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EFFECT OF ALLOPURINOL ON THE HEPATIC AND CEREBELLAR IRON, SELENIUM, ZINC AND COPPER STATUS FOLLOWING ACUTE ETHANOL ADMINISTRATION TO RATS

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An acute ethanol load (50 mmol/kg, i.p.) induces an increase in the total non-heme iron and in the low-molecular-weight non-heme iron complexes (LMW-Fe) content both in liver and cerebellum. This increase in LMW-Fe is associated with a decrease in some essential trace elements (selenium, zinc, copper) playing a role in the anti-oxidant system. These changes could contribute to the enhancement in lipid peroxidation which occurs at the hepatic and cerebellar level following the ethanol administration.

The administration of allopurinol prior to the ethanol load prevents the changes in non-heme iron and trace elements. This prevention may contribute to the protective effects of allopurinol on the ethanol-induced oxidative stress.

KEY WORDS: Ethanol, allopurinol, non-heme iron, trace elements, liver, cerebellum.

INTRODUCTION

Several reports have previously shown that an acute ethanol load elicits an enhanced lipid peroxidation in rat liver[1] and cerebellum.[2] These changes in lipid peroxidation reflect an oxidative stress, i.e. a disturbance in the prooxidant/antioxidant systems in favour of the former.[3] Oxygen-derived free radicals, such as the hydroxyl radical (·OH), are to be considered as cellular prooxidants. The biosynthesis of these highly oxidizing species requires the presence of suitable metal promoters, especially iron derivatives. The exact nature of the iron derivatives acting as catalysts is still a subject of debate. However a fraction of the cellular non-heme iron consisting in low-molecular-weight non-heme iron complexes (LMW-Fe) appears to represent the iron species catalytically active in initiating free radical reactions and lipid peroxidation.[4-6] The cells contain two groups of antioxidant systems able to prevent or limit damages induced by free radicals. The first one includes antioxidant substrates such as alpha-tocopherol and ascorbate. The second one comprises antioxidant enzymes such as Cu–Zn-superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) which requires selenium. In addition, selenium has biological activities and antioxidant effects not mediated by GSH-Px.[7,8] The present studies were undertaken to assess the effects of an acute ethanol load on total non-heme iron, LMW-Fe, selenium, zinc and copper in blood serum, liver and cerebellum.

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Having observed that allopurinol, which is altogether an inhibitor of xanthine oxidase and a direct scavenger of free radicals,[9] affords protection against the oxidative stress induced by acute ethanol at the hepatic and cerebellar level,[10] we also studied the effect of an allopurinol pretreatment on the ethanol-induced changes in the distribution of non-heme iron and trace elements.

MATERIALS AND METHODS

Allopurinol was purchased from Sigma Chemical Co and Chelex 100 was from Bio-Rad Laboratories. All other chemicals were of analytical purity.

Animals and Treatments

Male Sprague Dawley rats weighing about 180 g were fed a standard pellet diet (Iffa Credo, UAR) containing 100 ppm iron. Ethanol (50 mmol/kg body weight, i.p.) was administered as a 20 per cent (v/v) aqueous solution to overnight fasted animals. Allopurinol (146 μmol/kg, i.p.) was administered 16 hr and 20 mn prior to the ethanol load. Control animals were given the same volume of saline.

Tissue Preparations and Analyses

The rats being sacrificed by decapitation, the blood, cerebellum and liver were rapidly removed. Blood was rapidly centrifuged and the serum was collected and kept at + 4 °C until used for analyses. A sample of the liver and the cerebellum was washed, mopped up, frozen and kept in liquid nitrogen until used for the analysis of the non heme-iron and trace element contents. The remaining cerebellum and liver were used for the preparation of the cytosolic fraction.[11] Preparation of LMW-Fe was achieved by filtration of samples of hepatic and cerebellar cytosolic fractions through an YMT ultrafiltration membrane (cut off 30,000 daltons) in an Amicon-MPS 1 device.[12]

Non heme-iron and LMW-Fe were determined after trichloracetic and hydrochloric acid extraction in serum, liver and cerebellum by inductively coupled plasma atomic emission spectrometry (i.c.p.a.e.s.) according to [13]. In these tissues, zinc and copper were determined by flame atomic absorption spectrometry after acidic extraction and selenium was measured by furnace graphite atomic spectrometry with Zeemann correction. The same techniques were used for determination of zinc, copper and selenium in the serum after dilution (1:10) in 1 per cent nitric acid solution (v/v). Protein was assayed following Lowry *et al.*[14] Blood serum ethanol concentrations was determined by gas chromatography according to [15]. All solutions were prepared with Chelex 100-treated water.

Statistical Interpretation

Reported values are means + S.E., statistical analysis being performed using Student's t-test.

RESULTS

The mean *blood serum* ethanol concentration was 40 ± 3 mM 4 hours after the

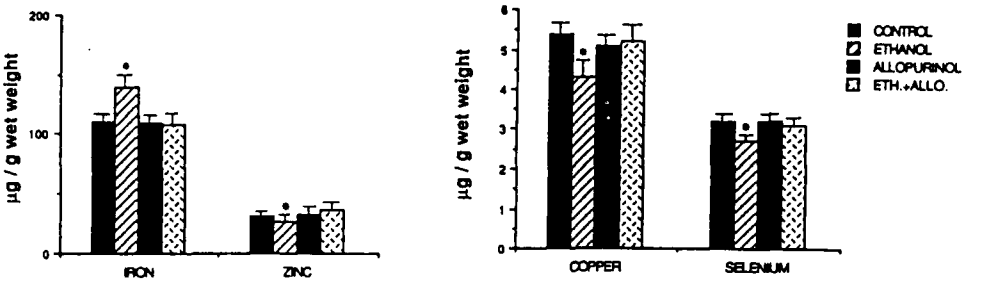

FIGURE 1 Effects of ethanol and/or allopurinol on liver non-heme iron and trace elements. Ethanol (50 mmol/kg, i.p.) was injected 4 hr before sacrifice. Allopurinol (146 µmol/kg, i.p.) was injected 16 hr and 20 mm prior to the ethanol treatment. Statistical significance: $*p < 0.01$ versus control.

ethanol load. The serum non-heme iron concentration was significantly decreased by the ethanol administration, the mean values being 14.5 ± 3.8 vs. 9.2 ± 0.8 µmol/l in 9 control rats and 7 ethanol-treated rats, respectively. The serum selenium concentration was also significantly decreased, the mean values being 6.2 ± 0.2 vs. 5.3 ± 0.3 µmol/l in 5 control rats and 5 ethanol-treated rats, respectively. However no significant changes were observed in the blood serum zinc and copper concentrations after the ethanol load (results not shown).

In the liver, ethanol elicited a significant increment in the non-heme iron content (+ 25 %) (Figure 1), associated with a significant enhancement in the cytosolic LMW-Fe (+ 100 %) (Figure 2). At the opposite, the selenium (− 10 %), zinc (− 25 %) and copper (− 24 %) contents were significantly decreased (Figure 1).

In the cerebellum, the ethanol load increased significantly the non-heme iron content (+ 34 %) (Figure 3) and the cerebellar cytosolic LMW-Fe (+ 200 %) (Figure 2). At the opposite, the selenium (− 48 %), zinc (− 20 %) and copper (− 24 %) contents were significantly decreased (Figure 3).

The pretreatment with allopurinol prevented the ethanol-induced decrease in blood serum non-heme iron and selenium levels as well as all changes in the cerebellar and hepatic non-heme iron and trace element contents (Figures 1 and 3). However, allopurinol administered alone did not affect these parameters (Figures 1 and 3).

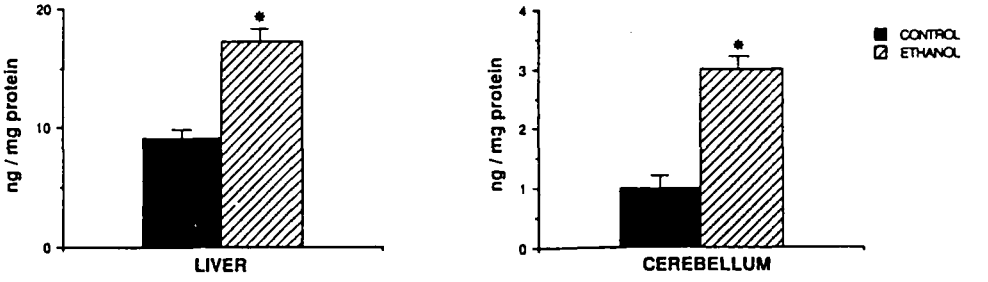

FIGURE 2 Effect of an acute ethanol load on the hepatic and cerebellar low molecular weight iron complexes (LMW-Fe). Ethanol (50 mmol/kg, i.p.) was injected 4 hr before sacrifice. Statistical significance: $*p < 0.01$ versus control.

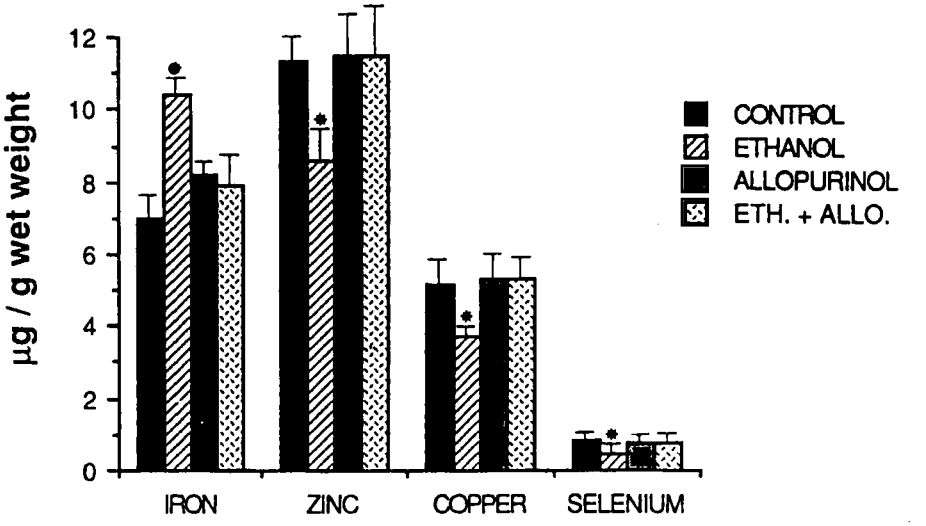

FIGURE 3 Effects of ethanol and/or allopurinol on cerebellar non-heme iron and trace elements. Ethanol (50 mmol/kg, i.p.) was injected 4 hr before sacrifice. Allopurinol (146 µmol/kg, i.p.) was injected 16 hr and 20 mn prior to the ethanol treatment. Statistical significance: $*p < 0.01$ versus control.

DISCUSSION

Few reports[16,17] have been concerned with the effects of an acute ethanol load on the non-heme iron content in the tissues and conflicting results have been reported. The present results show that an acute ethanol load elicits altogether a decrease in the total non-heme iron content of blood serum and an increase in total non-heme iron in the liver and cerebellum. This suggests that the ethanol load induces a shift in the repartition between the circulating and tissular iron content. The putative mechanisms implicated in the increase in tissular iron may involve changes in blood flow[18-20] and/or changes in cellular metabolism leading to an increase in the production of reducing equivalents. NADH which is produced during the metabolism of ethanol by alcohol dehydrogenase could be involved, as least partly, in these changes.[21]

Since LMW-Fe play a central role in the catalysis of free radical-mediated reactions and since Bacon *et al.*[22] have reported that lipid peroxidation is correlated to this iron fraction, it may be suggested that the increase in LMW-Fe presently found is an important contributing factor to the ethanol-induced oxidative stress in the liver and cerebellum. This suggestion is is accordance with the prominent role of LMW-Fe in oxidative damages.[23]

The present data show that the levels of zinc and copper are decreased in liver and cerebellum following the ethanol load. Since under these experimental conditions liver and brain Cu–Zn SOD activity is also decreased,[24,25] a positive correlation between the tissular copper content and Cu–Zn SOD can be suggested. The decrease of the zinc concentration in the same tissues could contribute to the ethanol-induced oxidative stress, zinc having by itself an antioxidant function.[26]

It is well known that selenium is present as selenocystein in the antioxidant enzyme GSH-Px, as well as in other selenoproteins.[27] These selenoproteins seem to play a role

in selenium transport,[27] protein synthesis[28] and especially in the antioxidant defence.[8] The presently reported decrease in the selenium level in serum, liver and cerebellum may affect GSH-Px and/or other selenoproteins. We have previously shown that an acute ethanol load does not modify the mitochondrial GSH-Px at the cerebellar level.[29] However the selenium present in GSH-Px represents only about 1/5 of the total Se present in brain tissue.[30] This raises the possibility that the ethanol-induced Se decrease (– 48 %) affects cerebellar selenoproteins involved in the antioxidant defence but different from GSH-Px.

Besides the decrease in antioxidant substrates such as alpha-tocopherol and ascorbate,[31] both the decrease in trace elements playing a role in the antioxidant defence and the increase in the cytosolic LMW-Fe content could contribute to the enhancement in cerebellar lipid peroxidation observed 4 hours after the ethanol load.[2]

We have previously shown[11,31] that allopurinol provides protection against the ethanol-induced hepatic and cerebellar oxidative stress. Using the same experimental conditions, we presently report that the pretreatment with allopurinol prevents the ethanol-induced changes in non-heme iron, zinc, copper and selenium. Moreover allopurinol prevents the ethanol-induced decrease in Cu–Zn SOD in brain.[25] This preventative effect of allopurinol could be linked to its activity as xanthine oxidase inhibitor, hydroxyl radical scavenger,[9] electronic transfer activator[32] or adenosine release enhancer.[33] Our data, albeit not conclusive about the mechanisms of the allopurinol effects, confirm its protective role in damage associated with free radical reactions.

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