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Serum Enzymes as Markers of Liver Injury in Long Term Ethanol Consumption

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Abstract

The effects of long-term ethanol consumption (10 weeks and 30 weeks) on the activities of the marker liver injury enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) were investigated in rat serum. The alcohol dehydrogenase (ADH) activity, the enzyme directly involved in the metabolism of ethanol, was also evaluated.

The serum ADH activities significantly increased after both periods of ethanol administration. The electrophoretic patterns showed a new anodic band with ADH activity in serum of 30 weeks ethanol treated rat.

The chronic ethanol consumption affected the aminotransferases and alkaline phosphatase activities in rat serum. The changes in the activities of these enzymes were more marked for the long periods of ethanol administration. At 30 weeks of ethanol consumption the electrophoretic patterns of AST and AP were also changed.

Keywords: chronic ethanol consumption, alcohol dehydrogenase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase

Introduction

The liver is the main organ involved in the oxidation of ingested ethanol, but other tissues, may contribute to the ethanol metabolism as well [1]. Ethanol is absorbed mainly in intestine, where it is channeled through the porta vein directly towards liver before passing through the circulatory system and the rest of the body. Three enzymatic systems are able to carry out ethanol oxidation: the alcohol dehydrogenase (ADH), the microsomal ethanol-oxidizing system (MEOS) and the catalase (CAT). ADH metabolizes most of the ingested ethanol. This enzyme catalyzes the oxidation of ethanol into acetaldehyde with the reduction of nicotinamide adenine dinucleotide (NAD^+). The MEOS system couples ethanol and nicotinamide adenine dinucleotide phosphate oxidation (NADPH) to the reduction of an oxygen molecule to form hydrogen peroxide and these transformations require cytochrome P-450 participation [2]. In the third system, the oxidation of a molecule of ethanol into acetaldehyde is coupled with the simultaneous decomposition of a hydrogen peroxide molecule in a reaction catalyzed by catalase. The contribution of catalase to ethanol metabolism has been long questioned because the ethanol elimination was not affected by the treatment with aminotriazole, a catalase inhibitor [3, 4].

The alcoholic liver injury appears to be generated by the effects of ethanol metabolites and the toxic effects of acetaldehyde may be induce by acetaldehyde-altered proteins [5]. Ethanol produces severe changes (fatty liver) that may be potentially reversible (alcoholic

hepatitis), or virtually irreversible (alcoholic cirrhosis) [6]. The duration and dose of ethanol administration are factors that influence the risk of liver injury [7].

The aim of this research was to assess the effects of ethanol consumption, on the activities of some serum enzymes that are markers for liver injury: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP). The alcohol dehydrogenase activity, the enzyme directly involved in the metabolism of ethanol, was also assessed. We have also studied the correlation between the changes and the duration of ethanol consumption.

Materials and Methods

Animals. Thirty-two healthy, male, Wistar rats weighing 140-160 g were housed two per cage under controlled conditions of a 12 hours light/dark cycle, 50% humidity and 24^{o}C. Before the experiments began, rats were monitored daily and had free access to water and standard pellet diet (10g/100g body weight/day). After one week of acclimation, the animals were randomly divided into two groups of sixteen each. Group 1, the control group, continued to receive water for fluid. Group 2, the ethanol-fed group, was treated daily with 1ml of 35% ethanol, equivalent to 2g/kg body weight, as an aqueous solution, using an intragastric tube. After 10 weeks, eight rats of each group were killed by cervical decapitation under light ether anesthesia and blood was collected. The remained rats of each group were sacrificed in the same conditions after 30 weeks. Blood samples were collected from both ethanol-treated and control groups.

Enzyme assays. The ADH (EC 1.1.1.1) activity was assayed using the method of Vallee (1978) [8] with a slight modification. Recording the changes in absorbency at 340 nm for 5 minutes after enzyme addition followed the conversion of NAD^+ to NADH, as a measure of the ADH activity. The results were calculated as units (U), one unit was expressed as μmole of NAD^+ consumed per minute.

Pyruvate, the product of ALT, was measured with lactate dehydrogenase as indicator enzyme and NADH [9]. The amount of oxaloacetate produced by AST action, was determined by enzymatic methods with NADH and malate dehydrogenase as indicator enzymes [10]. Both transaminases activities were expressed as U/l. One unit will cause the transformation of one μmole of NADH in a minute.

Total AP activity was measured by means of Walk procedure [11]. One unit of AP activity was calculated as μmole of 4-nitrophenol per minute. The extinction coefficient 18.5 cm^2 $\mu mole^{-1}$ of 4-nitrophenol in alkaline solution at 405 nm was used for the calculation. All activities of the enzyme were expressed as U/l.

Protein concentration in serum was determined by Lowry method [12].

Electrophoresis. The serum proteins were separated under non-denaturing conditions in 7.5% (w/v) polyacrylamide slab gel using a MIGET LKB Pharmacia apparatus. The fractions presenting alcohol dehydrogenase activities were separated by native electrophoresis, in 7.5% polyacrylamide gel, and detected using tetrazolium systems [13]. The AST isoezymes were detected by the specific and spontaneous reaction between oxaloacetate and Fast Blue BB [14]. Alkaline phosphatases were visualized following electrophoretic separatation using β-naphthyl phosphate as substrate and the colored reaction of β-naphtol and Fast Blue RR, using a method adapted from Rothe (1994) [15].

Statistical analysis. All values were expressed as means ± SEM. The differences between control and ethanol-treated experimental groups were compared by Student's t test using standard social science statistical packages. The results were considered significant if the value of p was less than 0.05.

Results

Table 1 summarizes the results concerning the enzyme′s activities in serum of control and ethanol treated rats.

Table 1. Serum enzymes activities after 10 and 30 weeks of ethanol treatment[1]

Enzyme (U/L)	10 weeks experiment		30 weeks experiment	
	Control	Ethanol	Control	Ethanol
ADH	67.8 ± 3.4	69.4 ± 5.1	59.4 ± 5.2 **	72.5 ± 6.7*
ALT	39.2 ± 2.6	49.5 ± 3.1*	32.5 ± 3.1	63.5 ± 4.7*
AST	60.7 ± 4.8	81.5 ± 5.3*	57.3 ± 2.8	102.1 ± 8.9*
AP	82.5 ± 3.2	129.3 ± 7.5*	75.6 ± 7.6	163.2 ± 10.5*

* Significantly different from control at P< 0.05.
** Significantly different between 10 weeks and 30 weeks controls, P< 0.05.
[1] Mean ± SEM

Figure 1 shows ADH isoenzyme patterns in serum of alcoholic rats as compared with the normal patterns. In **Figure 2** and **Figure 3** are presented the electrophotetic patterns of AST and AP, respectively, from normal and ethanol treated rats.

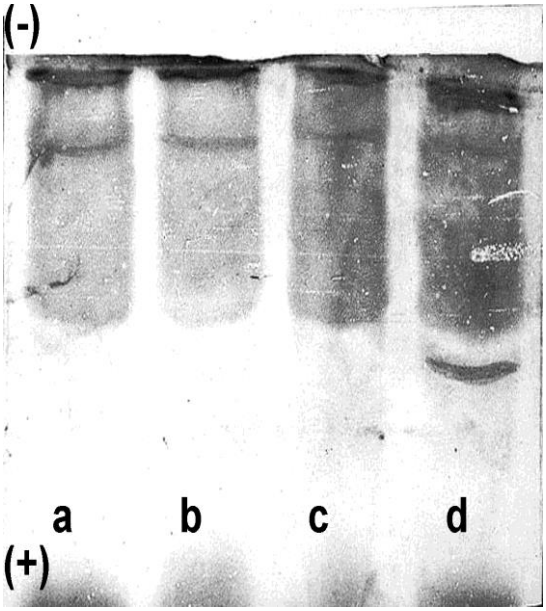

Figure 1. Isoenzymes of serum alcohol dehydrogenase: (a) control for 10 weeks; (b) 10 weeks ethanol exposure; (c) control for 30 weeks; (d) 30 weeks ethanol exposure.

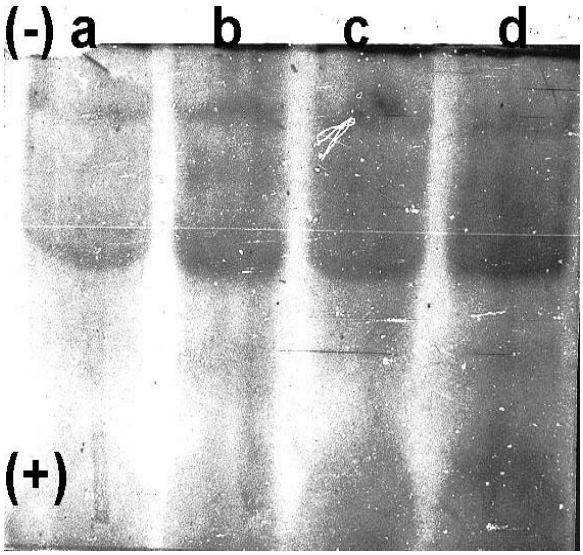

Figure 2. Electrophoretic pattern of serum aspartate amonotransferase: (a) control for 10 weeks; (b) 10 weeks ethanol exposure; (c) control for 30 weeks; (d) 30 weeks ethanol exposure.

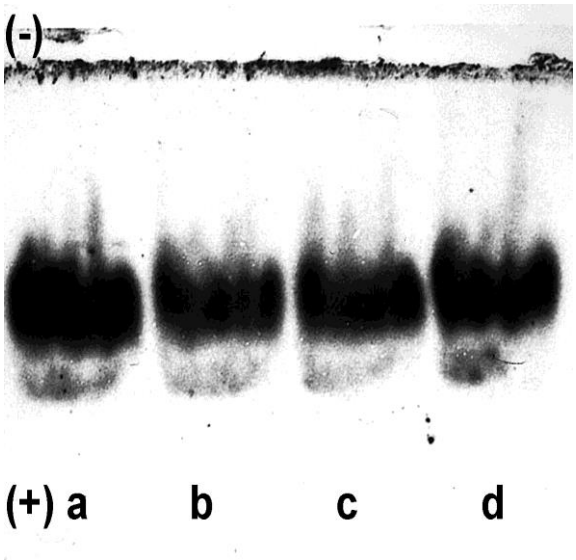

Figure 3. Polyacrylamide gel electrophoresis of alkaline phosphatase in rat serum: (a) 10 weeks ethanol exposure; (b) control for 10 weeks; (c) control for 30 weeks; (d) 30 weeks ethanol exposure.

Discussions

Elevated levels of serum enzymes are frequently associated not only with alcohol related organ damage, but also with excessive alcohol consumption and alcoholism without significant tissue injury.

Serum ADH activity decreased from 67.8 ± 3.4 in 10 weeks control rats to 59.4 ± 5.2 in 30 weeks control rats (**Table 1**). It has been shown that advanced age results in a decreased first, pass metabolism of ethanol with elevated serum ethanol concentrations. It is still unknown if this situation is due to age by itself or to others factors, like atrophic gastritis with

decreased activity of ADH [16]. No significant changes in serum ADH activity was observed after 10 weeks of ethanol administration, while after 30 weeks of treatment the activity of this enzyme increase by 122%. Alcohol dehydrogenase activity of human and animal blood serum was intensely investigated both in acute and chronic ethanol intoxication, but the results are still controversial. Our experimental results have indicated that the serum alcohol dehydrogenase activity was influenced by the duration of ethanol administration. Our results agree with the previous studies reporting a relatively low increase in the level of blood serum alcohol dehydrogenase in chronic alcoholic rats [17], while short-term ethanol ingestion (<1 month) had no significant effect on the activity of serum alcohol dehydrogenase [18].

The electrophoretic patterns revealed two alcohol dehydrogenase isoenzymes in the serum of control rats (**Figure 1 a, c)**, analog results being previously reported in human serum [19]. The intragastric ethanol administration for 10 weeks had no influence on these two ADH isoforms (**Figure 1 b**). In the serum of rats treated for 30 weeks, a third anionic band with alcohol dehydrogenase activity has been noticed (**Figure 1 d**). This new isoenzyme is, probably, a subunit of the oligomeric enzyme. The new electrophoretic band, with a lower alcohol dehydrogenase activity, may be due to the decrease of zinc level, a metal involved in the quaternary structure of the enzyme. Zinc level in alcoholic serum is known to be decreased [20]. The appearance of this new molecular form is correlated with the relatively low elevation of the ADH activity after 30 weeks of ethanol ingestion. These changes in ADH activity and isoforms may be due to the damage of liver or/and other organ induced by the long-term ethanol administration.

The chronic ethanol consumption affected the aminotransferases activities in rat serum. The changes in these enzyme′s activities were more marked for the long periods of ethanol administration. Thus, the ALT and AST activities increased by 26.9% and by 34.4% after 10 weeks of ethanol ingestion (**Table 1**). After 30 weeks of treatment, a two- fold increase (95.4%) in ALT activity and a 78.2% increase in AST activity was recorded (**Table 1**). Increases in serum aminotransferases activities during long periods of ethanol consumption were reported [21, 22]. An elevated level of serum aspartate and alanine aminotransferase can be considered as highly suggestive for the alcoholic etiology of liver injury.

The electrophoretic studies showed two bands with AST activity, one for the citoplasmatic isoenzyme and the other for the mitochondrial form. (**Figure 2**). For both periods of ethanol treatment, the activity of isoenzyme with anodic migration was more evident (**Figure 2 b,d**).

Compared to the control diet, chronic alcohol administration resulted in a significant enhancement of serum AP by 56.7% (10 weeks) and 115.8% (30 weeks), respectively. The up-regulation of this enzyme after chronic ethanol administration was previously reported. Thus, Nishamura and Teschke [23] reported an 80% increase in alkaline phosphatase activity in serum of rats fed for 6 weeks with an ethanolic diet.

Two bands with AP activity were found in serum of rats (**Figure 3**). In chronic ethanol administration the two isoform of this enzyme were more intense, and this observation may be related to hepatic disease (**Figure 3 a, d**). The presence of fast moving fraction of serum alkaline phosphatase (AP-1) in serum might be a sensitive indicator of liver damage induced by alcohol consumption [24].

Conclusion

The data obtained confirm the earlier observations concerning the dependence of risk of liver injury on the period of ethanol administration.

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