# Temporal Changes of the Lipid Peroxidation in Rats after Acute Intoxication by Ethanol

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A group of male rats were intoxicated within 24 h by three successive i.p. doses of ethyl alcohol (7.5 g per 1 kg of the body weight). In parallel, a control group of rats were dosed i.p. with a physiological saline. At time intervals of 0 h, 4 h, 24 h, and 48 h after the intoxication, the content of thiobarbiturate-reactive substances (TBARS) as product of lipid peroxidation within the liver and brain microsomes and mitochondria was followed.

In liver microsomes of the experimental rats there was a rapid increase (by 220%) in the content of TBARS during 4 h after the last application of ethanol, later on the level of lipid peroxidation decreased to the low original value. In other organelles examined only an insignificant increase in the content of TBARS was found. The results prove that an acute intoxication by ethanol does elicit an oxidative stress of the organism, expressed by a transiently increased production of TBARS. These oxidative and harmful changes for the cell structures are mostly located in the liver microsomes but a rapid repair of this damage follows. However, if such a short-term excessive abuse of alcohol is repeated more often, the above changes may lead to severe alcoholic injury to the liver tissue.

## Introduction

Most of harmful effects on health of ethyl alcohol is supposed to be caused by reactive oxygen species, especially by some oxygen and other free radicals (McCay *et al.,* 1992; Nordmann *et al.,* 1992). Consequently, their production within intracellular medium is connected with oxidative metabolism of ethanol, and in decisive measure it is thus concentrated in the liver (Lieber, 1991). Oxidative changes of polyunsaturated fatty acids, proteins and other biostructures, on the one hand (Nordmann *et al.,* 1990; Rouach *et al.,* 1987) as well as a decrease in the protective antioxidative potential, on the other hand (Chen *et al.,* 1992; Zidenberg-Cherr *et al.,* 1990), can be found during chronic intoxication by ethanol of experimental animals in various parts of CNS and liver. Both the results of experiments on animals and those of studying the health condition of people demonstrate that in etiopathogenesis of an alcohol-induced organ injury the oxidative destruction of membranes plays a prominent role, manifesting itself predominantly by the increased peroxidation of membrane lipids (Bautista and Spitzer, 1992; Rashba-Step *et al.,* 1993; Reinke *et al.,* 1990).

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Nevertheless, a certain part of clinical and experimental works do not confirm any implication of the free radicals in any detrimental effects (Inomata *et al.,* 1987). It appears that it is the conditions of the experiment in question, such as the kind of alcohol abuse model – whether a chronic or an acute one – the nutrition state of the experimental object as well as the volume and frequency of the alcohol doses applied, that essentially influence both the character and extent of an oxidative stress during alcohol intoxication (Nadkarni and D'Souza, 1988; Remmer *et al.,* 1989). The majority of controversial views is based on the results of long-term experiments finished off by one-shot measurements; lack of knowledge is apparent concerning the dynamics of the changes caused by large short-term doses of alcohol. Hence, we have established in some short-term alcohol fed animals the values of lipid peroxides within an intracellular medium, checking up those values repeatedly in the course of 48 h. The results of this experiment were corrected by the values obtained in a pair-fed control group.

## Materials and Methods

In our experiment, 48 male rats of laboratory strain (VELAZ Prague) of an average body weight of 230 g were used. One half of the animals

formed an experimental, the other half a control group.

The animals were fed with standard commercial diet for laboratory rats and supplied with water *ad libitum*, otherwise being kept in cages at 21 °C and having a cycle of 12 h for a day, and 12 h for a night.

*Application of alcohol*

30% solution (vol/vol) of ethyl alcohol in a physiological saline was injected i.p. in a dosis of 2.5 g of alcohol per 1 kg of body weight. The control group got i.p. the same volume of the pure physiological saline. Three doses were applied within 24 h: at 8.00 in the morning, at 12.00 at noon, and at 8.00 next day in the morning. Food was taken away from the animals 12 h before killing. The animals were decapitated in groups of 6 animals in the following four intervals: a) immediately after the last application of ethanol (alternatively, the pure saline) – 0 h; b) 4 h after; c) 24 h after; d) 48 h after it.

*Working up the experimental material*

Immediately after killing the animals, their liver and brain were taken out to be stored at a temperature of –25 °C. Having used the ultracentrifugal technique (Ayaz *et al.*, 1976), we managed to isolate mitochondria and microsomes from the above organs in the course of 24 h (1 g of tissue having been homogenized with 5 ml of saccharose solution).

The content of thiobarbiturate-reactive substances (TBARS) within the organelles was determined by a test with 2-thiobarbituric acid; establishing the corresponding values in mitochondria was performed after Ohkawa *et al.* (1979), those in microsomes being carried out by a metabolic activation in the presence of NADPH and $Fe^{2+}$ (Ready *et al.*, 1982). The protein content in the biological samples was estimated according to Lowry *et al.* (1951). In a parallel manner, we used a standard solution of malondialdehyde-bis-(diethylacetal) the results being expressed as the amount of malondialdehyde and related to 1 mg of protein. The values of lipid peroxides (more exactly, those of TBARS) found in the experimental group of animals were reduced by the values found in the controls. The level of ethanol in the blood serum was established by adopting the method of gas chromatography (which is a standard procedure in the practice of forensic medicine).

Statistical significance of the difference between the values obtained in the experimental group and those in control group was determined by means of Student's t-test.

## Results and Discussion

In Table I the lipid peroxides values (means ±S.D.) found in both experimental and control group are summarized.

Fig. 1 and 2 express the course of the time-bound changes of lipid peroxidation in the liver and brain organelles the values being presented as the difference, for the same organelles and time, between the experimental and control group.

All the data and four curves show an increase within the time interval of 4–12 h after the last application of ethanol to the animals, but a statistically significant change ($p < 0.001$) was found only in the liver microsomes where the maximum TBARS had been established 4 h after the last dosis of alcohol. The changes in the extent of lipid peroxidation in the liver microsomes were parallel with the changes of ethanol concentration in the blood of the experimental animals, the highest alcoholaemia being 0.62 ± 0.18 g/l.

Table I. Lipid peroxide content in the liver and brain organelles of ethanol-treated and control rats (nmol malondialdehyde per 1 mg protein, mean of six determinations ±S.D.) at four time points after the last ethanol application; control – physiological saline.

| Time [h] | Liver microsomes Experimental group | Liver microsomes Control group | Liver mitochondria Experimental group | Liver mitochondria Control group |
|---|---|---|---|---|
| 0 | 0.87 ± 0.32 | 0.37 ± 0.12 | 1.09 ± 0.55 | 0.48 ± 0.23 |
| 4 | 2.05 ± 0.33 | 0.64 ± 0.21* | 1.11 ± 0.38 | 0.52 ± 0.18 |
| 24 | 1.94 ± 0.41 | 0.71 ± 0.22 | 0.99 ± 0.40 | 0.48 ± 0.17 |
| 48 | 1.04 ± 0.36 | 0.42 ± 0.18 | 0.98 ± 0.34 | 0.45 ± 0.21 |
| **Time [h]** | **Brain microsomes Experimental group** | **Brain microsomes Control group** | **Brain mitochondria Experimental group** | **Brain mitochondria Control group** |
| 0 | 1.82 ± 0.55 | 0.82 ± 0.31 | 0.64 ± 0.28 | 0.23 ± 0.11 |
| 4 | 1.68 ± 0.42 | 0.88 ± 0.40 | 1.17 ± 0.45 | 0.45 ± 0.22 |
| 24 | 2.04 ± 0.78 | 0.94 ± 0.38 | 1.72 ± 0.58 | 0.77 ± 0.37 |
| 48 | 1.53 ± 0.49 | 0.71 ± 0.42 | 0.85 ± 0.32 | 0.34 ± 0.15 |

* Statistical significance between the experimental and control group ($p < 0.001$).

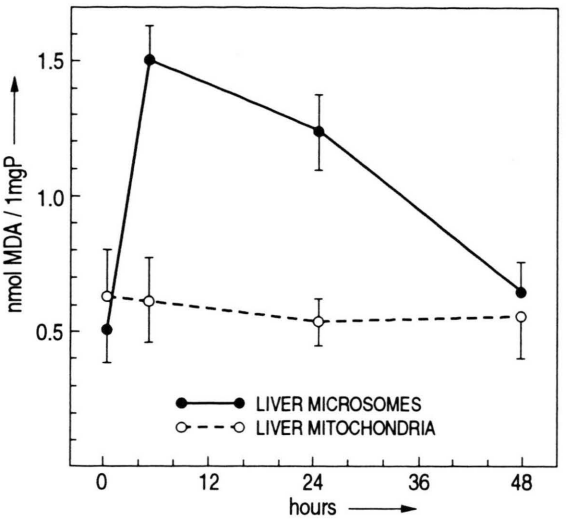


Fig. 1. Temporal changes of the lipid peroxide content within the liver organelles (nmol malondialdehyde related to 1 mg protein (= P) *vs.* time in hours after the last dose of ethanol). The values are corresponding to the average of the group ±S.D. and are expressed as the results of subtracting of the values of the pair-fed controls from those of the experimental group (intoxicated by ethanol).

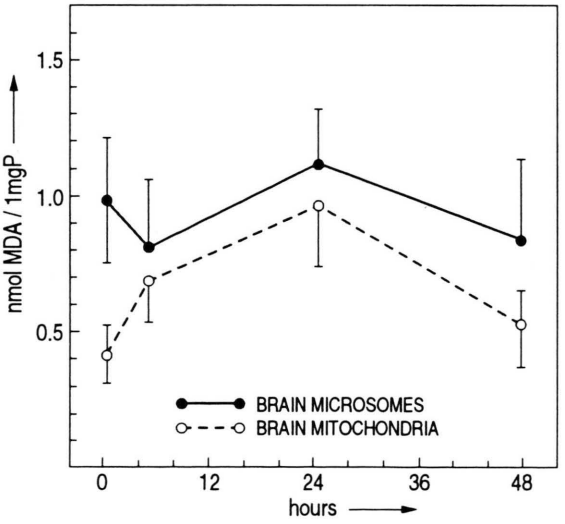


Fig. 2. Temporal changes of the lipid peroxide content within the brain organelles. Footnotes are the same as in Fig. 1.

The above results clearly show that in rats a short-term application of large doses of alcohol does result in an enhanced production of the lipid peroxides within the liver microsomes. This change has a time-limited course, and within a space of 24–48 h after the alcohol intoxication its physiological extent is renewed.

These findings prove analogous to some results ascertained in the experimental rats subjected to a regimen of chronic alcohol intoxication (Dicker and Cederbaum, 1987; Puntarulo and Cederbaum, 1988). Under long-term toxic effects of ethanol, the microsomal ethanol oxidizing system (MEOS) in the liver gets activated, operating then with the isoenzyme cytochrome P 450 2E1 (Ekström and Ingelman-Sundberg, 1989). An enhanced oxidation of the microsomal polyunsaturated fatty acids that was ascertained under these conditions is the consequence of a decreased activity of the enzyme NADPH cytochrome P 450 reductase as well as of an enhanced oxidation of NADPH resulting from the former (French *et al.*, 1993; Kato *et al.*, 1990); from the latter the production of reactive oxygen species (*e.g.* hydroxyl radical and superoxide anion) and the 1-hydroxyethyl radical is derived, both being effective agents destructing lipid and other oxilabile components of microsomal structures (Albano *et al.*, 1991; Nordmann *et al.*, 1992). Our results show that even a short-term massive intoxication of rats by ethanol causes a similar mechanism of an intensified lipid peroxidation within the liver microsomes.

This finding is at variance with the ascertainment of the authors (Remmer *et al.*, 1989) who, after an i.p. intoxication of animals by alcohol, did not find any changes in the expired quantity of ethan and *n*-pentan, nor in other indicators of the oxidative processes. On the contrary, our results are comparable to those of Uysal *et al.* (1989b), with the difference, however, that this group has proved the maximum increase in lipid peroxidation in the liver mitochondria. These and similar differences in the experimental results often appear owing to different arrangements of the experiments in question and as a result of diverse criteria applied to the evaluation of the pathobiochemical consequences of the toxic effect of ethanol.

Unlike the results published by other authors (Uysal *et al.*, 1989a), our data show that after the acute ethanol intoxication, organelles from the whole brain tissue were not oxidatively changed. This circumstance may have been caused by a

short-term application of the noxa as well as by the fact that in the brain the intensity of oxidizing catabolism of ethanol appears too small, so that even the production of reactive oxygen species proves little effective.

The oxidative changes in liver microsomes we have found as a consequence of a short-time excessive use of ethanol may etiologically be connected with the damaging to liver tissue on the same principle that comes into force in a chronic, long-term use of lesser doses of alcohol. Hence our assumption that a sufficient antioxidative protection of the organism (*e.g.* by means of some biologic antioxidants) might be able to moderate these harmful effects of alcohol in a desirable way, appears quite substantiated.


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