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RAPID COMMUNICATION

HEPATOTOXICITY OF TETRAHYDROAMINOACRIDINE IN ISOLATED RAT HEPATOCYTES: EFFECT OF GLUTATHIONE AND VITAMIN E.

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INTRODUCTION.

Alzheimer's disease is characterized by a progressive neurological degeneration, which possibly is due to a major loss of acetylcholine-producing neurons in certain parts of the brain (1). In 1986, Summers et al. (2) published the results of a clinical study in which patients who suffered from moderate to severe Alzheimer's disease, were treated for a period of one year with tetrahydroaminoacridine (THA), a centrally acting anticholinesterase. The results of this study were positive and encouraging. However, by the end of 1987, it was reported in Science (3), that another THA trial, sponsored by the National Institute of Aging (NIA, USA) was put on hold because treatment of patients with THA in this trial was accompanied by elevated levels of liver enzymes in blood, indicative of liver damage. Since no data on the hepatotoxicity of THA are available, we have investigated the toxicity of THA in isolated rat hepatocytes. We also investigated whether the presence of glutathione (GSH) or vitamin E could prevent such a toxicity.

MATERIALS AND METHODS.

9-Amino-1,2,3,4 tetrahydroacridinehydrochloride hydrate (THA) was purchased from Janssen, Beerse, Belgium and α tocopherol succinate (vitamin E) from Sigma. St. Louis, MO, USA.
Liver parenchymal cells were isolated by the collagenase perfusion technique (4) from male Wistar rats (weighing 200-250g), which had free access to food and water. Cells ($2\text{-}4\times10^6$ cells/ml) were incubated in Hanks'- HEPES buffer, pH=7.4, under an atmosphere of 95% O_2/ 5% CO_2 at 37°C in a rotary shaker, as described elsewhere (5). Cells were preincubated with or without vitamin E for 30 minutes. Diethyl maleate (DEM, 1 mM), to deplete GSH, and THA were added at the same time. DEM depleted GSH to 25% of the control value within 2 minutes (unpublished observations).
GSH was determined by the method of Saville (6); protein thiols were determined in the acid precipitated pellet with Ellmann's reagent (7). As a measure for lipid peroxidation, thiobarbituric acid reacting products were determined (8), while lactate dehydrogenase leakage from cells was used as an parameter for cell death (9).

RESULTS.

THA is toxic to isolated hepatocytes: at a concentration of 3 mM, cell death occurred after approximately 3 hours of incubation (fig.1). Dead cells more or less maintained their cellular morphology as judged by microscopic evaluation of trypan blue-stained cells. When a concentration of 10 mM THA was used, all cells died within one hour. However, at this concentration, THA completely disrupted the cellular structure. Therefore, all further experiments were done with 1-3 mM THA.
Lipid peroxidation increased later in time than cell death (fig.2), so that lipid peroxidation may have been a consequence of cell death (10). The cellular GSH content decreased at a moderate rate upon addition of THA to hepatocytes: this decrease was almost the same at 1 and 3 mM in the first 3 hours of incubation (fig.3). Modification of protein thiols paralleled with the induction of cell death at 3 mM THA (fig.4).

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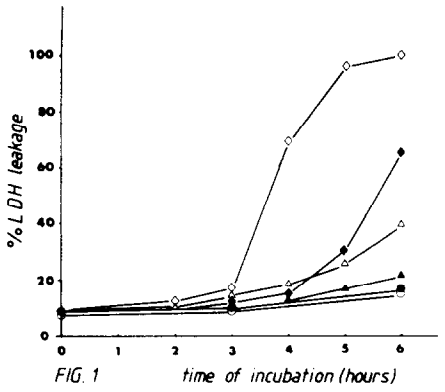

FIG. 1

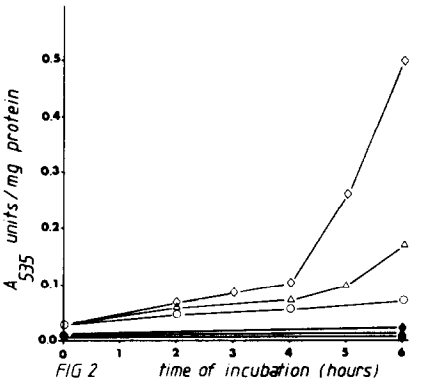

FIG 2

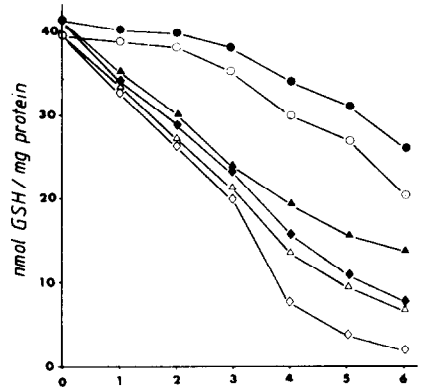

FIG 3

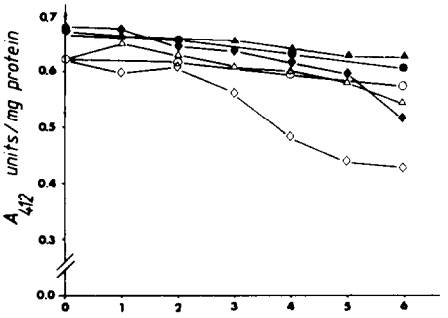

FIG 4

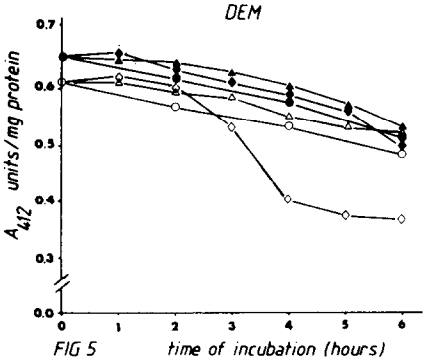

FIG 5

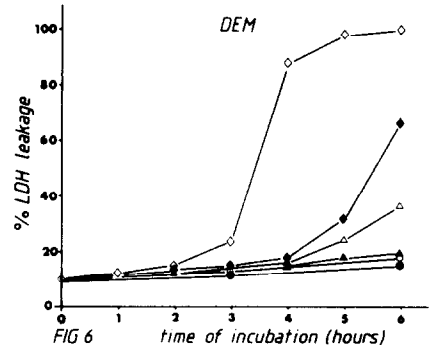

FIG 6

FIGURES.

Fig.1. Leakage of LDH from hepatocytes exposed to THA or THA plus vitamin E
Fig.2. Lipid peroxidation, as determined by the thiobarbituric acid positive material, in hepatocytes exposed to THA or THA plus vitamin E.
Fig.3. Content of GSH in hepatocytes exposed to THA or THA plus vitamin E.
Fig.4. Effect of vitamin E upon THA-induced protein thiol modifications in hepatocytes.
Fig.5. Effect of vitamin E on THA-induced protein thiol modifications in GSH-depleted (1mM DEM) hepatocytes.
Fig.6. Leakage of LDH from GSH-depleted (1 mM DEM) hepatocytes exposed to THA or THA plus vitamin E.

Legends for fig.1 t/m 6:
○ control (1 t/m 4) or 1 mM DEM (5 and 6), ● 100 ㎛M vitamin E, △ 1 mM THA, ▲ 1 mM THA plus vitamin E, ◇ 3 mM THA, ◆ 3 mM THA plus vitamin E.
For all figures: one experiment typical of three.

To investigate if the presence of GSH might prevent the toxicity of THA, we did the same experiments with GSH depleted cells. GSH was rapidly depleted by DEM (1mM) to about 25% of the control value (data not shown). This GSH depletion had no effect on either protein thiols or cell viability (fig.5, 6). In spite of the fact that GSH was depleted, no increased THA toxicity was observed; no change was observed in THA-induced protein thiol modification (fig.5) and also cell death occurred to the same extent as in GSH adequate cells (fig.6). Therefore, GSH does not protect against THA-induced cell death.
Vitamin E, a naturally occurring antioxidant (11), protects cells against some hazardous compounds (12). We preincubated cells with vitamin E which resulted in a strong decrease of THA toxicity, both in GSH adequate cells and GSH depleted cells (fig.1,6). Vitamin E as expected, prevented lipid peroxidation (fig.2) while protein thiols were maintained in their reduced form (fig.2,5). It slightly alleviated GSH depletion by THA (fig.3).

DISCUSSION.

THA seems to induce liver damage in Alzheimer patients, which may preclude the use of THA as a safe drug in the treatment of these patients. In our hepatocyte system, THA caused cell death which was preceeded by a slow decrease of the intracellular GSH content. However, this GSH decrease induced by THA was of minor importance because GSH depletion by DEM did not lead to increased toxicity. Hepatoxicity of some other drugs, like paracetamol, is prevented by the intracellular GSH and thus depletion of GSH by DEM or other GSH depletors results in an increase of such toxicity (13). In the case of THA, GSH depletion did not lead to increased toxicity. Thus, it is unlikely that elevation of the liver GSH content by e.g. N-acetyl cysteine, will lead to a protection against THA-induced toxicity. Elevation of the cellular vitamin E content seems to be more promising because vitamin E strongly delayed the toxicity of THA, irrespective of the intracellular GSH status. Since no data on the type of liver injury in Alzheimer patients have been published yet, it is hard to judge whether the presented results in isolated hepatocytes will be predictive for the human situation. It remains to be investigated whether this vitamin E effect provides a means to optimize the THA therapy in Alzheimer patients.

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