls test). AST = aspartate aminotransferase, γGT = gamma-glutamyltransferase, TCL = total cholesterol.

The HDL levels were altered in the MeHg group ($F_{(3,19)}= 12.57$, $p<0.001$) and the LDL level (figure 3) was altered in all the treated groups [EtOH ($F_{(3,19)}= 8.833$, $p<0.001$); MeHg ($F_{(3,19)}= 15.46$, $p<0.001$); EtOH+MeHg ($F_{(3,19)}= 6.901$, $p<0.001$)] that did not undergo the exhaustive swimming test (panels A and C). In groups subjected to the exhaustive swimming test (panels B and D), the HDL levels increased in the EtOH+MeHg group ($F_{(3,19)}= 12.29$, $p<0.001$), and the LDL levels reduced in the EtOH ($F_{(3,19)}= 6.300$, $p<0.001$) and EtOH+MeHg ($F_{(3,19)}= 8.709$, $p<0.001$) groups. The TGD levels were increased in all the treated groups [EtOH ($F_{(3,19)}= 17.13$, $p<0.001$); MeHg ($F_{(3,19)}= 18.39$, $p<0.001$); EtOH+MeHg ($F_{(3,19)}= 10.33$, $p<0.001$)] that were not subjected to the exhaustive swimming test (panel E). Rats that received EtOH+MeHg also showed altered TGD concentrations compared to rats that received only one toxicant [EtOH ($F_{(3,19)}= 6.803$, $p<0.001$); MeHg ($F_{(3,19)}= 8.063$, $p<0.001$)]. Animals that were exposed to the exhaustive swimming test (panel F) showed altered TGD levels [EtOH ($F_{(3,19)}= 4.110$, $p<0.05$); MeHg ($F_{(3,19)}= 8.951$, $p<0.001$); EtOH+MeHg ($F_{(3,19)}= 4.293$, $p<0.05$)].

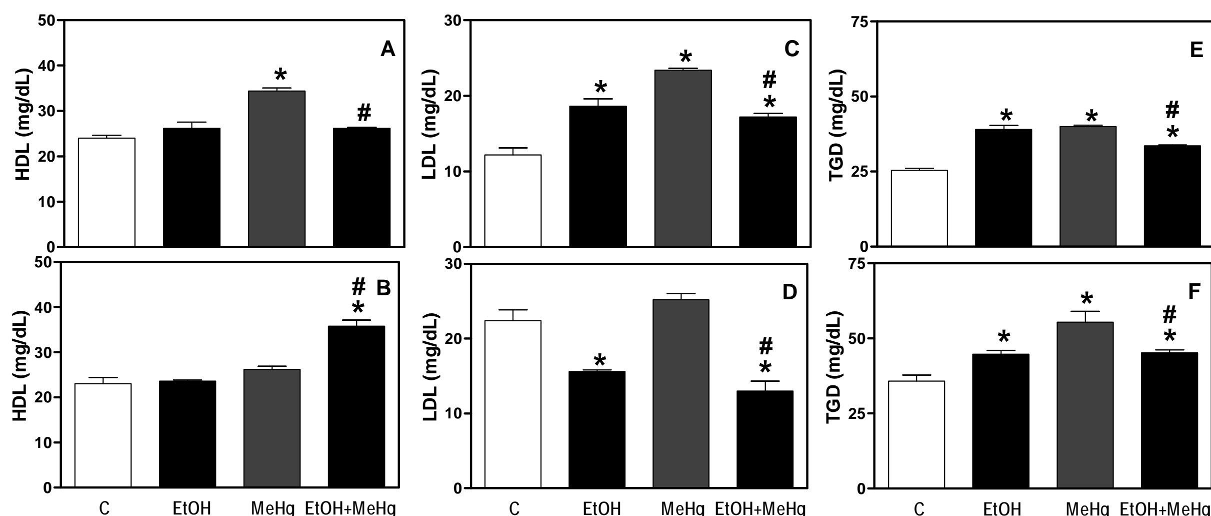

Figure 3 – Effects of EtOH and/or MeHg on HDL, LDL and TGD levels. Panel A, C and E: Dosage of biochemical levels in the blood samples from rat offspring not subjected to exhaustive swimming activity. Panel B, D and F: Dosage of biochemical levels in the blood samples from rat offspring subjected to exhaustive swimming activity. Each value represents the mean±SEM of 5 rats. *p<0.05 when compared to the Control group, treated with tap water. #p<0.05 when compared to the EtOH and/or MeHg groups (Newman-Keuls test). HDL = direct high-density lipoprotein-cholesterol without precipitation, LDL = direct low-density lipoprotein- cholesterol, without precipitation, TGD = triglycerides.

The GLC levels were increased in the MeHg group ($F_{(3,19)}$= 10.20, p<0.001) that was not subjected to the exhaustive swimming test (panel A). After the exhaustive swimming test (panel B), the glucose blood levels of the EtOH+MeHg group decreased significantly ($F_{(3,19)}$= 14.59, p<0.001). The urea [EtOH ($F_{(3,19)}$= 9.247, p<0.001); MeHg ($F_{(3,19)}$= 6.725, p<0.001); EtOH+MeHg ($F_{(3,19)}$= 14.80, p<0.001)] and CRT levels [MeHg ($F_{(3,19)}$= 8.083, p<0.001); EtOH+MeHg ($F_{(3,19)}$= 11.55, p<0.001)] in animals that did not undergo the exhaustive swimming test (panels C and E) were higher than the control group, except for the CRT levels of the EtOH group. As for animals that were submitted to the exhaustive swimming test (panels D and F), only the EtOH+MeHg group ($F_{(3,19)}$= 7.658, p<0.001) displayed an increase in their urea levels, while the EtOH group ($F_{(3,19)}$= 6.600, p<0.001) showed a decrease in their CRT levels (figure 4).

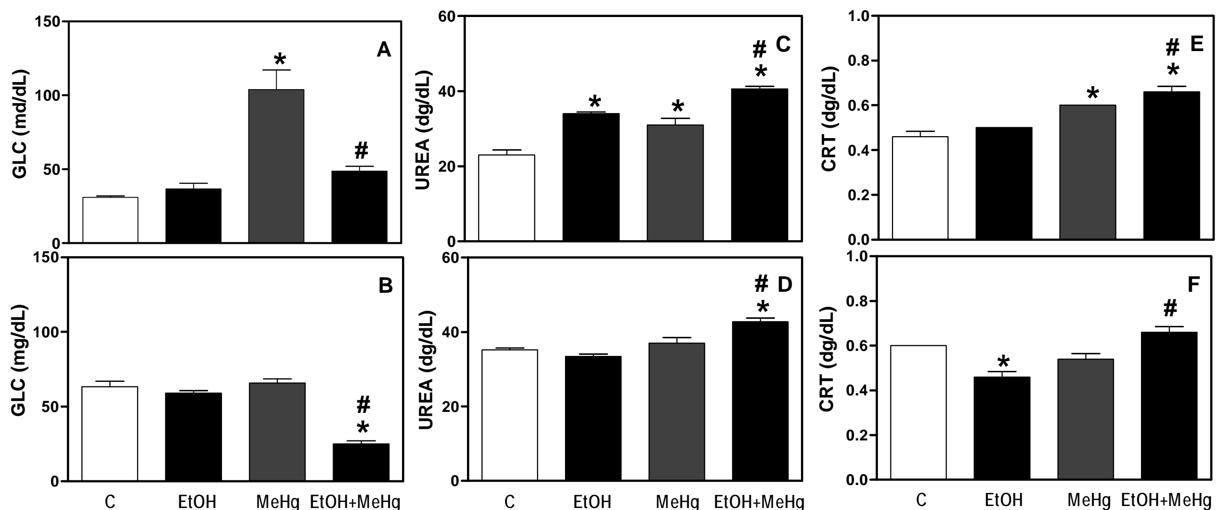

Figure 4 – Effects of EtOH and/or MeHg on GLC, Urea and CRT levels. Panel A, C and E: Dosage of biochemical levels in the blood samples from rat offspring not subjected to exhaustive swimming activity. Panel B, D and F: Dosage of biochemical levels in the blood samples from rat offspring subjected to exhaustive swimming activity. Each value represents the mean±SEM of 5 rats. *p<0.05 when compared to the Control group, treated with tap water. #p<0.05 when compared to the EtOH and/or MeHg groups (Newman-Keuls test). GLC = glucose, CRT = creatinine.

DISCUSSION

The results obtained from this study show that the adult rat offspring that underwent treatment with tap water (control), EtOH, MeHg or EtOH+MeHg in utero and did not perform exhaustive swimming test displayed alterations in some studied biochemical parameters. The alterations depended on the treatment group. When the same biochemical parameters were evaluated in offspring subjected to the exhaustive swimming test, it was observed that the levels of the CPK and CKMB enzymes were higher in the offspring that received only MeHg, compared with those that received EtOH or EtOH+MeHg and were subjected to the same conditions. These results suggest that the adult rat offspring that received only MeHg during their intra-uterine lives were more susceptible to acute myocardium infarction after exhaustive activity.

The biochemical parameters and enzymes we analyzed could be used to evaluate hepatic cellular damage. In this study, the main biochemical alteration in the liver was the levels of AST, which was increased in the MeHg and EtOH groups, compared to the control group; this increase was apparent in rats whether or not they were subjected to the exhaustive swimming test. Rats in the EtOH group showed decreased levels of γGT after performing the test. This result suggests that the offspring that received only MeHg during their intra-uterine lives displayed a higher level of hepatic damage when compared to animals that received only EtOH under the same conditions. This conclusion is supported by the reduced level of γGT.

The glucose blood level of rats in the MeHg group was increased, demonstrating the hyperglycemic state of this group. Based on the derived lipid substances, an important factor in the predisposition to myocardial disease, rats in MeHg group that was not exposed to the swimming test displayed an increase in all parameters (TCL, LDL, TGD and HDL). Despite the fact that HDL is an important substance in the prevention of circulatory system impairment, increases in all endogenous lipid parameters are a risk factors for the heart. Also, the fact that urea and CRT levels in the MeHg group were higher than in the control group suggests possible kidney alterations in rats that received MeHg.

The indirect determination of the alcohol concentration in the blood stream of the dams and offspring was performed. No statistical difference between the groups that received EtOH was demonstrated. However, when we evaluated the mercury concentration in the dams and offspring undergoing the same treatments used in this study, we confirmed our hypothesis of a possible protective effect of EtOH in animals that received EtOH+MeHg, since the offspring and even the dams of the EtOH+MeHg group displayed reduced mercury concentrations when compared to the animals that received only MeHg[28]. This effect maybe reflected in the absence of an increase in the level of CPK, CKMB, and AST in the EtOH+MeHg group after the exhaustive swimming test. These data reinforce our hypothesis that EtOH used during intra-uterine life in combination with other oxidative products like MeHg may have a cardio protective effect, even though the probable mechanisms of this cardio protection are not clearly understood.

According to Maia et al.[28], even though our study was conducted with pregnant rats, alterations in the behavioral response of the offspring does not seem to reflect the variations caused by the metabolism of EtOH, since the animals received EtOH during their intrauterine lives and the behavioral experiments were carried out during adulthood. Based on the mercury concentration determined in hair samples from the rats, our data showed that the mercury levels were significantly reduced when MeHg was combined with EtOH. Also, the results did not allow us to draw any conclusions about possible pharmacokinetic and/or pharmacodynamic interferences in the alteration of behaviors observed here.

When compared to the MeHg group, the group of offspring that received EtOH+MeHg during their intra-uterine lives and were later forced to perform exhaustive physical activity demonstrated no increase in the levels of CPK, CKMB, LDH, AST and γGT enzymes, which is indicative of lesions in the cardiac cells. The endogenous biochemical products that are important for good health, such as LDL and glucose, were shown to increase. Both of these observations suggest the possibility of the hypothesis stated earlier; i.e. that EtOH, in association with MeHg, can be an important factor in reducing the oxidative action of MeHg on the cardiac muscle, consequently reducing the risk of acute infarction of the myocardium in these animals.

HDL is another biochemical parameter that is relevant to quality of life and has been demonstrated to have beneficial effects on the cardiovascular system. This benefit comes from its ability to remove cellular cholesterol. Additionally, HDL acts as antithrombotic, anti-inflammatory, and as antioxidant compounds, and these properties promote endothelial health[29]. The HDL level was increased in the EtOH+MeHg group after the exhaustive swimming test. In our studies we also wanted to evaluate the use of CKMB, LDH, CPK, AST, and γGT levels in the peripheral blood, as indicators of acute infarction of the myocardium, as these enzymes usually appear at higher concentrations in circulating blood when myocardial cells are subjected to oxidative stress[30-32]. The liberation of these enzymes and their detection in the blood, allows us to produce a biological estimation of the size of the area of infarction of the cardiac muscle, as well as the onset of the cell lesion, even before these alterations can be detected microscopically[30,33].

While CKMB is known to be an important indicator for acute myocardial infarction, this enzyme is no longer the only factor in the determination of pathology. Other substances, such as troponine I[32] and the natriuretic peptides (BNP), and their fractions (NT-pro BNP)[34], have been shown to be important biochemical and immunological indicators, which are more sensitive and specific in the characterization of the risks of myocardial infarction, size of the infracted area, and even the possible factors that can generate this pathology, such as in cases of cardiac insufficiency[31,32,34]. However, in our studies, it was not possible to evaluate any of these substances.

The specific mechanism of action of MeHg in the cardiac muscle is not totally clear. However,

several mechanisms have been proposed to explain its action. The first mechanism is connected to the transportation of MeHg by the erythrocytes (90% of the total); this facilitates its dissemination through the whole body and into cell groups. The second is related to the fact that MeHg is liposoluble, and thus builds up in the embryo and interferes with its development [35-39]. A third mechanism is related to the fact that MeHg is able to consistently alter the intracellular homeostasis of Ca^{++} in various types of cells[40,41]. This last mechanism is already well documented in causing cell death in the granule layer of the cerebellum induced by MeHg[41]. In this case, it has been observed that MeHg causes a biphasic growth, which is also a characteristic of intracellular Ca^{++} release, which involves a "first phase" of the initial liberation of intracellular Ca^{++} from one or more cytoplasmic organelles occurs, followed by a "second phase", related to the secondary inflow of extracellular Ca^{++} [40-42].

This evidence suggests that mitochondria significantly contribute to both phases. During the first phase, an increase in intracellular Ca^{++} occurs, followed by the subsequent death of the cell. This death is a result of the opening of the transition channels of the mitochondria's permeability (TCMP), although the mechanism involved is not yet totally elucidated[42,43]. Studies suggest that the elevation of Ca^{++} in the mitochondria could be associated with its intracytoplasmic excess, which results from its liberation from the straight endoplasmatic reticule (SER) after the action of the MeHg on this organelle. Thus, when the mitochondria becomes excessively full of Ca^{++}, more TCMPs are opened[43], resulting in the increase in cytoplasmic Ca^{++}, which continues for many minutes until the moment when the inflow of extracellular Ca^{++} ceases to occur[42,44]. Therefore, we believe that this could also be a possible mechanism associated with the toxic effect of MeHg in the cardiac muscle, even though this phenomenon was not investigated in this study.

Consequently, our results showed that the intra-uterine exposure of offspring to MeHg, through mothers who received the MeHg, is able to directly interfere with the cardiac function of these offspring. Also, the offspring from dams that received EtOH and MeHg concurrently (EtOH+MeHg) during pregnancy, showed that EtOH has an important "cardio protective effect" against the risk of acute myocardial infarction, even in animals subjected to exhaustive physical activity.

Based on the obtained results, this study demonstrated not only an experimental biochemical evaluation of animals exposed to EtOH, MeHg or a combination of both substances, but also an evaluation of the risk of acute infarction of the myocardium in the offspring of animals that were subjected to EtOH, MeHg or EtOH+MeHg in utero; thus making it possible to assess the cardiac risks of these substances on a long-term basis in future generations. Therefore, this study shows that the results seen with adult offspring rats that received EtOH, MeHg and/or EtOH+MeHg, could at least be similar to what observed in human beings, in whom MeHg could increase the risk in various physiological functions, particularly in the cardiovascular system.

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