INVESTIGATION OF THE ACTION OF SPOROFUSARIN ON CELL MEMBRANES OF THE ISOLATED PERFUSED LIVER

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The effect of sporofusarin (a myoctoxin produced by Fusarium sporotrichiella v. sporotrichioides) on the functional activity and permeability of cell membranes of the isolated perfused rat liver was studied. Sporofusarin in a final concentration of $5.9 \cdot 10^{-5}$ M was found to reduce the rate of bile formation, urea synthesis, and oxygen consumption and also to cause an earlier and severe disturbance of permeability of the lysosomal and plasma membranes of the hepatocytes (an increase in activity of the enzymes β-acetylglucosaminidase, β-glucuronidase, arylsulfatases A and B, and β-galactosidase in the supernatant of a liver homogenate and in the perfusion fluid). The depression of liver function by sporofusarin is considered to be the result of damage to the membraneous structures of the cell and, in particular, of the lysosomes.

KEY WORDS: Mycotoxin (sporofusarin); permeability of membranes; lysosomes; liver function (bile formation, urea synthesis).

Poisoning with sporofusarin is known to be a basic component of the disease known as "alimentary toxic aleukia," connected with the consumption as food of grain covered with snow in the winter and infected with the microscopic mold Fusarium sporotrichiella. Despite many investigations of the pathogenesis of sporofusarin poisoning, the mechanism of action of this highly toxic metabolite has itself received little study [1, 2, 7]. In previous investigations the writers demonstrated the marked injurious action of sporofusarin on lysosomal membranes [6, 14].

The object of the investigation described below was to study the effect of this poison on the isolated perfused liver.

EXPERIMENTAL METHOD

Perfusion was carried out with a system consisting of a specially made roller pump with controllable output, a bubble oxygenator, a thermostatically controlled chamber for the isolated liver, and a measuring unit (radiometer ABC-1) permitting constant monitoring of the perfusion condition (Fig. 1). The following parameters were maintained: rate of perfusion 12-15 ml/min; temperature 36.5 ± 0.5° C; pressure 90-110 mm water; pH 7.4 ± 0.5; partial pressure of oxygen 250 ± 50 mm Hg [8, 10]. Hanks' salt solution was used as the perfusion medium, with the addition of 5% $NaHCO_3$ (to pH 7.4), bovine albumin (to a final concentration of 3%), and a mixture of L-alanine, L-arginine, and L-glutamine (in concentrations of 30, 1.4, and 0.6 mg/100 ml respectively), ensuring satisfactory conditions for urea synthesis [9, 16]. The total volume of circulating perfusion medium was 100 ml. The perfusion fluid was oxygenated with carbogen (95% O_2 + 5% CO_2).

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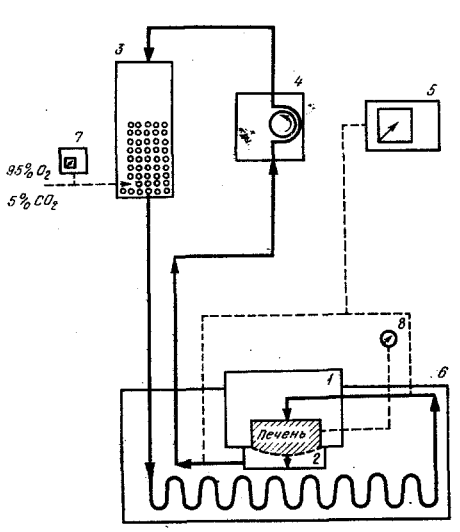

Fig. 1

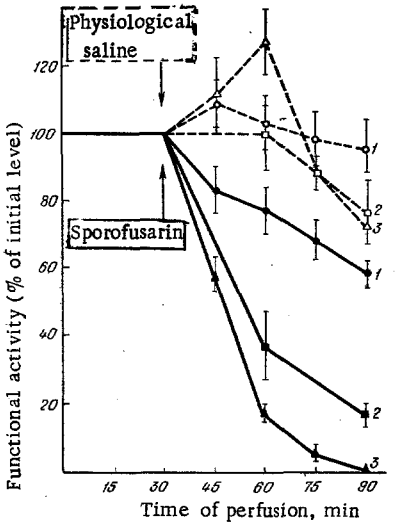

Fig. 2

Fig. 1. Block diagram of the system used for perfusion of isolated rat liver: 1) thermostatically controlled chamber; 2) collecting chamber; 3) oxygenator; 4) perfusion roller pump; 5) monitoring unit (pO_2, pH, temperature, pressure in the system); 6) thermostat; 7) rotameter; 8) meter of bile secretion.

Fig. 2. Effect of sporofusarin on function of isolated perfused liver: 1) rate of oxygen consumption; 2) rate of urea synthesis; 3) rate of bile formation.

Experiments were carried out on male Wistar rats weighing about 250 g and kept on an ordinary diet. The animals were deprived of food 24 h before the experiment. The liver was isolated from the rat (under general ether anesthesia) by the microsurgical technique of Hems et al. and Lambert [11, 12]. After cannulation of the portal vein and bile duct the isolated liver was washed out with Ringer's solution at 20° C for 10 min to remove blood, after which it was connected to the perfusion system. At the end of the period of adaptation (30 min) 1 ml physiological saline (control) or sporofusarin solution was injected directly into the portal vein of the liver, and the perfusion was continued after an exposure of 3 min. The final concentration of sporofusarin in the perfusion fluid was $5.9 \cdot 10^{-5}$ M. Before the beginning of perfusion and 60 and 90 min later, liver biopsy was carried out by excision of a piece of tissue weighing about 100 mg, followed by ligation. Meanwhile samples of perfusion fluid (1 ml) were taken every 15 min during perfusion.

The state of liver function was assessed from the rate of formation of bile and of urea synthesis [15] and the oxygen consumption. Samples of liver were homogenized under standard conditions in 0.25 M sucrose solution containing 0.001 M EDTA (pH 7.4). In order to determine nonsedimented enzyme activity, part of the homogenate was centrifuged at 100,000 g for 30 min.

Liver homogenates, supernatant, and samples of the perfusion fluid were tested for their protein content and activity of five lysosomal enzymes; β-acetylglucosamidase (EC 3.2.1.30), β-glucuronidase (EC 3.2.1.31), arylsulfatases A and B (EC 3.1.6.1), β-galactosidase (EC 3.2.1.23), and β-glucosidase (EC 3.2.1.21). Activity of these enzymes was determined by spectrophotometric micromethods based on the use of the ultramicrosystem of biochemical analysis developed by Pokrovskii et al. [3-5]. The protein content was determined by Lowry's method [13].

EXPERIMENTAL RESULTS AND DISCUSSION

It should be clear from Fig. 2 that changes in the parameters of function of the isolated liver in the control differed only slightly from the original level, the oxygen consumption after perfusion for 90 min was reduced by only 5.1%; the rate of urea synthesis and bile formation by 25 and 28.5%, respectively. Meanwhile, sporofusarin caused a sharp decrease in liver function. The rate of bile formation 15 min after injection of the toxin was reduced by 42.8% and after 30 min by 83.9%. By the end of the 60th minute bile secretion had ceased completely. The rate of urea synthesis also was sharply depressed by sporofusarin

TABLE 1. Effect of Sporofusarin on Total and Nonsedimented Activity of Lysosomal Enzymes of Isolated Perfused Rat Liver (mean data from four to five experiments)

Type of activity	Time of perfusion (in min)	β-acetylglucosamidase		β-glucoronidase		Arylsulfatases A and B		β-galactosidase		β-glucosidase	
		control	experiment	control	experiment	control	experiment	control	experiment	control	experiment
Total activity (in μmoles/min/g protein)	30	11,3	11,2	5,5	4,9	10,2	9,0	0,48	0,46	2,00	2,04
	60	9,8	9,8	4,2	4,5	10,0	10,0	0,43	0,38	1,34	1,80
	90	10,3	10,0	4,9	4,5	11,0	10,3	0,47	0,34	0,74	1,35
Nonsedimented activity (in % of initial level)	30	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	0	0
	60	241,0	294,7	163,0	247,2	156,3	239,6	147,1	216,7	0	0
	90	229,5	394,7	190,1	367,9	230,0	327,1	176,5	266,7	0	0

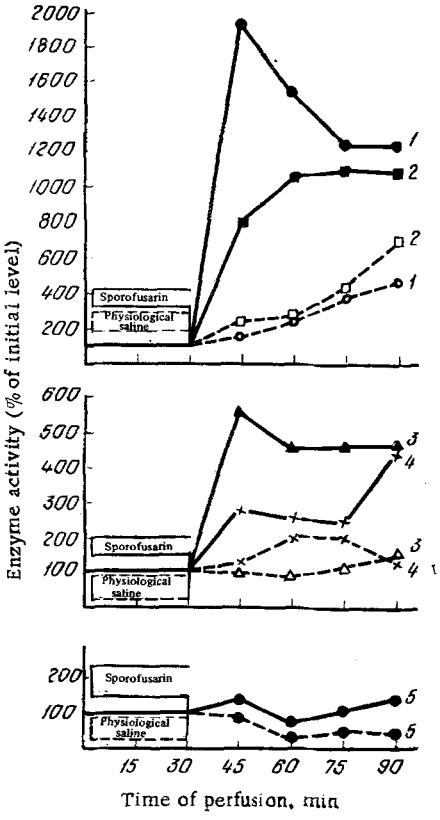

Fig. 3. Effect of sporofusarin on liberation of lysosomal enzyme of isolated liver into perfusion fluid: 1) β-acetylglucosamidase; 2) β-glucuronidase; 3) arylsulfatases A and B; 4) β-galactosidase; 5) β-glucosidase.

(by 84% of its initial level 60 min after injection of the toxin). The oxygen consumption was considerably reduced by the end of perfusion to 58.2% of its initial level.

To assess the effect of sporofusarin on the stability of the lysosomal membrane the total and nonsedimented activity of the lysosomal enzymes was determined in the liver tissues. As Table 1 shows, during perfusion of the isolated liver no significant changes were observed in the total activity of the lysosomal enzymes except β-glucosidase, the activity of which was reduced. As regards nonsedimented activity of the lysosomal enzymes, sporofusarin caused a sharper increase in the activity of most enzymes studied in the supernatent; during perfusion (90 min) the nonsedimented activity of the lysosomal enzyme was 1.5-2 times higher than the control level. The exception was membrane-bound β-glucosidase; activity of this enzyme in the supernatant could not be detected in either the experimental nor the control series.

To study the effect of sporofusarin on the permeability of the tissue-blood barrier, the activity of the lysosomal enzymes was determined in the perfusion fluid also (Fig. 3). Sporofusarin caused a much greater and earlier liberation of enzymes from the hepatocytes into the perfusion fluid. The β-acetylglucosamidase activity in the perfusion fluid 15 min after injection of sporofusarin was 20 times higher than initially. The increase in activity of β-glucuronidase (by eight times), arylsulfatases A and B (by five and one-half times), and β-galactosidase (three times) during perfusion was rather smaller. No significant change in the activity of only the membrane-bound lysosomal enzyme β-glucosidase was found in the perfusion fluid.

It must be emphasized in particular that the liberation of enzymes into the perfusion fluid was observed very soon after the injection of sporofusarin, whereas in the control experiments a low level of enzyme activity in the perfusion fluid was observed only at the end of perfusion.

It can be concluded from the results of these experiments that the use of the technique of the isolated perfused rat liver to study the mechanism of the toxic action of poisons and, in particular, of sporofusarin, provides a very convenient model for recording their effect simultaneously on the function of the organ and on the stability of the membraneous structures of its cells.

It has now been shown that, besides its marked inhibitory action on the indices of liver function studied above and, in particular, on urea synthesis and bile formation, sporofusarin also disturbs the permeability of both lysosomal and plasma membranes of the hepatoxytes (increased enzyme activity in the supernatant

of the liver homogenate and in the perfusion fluid). It is particularly interesting to note that the action of sporofusarin in damaging membrane was manifested sooner than its effect on liver function. As a result it can be postulated that the depression of liver function by sporofusarin is the result of disturbance of the membranous structures of the cells and, in particular, of the lysosomes.

Finally, it is important to emphasize that in experiments with the isolated liver the harmful action of sporofusarin on the membranes was manifested by the poison in a concentration ($5.9 \cdot 10^{-5}$ M) comparable with the minimal concentration used in experiments in vitro ($1.6 \cdot 10^{-5}$ M) and much less than the calculated concentration in the blood of rats following injection of sporofusarin in a dose sufficient to induce an acute toxic effect in vivo ($3.0 \cdot 10^{-4}$ M). The results thus confirm the hypothesis [6] that injury to the lysosomal membranes by sporofusarin is an important component in the mechanism of its toxic action.

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