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Does a single dose of alcohol cause oxidative stress in the pancreas?

Tek doz alkol pankreasta oksidatif strese yol açar mı?

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ÖZET: *Pankreatit etyolojisinde kronik alkol alımının rolü iyi bilinmekle birlikte akut yüksek doz alkolün de pankreatit yapabileceğini gösteren az sayıda çalışmada mevcuttur. Deneysel pankreatitlerde serbest oksijen radikallerinin önemli rol oynadıkları gösterilmiştir.*
Çalışmamızda akut yüksek doz alkolün pankreasta (serbest radikale bağlı oksidatif stresin indirekt göstergeleri olan) malondialdehid (MDA) oluşumu ve glutatyon (GSH) azalmasına neden olup olmayacağını araştırdık. Ayrıca eğer oluşursa, histopatolojik hasarı tesbit etmek istedik.
Çalışmada kullanılan ratlar iki gruba ayrıldı. Kontrol grubuna (5 rat) herhangi bir kimyasal madde verilmedi. Çalışma grubuna (10 rat) ise tek doz etanol verilerek (0.5 gr/kg vücut ağırlığı, etanol % 40 vol/vol, intraperitoneal) pankreasta protein (0.54 ± 0,10 ve 0,54 ± 0,13), MDA (6,52 ± 1.04 ve 5.34 ± 0,55) ve GSH düzeyleri (0.14 ± 0,06 ve 0,14 ± 0,08) ölçüldü ve anlamlı bir değişme olmadığı gözlendi. Işık mikrobik incelemede de histopatolojik hasar görülmedi.
Sonuç olarak parenteral yolla tek doz alkol pankreasta oksidatif strese ve histopatolojiik hasara neden olmamaktadır.

Anahtar Kelimeler: **Alkol, pankreas, malondialdehid, glutatyon**

SUMMARY: *In the etiology of pancreatitis, the causative role of chronic alcohol ingestion is well established, however, there are also rare studies indicating pancreatitis due to acute large doses of ethanol. Oxygen free radicals are shown to have an important role in the pathogenesis of experimental pancreatitis.*
Our aim was to investigate whether a single dose of ethanol causes malondialdehyde (MDA) formation and glutathione (GSH) depletion which are indirect indicators of free radical-mediated exidative stress. And in the case that this occured, we wanted to asses histopathologic damage after alcohol.
In the study, rats were used and divided into two groups. Control group (5 rats) did not receive any chemicals. After a single dose of ethanol (0,5 gr/kg bw. of ethanol, % 40 vol/vol, intraperitoneally) had been infused to the study group (10 rats) of animals, no significant change was observed in protein (0.54 ± 0.10 vs. 0.54 ± 0.13), MDA (6.52 ± 1.04 vs. 5.34 ± 0.55) and GSH levels (0.14 ± 0.06 vs. 0.14 ± 0.08) in pancreas. Also, there was no histopathologic change after ethanol by light microscopy. In conclusion, a high single dose of ethanol given with parenteral route does not cause oxidative stress and histopathological damage in pancreas.

Key Words: **Alcohol, pancreas, malondialdehyde, glutathione**

CHRONIC alcohol abuse is the most important cause of pancreatitis in western countries. Generally, after several years of alcohol abuse, attacks of pancreatitis may be seen (1). But some reports suggest the possibility of alcohol induced pancreatitis without prior history of chronic alcohol abuse (2). Acute parenteral ethanol administration may result in the increase, decrease, or no change in pancreatic exocrine secretion (3).

While ethanol increases activity of proteolytic and lysosomal enzymes, it decreases trypsin inhibiting capacity (4, 5). Ethanol may alter the fluidity and permeability of lipoprotein membranes of cells and intracellular organels (6).

By triggering premature proteolytic enzyme activation and altering transport mechanismis in the golgi-complex, ethanol may lead to acinar cell destruction (1).

Oxygen free radicals are potent intermediate metabolites having an important role in the pathogenesis of several disease states (7-10). The offending molecules are superoxide (O^{-2}), hydrogen pe-

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Table 1. Protein levels of alcohol-treated and control groups

Control Group (n=5)	*Alcohol Group (n=10)*
0.49	0.47
0.56	0.40
0.72	0.79
0.51	0.64
0.41	0.46
	0.34
	0.67
	0.62
	0.58
	0.49
Mean 0.54±0.10	*0.54±0.13*

Table 2. MDA levels of alcohol-treated and control gruops

Control Group (n=5)	*Alcohol Group (n=10)*
7.59	5.34
7.88	5.00
5.74	5.68
5.17	6.13
6.24	6.59
	5.40
	5.00
	5.12
	5.05
	4.90
Mean 6.52±1.0	*5.38±0.55*

roxide (H_2O_2) and hydroxyl (OH*) radicals. Through the effect of free radicals, lipid peroxidation which results in malondialdehyde (MDA) formation and glutathione (GSH) depletion occur (11). In experimental models of pancreatitis it was shown that free radicals play important roles in tissue injury (12, 13). Our aim was to investigate whether a single dose of ethanol can induce lipid peroxidation, GSH depletion and inflammation in the pancreas.

MATERIALS AND METHODS

Female Wistar rats weighing 150-200 gr were used in all experiments. The animals were divided into two groups. Five animals served as control. The ethanol-treated rats (10 animals) received a single dose of ethanol (0,5 gr/kg b.w. of ethanol 40 % V/V, intraperitoneally) (13). The control group did not receive any treatment.

The ethanol-treated animals were killed 24 hours after ethanol administration and their pancreases were rapidly removed, frozen in liquid N_2 and kept at -70°C until analysis. The control animals were killed and their pancreases were exposed to the same procedure. Tissues were homogenized with 0,15M KCL to make a %10 homogenate.

Protein levels were measured by the Lowry method in the homogenate and lipid peroxidation was determined by malondialdehyde (MDA) formation, using the Thiobarbituric acid method (14,15). The results were expressed as nanomoles MDA per mg of protein.

For the determination of Glutathione, centrifuged (GSH) a piece of tissue homogenized with 1 ml metaphosphoric acid and centrifugated 3500 rpm for 10 minutes. 0,2 ml of supernatant was added to 0,3 ml of 1 M KPO4 (pH: 8), 2,4 ml of Ellman reactive and 0,1 ml distilled water. Thiol groups were assayed by the Ellman method (14).

After tissue specimens had been stained with Hematoxylen-Eosin, they were examined by light microscopy.

Results were analyzed by using Mann-Whitney U test.

RESULTS

Mean protein concentrations in pancreatic tissue were 0,54 ± 0,10 mg/ml in control animals. Protein values of alcohol-treated group were 0,54 ± 0,13 mg/ml.

There was no significant difference between the two groups. ($P > 0.05$) (Table I). Pancreatic MDA values were 6,52 ± 1,04 nmol MDA/mg protein in the control group. After alcohol treatment, MDA values were 5,37 ± 0,55. (Table II) This difference is insignificant ($P > 0.05$)

GSH concentrations of the alcohol group were 0,14 ± 0,08 μmol GSH/mg protein. The control group's values were 0,14 ± 0,06, and the difference is again insignificant ($P > 0,05$) (Table III). After staining with Hematoxylin - Eosin, pancreatic tissue specimens of both groups were examined by light microscopy. No histopathological alteration was observed after alcohol administration.

DISCUSSION

There are several hypotheses regarding the pat-

Table 3. GHS levels of alcohol-treated and control groups

Control Group (n=5)	*Alcohol Group (n=10)*
0.24	0.23
0.16	0.29
0.08	0.09
0.14	0.06
0.09	0.11
	0.04
	0.15
	0.08
	0.24
	0.07
Mean 0.14±0.08	*0.14±0.06*

hogenesis of alcohol-induced pancreatic injury. One of them is the toxic-metabolite theory (1). According to this theory ethanol has a direct deleterious effect on the pancreas (4). While ethanol - induced acute injury of liver can be elicited easily, it is difficult to achieve this in the pancreas. Indeed, the majority of the results of many previous studies favor the above statement above (2, 16).

In previous studies to create experimental models of acute alcohol-induced injury of pancreas, ethanol ingestion was required for several weeks (4,12). The existence of some reports suggesting the acute injuring effect of ethanol on the pancreas has encouraged us to try pancreatic injury with a single dose of ethanol (2, 6). In our study, no histopathological change was observed after alcohol treatment.

Using ethanol in the same way and with the same dose, Battiston et al demonstrated GSH depletion in liver (17) GSH is pivotal to molecule detoxifying reactive toxic substances and free radicals. GSH levels may be an indirect indicator of toxic metabolite burden and detoxifying capacity in any tissue (17).

Many authors showed that oxygen free radicals are important mediators in pancreatic inflammation (11-13). In their studies, macroscopic and histopathological changes of the pancreas were induced by using strong stimuli such as longer and higher doses of ethanol consumption, caerulein infusion and bile duct ligation (12). There was no change in the level of GSH in the pancreas after ethanol in our study.

Ethanol may cause lipid peroxidation which results in MDA formation in the liver (18). Also, in experimental models of pancreatitis using strong stimuli MDA production has been demonstrated (11, 12). Thus our MDA values are not in accord with the literature. Probably, the stimulus used in our study was much weaker than the others. Alcohol is largely metabolized in teh liver. The liver has two major ethanol metabolizing systems; the alcohol dehydrogenase (ADH) and microsomal ethanol oxidising system (MEOS) (19). A pancreatic ADH was described in recent years (20). It is unknown whether MEOS is present in the pancreas. ADH is most abundant in hepatocytes (19). Through the effect of ADH and MEOS, ethanol is converted to acetaldehyde which is the most toxic product of the ethanol metabolism (21). Acetaldehyde itself is highly reactive and induces free radical production (18). Ethanol-induced tissue damage is possibly due to acetaldehyde and its degradation products rather than ethanol itself (21).

The cause of the different effects of ethanol on the liver and pancreas may be related to the different levels of ethanol metabolizing enzymes in the two organs. In our opinion, because of the low levels of ADH in the pancreas, small amounts of acetaldehyde which are insufficient to cause tissue damage must be produced. In the light of these findings, it seems that histopathological destruction and free radical production are closely interrelated and concomitant. An agent not causing histopathologic damage in pancreas seems not to induce free radical production or vice versa.

In conclusion, ethanol did not cause free radical production, and tissue injury in the pancreas because of the low level of ethanol metabolizing enzymes and consequently there were small amounts of acetaldehyde production. But it may be possible to cause lipid peroxidation and GSH depletion by using alcohol in higher doses and for a longer duration.

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