PHARMACOKINETICS OF ANTIPYRIN, NIFEDIPINE, AND DIAZEPAM IN EXPERIMENTAL MYOCARDIAL INFARCTION

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The pharmacokinetic parameters of lipid-soluble drugs are largely determined by the functional state of the microsomal monooxygenases of the liver [9]. Previous investigations have shown that acute myocardial infarction weakens hydroxylating activity and reduces the content of the main component of the monooxygenases, namely cytochrome P-450 [3]. However, considering the modern view of the multiplicity of forms of cytochrome P-450 [4], it is interesting to study the biotransformation of individual drugs used in the pharmacotherapy of myocardial infarction or as test preparations to assess activity of the liver microsomal enzymes in various pathological states [2].

The aim of this investigation was to study the pharmacokinetics of antipyrin, nifedipine, and diazepam in the course of acute coronary occlusion leading to myocardial infarction in rats.

METHODS

Experiments were carried out on male Wistar rats weighing 170-230 g. A model of acute myocardial infarction (AMI) was created as described by the authors previously [3]. There were two series of experiments. In series I the pharmacokinetics of antipyrin (AP), nifedipine (NF), and diazepam (D) was studied on the 7th, 14th, and 21st days of the recovery period after AMI. AP was injected intraperitoneally (in a dose of 18 mg/kg), NF (Cordafen) was given by the intragastric route (10 mg/kg), and D (Relanium (intraperitoneally (20 mg/kg). Blood was taken from the caudal vein 0.5, 1, 1.5, and 2 h after administration of AP and NF and 2, 2.5, 3, and 3.5 h after injection of D. Plasma levels of AP [5], NF [13], and D [7] were determined by reverse phase high-performance liquid chromatography (HPLC) with ultraviolet detection on a "Milikhrom-1A" chromatograph. In the experiments of series II the concentration of cytochrome P-450 was determined and metabolism of amidopyrine, aniline, and diazepam in the liver microsomes was assessed. The microsomal fraction was isolated on the 7th, 14th, and 21st days of AMI by differential centrifugation [1]. The microsomal fraction was isolated on the 7th, 14th, and 21st days of AMI by differential centrifugation [1]. Metabolism of amidopyrine and aniline and the cytochrome P-450 concentration in the microsomes were determined as described by the authors previously [10]. Microsomal metabolism of diazepam (35 μmoles in the incubation medium) was estimated on the basis of its disappearance from the incubated mixture and the rate of formation of its metabolites: oxazepam, 3-hydroxydiazepam, and N-desmethyldiazepam. The concentration of D and its metabolites was determined by HPLC [7]. The protein concentration in the microsomes was determined as in [12]. The pharmacokinetic parameters of the drugs were calculated by means of the usual equations [6, 9]. The experimental results were subjected to statistical analysis by Student's t test.

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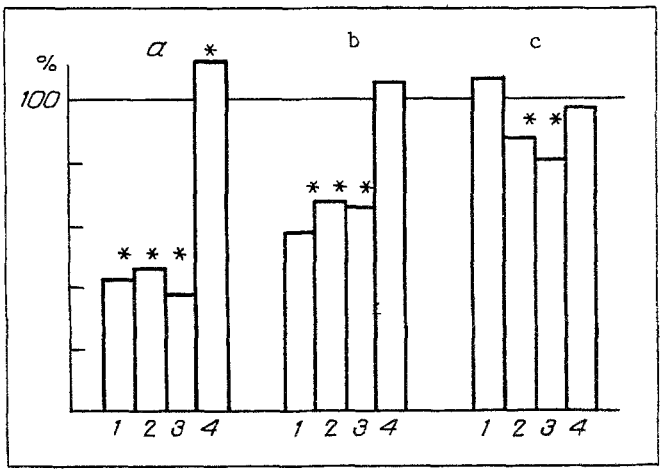

Fig. 1. Change in microsomal activity of monooxygenase enzyme of the liver by the 7th (a), 14th (b), and 21st (c) day of acute myocardial infarction in rats (in % of control, assumed to be 100%). 1) Cytochrome concentration P-450; 2) rate of N-demethylation of amidopyrine; 3) rate of n-hydroxylation of aniline; 4) rate of diazepam hydroxylation. *) Probability of distinction with control ($p < 0.05$).

TABLE 1. Pharmacokinetics of Antipyrin and Time Course of Acute Coronary Occlusion with Myocardial Infarction in Rats (M ± m, n = 6)

Parameter	Experimental conditions	Time after ligation of coronary artery, days			Control (intact animals)
		7-e	14-e	21-e	
$T_{1/2}$, min	Myocardial infarction	411,4±56,9*	243,4±18,8*	203,8±11,3*	166,5±8,3
	Mock operation	164,3±6,4	175,8±14,2	—	
Cl, ml/min	Myocardial infarction	0,94±0,11*	1,2±0,09*	1,1±0,05*	2,3±0,1
	Mock operation	2,5±0,13	2,7±0,15*	—	
K_{el} in 1 h	Myocardial infarction	0,11±0,02*	0,17±0,01*	0,20±0,01*	0,26±0,01
	Mock operation	0,25±0,01	0,24±0,02	—	

Notes. Here and in Tables 2-4, an asterisk indicates significance of differences with control ($p < 0.05$). $T_{1/2}$) Half-elimination time; Cl) clearance; K_{el}) elimination constant.

RESULTS

The investigation showed that the pharmacokinetics of AP, NF, and D in the recovery period after AMI changed in different ways. The greatest changes occurred in the pharmacokinetics of AP (Table 1). On the 7th day the half-elimination period ($T_{1/2}$) of AP was lengthened by 2.5 times, and it still remained 46 and 22% above the control value, respectively, on the 14th and 21st days of AMI. The clearance (Cl) and elimination constant (K_{el}) of AP also showed the greatest fall on the 7th day and still remained below the control levels on the 14th and 21st days of the recovery period. The pharmacokinetics of NF in the postinfarction period underwent less-marked changes. For instance, $T_{1/2}$ of NF on the 7th day was 75.7% higher than in the control. K_{el} at this period amounted to 58% of the control (Table 2). On the 14th and 21st days of AMI $T_{1/2}$ and K_{el} did not differ significantly from the control. Cl of NF did not change throughout the recovery period. The study of the pharmacokinetics of D revealed no significant changes throughout the post-infarction period. Comparison of changes in the pharmacokinetics of the drugs and the decrease in activity of the mono-oxygenases of the liver in the recovery period after AMI showed that only slowing of the rate of AP elimination correlated with the decrease in the cytochrome P-450 concentration and the allowing of N-demethylation

TABLE 2. Pharmacokinetics of Nifedipine in Recovery Period after Acute Myocardial Infarction in Rats (M ± m, n = 6-8)

Parameter	Time after ligation of coronary artery, days			Control (intact animals)
	7th	14th	21st	
$T_{1/2}$, min	54,3±7,4*	47,4±10,7	53,8±18,3	30,9±4,2
Cl, ml/min	14,7±3,8	8,6±0,9	12,9±4,9	8,2±0,9
K_{el} in 1 h	0,8±0.09*	1,0±0,2	1,0±0,3	1,4±0,2

TABLE 3. Pharmacokinetics of Diazepam in Recovery Period of Acute Occlusive Myocardial Infarction in Rats (M ± m, n = 6-8)

Parameter	Experimental conditions	Time after ligation of coronary artery,		Control (intact animals
		7-e	14-e	
$T_{1/2}$, min	Myocardial infarction	0,98±0,08	0,89±0,12	1,3±0,48
	Mock oper	1,28±0,13	1,05±0,2	
Cl, ml/min	Myocardial infraction	1,9±3,8	6,7±1,8	5,5±2,6
	Mock operation	4,4±1,1	6,5±1,2	
K_{el} in 1 h	Myocardial infraction	0,73±0,07	0,83±0,1	0,98±0,26
	Mock operation	0,46±0,03	0,84±0,13	

TABLE 4. Rate of Formation of Diazepam Metabolites in Liver Microsomes in Rats with Acute Occlusive Myocardial Infarction (M ± m, n = 6)

Metabolite	Experimental conditions	Time after ligation of coronary artery, days			Control (intact animals)
		7-e	14-e	21-e	
Oxazepam	Myocardial infarction	83,2±8,2* (18)	79,7±4,1* (18)	80,4±2,3* (16)	51,3±2,3 (13)
	Mock operation	47,4±4,5 (17)	—	—	
3-hydroxydiazepam	Myocardial infarction	201,5±16,7 (43)	211,5±6,8* (48)	239,3±12,8* (49)	164,8±8,9 (42)
	Mock operation	121,5±6,6* (45)	—	—	
N-desmethyldiazepam	Myocardial infarction	184,5±7,9 (39)	147,2±7,2* (34)	173,2±7,9 (35)	180,0±13,6 (45)
	Mock operation	103,0±7,5* (38)	—	—	

Notes. Rate of formation of diazepam metabolites shown in μmoles/min/mg microsomal protein. Numbers in parentheses indicate content of metabolite as a percentage of their total content, taken as 100%; —) metabolite not studied.

of amidopyrine and of *p*-hydroxylation of aniline in the liver microsomes (Fig. 1). The less-marked change in the pharmacokinetics of NF was evidently connected with the existence of an isoform of cytochrome $P\text{-}450_{NF}$ [11], responsible for nifedipine metabolism, and evidently more resistant to damage in this pathological condition. The absence of changes in the pharmacokinetics of D after AMI provided the basis for a study of activity of its metabolism in isolated liver microsomes in vitro (Table 3). The results showed that the rate of metabolism of D on the 7th day of AMI was 16% above the control level, whereas on the 14th and 21st days it did not differ from the control value. A study of the rate of formation of metabolites of D in the liver microsomes showed that on the 7th day of AMI the rate of oxazepam (OX) production was increased by 63%. The rate of formation of 3-hydroxydiazepam (3-HD) and of N-desmethyldiazepam (NDD) was unchanged. On the 14th day of AMI, despite the continued high rate of OX production (155% of the initial level) the rate of formation of 3-HD was increased (by 28%) whereas the rate of formation of NDD was reduced by 20%. On the 21st day the rates of formation of OX and 3-HD still remained 57 and 45% higher, respectively, than the control, whereas the rate of NDD production was the same as in the control. In the post-infarction period the profile of D metabolites showed a considerable change (Table 4). At all times of AMI studied, a lasting increase

was observed in the relative content of OX and a decrease in that of NDD. The relative content of 3-HD was increased on the 14th and 21st days of the recovery period. The results indicate that the cytochrome P-450 isozyme involved in metabolism of diazepam [8] not only is not inhibited but, on the contrary, on the 7th day of AMI it is activated, and helps to maintain the rate of elimination of D in the post-infarction period.

Differences in disturbance of elimination of antipyrin, nifedipine, and diazepam in myocardial infarction are thus probably linked with differences in the degree of damage to individual cytochrome P-450 isozymes, which oxidize these drugs.

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