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COMPARATIVE EFFECT OF A SERIES OF HYPOLIPIDEMIC COMPOUNDS ON THE RELEASE OF ENZYMES FROM ISOLATED HEPATOCYTES.

K.AGUIAR, M.M.GALTEAU, G.SIEST, S.FOURNEL, J.MAGDALOU

Centre du Médicament, Unité associée au CNRS n°597, Faculté des Sciences Pharmaceutiques et Biologiques, 30 rue Lionnois, 54000 Nancy (F)

INTRODUCTION

Isolated hepatocytes have been used to study the metabolism of several compounds such as acetaminophen (1) or biphenyl (2). We used this model to study a series of structurally related chemicals, and to select among this series the less "membrane-aggressive" compounds.

MATERIALS AND METHODS

Male Wistar rats (180–200 g) were purchased from Iffa-Credo (St Germain sur l'Arbresle/F). They were allowed food and water ad libitum. Hepatocytes were isolated by the method of Van Bezooijen (3) except that hyaluronidase was omitted from the collagenase solution. The viability of isolated cells was determined by the Trypan blue exclusion test. The incubation medium was constituted of Krebs-Ringer-bicarbonate buffer (pH 7,4) containing 0,5 % (w/v) bovine albumin, and 5 mM glucose. Chemicals were added to incubation flasks containing 2 ml of cell suspension (2 x 10^6 cells/ml) with shaking at 37°C. Control incubation was conducted under the same conditions without any chemicals. Lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) activities were measured using optimized UV technics (LDH Boehringer 124915, AST BioMérieux 61341) adapted to a fast centrifugal analyser (Cobas, Roche Bioelectrique France). Reduced glutathione (GSH) was estimated by the method of Hissin and Hilf (4).

RESULTS AND DISCUSSION

The series we chose to test was known for its lipid lowering properties in humans and in rats i.e. F 1379 [4-(2'-chlorobiphenyl)-4-oxo-2-methylene butyric acid]. The structural analogs were : F 1550 [4-(2'-chlorobiphenyl)-4-oxo-2-methyl butyric acid], F 1594 (4-biphenyl-4-oxo-2-methyl butyric acid), F 1377 (4-biphenyl-4-oxo-2-methylene butyric acid). The release of lactate dehydrogenase and of aspartate aminotransferase was measured as a function of time and of drug concentration. The intracellular reduced glutathione was also measured.

Fig.1 indicates the results obtained for LDH and AST release after 2 h incubation in the presence of these different compounds (final concentration = 1 mM).

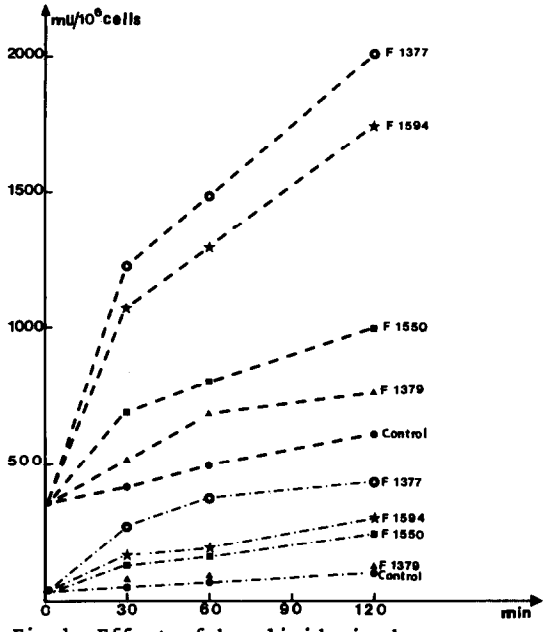

Fig.1. Effect of hypolipidemic drugs on LDH (---) and AST (-·-·-) release (n = 4)

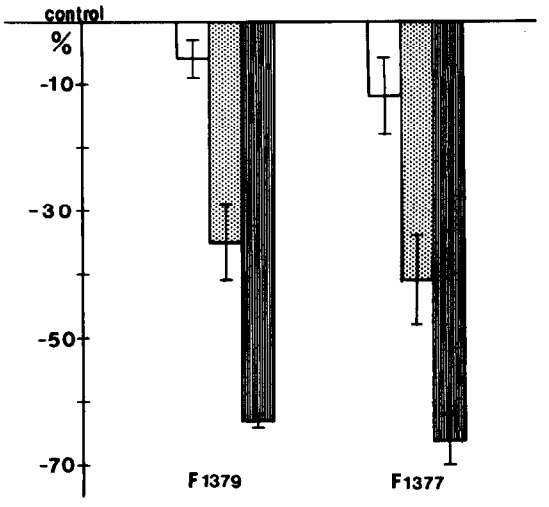

Fig.2. Modifications of GSH content.
Concentrations of drugs
0,1 mM 0,5 mM 1 mM

When no drug is added to the hepatocytes, LDH and AST are slightly released in extracellular medium. At a concentration of 1 mM, the release is obvious, especially for LDH which is more sensitive than AST. The location of LDH is exclusively cytoplasmic whereas AST is partly located in the mitochondrial matrix and partly in the cytoplasm. The compounds which are chlorinated in position 2 (F 1379, F 1550) provoke less LDH and AST release than the non-chlorinated molecules (3 to 5 times less). Two compounds from each group, F 1379 and F 1377, both diminished the intracellular GSH (Fig.2). The percentage of decrease is however similar for the two compounds. No difference occurs due to the chlorine in position 3. From these results, no direct correlation exists between enzyme release and glutathione decrease. At a final concentration of 0.5 mM, LDH and AST are not released with F 1379 or F 1377 ; but, GSH is already diminished by 35 % and 41 % respectively.

In conclusion, enzyme release from isolated hepatocytes seems to be a good index of cell sensitivity to different compounds. We observed high differences; and, we were able to "classify" the molecules as a function of their membrane effect in the conditions we used. It must be noticed that the concentrations used are very high compared to the doses administered to humans.

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