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SHORT COMMUNICATIONS

Taurine protection of lymphoblastoid cells from iron-ascorbate induced damage

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Taurine is a sulfur-containing amino acid present in high concentrations in animal tissues [1]. In lymphocytes, taurine is the most abundant amino acid, accounting for more than 50% of the total free amino acid pool [2, 3]. Despite its high concentrations, the physiological role of taurine in cells remains unknown.

Lymphocytes seem to have a limited ability to form taurine and probably depend on that taken up from plasma to maintain their high endogenous levels. In human lymphocyte-derived cultured lymphoblastoids, an active taurine transport system has been identified [4]. When such cells are cultured in a medium supplemented with serum, the latter provides taurine to them; but these same cells growing in a chemically-defined, taurine-free medium become progressively depleted of this amino acid. Addition of taurine to the chemically-defined medium results in an increase in the number of viable cells in the culture [5]. The mechanism of this effect of taurine has not yet been clarified, but recent experimental evidence on its effects as a membrane stabilizer in biological preparations [6–8] raises the possibility of an effect mediated through a protective action on cell membranes.

In a number of experimental conditions in which taurine has demonstrated a protective effect on cell membranes, damage has been related to the occurrence of lipid peroxidation [8, 9]. The peroxidation of endogenous phospholipids in cell membranes occurs during physiological situations, e.g. phagocytosis, biosynthesis of prostaglandins and aging [10]. In cells growing in a chemically-defined medium, a number of agents able to counter peroxidation may be absent, making cells more susceptible to membrane damage and resulting in decreased cell viability.

In this study, we have investigated the effect on the viability of cultured lymphoblastoid cells of long aerobic incubation and of ferrous sulfate and ascorbate as peroxidative agents. The effect of taurine as protective agent was explored.

Methods

Lymphoblastoid cell lines used in this study were initiated as normal controls for diagnostic purposes and have been cultured for many years. They were maintained in RPMI 1640 medium (Gibco) supplemented with glutamine, penicillin, streptomycin and 16% fetal calf serum. Other cells were maintained in a biochemically-defined, serum-free medium consisting of RPMI 1640 supplemented with 5 μg/ml insulin, 5 μg/ml transferrin, 3×10^{-5} M $CuSO_4$ and 5×10^{-5} M $FeSO_4$.

Cells were concentrated by centrifugation and resuspended in a Krebs-bicarbonate medium (pH 7.4) containing in mmoles/l: NaCl, 118; KCl, 3.0; $CaCl_2$, 1.0; $MgSO_4$, 1.18; KH_2PO_4, 12.5; $NaHCO_3$, 12.0; and glucose, 10. Cells were incubated at 37°, with shaking, in the presence of the compounds tested and during the times indicated for each experiment. After incubation, the number of viable cells was determined by Trypan blue (0.5% in saline solution) exclusion. Viability is expressed as a percentage of cells excluding Trypan blue after enumerating approximately 400 cells for each determination. The number of viable cells in the culture bottle before incubation was calculated for each experiment.

Malondialdehyde formation was measured by the amount of thiobarbituric-acid reacting material as previously described [9].

Accumulation of labeled sodium and calcium was measured by incubating cells in a Krebs-bicarbonate medium (1 ml final volume) containing ^{22}Na (New England Nuclear), 1.0 μCi/ml, or $^{45}Ca^{2+}$ (New England Nuclear), 2.5 μCi/ml. After 30 min of incubation, 0.3-ml samples were withdrawn and centrifuged. Cells were washed and solubilized with NCS (Tissue solubilizer, Amersham), and the radioactivity accumulated was measured by scintillation spectrometry.

Results and discussion

When cells growing in a culture medium supplemented with 16% bovine fetal serum were transferred to a Krebs-bicarbonate medium, a time-dependent decrease in cell viability was observed. After 30 min of incubation, cell viability decreased from 80% to 65% and after 60 and 90 min of incubation cell viability decreased to 60% and 56% respectively. This deleterious effect was prevented when taurine, at concentrations of 5–10 mM, was present in the incubation medium.

Exposure of cells to 0.2 mM ferrous sulfate and 0.4 mM ascorbate caused a further decrease of cell viability, from 80% to 42% after 30 min of incubation (Fig. 1, Table 1). Cells exposed to iron-ascorbate appeared often swollen and always showed crenations and protrusions. The effect of iron-ascorbate was very similar in cells grown in a chemically-defined medium. These cells showed a lower viability, 48%, and exposure to iron-ascorbate decreased this value to 21%.

The presence of 1–10 mM taurine protected cells from the injurious effect of iron-ascorbate, maintaining cell viability at values close to those observed prior to incubation. The maximum effect of taurine was observed at a concentration of 5 mM. Taurine also counteracted cell swelling and cell shape distortion caused by iron-ascorbate. From other amino acids tested, only hypotaurine had a protective effect similar to that of taurine. Glycine and β-alanine showed only a slight effect, whereas glutamic acid and cysteine were ineffective.

The iron-ascorbate system is known to induce lipid peroxidation in a number of biological preparations, and this has been considered as a possible cause of membrane damage and cell death [11, 12]. In our preparation, iron-ascorbate also induced a marked increase in lipid peroxidation as measured by the formation of malondialdehyde (Fig. 2). The presence of taurine in the incubation medium, in conditions which preserve cell viability, failed, however, to decrease lipid peroxidation (Fig. 2). This result suggests that taurine protective action is unrelated to the mechanisms preserving lipid peroxidation but it may be associated with some additional factors causing death in cells having membranes injured by peroxidation processes.

It has been reported that membrane damage caused by lipid peroxidation is often accompanied by a disruption of the membrane permeability barrier resulting in enhanced water and ion accumulation [6, 13–15]. Ion overloading may thus be the primary cause of cell death. This notion is

supported by the observation that cells incubated in a Tris-sucrose medium free of ions are unaffected by iron-ascorbate (Table 1). A less severe deleterious effect was observed in cells incubated in a sodium-free medium or in a chloride-free medium (Table 1), and little damage occurred in cells incubated in a calcium-free medium (Table 1).

The protective effect of taurine may thus be exerted on the control of ion permeability, since taurine effects on ionic fluxes are well documented in other preparations [16, 17]. To explore more directly this possibility, the accumulation of labeled sodium and calcium by cells exposed to iron-ascorbate in the presence of taurine was

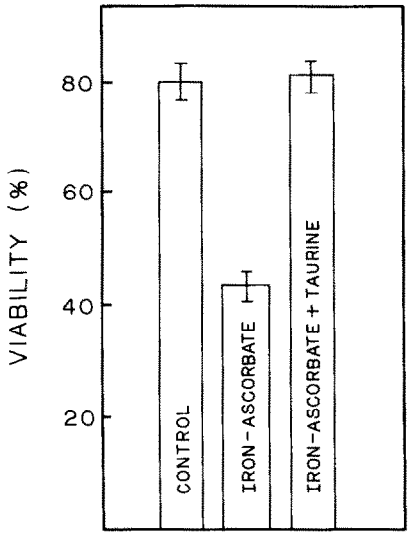

Fig. 1. Effect of taurine (5 mM) on the viability of cells exposed to 0.2 mM ferrous sulfate and 0.4 mM ascorbate. Cells were incubated for 30 min in a Krebs-bicarbonate medium containing the indicated additions. Values are the means ± S.E.M. of six to eight separate experiments.

Table 1. Effect of ionic compositon of the incubation medium on viability of lymphoblastoids exposed to iron-ascorbate*

Medium	Viability (%)
Control (prior incubation)	80 ± 5
Iron-ascorbate	
Krebs-bicarbonate	42 ± 5
Sodium-free	61 ± 6
Chloride-free	60 ± 7
Calcium-free	71 ± 7
Bicarbonate-free	40 ± 5

* The number of viable cells was determined before incubation or after 30 min of incubation in the presence of 0.2 mM ferrous sulfate and 0.4 mM ascorbate. Choline chloride replaced sodium chloride and potassium bicarbonate replaced sodium bicarbonate in the sodium-free medium. Sodium isethionate replaced sodium chloride in the chloride-free medium and 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) replaced bicarbonate. Calcium-chloride was omitted in the calcium-free medium and water used was passed through Chelex. Results are the means ± S.E.M. of seven to ten separate experiments.

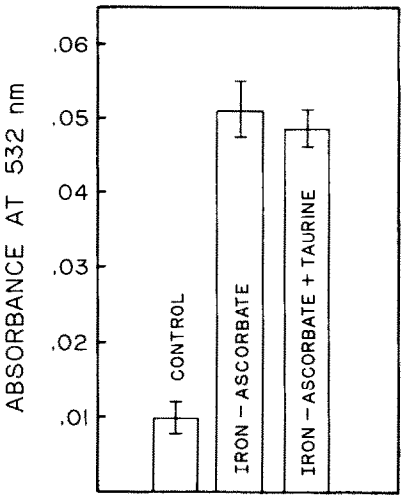

Fig. 2. Effect of iron-ascorbate on thiobarbituric acid-reactive material formed by lymphoblastoids in the presence or absence of 5 mM taurine. Results are the means ± S.E.M. of four separate experiments.

Table 2. Effect of iron-ascorbate on $^{45}Ca^{2+}$ accumulation by human lymphoblastoids in the presence or absence of taurine*

Condition	$^{45}Ca^{2+}$ accumulation (nmoles × 10^6 cells)
Control	3.15 ± 0.31 (4)
Taurine (5 mM)	2.32 ± 0.17 (4)
Iron-ascorbate	15.34 ± 1.10 (4)
Iron-ascorbate plus taurine	5.21 ± 0.16 (4)

* Cells were incubated in a Krebs-bicarbonate medium containing 2.5 mM $CaCl_2$ and 2.5 μCi/ml $^{45}Ca^{2+}$. Ferrous sulfate concentration was 0.2 mM and ascorbate 0.4 mM. Incubation time was 30 min. Results are the means ± S.E.M. of four separate experiments.

examined. Iron-ascorbate did not markedly increase sodium accumulation (results not shown) but caused a large increase of $^{45}Ca^{2+}$ uptake (Table 2). This effect was clearly counteracted by taurine (Table 2).

It has been suggested recently that cell death produced as a consequence of lipid peroxidation in other preparations may be due to increased calcium influx through membranes [18]. This calcium accumulation has been proposed as the feature which converts an initially non-lethal damage into irreversible cell injury [19]. This may be true not only for lipid peroxidation but also for a number of agents causing disruption of the integrity of plasma membrane [19].

According to the above interpretation, the prevention by taurine of enhanced calcium influx occurring in the presence of iron-ascorbate may constitute the mechanism of its protective action on lymphoblastoid viability. Taurine effects preserving cell membrane integrity have also been observed in other preparations such as retinal rod outer segments exposed to illumination or to ferrous sulfate [6] as well as in ischemic heart or in heart perfused with a calcium-free medium followed by a calcium-containing medium (calcium paradox) [20]. *In vivo*, taurine is required to maintain the morphological and functional integrity of retinal photoreceptors [21, 22].

In summary, this work presents evidence of a protective effect of taurine on viability of lymphoblastoids in conditions of cell damage produced by a model of lipid peroxidation. Taurine effects seem to occur through an action on calcium permeability through the injured membranes.

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Metabolism of mandelonitrile in the rat

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Mandelonitrile, $C_6H_5CH(OH)CN$, arises from the hydrolysis of amygdalin, found in apricot pits and bitter almonds, and of laetrile, a drug controversially used in cancer chemotherapy [1]. Mandelonitrile is also used for the treatment of urinary tract infection [2]. Amygdalin has been shown to release hydrogen cyanide *in vivo* in rodents [3] and dogs [4], and it has been postulated that mandelonitrile may be an intermediate in this conversion. These reactions are catalyzed by the "emulsin" complex of enzymes in plants [5] and by the gut microflora [6], but may also be non-enzymic [7].

The present studies were designed to elucidate the possible metabolic pathways of mandelonitrile in the rat. Metabolites were identified and quantitatively determined to establish the relevant importance of these pathways with reference to the toxicity of mandelonitrile.

Materials and methods

Chemicals. *dl*-Mandelonitrile, b.p. 170°, density 1.24 g/ml, was obtained from Albright & Wilson Ltd., Warley, West Midlands, U.K.

Animal preparation. Male and female Wistar rats (Birmingham Wistar strain), weighing 200–350 g, were maintained on a diet as previously described [8]. The animals were housed in metabolism cages which allowed the separate collection of urine and faeces. Mandelonitrile was administered to adult rats as a solution in corn oil ("Mazola") at doses of 30 mg/kg (0.2 ml) orally by stomach tube. Urine was collected for 24 hr before dosing and for successive 24-hr periods up to 4 days.

Metabolite identification. Metabolites were identified in 10-μl aliquots of urine and acidic ethereal extracts by paper chromatography (Whatman 3MM) using butan-1-ol/ethanol/aq. NH_3 (sp. g. 0.88)/water (10:10:1:4, by vol.) as solvent [9] and by TLC on plates coated with silica gel G (0.25 mm thick) containing a fluorescent indicator (E Merck AG, Darmstadt, West Germany) using propan-2-ol/aq. NH_3 (sp. g. 0.88) (4:1, v/v) [10]. Mandelic acid and benzoic acid were detected by 8-hydroxyquinoline sulphate [11] and hippuric acid by *p*-dimethylamino-benzaldehyde in acetic anhydride (10%, w/v) [12]. Cyanide (CN^-) and thiocyanate (SCN^-) were detected as violet spots against a