THE EFFECT OF ETHANOL TREATMENT ON SURFACE ALKALINE PHOSPHODIESTERASE I ACTIVITY OF CULTURED HUMAN MESANGIAL CELLS

Vladisav Stefanović, Predrag Vlahović, Marina Mitić-Zlatković

Institute of Nephrology and Hemodialysis, Faculty of Medicine, Niš, Yugoslavia

Summary. Human mesangial cells grown in culture have been employed to investigate the interaction between exposure to ethanol and alkaline phosphodiesterase I activity at the plasma membrane of intact cells. Chronic exposure of human mesangial cells to ethanol induced a dose-dependent rise in enzyme activity. Similar effect was observed after the treatment of mesangial cells with methanol. Short-term (1-3 h) exposure to ethanol was also found to cause a dose-dependent stimulation in enzyme activity, but an inhibition at concentrations higher than 0.5%. A possible mechanism involving a change in membrane fluidity was proposed for the observed effects of ethanol on mesangial cell surface alkaline phosphodiesterase I.

A direct role for ethanol in the pathogenesis of IgA nephropathy was previously suggested, and his study opens a way to understand mesangial cell membrane changes upon ethanol exposure.

Key words: Ethanol, mesangial cell, alkaline phosphodiesterase I

Introduction

Alcoholism is a major problem in many countries. Several in vitro studies have elucidated effects of ethanol on the nervous system (1-4). An increased Na⁺⁺K⁻⁻-ATPase activity in erythrocytes from alcoholics was demonstrated, however, erythrocytes incubated in vitro with ethanol failed to show similar pump changes (5). The plasma membrane Ca²⁺-ATPase from human erythrocytes can be stimulated by ethanol, with a maximum velocity of the enzyme about 2.4 fold higher than in control (6). Ca²⁺ transport by inside-out vesicles was also stimulated by ethanol, showing the same concentration dependence as the Ca²⁺-ATPase activity.

Effect of ethanol on kidney function is poorly understood. In a water diuretic anesthetized rat model ethanol infusion was associated with an immediate increase in urine flow and fractional sodium excretion, which had returned to control level by 20 min despite a further increase in the plasma ethanol concentration. The urinary excretion of potassium, calcium and magnesium was not altered nor was glomerular filtration rate or renal plasma flow (7). Administration of ethanol to rats was associated with induction of cytochrome P450 enzymes not only in liver but also in kidney, principally in tubular cells (8,9). Chronic ethanol consumption in rats for 10 weeks increased renal (Na⁺⁺K⁻⁻)-ATPase activity, compared to acute inhibition by ethanol in vitro (10). Alcohol was found to inhibit rat liver and renal 11-beta-hydroxysteroid dehydrogenase activity, with plasma aldosterone levels significantly lower than in controls (11).

Recently, using the intragastric ethanol infusion model of IgA nephropathy, the hypothesis that mesangial changes commence prior to the deposition of IgA, was investigated (12). Using molecular techniques on cultured contractile mesangial cells a threefold increase in interleukin-6 mRNA expression in mesangial cells incubated with ethanol was demonstrated. These mesangial changes suggested a direct role for ethanol in the pathogenesis of IgA nephropathy.

The aim of this study was to investigate in vitro effect of ethanol on alkaline phosphodiesterase I activity in cultured human mesangial cells.

Materials and Methods

Materials. The following materials were purchased from the corresponding suppliers: cell culture medium and 0.05% trypsin-0.02% EDTA from Flow Laboratories (Irvine, Ayrshire, UK); fetal calf serum (FCS), penicillin G and streptomycine sulfate from Gibco (Grand Island, N.Y., USA); plastic flasks and dishes from Nunc (Roskilde, Denmark); collagenase, and p-nitrophenyl thymidine 5'-monophosphate from Sigma (St.Louis, Mo., USA).

Cell Culture. Human glomerular mesangial cells were isolated and characterized as previously described (13). Glomeruli were prepared by differential sieving and centrifugation from the cortex of human cadaver kidneys judged to be unsuitable for transplantation. After collagenase treatment, the digested glomeruli were seeded in Petri dishes and cultured in RPMI 1640 medium supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 ug/ml streptomycin and 10% FCS, and
buffered with 20 mM HEPES at pH 7.4. Cell cultures were grown at 37°C in a humidified 5% CO₂-95% air atmosphere. Mesangial cells which grew from glomeruli fragments reached confluence after 3-5 weeks. They were purified by successive subcultures and were studied after 3-5 passages. Mesangial cells were identified by light microscopy and indirect immunofluorescence. They have a stellate appearance, overgrow each other, and show a network of intracellular fibrils of myosin. They were not positive for anti-von Willebrand factor, antitroponin and anticytokeratin.

Alkaline Phosphodiesterase Assay. Surface activity was determined on cultures of mesangial cells in 24-well plates. Cells were rinsed with the substrate-free incubation solution containing 100 mM glycine buffer, pH 9.0, 100 mM NaCl and 2 mM zinc acetate. Incubation was started upon addition of 1.5 mM p-nitrophenyl thymidine-5'-monophosphate to the medium. Incubation was terminated by addition of 0.1 M NaOH and OD at 400 nM was determined.

Cell protein was estimated according to the method of Lowry et al (14). Enzyme activity was expressed as nmol of p-nitrophenol liberated per min and per mg of cell protein. Values are given as means ± SEM. Comparisons between groups were analysed using Student’s t-test and analysis of variance.

Results

Short-term effects of ethanol
The addition of ethanol for 1 and 3 hours to mesangial cell culture produced an increase of alkaline phosphodiesterase I activity of 27 and 57% at the 0.250% concentration (Fig.1). Further increase in ethanol concentration had an inhibitory effect, the activity being 77 and 62% from the control (p<0.001) at 1 and 3 hours of treatment with 1% ethanol, respectively.

Long-term effects of ethanol
Dose-effect curves, after 24, 48 and 72 hours of ethanol and 24 hours of methanol treatment are presented in Fig.2. Both ethanol and methanol treatments produced a significant increase in alkaline phosphodiesterase activity, maximal at 1% concentration (p<0.001).

The increase was near maximal after 24 hours and did not change over the 3 days. During these experiments there was no difference in the cell number or cell volume between ethanol-treated cells or controls.

Discussion
Alkaline phosphodiesterase I (EC 3.1.4) is a membrane bound enzyme that catalyses hydrolysis of polyribonucleotides that have a free 3'-hydroxyl group, sequentially liberating 5'-nucleoside monophosphates.

Cell surface antigen PC-1, which is characteristic of murine B cells has been identified as alkaline phosphodiesterase I (15). This enzyme has been described in several mammalian cells including kidney. In kidney alkaline phosphodiesterase I is present in the proximal tubule, essentially in the brush border membranes, and also in the distal tubule segments and the glomerulus (16). Ecto-alkalinephosphodiesterase I activity was demonstrated in both mesangial and epithelial glomerular cells, and its control of expression was studied (17,18). A specific role of alkaline phosphodiesterase in kidney disease was recently investigated (19).

In this paper acute and chronic culture with ethanol produced an increase in human mesangial cell ecto-alkaline phosphodiesterase I activity. This effect was recorded over 72 hours of culture and was reproducible. Another alcohol, methanol also produced dose-dependent increase in ecto-alkaline phosphodiesterase activity. Stimulation of enzyme activity by different alcohols was previously observed for the plasma membrane.
Ca\textsuperscript{2+}-ATPase (6). Other short chain alkyl alcohols (methanol, n-propanol and n-butanol) stimulated the Ca\textsuperscript{2+}-ATPase activity to the same extent than ethanol but with different efficacy.

The mechanism of increase of the ecto-alkaline phosphodiesterase I activity upon ethanol treatment was not studied. However, a similar increase in the sodium pump enzyme activity was related to the change in membrane fluidity (20). This is a real possibility, especially for the short term effects of ethanol upon mesangial cell plasma membrane. The ethanol-stimulated increase in sodium pump enzyme in HeLa cells was inhibited by cycloheximide (21). The reversal of ethanol effect indicated that the mechanism requires protein synthesis and is presumably therefore enzyme induction. Induction of alkaline phosphodiesterase I by chronic ethanol treatment is also possible.

A direct role of ethanol in the pathogenesis of IgA nephropathy was previously suggested (12). Major changes in this nephropathy are in glomerular mesangium. The possible ecto-enzyme changes of mesangial cells could play a role. The importance of an increase in mesangial cell ecto-alkaline phosphodiesterase I activity upon ethanol treatment remains to be elucidated. Further studies of this and other nucleotide splitting plasma membrane enzymes in the ethanol model of IgA nephropathy should be performed.

References

UTICAJ TRETIRANJA ETANOLOM KULTIVISANIH HUMANIH MEZANGIJSKIH ĆELIJA NA AKTIVNOST EKTO-ALKALINE FOSFODIESTERAZE I

Vladisav Stefanović, Predrag Vlahović, Marina Mitić-Zlatković

Institut za nefrologiju i hemodijalizu, Medicinski fakultet Niš

Kratak sadržaj: Humane mezangijske Ćelije u kluturi su korišćene za ispitivanje uticaja etanola na aktivnost alkalne fosfodiesteraze I u Ćelijskoj membrani. Hronično izlaganje mezangijskih Ćelija etanolu dovodilo je do dozno-zavisnog porasta enzimske aktivnosti. Sličan efekat dobijen je izlaganjem mezangijskih Ćelija metanolu. Kratkotrajno izlaganje (1-3 h) etanolu takođe je dovodilo do stimulacije enzimske aktivnosti zavisne od doze, međutim, u dozama većim od 0,5% nađena je inhibicija enzimske aktivnosti. Za objašnjenje uticaja etanola na alkalnu fosfodiesterazu I mezangijskih Ćelija, kao mogući mehanizam delovanja predložena je promena u fluidnosti plazme membrane. Prethodne studije sugerisale su ulogu etanola u nastanku sekundarne IgA nefropatije, a ovo ispitivanje otvara mogućnost za razumevanje pomena u plazma membrani mezangijskih Ćelija pod dejstvom etanola.

Ključne reči: Etanol, mezangijske Ćelije, alkalna fosfodiesteraza I