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TAURINE PROTECTS AGAINST THE CYTOTOXICITY OF HYDRAZINE, 1,4-NAPHTHOQUINONE AND CARBON TETRACHLORIDE IN ISOLATED RAT HEPATOCYTES

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Abstract—Exposure of rat hepatocytes to hydrazine, carbon tetrachloride or 1,4-naphthoquinone results in cytotoxicity determined as uptake of Trypan blue and leakage of lactate dehydrogenase (LDH). After exposure of hepatocytes to hydrazine and 1,4-naphthoquinone, ATP was also measured and was found to be depleted. Addition of the β-amino acid taurine to the hepatocyte incubation buffer partially protects the cells against the cytotoxicity of these three different cytotoxic compounds, as indicated by Trypan blue uptake and LDH leakage. Taurine also reduces the depletion of ATP caused by 1,4-naphthoquinone but not hydrazine. It is suggested that taurine may have a cytoprotective effect *in vitro* and may be a useful tool for the investigation of mechanisms of cytotoxicity.

We have shown recently: (i) that depletion of liver taurine in rats increases their susceptibility to the hepatotoxicity of carbon tetrachloride [1]; (ii) that there is a correlation between liver taurine and urinary taurine and between urinary taurine (as a predictor of liver taurine level) and degree of susceptibility to various hepatotoxic compounds [2]. Other workers have shown that taurine protects against pulmonary damage caused by bleomycin [3, 4] and against carbon tetrachloride-induced lipid peroxidation [5]. These data suggest that taurine may have a protective effect *in vivo*. Studies in lymphoblastoid cells *in vitro* [6, 7] demonstrated that the presence of extracellular taurine (5 mM) was able to protect cells against iron-ascorbate-induced calcium accumulation and cytotoxicity and 20 mM taurine co-administered with zinc chloride protected lymphoblastoid cells against retinol and retinoic acid toxicity. Studies in hepatocytes *in vitro* have demonstrated that taurine can exert a protective effect by decreasing lipid peroxidation due to hyperoxygenation thus preventing cell death [8]. Taurine is also believed to protect neutrophils against the toxic effects of hypochlorous acid generated *in situ* [9] and against bronchiolar damage induced by nitrogen dioxide [10]. The protective effects in other systems have been suggested to involve membrane stabilizing properties [11, 12].

The objective of this study was to determine if taurine would protect isolated rat hepatocytes against the cytotoxic effects of three different toxic compounds. The three compounds used, carbon tetrachloride, hydrazine and 1,4-naphthoquinone are all cytotoxic but probably via different mechanisms. The mechanism by which hydrazine is cytotoxic is not clearly understood. However, hydrazine interferes in a number of crucial metabolic processes [13] and the cytotoxicity certainly involves depletion of ATP [14]. 1,4-Naphthoquinone causes redox cycling and oxidative stress which will result in oxidation of critical thiol groups and is thought to be toxic via this mechanism [15]. Plasma membrane changes such as blebbing also occur, reflecting possible loss of membrane function [15]. Carbon tetrachloride is toxic via a reactive free radical metabolite produced in the smooth endoplasmic reticulum which causes lipid peroxidation [16–18]. This gives rise to breakdown of lipids, particularly unsaturated phospholipids which will damage intracellular membranes and the plasma membrane. The breakdown products such as reactive aldehydes spread throughout the cell and cause other damage and effects such as increased membrane permeability. Carbon tetrachloride may also have a significant direct solvent effect on cell membranes in isolated cells.

MATERIALS AND METHODS

Male, Sprague–Dawley rats (200–300 g) obtained from Glaxo Group Research were used to prepare hepatocytes. Hydrazine hydrate (100%) was obtained from the Sigma Chemical Co. (Poole, U.K.), 1,4-naphthoquinone (99%) was obtained from Fluka Chemie AG (Buchs, Switzerland) and carbon tetrachloride (99.8%) was obtained from BDH (Lutterworth, U.K.). Luciferase, NADH, dimethyl sulphoxide (DMSO†), pyruvate and collagenase (Type 1) were obtained from Sigma.

Isolation of rat hepatocytes

The technique used was essentially the two-step perfusion method [19]. Hepatocyte preparations were suspended in Krebs–Henseleit buffer. Except for studies with carbon tetrachloride cells were

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† Abbreviations: LDH, lactate dehydrogenase; DMSO, dimethyl sulphoxide.

gassed with 95% O_2/5% CO_2 and were incubated in rotating siliconized round-bottomed flasks (100 mL) at a live cell density of 2×10^6/mL (20 mL). Cells were preincubated for 30–60 min before addition of the compounds. Initial viability was 87 ± 2%. Samples were taken just prior to the addition of taurine, then 1, 2 and 3 hr after, for measurement of Trypan blue uptake, lactate dehydrogenase (LDH) leakage and ATP content. An aliquot (0.5 mL) was added to trichloroacetic acid (0.5 mL, 20%, 4°) and frozen at −80° for later analysis of ATP. Taurine was added to cell suspensions dissolved in Krebs–Henseleit buffer immediately before the addition of the cytotoxic compounds.

Carbon tetrachloride studies

In studies with carbon tetrachloride (CCl_4) an aliquot of cell suspension (2 mL) was transferred to 25-mL Erlenmyer flasks fitted with a centre well. Taurine dissolved in Krebs–Henseleit buffer was added to the cell suspensions to give final concentrations between 0.05 and 20.0 mM with no addition being greater than 200 μL. Carbon tetrachloride was added directly to the centre wells (0.0, 7.5, 10 or 15 μL) and allowed to diffuse into the flasks, which were sealed. Flasks were prepared in triplicate. These were placed in an oscillating water bath at 37° moving at 2 cycles/sec. LDH leakage into buffer was determined after 45–50 min of incubation (see below).

Studies with 1,4-naphthoquinone and hydrazine

1,4-Naphthoquinone was dissolved in DMSO and added to hepatocytes to give final concentrations of 20–200 μM. Control cells were also exposed to the same DMSO concentration (0.1%). Hydrazine was added to cell suspensions dissolved in degassed water to give final concentrations of 12–24 mM. Viability, LDH leakage and ATP were measured in aliquots of cell suspension taken at hourly intervals (see below). The cells were incubated for 3 hr.

Assessment of viability of isolated hepatocytes

Trypan blue uptake. The viability of isolated hepatocytes was measured using the uptake of the dye Trypan blue by damaged cells.

LDH leakage. Leakage of the cytosolic enzyme, LDH, into the suspension buffer was used as a marker of cell membrane integrity. LDH was measured by the method of Bergermeyer [20] as described previously [13].

ATP determination. The assay for ATP in isolated hepatocytes used the detection of luciferase-linked bioluminescence [21] in trichloroacetic acid extracts of hepatocytes as described previously [13, 14].

Statistical analysis

Statistical significance was assessed using the Student's *t*-test to compare treated with control values.

RESULTS

Carbon tetrachloride

Hepatocytes exposed to the vapour from 7.5, 10 and 15 μL CCl_4 showed a decline in viability with time compared with controls. However, only the cells exposed to 15 μL CCl_4 showed a marked loss of viability (70%) and leakage of LDH (35%). Therefore, this exposure level of CCl_4 was subsequently used.

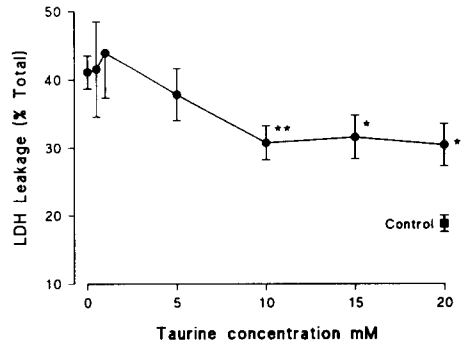

Fig. 1. Effects of various concentrations of taurine on carbon tetrachloride cytotoxicity measured as LDH leakage in isolated hepatocytes. Hepatocytes were exposed to the vapour from 15 μL of carbon tetrachloride for 45 min. (■) Control, not exposed to carbon tetrachloride. Values are means ± SEM, N = 5. *P < 0.05; **P < 0.01 compared to control with no taurine added.

Addition of taurine to the incubation buffer in the range 0.05 to 20 mM showed a dose-related protection against cytotoxicity measured as leakage of LDH after 45 min (Fig. 1). This protection became statistically significant at taurine concentrations of 10 mM. The protection also seemed to be maximal at this concentration as there was no further protection even at a taurine concentration of 20 mM. The protection afforded by taurine did not however reduce LDH leakage to control levels (Fig. 1).

1,4-Naphthoquinone

1,4-Naphthoquinone caused a dose-related decrease in hepatocyte viability (Fig. 2a), a corresponding increase in LDH leakage (Fig. 2b) and a decrease in ATP after 180 min of incubation (Fig. 2c). For each parameter there was a significant difference from the control group at exposure concentrations of 100 and 200 μM.

In the presence of 15 mM taurine, the cytotoxicity was attenuated with the dose–response relationship being shifted to the right with a statistically significant difference in viability, LDH leakage and ATP depletion compared to 1,4-naphthoquinone alone at an exposure level of 100 μM (Fig. 2a, b and c). However with all three parameters measured, the cells exposed to the highest concentration of 1,4-naphthoquinone (200 μM) were not protected against cytotoxicity or ATP depletion. Therefore, for the next studies, an exposure concentration of 100 μM was used.

When different concentrations of taurine were used it was found that even 5 mM taurine significantly reduced cytotoxicity as measured by Trypan blue uptake at all three time points (Fig. 3a), but LDH leakage was only significantly reduced for the first

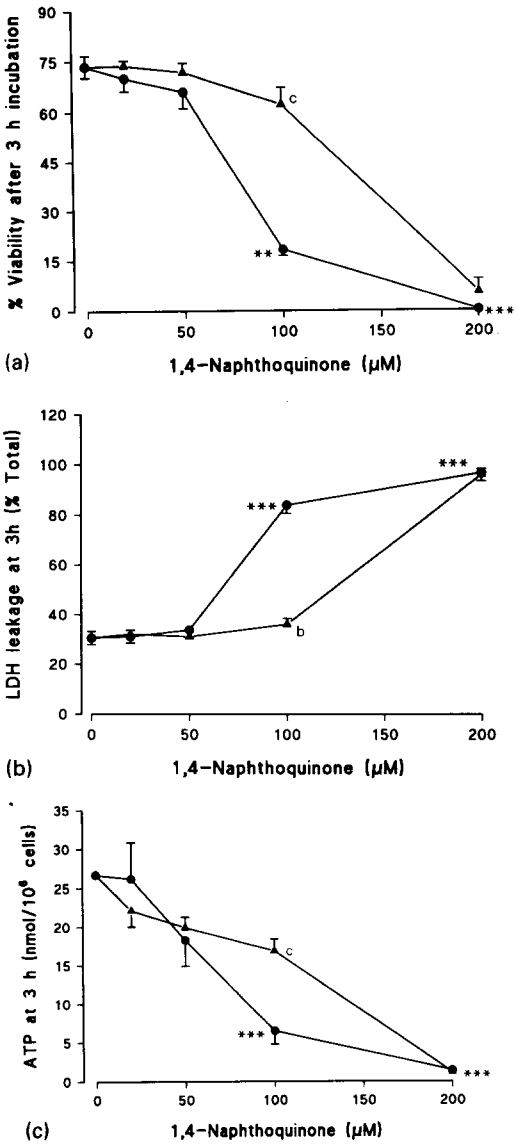

Fig. 2. Effect of taurine on the dose-dependent cytotoxicity of 1,4-naphthoquinone in isolated hepatocytes. (a) Loss of viability measured as uptake of Trypan blue; (b) leakage of LDH; (c) depletion of ATP. (●) 1,4-Naphthoquinone alone; (▲) 1,4-naphthoquinone plus taurine (15 mM). Values are means ± SEM, N = 3–4. $^{**}P < 0.01$; $^{***}P < 0.001$ compared to control. $^{b}P < 0.01$; $^{c}P < 0.001$ compared to 1,4-naphthoquinone alone.

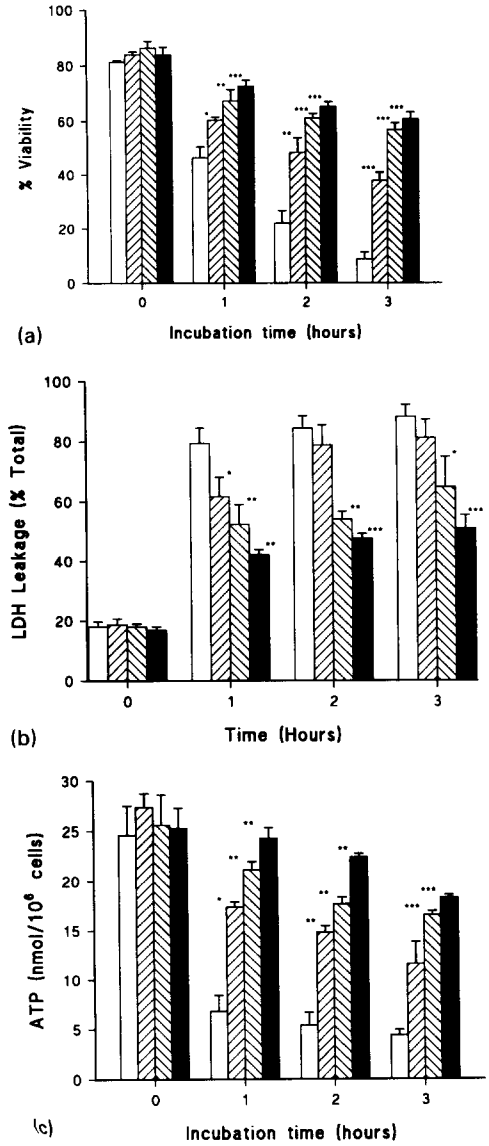

Fig. 3. Effect of various concentrations of taurine on the time course of 1,4-naphthoquinone cytotoxicity in isolated hepatocytes. (a) Loss of viability measured as uptake of Trypan blue; (b) leakage of LDH; (c) depletion of ATP. (□) No taurine; (▨) 5 mM taurine; (▧) 10 mM taurine; (■) 15 mM taurine. All hepatocytes were exposed to 100 μM 1,4-naphthoquinone. Values are means ± SEM, N = 3–4. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ when compared to hepatocytes exposed to 1,4-naphthoquinone in the absence of taurine.

hour by 5 mM taurine (Fig. 3b). However, 10 and 15 mM taurine both significantly reduced LDH leakage at all three time points (Fig. 3b). Similarly ATP depletion was attenuated by the presence of all three concentrations of taurine after 1 and 2 hr, but only 10 and 15 mM taurine afforded a statistically significant protection at 3 hr (Fig. 3c). Thus, with all three parameters the protection afforded by taurine was dose dependent.

The time course of the cytotoxicity of 1,4-naphthoquinone (100 μM) in the presence and absence of taurine also revealed that taurine (15 mM) had a significant protective effect, with both Trypan blue uptake (Fig. 4a) and LDH leakage (Fig. 4b) being significantly reduced at 2 and 3 hr compared to cells exposed to 1,4-naphthoquinone alone. Furthermore, in cells exposed to 1,4-naphthoquinone plus taurine, LDH leakage was not significantly different from that in control cells and control cells plus taurine at all time points. ATP depletion caused

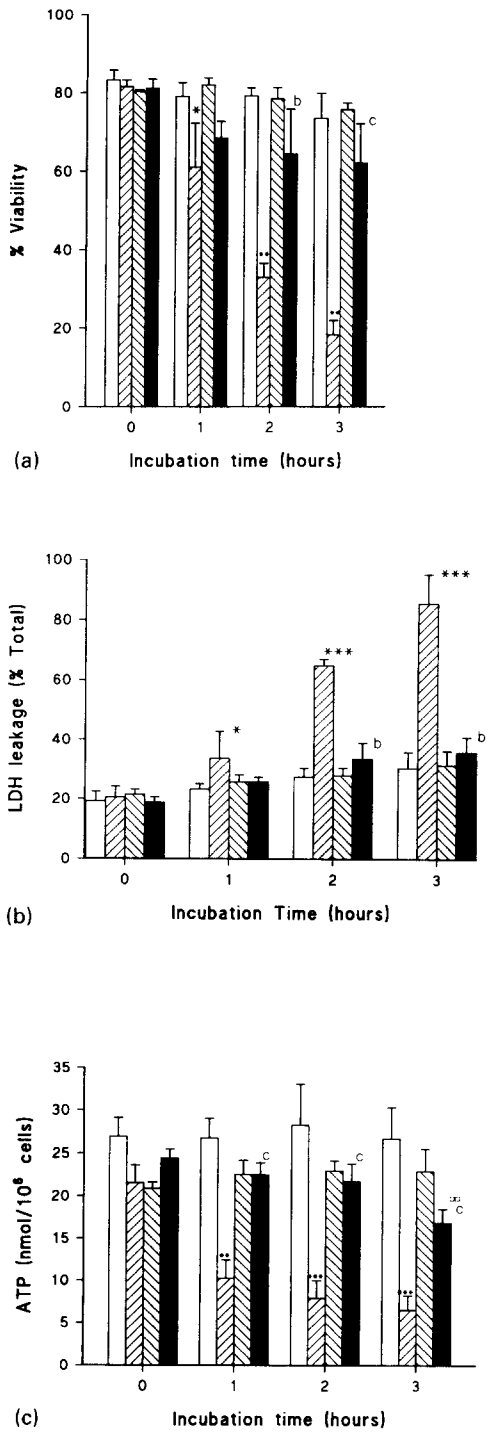

Fig. 4. Effect of taurine on the time course of 1,4-naphthoquinone cytotoxicity in isolated hepatocytes. (a) Loss of viability measured as Trypan blue uptake; (b) leakage of LDH; (c) depletion of ATP. (□) Control; (▨) 100 μM 1,4-naphthoquinone; (▧) 15 mM taurine; (■) 100 μM 1,4-naphthoquinone plus 15 mM taurine. Values are means ± SEM, N = 3–6. *P < 0.05; **P < 0.01; ***P < 0.001 when compared to control hepatocytes. [b]P < 0.01; [c]P < 0.001 when compared to hepatocytes exposed to 1,4-naphthoquinone alone. □□P < 0.01 when compared to hepatocytes under the same conditions but after 2 hr of incubation.

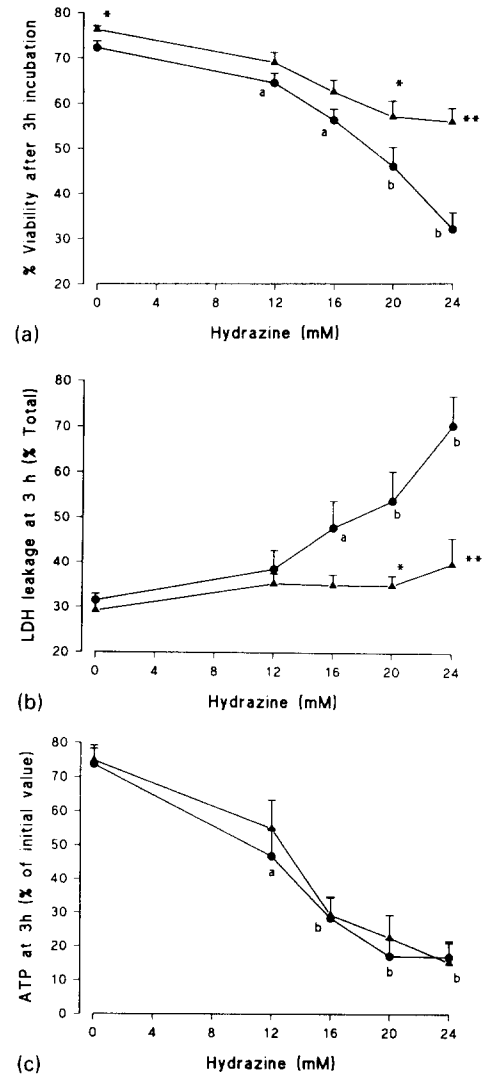

Fig. 5. Effect of taurine on the dose-dependent cytotoxicity of hydrazine in isolated hepatocytes. (a) Loss of viability measured as Trypan blue uptake; (b) leakage of LDH; (c) depletion of ATP. (●) Hydrazine alone; (▲) hydrazine plus taurine (15 mM). Values are means ± SEM, N = 4–7. *P < 0.01; **P < 0.01 compared to hepatocytes exposed to hydrazine alone. [a]P < 0.05; [b]P < 0.001 compared to control hepatocytes.

by naphthoquinone was also significantly ameliorated by taurine compared with cells treated with 1,4-naphthoquinone alone at 1, 2 and 3 hr, although even in the presence of taurine ATP was still significantly depleted after exposure for 3 hr when compared to cells exposed for 2 hr or control cells plus taurine (Fig. 4c).

Hydrazine

Hepatocytes incubated with various concentrations of hydrazine showed a dose-dependent loss of cell viability (Fig. 5a) and an increase in leakage of LDH (Fig. 5b). Trypan blue uptake and LDH leakage

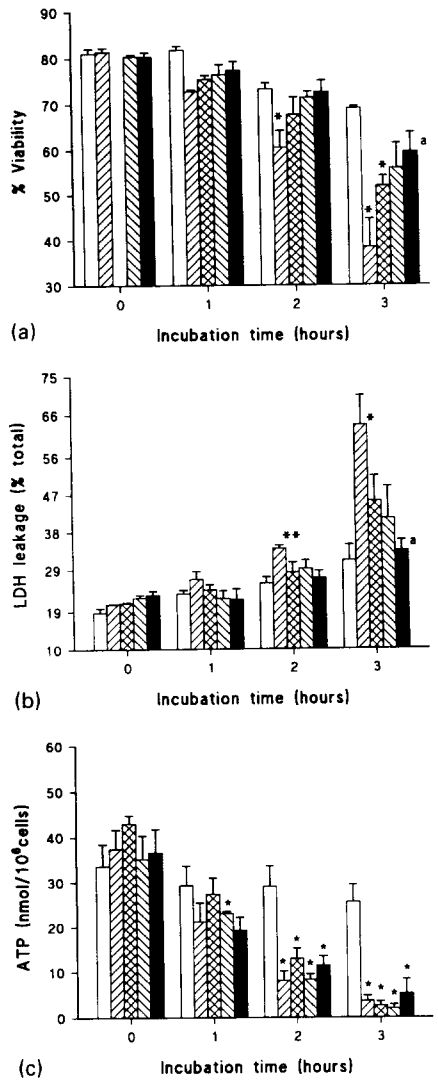

Fig. 6. Effect of various concentrations of taurine on the time course of hydrazine cytotoxicity in hepatocytes. (a) Loss of viability measured as uptake of Trypan blue; (b) leakage of LDH; (c) depletion of ATP. (□) Control; (▨) 24 mM hydrazine; (▩) 24 mM hydrazine plus 5 mM taurine; (▧) 24 mM plus 10 mM taurine; (■) 24 mM hydrazine plus 15 mM taurine. Hepatocytes were exposed to 24 mM hydrazine. Values are means ± SEM, N = 4. *$P < 0.05$; **$P < 0.01$ compared to control hepatocytes. [a]$P < 0.05$ compared to hepatocytes exposed to hydrazine alone.

were significantly reduced by 15 mM taurine after exposure to hydrazine at concentrations of 20 and 24 mM (Fig. 5a and b). Hydrazine also caused a dose-dependent decline in ATP, but the presence of taurine (15 mM) had no significant effect on depletion at any hydrazine concentration (Fig. 5c). After exposure to a single cytotoxic concentration of hydrazine (24 mM) there was a time-dependent loss of viability measured by Trypan blue exclusion and LDH leakage (Fig. 6a and b). However, the cytotoxicity of hydrazine measured by dye uptake and LDH leakage was *reduced* in a concentration-dependent manner by the addition of different concentrations of taurine (5, 10 and 15 mM) to the incubation buffer, although this was only statistically significant after 15 mM taurine. However, addition of taurine to the incubation buffer at any of these concentrations failed to protect significantly the hepatocytes from hydrazine-induced ATP depletion at any time point.

DISCUSSION

These studies have shown that taurine is able to protect against the cytotoxicity of carbon tetrachloride, 1,4-naphthoquinone and hydrazine *in vitro*, and that this protection is dose related. After exposure to cytotoxic concentrations of each of these compounds, viability as indicated by LDH leakage and/or Trypan blue uptake was significantly improved by the presence of taurine. Protection against carbon tetrachloride cytotoxicity was maximal at 10 mM whereas against 1,4-naphthoquinone and hydrazine cytotoxicity there was greater protection at a taurine concentration of 15 mM. After exposure to 1,4-naphthoquinone (100 μM), ATP depletion, which may be partly a consequence of cytotoxicity, was also significantly attenuated by taurine in a dose-related manner.

The protection of other types of isolated cell by taurine has previously been demonstrated by Pasantes-Morales *et al.* [6, 7] where similar concentrations of taurine were used to reduce the cytotoxicity of retinol and iron-ascorbate to lymphoblastoid cells. They demonstrated that taurine (5 mM) provided complete protection against the loss of viability of lymphoblastoid cells exposed to 0.2 mM ferrous sulphate and 0.4 mM ascorbate (Trypan blue exclusion). They also showed that taurine (5 mM) reduced the accumulation of Ca^{2+} caused by ferrous sulphate and ascorbate to levels similar to those in controls after 30 min incubation. However, this concentration of taurine did not prevent lipid peroxidation due to these concentrations of ferrous sulphate and absorbate. Similar work with lymphoblastoid cells exposed to retinol and retinoic acid demonstrated that taurine (20 mM) combined with zinc chloride (100 μM) was able to reduce cell death almost to control levels [7]. Taurine and zinc alone afforded some protection and when combined with α-tocopherol, viability was the same as for control cells. It was noted that taurine and zinc or taurine and zinc combined with α-tocopherol were able to reduce retinol-induced cell swelling after 90 min incubation. This protection was believed to result from stabilization of the cell membrane, preventing flux of ions and water. Taurine did not however affect lipid peroxidation in these experiments. Incubations for a longer period still resulted in cell death, despite the fact that the cells had not swollen, but maintained the same volume as control cells. The additive effects of these three compounds suggested that their mechanisms of protection were different. Taurine has been shown to stabilize membranes, reduce cellular calcium accumulation, remove HOCl by forming the stable taurochloramine and help to maintain the osmotic

potential of cells. Thus, any or all of these mechanisms might have been involved in the protection of lymphoblastoid cells.

In the present studies the leakage of LDH and uptake of Trypan blue caused by three different cytotoxic agents was shown to be reduced by taurine. As both these parameters reflect membrane damage, the effect of taurine may be due to stabilization of the hepatocyte membrane.

In hepatocytes exposed to hydrazine and 1,4-naphthoquinone where both LDH leakage and Trypan blue uptake were measured, the two measurements were in agreement in that protection occurs at the same taurine concentration (15 mM) and the same exposure times. In the case of 1,4-naphthoquinone, ATP depletion is also complementary to the other two parameters. However, ATP was still significantly depleted in cells significantly protected by taurine (Fig. 4c). Thus, ATP depletion may be only partially a *result* of the process of cell death and therefore is only partly attenuated by taurine. In the case of hydrazine the situation is different. Hydrazine also depletes hepatocytes of ATP but this occurs at concentrations which are not cytotoxic (12 mM) (Fig. 5a–c). Indeed, it has been shown previously that hydrazine causes ATP depletion at the lower concentration of 8 mM which is clearly not cytotoxic [14] and it was concluded that ATP depletion was therefore a possible *cause* of the cytotoxicity of hydrazine rather than a consequence. If ATP depletion is a *result* of cytotoxicity and cell death then it should be reduced by taurine as taurine protects against cytotoxicity (Fig. 5a and b). The lack of effect of taurine on hydrazine-induced ATP depletion, which still occurred even in protected cells where viability (Trypan blue uptake and LDH leakage) was not significantly different from that of control cells, may therefore reflect the role of ATP depletion in the underlying mechanism of toxicity of hydrazine. This is consistent with our previous data which showed that hydrazine caused ATP depletion in hepatocytes in the absence of cytotoxicity [14] (Waterfield and Timbrell, 1992, unpublished observations). The data also shows that depletion of ATP by hydrazine to 10–15% of normal cellular levels does not lead to cell death in the presence of taurine. Further changes in the cell must therefore be necessary for cell death to occur and taurine presumably protects against these changes.

The mechanism by which taurine was able to protect hepatocytes from the three toxic compounds in the current studies may be the result of one or more mechanism either individually or in combination. It would appear that the plasma membrane was made less "leaky" by the addition of taurine as both LDH leakage and Trypan blue uptake were reduced by taurine. This may be an effect either on the surface of the plasma membrane or one initiated intracellularly. The observation that a reduction in cell death was achieved by using similar but not identical concentrations of taurine against three different compounds, might suggest that the mechanism of protection involves one of the fundamental events leading to cell death. This protection could involve the maintenance of membrane integrity, a reduction in membrane permeability or reduction in ion fluxes. All of these have been ascribed to taurine [9, 11, 12]. Taurine is also postulated to be able to influence Ca^{2+} levels in cells [8, 22]. As an increase in intracellular Ca^{2+} level is believed to be an important event in cell death, this might be one point at which taurine acts protectively. Recent studies have been carried out on the protective effects of cyclosporin A against the toxicity of several compounds causing oxidative stress in isolated hepatocytes. These studies concluded that the mechanism of protection may involve prevention of mitochondrial Ca^{2+} cycling and mitochondrial damage [23]. Taurine could also protect in a similar manner, as has been suggested previously in relation to myocardial damage [24]. Taurine might thus reduce ATP depletion due to calcium-induced mitochondrial damage after exposure to 1,4-naphthoquinone. However hydrazine directly affects mitochondrial function [25], inhibits the mitochondrial enzyme succinate dehydrogenase and also may deplete ATP via other mechanisms [13]. Consequently, the lack of protection by taurine against ATP depletion due to hydrazine could be due to the absence of involvement of calcium. Further work is being carried out to determine the mechanism by which taurine protects against cytotoxicity.

This study has shown that taurine is able to protect hepatocytes against cytotoxicity caused by different compounds. Furthermore, in the presence of taurine the process of cell death induced by hydrazine was separated from ATP depletion. Taurine may thus prove to be a useful tool for probing the mechanisms of toxicity of compounds in isolated cells.

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REFERENCES

1. Waterfield CJ, Turton JA, Scales MDC and Timbrell JA, Reduction of liver taurine in rats by *B*-alanine treatment increases carbon tetrachloride toxicity. *Toxicology* **77**: 7–20, 1993.
2. Waterfield CJ, Turton JA, Scales MDC and Timbrell JA, The correlation between urinary and liver taurine levels and between predose urinary taurine and liver damage. *Toxicology* **77**: 1–5, 1993.
3. Oja SS and Kontro P, Taurine. In: *Handbook of Neurochemistry* (Ed. Lajtha A), pp. 501–533. Plenum Press, New York, 1982.
4. Wang Q, Giri SN, Hyde DM and Nakashima JM, Effects of taurine on bleomycin-induced lung fibrosis in hamsters. *Proc Soc Exp Biol Med* **190**: 330–338, 1989.
5. Nakashima T, Takino T and Kuriyama K, Therapeutic and prophylactic effects of taurine administration on experimental liver injury. In: *Sulphur Amino Acids: Biochemical and Clinical Aspects* (Eds. Kuriyama K, Huxtable RJ and Iwata H), pp. 449–459. Alan R. Liss, New York, 1983.
6. Pasantes-Morales H, Wright CE and Gaull GE, Taurine protection of lymphoblastoid cells from iron-ascorbate induced damage. *Biochem Pharmacol* **34**: 2205–2207, 1985.
7. Pasantes-Morales H, Wright CE and Gaull CE, Protective effect of taurine, zinc and tocopherol on

retinol-induced damage in human lymphoblastoid cells. *J Nutr* **114**: 2256–2261, 1984.

8. Nakashima T, Seto Y, Toshikazu N, Shima T, Iwai Y, Zeizo K, Okanque T and Kashima K, Calcium-associated cytoprotective effect of taurine on the calcium and oxygen paradoxes in isolated rat hepatocytes. *Liver* **10**: 167–172, 1990.
9. Wright CE, Lin TT, Lin YY, Sturman JA and Gaull GE, Taurine scavenges oxidised chlorine in biological systems. In: *Taurine: Biological Actions and Clinical Perspectives* (Eds. Oja SS, Ahtee L, Kontro P and Paasonen MK), p. 137. Alan R. Liss, New York, 1985.
10. Gordon RE, Shacked AA and Solano DF, Taurine protects hamster bronchioles from acute NO_2-induced alterations—a histologic, ultrastructural and freeze-fracture study. *Am J Pathol* **125**: 585–600, 1986.
11. Gaull GE, Pasantes-Morales H and Wright CE, Taurine in human nutrition: overview. In: *Taurine: Biological Actions and Clinical Perspectives* (Eds. Oja SS, Ahtee L, Kontro P and Paasonen MK), p. 3. Alan R. Liss, New York, 1985.
12. Huxtable RJ, Physiological actions of taurine. *Physiol Rev* **72**: 101–163, 1992.
13. Ghatineh S, Morgan W, Preece NE and Timbrell JA, A biochemical and NMR spectroscopic study of hydrazine in the isolated rat hepatocyte. *Arch Toxicol* **66**: 660–668, 1992.
14. Preece NE, Ghatineh S and Timbrell JA, Course of ATP depletion in hydrazine hepatotoxicity. *Arch Toxicol* **64**: 49–53, 1990.
15. O'Brien PJ, Molecular mechanisms of quinone cytotoxicity. *Chem Biol Interact* **80**: 1–41, 1991 (published erratum, *Chem Biol Interact* **81**: 219, 1992).
16. Poli G, Cheeseman K, Slater TF and Dianzani MU, The role of lipid peroxidation in CCl_4-induced damage to liver microsomal enzymes: comparative studies *in vitro* using microsomes and isolated liver cells. *Chem Biol Interact* **37**: 13–24, 1981.
17. Rechnagel RO and Glende EA Jr, Carbon tetrachloride hepatotoxicity: an example of lethal cleavage. *CRC Crit RevToxicol* **2**: 263–297, 1973.
18. Waller RL, Glende EA Jr and Rechnagel RO, Carbon tetrachloride and bromotrichloromethane toxicity: dual role of co-valent binding of metabolic cleavage products and lipid peroxidation in depression of microsomal calcium sequestration. *Biochem Pharmacol* **32**: 1613–1617, 1983.
19. Moldeus P, Hogberg J and Orrenius S, Isolation and use of liver cells. *Methods Enzymol* **51**: 60–71, 1978.
20. Bergermeyer HU, Bernt E and Hess B, Lactate dehydrogenase (II 2a). In: *Methods of Enzymatic Analysis* (Ed. Bergmeyer H), pp. 736–743. Academic Press, New York, 1965.
21. Stanley PE and Williams SG, Use of the liquid scintillation spectrometer for determining adenosine triphosphate by luciferase enzyme. *Anal Biochem* **29**: 381–392, 1969.
22. Huxtable RJ, Physiological actions of taurine. *Physiol Rev* **72**: 101–163, 1992.
23. Kass GEN, Juedes MJ and Orrenius S, Cyclosporin A protects hepatocytes against prooxidant-induced cell killing. A study of the role of mitochondrial Ca^{2+} cycling in cytotoxicity. *Biochem Pharmacol* **44**: 1995–2003, 1992.
24. Azuma J, Hamaguchi T, Ohta H, Takihara K, Awata N, Sawamura A, Harada H, Tanaka Y and Kishimoto S, Calcium overload-induced myocardial damage caused by isoproterenol and by adriamycin: Possible role of taurine in its prevention. *Adv Exp Med Biol* **217**: 167–179, 1987.
25. Hadler HI and Cook GL, A requirement for Pi for the transitory uncoupling of rat liver mitochondria by hydrazine when β-hydroxybutyrate is the substrate. *J Environ Pathol Toxicol* **1**: 419–432, 1978.