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Biochemical Pharmacology, Vol. 42, No. 3, pp. 702–704, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Drug oxidation and N-acetylation in rats pretreated with subtoxic doses of streptolysin O

(*Received* 24 *September* 1990; *accepted* 23 *March* 1991)

The hemolytic, cardiotoxic and lethal streptolysin O (SLO) possesses, when given in low, sublytic doses, notable immunological properties [1–4]. Further, previous studies have shown that SLO increases the acetylation rates of sulfamethazine and p-amino benzoic acid (PABA) in rats and rabbits [5, 6]. The effect of SLO on drug acetylation might be associated with the reticuloendothelial cell system whose primary function is the immunological response and which is involved in the polymorphic drug N-acetylation also [7–10]. There is evidence that morphological and functional changes of this cell system result in altered N-acetylation capacity [9, 11–15]. Whether it also influences the oxidative drug metabolism is not known.

The present study was performed to examine whether SLO when given to rats in sublytic doses would be capable of influencing the activities of selected hepatic microsomal monooxygenases and the cytosolic *N*-acetyltransferase [16].

Materials and Methods

Animals and pretreatment. All experiments with SLO (Imuna, Czechoslovakia) were performed in male Wistar albino rats (200–275 g) purchased from Versuchstierzucht Schönwalde/Berlin. They were housed under controlled conditions in groups of six to eight animals with free access to standard diet and water for at least 14 days prior to the study. After randomization, groups of 12 animals were treated intravenously (tail vein) in shallow ether narcosis with 100 hemolytic units (HU)/kg body weight 1 day (acute pretreatment) or for 5 days (subacute treatment) before being killed, respectively (injection volume 1.0 mL/kg). Controls were administered equivalent volumes of saline at corresponding times. The interval between the last pretreatment dose and the beginning of both studies was generally 24 hr.

Biochemical protocol. After light ether anesthesia of the animals, blood was taken by puncture of the retrobulbar venous plexus for determination of γ-glutamyltransferase (GT), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and creatinine. After decapitation, the abdominal wall was opened to insert a cannula into the portal vein. The liver was quickly removed, freed from connective tissue, weighed and perfused with ice-cold saline. Cytosolic and microsomal fractions of rat livers were prepared by gradient centrifugation. Microsomal cytochrome P450 was determined by the method of Greim [17] and protein levels according to Eggstein and Kreutz [18]. Activities of aminopyrine demethylase and aniline hydroxylase were measured in 1 mL phosphate buffer containing 2 mg/mL microsomal protein, a NADP-regenerating system (3.1 mmol/L glucose-6-phosphate, 0.4 mmol/L NADP, 3 I.U. glucose-6-phosphate dehydrogenase), 13.3 mmol/L niacin, and the substrates (aminopyrine 10 mmol/L and aniline 200 mmol/L, respectively). The pH of the solution was adjusted to 7.4 and 7.8, respectively. Enzyme activities were expressed as amounts of formaldehyde and *p*-aminophenol formed per min and mg microsomal protein [19, 20]. *N*-Acetyltransferase activities were determined with procainamide and *N,S*-diacetylcysteamine as substrates [21]. Aliquots of the cytosolic fractions (about 5 mg protein) were incubated in 1 mL samples (phosphate buffer pH 7.4) with *N,S*-diacetylcysteamine (3.1 mmol/L) and procainamide (4 mmol/l) at 37° for 30 min. The reaction was terminated with NaOH (2.5 mol/L) and the metabolite *N*-acetylprocainamide was measured with a gas chromatographic method [22]. After addition of 100 μL internal standard (0.3 mol/L butyrylprocainamide), the samples were extracted twice with 3 mL ethylacetate. After re-extraction into 1 mL 0.1 mol/L HCl, the samples were washed twice with 0.2 mL 2.5 mol/L NaOH and extracted again with ethylacetate. The organic phase is evaporated to dryness and after that redissolved in 50 μL methanol.

Chromatographic conditions. Apparatus: Hewlett-Packard 5830 A equipped with N-P-FID; column: 4 ft × 2 mm i.d., glass, 3% OV-17 on Chromosorb WAW-DMCS; gases: N_2 30 mL/min, H_2 3 mL/min, air 50 mL/min; temperature: thermostat 280°, injector 300°, detector 300°. ASAT, ALAT, γ-GT and creatinine in serum were

Table 1. Hepatic microsomal monooxygenase system and N-acetylation after acute and subacute pretreatment of rats with SLO

	Acute pretreatment		Subacute pretreatment	
	Control	SLO	Control	SLO
Body weight (g)	248 ± 20	240 ± 11	238 ± 16	220 ± 18*
Relative liver weight (%)	3.84 ± 0.27	4.10 ± 0.28*	3.06 ± 0.48	3.35 ± 0.28*
Creatinine (μmol/L)	30.9 ± 4.8	35.5 ± 5.3	37.0 ± 9.9	49.1 ± 5.6*
γ-GT (nmol/sec · L)	59 ± 22	82 ± 21*	31 ± 15	39 ± 40
ALAT (nmol/sec · L)	794 ± 59	880 ± 402	682 ± 165	678 ± 145
ASAT (nmol/sec · L)	961 ± 101	896 ± 201	838 ± 176	1257 ± 300*
Cytochrome P450 (nmol/mg protein)	1.10 ± 0.12	0.72 ± 0.13*	1.09 ± 0.21	0.74 ± 0.15*
Aminopyrine-*N*-demethylase (nmol/min · mg)	3.24 ± 0.09	2.41 ± 0.27*	3.25 ± 0.15	1.98 ± 0.62*
Aniline hydroxylase (nmol/min · mg)	0.24 ± 0.03	0.17 ± 0.01*	0.26 ± 0.01	0.24 ± 0.05
N-Acetyltransferase (nmol/min · mg)	17.7 ± 2.7	22.6 ± 2.6*	16.7 ± 4.1	29.4 ± 5.7*

Values are means ± SD.
* $P < 0.05$.

measured according to AB-2 (G.D.R.) with AGP 5040 (Eppendorf) and flow stream apparatus (MLW, Medingen).

Statistics. Means ± standard deviations were given. Differences between two random samples were analysed with Wilcoxon's signed rank test and $P < 0.05$ as level of significance.

Results and Discussion

Acute SLO pretreatment of male rats did not influence the laboratory data of creatinine, ALAT and ASAT, but increased γ-GT. Subacute premedication for 5 days tended to increase moderately the values of creatinine and ASAT probably as signs of the beginning organ toxicity of SLO. Both dosage regimens caused significantly higher relative liver weights and depressed markedly all parameters of microsomal drug oxidation except for the activity of aniline hydroxylase after subacute premedication (Table 1). All effects on phase I metabolism are most likely related to the non-specific membranal binding properties of SLO [4, 23]. This has to be considered even though the two monooxygenases studied are not selective with regard to a specific cytochrome P450 isozyme. However, aniline hydroxylase did not respond after subacute pretreatment, an observation which might account for some specificity of SLO on the monooxygenase system. Contrary to the depressive effect of SLO on the microsomal enzymes the hepatic cytosolic *N*-acetyltransferase activities were significantly enhanced after both dosage regimens. These findings of the biochemical study were in agreement with the results of hitherto reported pharmacokinetic experiments. Increased acetylation rates of substrates of the *N*-acetyltransferase (sulfamethazine, isoniazid) were observed in rabbits and rats after SLO pretreatment [5, 6]. The higher acetylation rates in these studies might have been caused by stimulation of the hepatic *N*-acetyltransferase. However, the mechanism of the increased N-acetylation capacity in animals is not clear. One might speculate that the phenomena are attributed to the effects of SLO on the reticulo-endothelial cell system (RES). There are several hints in the literature. Firstly, SLO in low doses is known to stimulate immunological functions of the RES [1, 2, 23]. Secondly, hepatic sinusoidal and other cells of this system are important places of drug acetylation [7, 9]. Further, immune stimulation with zymosan or Freud's adjuvant facilitated drug acetylation rates in rats [12, 14, 15]. Peculiarities of drug acetylation rates were also observed in patients with malignant lymphomas and acute and chronic leukemia [11, 13, 24].

The whole matter in discussion is confusing insofar as the observations reported were made with different forms of *N*-acetyltransferase. In rats procainamide, sulfamethazine or isoniazid are metabolized by the monomorphic *N*-acetyltransferase, whereas in man and rabbits, they are metabolized by the polymorphic *N*-acetyltransferase [16, 25, 26]. More detailed studies should be done to verify the hypothesis that the phenomena in drug N-acetylation after sublytic doses of SLO might have been caused by the reagibility of the RES.

In summary, SLO in sublytic doses (100 HU/kg body wt) depressed the hepatic microsomal cytochrome P450 and aminopyrine-*N*-demethylase but increased significantly the cytosolic *N*-acetyltransferase after acute and subacute (5 days) pretreatment of male Wistar rats. Aniline hydroxylase was reduced after acute and unchanged after subacute pretreatment.

The effects of SLO on the oxidative enzymes and drug N-acetylation might have been caused by the membranal and immunological properties of the toxin.

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Biochemical Pharmacology, Vol. 42, No. 3, pp. 704–707, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Effect of experimental cirrhosis on cholephilic dye metabolism and excretion in the rat

(*Received* 5 *December* 1990; *accepted* 2 *April* 1991)

The metabolism and hepatic excretion of a large number of drugs is significantly impaired in cirrhotic patients and animals with experimental cirrhosis [1, 2]. Perturbations in hepatic enzyme activity, hepatic hemodynamics and drug binding are believed to be responsible for changes in drug elimination. The relative importance of these factors, however, is unknown and the problem is further complicated by the variable effects of cirrhosis on the pathways of liver metabolism.

The cholephilic dye sulfobromophthalein (BSP) has been found to be a useful tool for studying hepatobiliary function, as its biliary transport maximum (T_{max}) is known to be modified in various experimental and pathological conditions. Both alterations in the conjugation capacity [3, 4] or in the transport mechanisms *per se* [5] have been found to affect the biliary excretion of the dye. In the present study we have explored the conjugation and hepatobiliary transport of BSP in rats rendered cirrhotic