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Effect of Lipid Peroxidation on Glucose Transport in Astrocytes: Potentiation by Ethanol

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Astrocyte cells derived from rat brain cells (BRA-1 cells) have been cultured in the laboratory for many years. After several days of cultivation, the normal medium (F-10 + 10% FBS) was replaced by another one composed with F-10 and different concentration of ethanol (0, 0.75, and 2%). The cultured cells were allowed to grow for 24 hours or more. Using phase-contrast microscopy to observe the morphological changes of these cells, we found that at higher concentrations of ethanol (2%), the astroglial cells contracted their processes and shrank into polygonol cells. But those of the lower concentrations appeared to find no dramatic changes. Lipid-peroxidation was investigated by thiobarbituric acid (TBA) method. The cells treated with higher concentrations of ethnol were more labile to lipid peroxidation when sample were incubated in the presence of Fe^{2+} at 37°C, where as treated with 0.75% ethanol were also more vulnerable to lipid peroxidation than control, resulting in a dose-dependent increase in the amount of malonyldialdehyde (MDA) formed. The concentration dependent inhibtion of glucose transport using ^{14}C-deoxyglucose was potentiated by ethanol treatment. Result from this study indicated that a change in membrane structure due to acute ethanol treatment may result in an alteration of metabolic activities.

Keywords: Astrocyte cells, Ethanol, Lipid peroxidation, Glucose transport

There is increasing awareness that cells in culture can be a good model for studying the effects of ethanol on membrane structure and function[1]. In previous studies with primary rat brain astrocytes, we have reported the effects of ethanol on morphological appearance of these cells[2]. Ethanol exposure also altered the membrane lipid metabolism of these cells[3].

Glucose transport is an important aspect of cerebral glucose ultilization[4,5]. This type of facilitated transport has been examined using cultured cells of neural origin[6,7,8,9].

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Since cells are constantly exposed to high levels of oxygen and are subject to various types of oxidative insults, the extent of oxidative stress to this transport system has not been clearly elucidated. Using the electron spin resonance (ESR) techniques, it is now possible to use spin trapping agents to assess the extent of free radical generation in tissue and organ [10]. With the in vitro system, we have also demonstrated that ethanol participates in the free radical reactions in the formation of hydroxyethyl free radicals[8]. Because the hydroxyethyl free radicals have a longer half-life, they may cause more cellular damage than other types of reactive oxygen species (H_2O_2, O_2 · or · OH). An objective of this study is to examine the effect of ethanol on lipid peroxidation activity in the astrocytes in culture and to further explore whether the peroxidative insult may alter the glucose transport activity of these cells.

MATERIALS AND METHODS

Primary astrocyte preparation

The primary astrocye culture was derived from neonatal rat brain as described elsewhere [11]. Briefly, astrocyte-like cells were isolated from cerebral cortices of three-day-old rats and were maintained in our laboratory by continuous passage. All monolayer cultures were grown in 6 x10 mm plates in a medium containing 10% fetal calf serum in F-10 (Gibco, USA). Cells were grown in an incubator maintained at 37°C and in an atmosphere of 95% air and 5% CO_2. Using immunocytochemical staining techniques[12], these cells showed positive staining for glial specific filrilary protein (GFAP).

Assay of lipid peroxidation activity

Lipid peroxidation was assayed by the thiobarbituric acid (TBA) reagent after incubating the cell membranes with ADP, H_2O_2 and Fe^{2+} in the presence of different concentrations of ethanol. The reaction mixture for generation of free radicals was similar to that used in the spin trapping study[10] except that the spin trapping agent was omitted. The malonyldialdehyde (MAD) formed was complexed with thiobarbituric acid (TBA) and analyzed spectrophotometrically at 540 mm[4].

Uptake of 2-deoxyglucose (2-DG)

The ability of cells to take-up glucose was measured using the glucose analog 2-deoxy-D-glucose (2-DG). The procedure was a slight modification of that described by Cummins et al.[7]. Briefly, one milliliter of cell suspension was first preincubated at 37°C prior to adding 1mM (1 μ Ci) of ^{14}C-2-deoxy-D-glucose (New England Nuclear, Boston, MA) to initiated the transport reaction. The mixture was incubated for various time periods and reaction was terminated with 8 ml of ice-cold isotonic 0.32 M sucrose medium. The cells were sedimented in a refrigerated centrifuge at 5000 rpm for 10 min, washed twice and then transferred to 10 ml Aquasol (NEN, Boston, MA) for measurement of radioactivity with a Beckman scintillation counter.

RESULTS

Growth of RBA-1 cells

A rat brain astrocyte cell line (RBA-1 cells) derived from rat cerebrum was successfully grown in T-75 Falcon flask with defined medium. These cells exhibited morphology of the fibrous astrocytes as shown in Fig la. After subculturing the cells for 7 days, ethanol in concentrations varying from 0.5% to 2.0% (V/V) was added. The cultures were observed and photographed under an inverse phase contrast microscope and camera assembly 48 hrs later. Figure 2 shows a dose-dependent

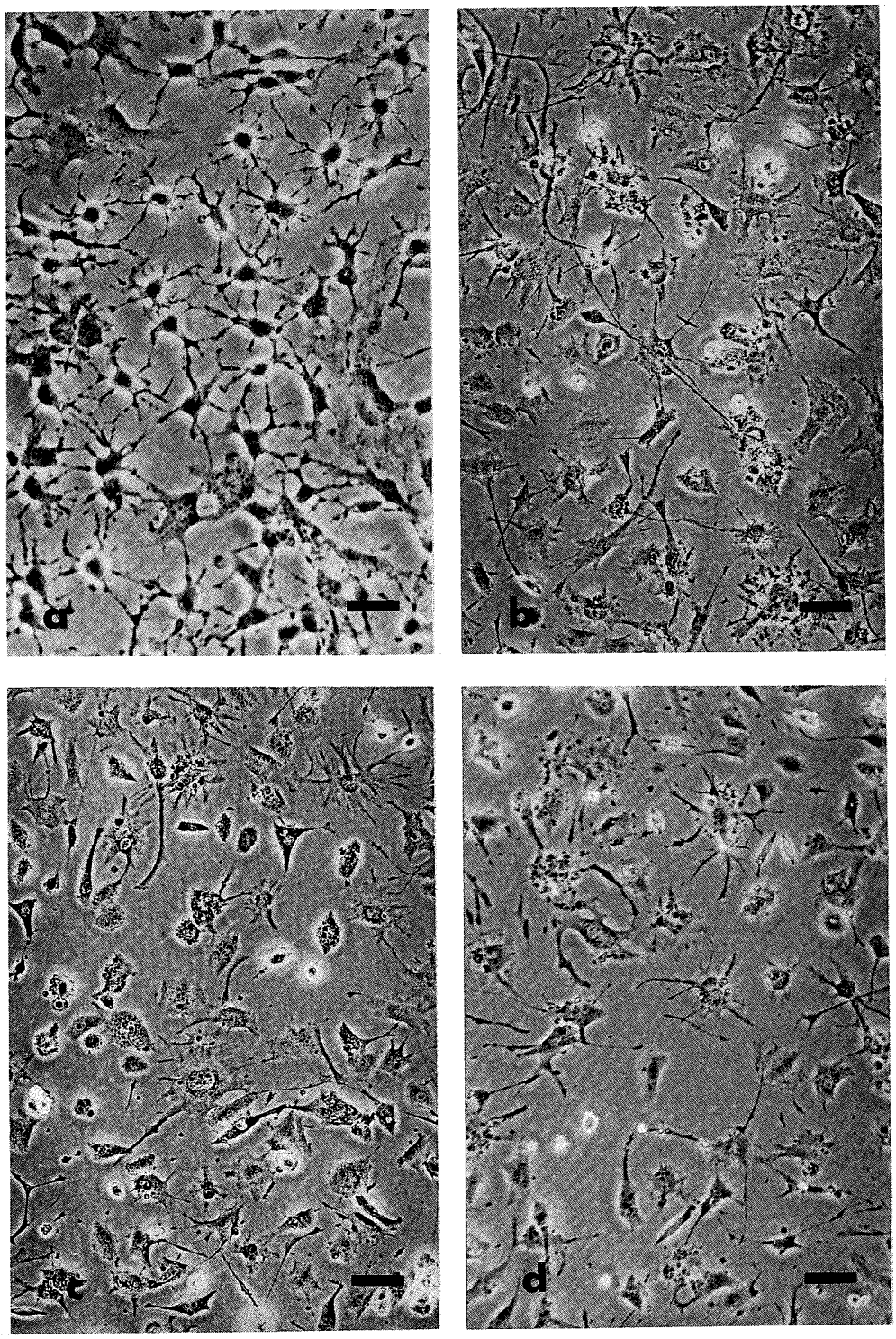

Fig 1: Photomicrographys of RBA-1 cells after adding ethanol treatment. Pictures were taken 48 hours after increasing concentration of ethanol to the medium. A: control without ethanol added; B, C and D: in the presence of 0.5, 0.75 and 2.0% ethanol, respectively. Bars equal 50 μm.

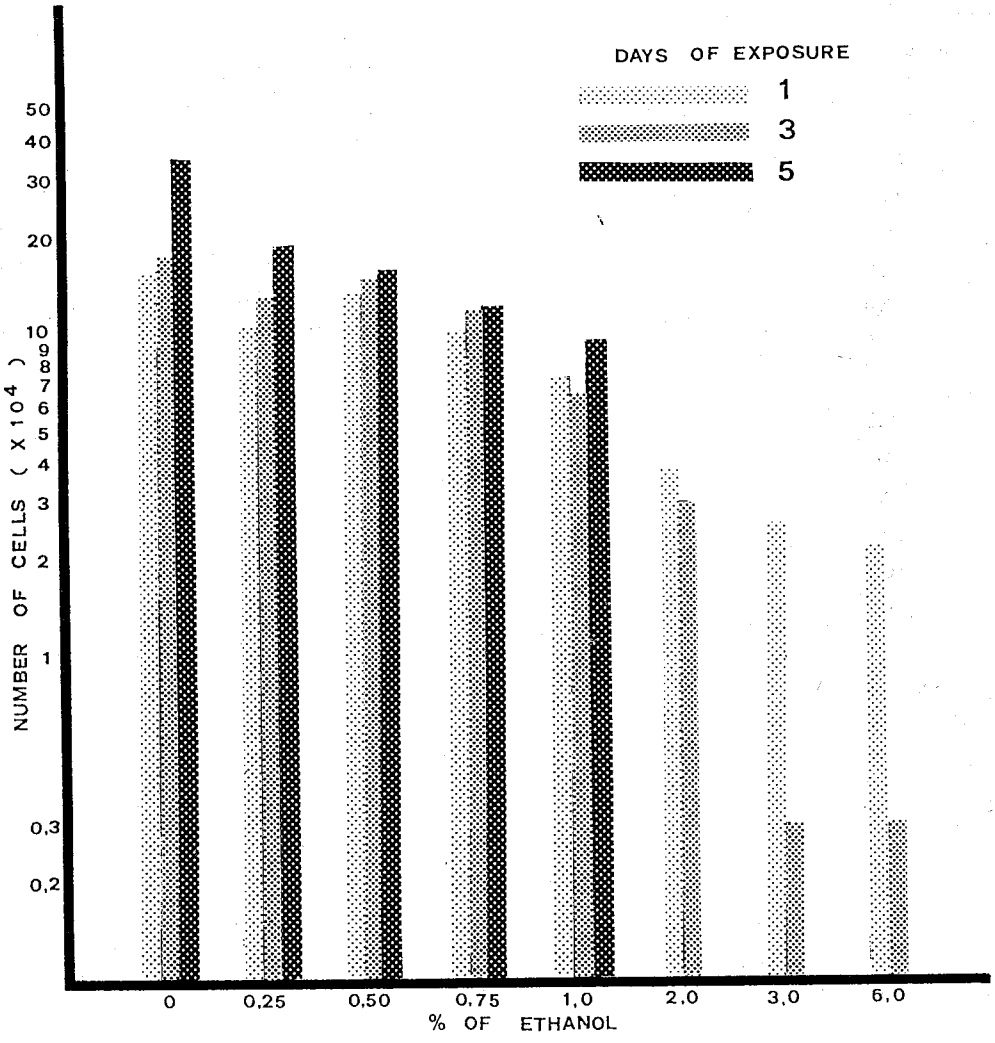

Fig 2: Growth of astrocyte cells in the presence of different concentration of ethanol. Data is derived from triplicate culture for each individual condition.

reduction of glial cell number with increasing ethanol concentration. At ethanol concentration less than 0.5%, there was minimal reduction in cell number and surviving cells exhibited elaborate cytoplasmic processes of varying sizes and lengths. As the ethanol concentration increased to 0.75%, there was an obvious reduction of cell number as shown in Figure 1c. The gross morphological appearance of the surviving cells was also altered due to ethanol exposure. For example, cultures treated with 2.0% ethanol show much less elaborate outgrowing processes (Fig lb), and there are more of the fusiform-shaped glial cells. Most of the dead cells were detached from the culture flask.

Lipid peroxidation

When astrocytes were incubated at 37°C for 5 min with different concentration of ethanol and in the presence of the free radical generating system, there was a dose-dependent increase in the amount of MAD formed (Fig 3).

Glucose transport

The uptake of 2-DG by astrocytes was time dependent and was saturable with increase concentration of 2-DG. When cells were exposed to the free radical generating agents, washed and subsequently tested for the glucose transport activity, the net rate of 2-DG transport was significantly reduced by about 50% as compared with the control cells which were not rendered the free radical generation system (Fig 4). Ethanol added to the system alone inhibited 2-DG transport of astrocytes cells in a concentration dependent manner. However, addition of ethanol to the incubation medium containing the hydroxyl free radical generating system resulted in further inhibition of the 2-DG transport activity (Fig 5).

DISCUSSION

We have previously shown with mannalian tissues and membranes that ethanol could directly participate in free radicals(13,14). Besides measurement using the spin trapping technique, generation of these free radicals can be indirectly related to the lipid peroxidation process. The increase in MDA formation was correlated to the decrease in activity of $(Na^+ + k^+)$ -ATPase, an enzyme known to depend greatly on membrane structural integrity for proper functioning[13]. In the present study, it is apparent that the enhancement in free radical generation in astrocytes induced by ethanol can also be demonstrated using the MDA assay procedure. Since MDA is a bifunctional molecule, it can cross-link with different macromolecules (proteins, lipids, nucleic acid) and in turn, may lead to various types of cellular damage.[15]

Several possibilites can be offered to explain the inhibitory effect of ethanol on the 2-DG transport system. The most direct explanation is an inhibition due to structural perturbation of the membrane by ethanol. On the other hand, this may also be due to cross-linking of the carrier protein by MDA. This modification may be one of the mechanisms by which lipid peroxidative damage may alter membrane structure, thereby leading to the decrease in glucose transport activity observed in this study. This notion is further supported by the observation that greater inhibitory effect could be observed when ethanol was added to the free radical generating systems.

Because glucose transport is important in the energy metabolism of the nervous system, the carrier mediated transport of 2-DG has been widely studied[4,5,6,7,8,9]. It is not surprising that this transport system is also dependent on the physical state of the membrane structure[10] and ethanol may perturb this transport system similarly to that for

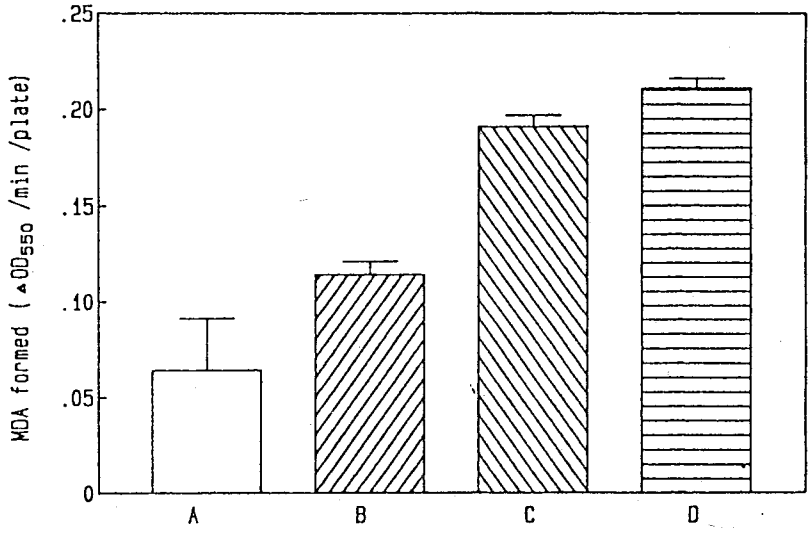

Fig 3: Effect of ethanol on lipid peroxidation of astrocytes. Cells were incubated at 37°C for 5 min with different concentrations of ethanol in the presence of free radical generation system as described in Methods. Lipid peroxidation was measured as the amount of malonyldialdehyde (MDA) produced through thiobarbituric acid reaction. A: control without ethanol; B, C and D: incubated with 0.38, 0.75 and 2.0% (V/V) ethanol respectively. Data are the mean ± SEM.

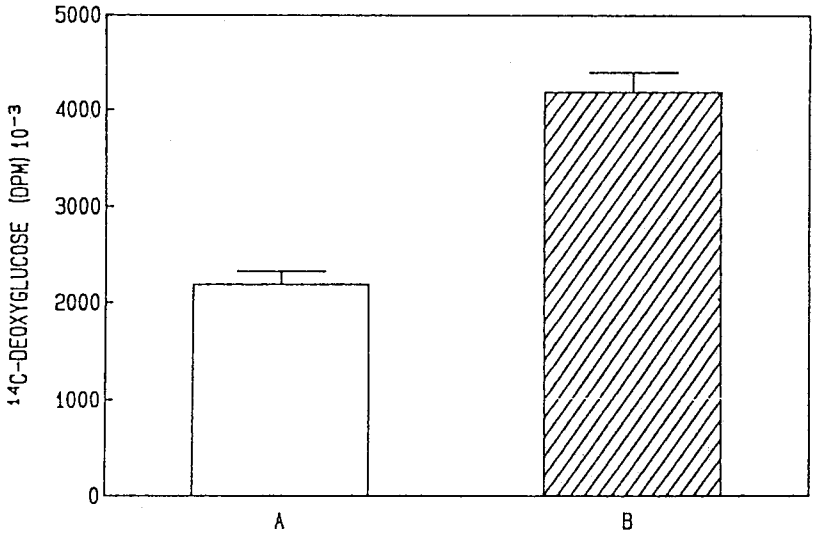

Fig 4: Effect of lipid peroxidation on 2-DG transport in astrocyte cell culture. Reaction was carried out in the presence (A) or absence (B) of free radical generating system as described in Methods. Values are the mean ± SEM.

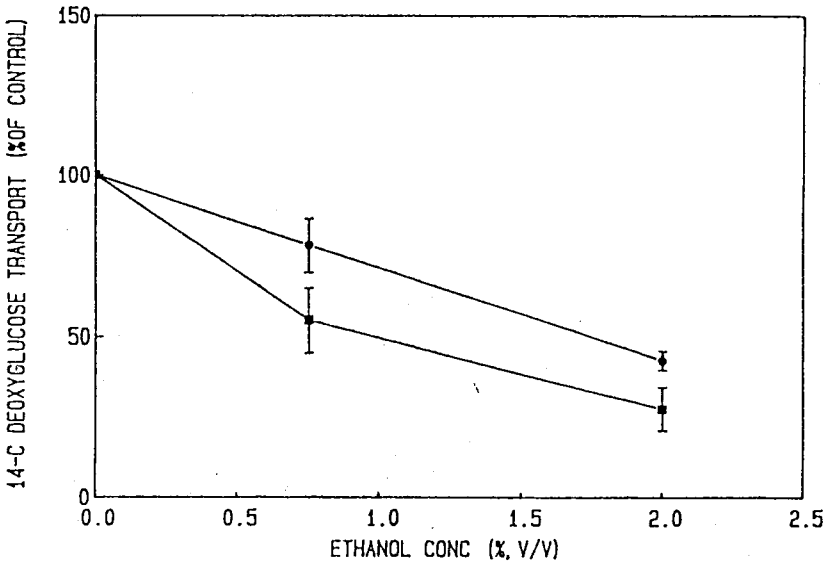

Fig. 5. Effect of ethanol on 2-DG transport in astrocyte cell culture. The transport activity was assayed in the presence of different concentrations of ethanol with (■) or without (●) the free radical generating system as described in Methods. Data are the mean ± SEM.

Na^+, K^+ -ATPase[16,17].

There is little doubt that cell culture is an excellent model for studying the effects of ethanol. This pertains specially to mechanisms associated with the membrane[1]. Freund and colleagues[18] have shown that ethanol, albeit at rather high concentrations, impaired the in vitro incorporation of DNA precursors into mouse spleen cells. On the other hand, Syapin et al[19] found that Mg^{2+} -ATPase and ($Na^+ + K^+$) -ATPase activities of hamster astroblasts and neuroblastoma cells were altered at lower ethanol concentrations (100 mM). The decrease in ATPase activity following acute exposure of ethanol and the increase following chronic exposure in these cells are consistent with the data obtained with brain synaptic plasma membranes[17]. Apparently, there are inherent differences in cellular response to ethanol perturbation with respect to different culture cell systems[20].

It is necessary to consider that the neurons in brain are constantly exposed to exogenous pro-oxidative insults such as radiation, drugs and endogenous reactive oxygen generation system. Although catalase, superoxide dismutase, glutathione peroxidase and vitamin E are known protective mechanisms to combat these insults, the significance of these various enzymic systems in protecting oxidative insult due to ethanol remains to be further investigated.

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酒精對組織培養之大白鼠腦神經膠細胞之影響——形態，生長，Lipid Peroxidation，Glucose Transport——

劉生明　周德程*　孫永光

組織培養所得之大白鼠腦神經膠細胞（RBA-1 cells）經數天培養後，將正常培養液加入不同濃度的ethanol，濃度為0.75%，2.0%及對照組。利用光學顯微鏡觀察細胞外形的變化及生長的影響；在高濃度（2.0%）的ehtanol可以使細胞產生胞突收縮，形成多角形或圓形細胞，而較低濃度時並無發現顯著形態改變，且ethanol濃度愈高細胞生長愈受抑制。藉著thiobarbituric acid（TBA）呈色法定量malonyldialdehyde（MDA）之形成，以及利用 ^{14}C-deoxyglucose測試不同濃度ethanol對細胞膜glucose transport之影響，結果顯示ethanol對細胞膜之lipid-peroxidation呈加強作用，並隨著ethanol濃度之增加而降低細胞膜對glucose transport之能力。本實驗結果顯示：在高濃度之ethanol存在下，除了使細胞形態構造改變外，亦影響細胞之生化代謝活性，這些包括了降低細胞膜對glucose的運送能力及加強細胞膜lipid-peroxidation之活性。

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