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SHORT COMMUNICATION

Effect of Short-term Ethanol Intake on Antibody-dependent Cell-mediated Cytotoxicity (ADCC) In Rats

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Summary

The present study was designed to evaluate the effect of short ethanol intake on ADCC activity in blood and spleen mononuclear cells.Wistar rats were fed a standard diet and drank 0.1 M ethanol solution for three days. Glucose and water controls were used in this experiment. Increased ADCC activity was found in ethanol consuming rats in the blood and spleen as compared to both controls. Our findings support the assumption that ADCC may play an important role in liver disease of alcoholics.

Key words

Ethanol intake - Rats - ADCC

Ethanol is a dietary component with a multiform effect on the immune system. Many investigators have observed different immunologic abnormalities: allergy to skin tests (Straus *et al.* 1971), reduced lymphocyte transformation (Zetterman *et al.* 1973), increased susceptibility to infection (Adams *et al.* 1984, Straus and Berenyi 1973) and also changes in ADCC activity (Stacey, 1984). ADCC is an important mechanism in host defence against various pathogenic microorganisms (Shore *et al.* 1974), parasites (Butterworth *et al.* 1975) and tumours (Hakala *et al.* 1974, Feldman *et al.* 1975). Recent findings suggest a possible role of enhanced ADCC in autoagressive disease, e. g. liver damage in alcoholics (Ishimara and Matsuda 1990).

Adult male Wistar rats (Velaz Prague) weighing between 380-400 g were kept in groups and fed an LD diet (15. 7 MJ/kg, Velaz, Prague). Before the experiment the animals were housed singly and divided into three groups (n=5): ethanol, glucose and water. Their feeding was determined in nutritional pilot experiments to eliminate the energetic influence of ethanol. Thus, the diet regime of the experimental groups was comparable to that of normal rats as far as the energetic intake is concerned. In pre-experiments we found that rats from ethanol group with free access to ethanol (0. 1 M) and to LD diet ate more on day 1 as compared to both controls. On day 3 their energetic input was the same as in the control groups. A diet regime of glucose as control of the energetic content of liquid and solid parts was found comparable to the ethanol group. However, the animals ate less and consequently, their energy input was below normal values. So, their diet regime was set up in such a way that the total energy input was divided into approximately the same parts between liquid and solid constituents of the food. Accordingly, the input did not differ from the water control. Water control had an LD diet and water *ad libitum*. Fluid and food consumption as well as body weight were recorded daily for each animal. All animals were kept under natural lighting conditions at 22-25 °C. After three days the animals were anaesthetized with pentobarbital (40 mg/kg b. w.). Blood samples and the spleen were asepticaly removed from the rats.

Effector cells

Rat blood was collected in heparin (15 IU/ml) and diluted 1:2 with phosphate buffered saline (PBS). The spleens were minced and gently passed through a nylon net. Mononuclear cells were separated on Ficoll-Verografin, rinced twice with PBS and resuspended in a tissue culture medium (TCM). TCM contained MEM supplemented with 7 % bovine serum, 100 μU/ml penicilin, 100 μg/ml streptomycin, 2 mM glutamine, 22.3 mM $NaHCO_3$ and 10 mM HEPES. Cell suspensions were counted and adjusted to

Table 1
Energy intake in the solid and fluid parts of diet and total energy intake in the experimental days (kJ/rat/day)

Part of diet	Group	Day 1st	2nd	3rd
Solid	Et	356.8 ± 13.0	272.3 ± 14.0	194.1 ± 32.4
	G	156.5 ± 53.6	193.8 ± 38.1	178.4 ± 22.8
	W	328.6 ± 27.0	349.5 ± 23.9	276.5 ± 63.3
Liquid	Et[§]	46.9 ± 9.8	51.5 ± 5.0	40.2 ± 1.3
	G	162.4 ± 0.0	178.2 ± 0.0	139.2 ± 0.0
	W	0.0	0.0	0.0
Total	Et	403.7 ± 11.9	323.8 ± 14.2	324.2 ± 33.0
	G	322.5 ± 49.8	372.0 ± 38.2	317.6 ± 22.0
	W	284.8 ± 39.1	339.6 ± 4.4	280.0 ± 37.6
Significance of differences				
	Et vs. G	0. 05	0. 02	N. S.
	Et vs. W	0. 50	N. S.	N. S.
	G vs. W	N. S.	N. S.	N. S.

Abreviations: Et = ethanol, G = glucose, W = water group; N. S. = not significant. Values are the mean ± S.E.M., each group n = 5 animals. [§] average of 0.1 M ethanol was: 8.0 ± 0.9; 10.7 ± 0.9; 7.3 ± 0.9 g/100 g b.w. on the 1st, 2nd and 3rd day, respectively

Table 2
Energy intake in solid and liquid parts of diet and total energy intake (kJ/rat/day)

Group	Portion of diet Fluid	Solid	Total
ET	46.2 ± 7.6	274.4 ± 71.7	320.6 ± 74.5
G	159.9 ± 16.6	179.3 ± 40.4	336.2 ± 45.5
W	0.0	301.6 ± 43.8	301.6 ± 43.8

No significant differences were found in total energy intake among the groups. Abbreviations - see Tab. 1

$8x10^6$/ml. The viability was 98 % determined by trypan blue.

Target cells

Chicken red blood cells (CRBC) served as targets. $3x10^7$ CRBCs were labelled for 1 h at 37 °C with 3.7 MBq of $Na_2{}^{51}CrO_4$ in PBS. After two washings in PBS, the cells were counted and adjusted to $8x10^5$/ml in TCM.

Antisera – xenogenic rabbit antiserum against CRBC was used.

ADCC *in vitro* system

ADCC was studied by a ^{51}Cr-release method. Our system contained 100 μl of effector cell suspension ($8x10^5$ cells), 50 μl of ^{51}Cr labelled CRBC suspension ($4x10^4$) and 50 μl of antiserum (200x diluted) in each well of a round bottom microtitre plate. The ratio of effector to target cells was 20:1. This represents experimental release (E). Each experiment included

control wells. Spontaneous ^{51}Cr release (S) contained the target cells and effector cells with nonimmune rabit serum. Maximal ^{51}Cr release (M) contained target cells and 3 % Triton X-100 . The samples were measured in duplicate. The percentage of specific ^{51}Cr release in ADCC was calculated according to the formula:

$$\% \text{ ADCC} = (E\text{-}S)/(M\text{-}S) \times 100$$

The differences in energy intake were analysed by Student's t-test in the case of agreement of standard deviations. In the case of disagreement of standard deviations the Welch method was used. The differences between groups in ADCC activity were analysed by the Mann-Whitney's test.

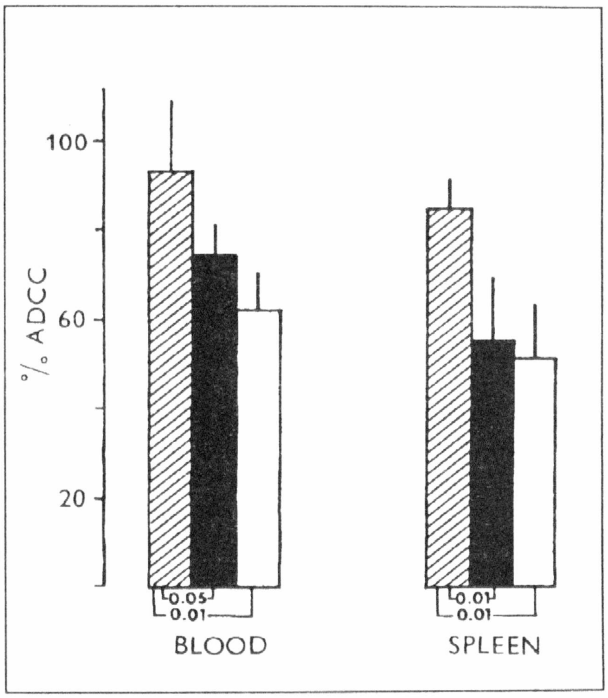

Fig. 1

Effect of ethanol (0.1 M) on ADCC activity (%) in organs of rats. Each column is the mean ± S.E.M. of two experiments (n = 5) performed in duplicate. The numbers under the columns are statistical significance levels.

Tab. 1 presents the energy intake of the solid and fluid part of the diet and total energy intake on each experimental day. No significant differences in total energy intake between the glucose and water groups were found throughout the experiment. On the 1st day, a significantly higher energy intake was found in the ethanol group compared to both controls. On the 2nd day, there was no significant difference between the ethanol and water groups and the significance of the difference between ethanol and glucose fell ($p < 0.02$). On the 3rd day, we found no significant differences among the experimental groups.

Fig. 1 shows that, in the ethanol group, ADCC was increased in the blood and spleen compared to both controls. There was no difference in ADCC between the water and glucose groups.

In alcoholics, the changes of liver functions are undoubtly the most often followed changes. Their impairment is considered to be a result of an altered immune system, mainly NK and ADCC activity. Therefore, our work tried to elucidate the effect of ethanol on the ADCC reaction. Our results show that ADCC increases in the blood and spleen. To find the effect of ethanol on ADCC activity, adequate control groups are needed. When comparing the results of various studies relating to this problem, the ethanol dose used is of importance. The first factor will be discussed in more detail. Ethanol is a diet component which has specific properties and those common with other substances. To eliminate the latter, saccharide controls are often used. On the other hand, saccharide controls involve the risk of inhibition of CMC (cell-mediated cytotoxicity) activities, as in the case of NK activity for various glycides (Ades and Hinson 1983, Vose *et al.* 1983). The ADCC reaction was found to act similarly (Orthaldo *et al.* 1984), that means that saccharides could cause a transient increase in ADCC values in the ethanol group. The use of water control may avoid this effect. In our work both controls were used. Fig. 1 shows that ADCC activity was equal in both controls. This suggests that enhanced ADCC in the ethanol group is caused exclusively by the specific properties of ethanol. Besides this, ADCC can be changed owing to different energy inputs in the controls. Table 1 presents the energy input in detail. It follows from this that no differences in mean energy input were found during the experiment. The increase in ADCC in the ethanol groups found in our study is also in agreement with the changes of other CMC, T cell and NK activity (Mufti *et al.* 1988, Saxena *et al.* 1981). The mechanism of ethanol on ADCC reaction is not clear yet. However, it is known that alcohol modifies FcR expression on effector cells (Bagasra *et al.* 1988) and subpopulations of T lymphocytes (Ishimaru and Matsuda 1990).

This study provides an additional link between alcohol and the immune system. Increased ADCC activity induced by ethanol may enhance (or even initiate) liver damage in alcoholics. In view of the wide incidence of alcoholic cirrhosis, our findings could contribute to understanding the way in which CMC modulate liver functions and its structure.

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