ACTIVITY OF LYSOSOMAL ENZYMES IN DRUG-INDUCED LIVER DAMAGE

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Lysosomal enzymes (LE) are invariable participants in all processes of cellular injury [7, 11]. This applies fully also to liver pathology: changes in LE activity are found in viral and toxic liver damage [1, 5, 6, 9, 13-15].

The aim of this investigation was to study the role of LE in the development of drug-induced liver damage.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing on average 150-200 g. Two groups of animals each received a single dose of CCl_4 after 24 and 48 h, respectively; the remaining groups received drugs in the form of courses lasting 1 and 2 weeks (the names of the drugs, their doses, and modes of administration are given in Tables 1 and 2). After sacrifice of the animals liver tissue homogenates and, in some groups also, blood serum were subjected to biochemical investigation. Free activity of the following LE was determined: acid phosphatase by the method of Bessey et al. and in the modification of Levitskii et al. [4], cathepsins at pH values of 3.5, 4.3, and 5.5 by Anson's method in Levitskii's modification [3], and acid nucleases: RNase by the method of Konovets and Levitskii [2] and DNase by the method of Samoilyuk et al. [8]. The protein concentration was determined by the method of Lowry et al. in Levitskii's modification [3]. To determine activity of transaminases (ALT and AST) and of gamma-glutamyl transferase (GGT) kits of reagents were used (Lachema, Czechoslovakia).

EXPERIMENTAL RESULTS

To obtain a key to the interpretation of the enzyme shifts induced by drugs, we first investigated free LE activity in the liver and in the blood serum in toxic hepatitis induced in rats by administration of CCl_4, which is rightly regarded as a model of hepatotoxicity [14]. Like some other investigators, we limited our efforts to the determination only of free LE activity, which was interpreted as an indicator of lysosomal stability [9]. It will be clear from Table 1 that as early as 24 h after administration of CCl_4 free acid phosphatase (AP) activity and cathepsin activity determined at pH 5.5 (C-5.5) in the liver tissue were significantly depressed, whereas cathepsin activity determined at pH 3.5 (C-3.5) was raised. After 48 h these same shifts, while remaining significant, were somewhat reduced, but activity of acid nucleases was increased, especially that of DNase, which showed a significant and almost fourfold rise compared with the control. Activity of most LE in the blood serum 24 h after administration of CCl_4, however, still remained significantly increased.

The drugs tested were conventionally divided into two groups. The first group consisted of known hepatotoxic agents: cinchophen (synonym Atophan), used to create an experimental model of hepatitis, and butadione, the adverse effect of which on the liver has been reported in the literature [10, 12] and demonstrated by ourselves experimentally by biochemical and morphological investigations of the liver.

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TABLE 1. Activity of Lysosomal Enzymes in Liver Homogenates and Blood Serum of Rats after a Single Dose or Course of Hepatotoxic Agents Administered by the Intragastric Route (in % of control)

Name of drug	Daily dose, ml/kg	Exposure	Acid phosphatase		Cathepsin 3.5		Cathepsin 5.5		Acid RNase		Acid DNase	
			liver	blood serum	liver	blood serum	liver	blood serum	liver	blood serum	liver	blood serum
CCl_4	5	24 h	14***	255***	195*	226*	14**	805*	84	124	134	649*
		48 h	29***	338***	176*	122	24**	83	221	203	395*	666
Ginchophen	500 mg/kg	1 week	61***	134	66*	652***	31**	507	155**	101	105	273
		2 weeks	42***	1251	133*	196**	15**	115	102	108	110	15*
Butadione	150 mg/kg	2 weeks	99	57	202***	119	17*	187	134	56	189	654

Legend. Here and in Table 2: *p < 0.05, **p < 0.01, ***p < 0.001.

TABLE 2. Specific Enzyme Activity in Liver Homogenates after Course (2 weeks) of Nonsteroid Anti-Inflammatory Agents Administered by the Intragastric Route (in % of control)

Name of drug	Daily dose, ml/kg	Transaminase		GGT	Acid phosphatase	Cathepsins		
		ALT	AST			pH 3,5	pH 4,3	pH 5,5
Butadione	150	35***	27***	49**	54***	82	48**	97
Aminopyrine	100	53**	52***	54**	78**	60**	24***	79
Indomethacin	2,5	61**	48***	44**	54***	53***	21***	137*
Ibuprofen	50	67**	54***	54**	68***	58***	45***	104
Naproxen	50	42***	45***	83	61***	85	44**	200**
Diclofenac	5	149**	84	85	52***	86	41***	159**

After a course of cinchophen, changes in LE activity (except nucleases) similar to those caused by CCl_4 were found in the liver and blood serum; after a course of butadione, similar significant changes affected only free C-3.5 and C-5.5 activity in the liver tissue (Table 1).

Data given in Table 1 demonstrate marked changes in free LE activity in the liver tissue which are in opposite directions: (a) an increase, evidence of injury to intracellular (lysosomal) membranes, and possibly an early enzymologic reaction of the liver cells to the action of hepatotoxins, and (b) a decrease in activity, usually explained by "leakage" of enzymes from the damaged cells. However, irrespective of the direction of these two types of changes, they were the result of a disturbance of integrity of the lysosomes, for in both cases raised blood enzyme levels were observed.

The second group consisted of drugs which also are nonsteroid anti-inflammatory agents (NSAIA; Table 2), but their adverse effect on the liver either is observed only in individual cases, or is unknown, and in clinical practice any hepatotoxicity they may possess is usually disregarded [10, 12].

It will be clear from Table 2 that most NSAIA studied significantly depressed transaminase activity in liver tissue [the exception was diclofenac (synonym Voltaren), which caused a moderate and significant increase in ALT activity], and many of them (except naproxen and diclofenac) significantly reduced GGT activity. These data, and also the results of our clinical investigations (hypertransaminasemia in some patients treated with the above-mentioned NSAIA), are evidence of the hepatotoxicity of these drugs. Changes in free LD activity in the liver tissue – reduction of EP and C-4.3 activity, and also in some cases, depression of C-3.5 activity, but in other cases an increase in C-5.5 activity – can be interpreted as further proof of drug-induced hepatotoxicity revealed by this investigation.

On the basis of the results of this investigation it can be postulated that lysosomes play a role in the development of drug-induced liver damage. LE may perhaps bring about cytolysis, initiated by the hepatotoxin.

This fact of the hepatotoxicity of a number of NSAIA, established by these experiments, confirms the importance of a close experimental study of the effect of drugs, including some well known ones, on the state of the liver (experimental testing for hepatotoxicity),

and it justifies the recommendation that LE activity under these circumstances be tested both in liver tissue and in blood serum.

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EFFECT OF POLYMETHYLENE DERIVATIVES OF 4-AMINOPYRIDINE ON HIPPOCAMPAL NEURON FUNCTION

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Among drugs used previously to correct cognitive disorders in senile dementia of Alzheimer type (SDAT), the most effective has proved to be tacrine [13], which is an aminoacridine derivative of 4-aminopyridine, a substance well known as blocker of potassium channels of a particular type [14]. It has been suggested that the high efficacy of tacrine (tetrahydroaminoacridine) may be due to its special pharmacologic spectrum, in which anticholinesterase activity is combined with ability to block potassium channels [13]. A similar spectrum is possessed also by substances belonging to a different class of polymethylene derivatives of 4-aminopyridine, namely the aminoquinoline class [1], among which special attention has been drawn to amiridine [9-amino-2,3,5,6,7,7'-hexahydro-IH-cyclopenta(B)-quinoline], a compound synthesized in the USSR and recommended for clinical use as a stimulator of conduction of excitation in nerve and muscle tissue. Amiridine has been shown to facilitate the learning process in animals with models of congenital and acquired memory disturbances [8]. Neurochemical changes linked with SDAT are most marked

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