MOLECULAR-BIOLOGICAL PROBLEMS OF DRUG DESIGN AND MECHANISM OF DRUG ACTION

EFFECT OF ANTIORTHOSTATIC HYPOKINESIA ON ACETAMINOPHEN PHARMACOKINETICS AND ITS DISTRIBUTION IN SALIVA OF HEALTHY VOLUNTEERS

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The pharmacokinetics of acetaminophen in a group of healthy volunteers has been studied after a single peroral administration at a dose of 625 mg under normal and antiorthostatic hypokinesia (head-down bed rest) conditions. A highly significant correlation has been established between the concentrations of acetaminophen in the blood plasma and saliva of volunteers and also between the pharmacokinetic parameters calculated from the dynamics of drug distribution in plasma and saliva. A tendency toward increased concentration of acetaminophen in saliva compared to blood plasma of volunteers; decreased time of attaining the maximum drug concentration, relative bioavailability, time of half-elimination, and mean retention time in the organism; and increased general clearance has been observed.

Key words: acetaminophen, pharmacokinetics, plasma, saliva, orthostatic hypokinesia.

The consequences of space flight (SF) can cause complicated changes in the activity of many organs and the functioning of their systems and restructure the course of important metabolic processes. This undoubtedly has an important influence on the absorption, distribution, and elimination of drugs used under SF conditions for medical purposes.

Then, the changes of the pharmacokinetic parameters of the drugs can transform the expected therapeutic activity relative to the onset and duration of the effect or cause undesirable side reactions.

Pharmacokinetics of drugs are studied using frequent fraction collection and subsequent analysis of blood samples. This limits the application of this method during SF. Therefore, non-invasive methods based on the collection and analysis of biological fluids, in particular, saliva, provide alternatives for estimating drug concentrations. It is known that therapeutic concentrations of acetaminophen under ordinary conditions can be determined in saliva and that the ratio of its contents in saliva and plasma is constant [1].

Our goal was to determine the correlation between acetaminophen in biological fluids (saliva and blood plasma) under ordinary and simulated SF conditions (common SF model of antiorthostatic hypokinesia).

EXPERIMENTAL PART

The pharmacokinetic study included seven practically healthy volunteers (male) from 38 to 47 years old. Study procedures were approved by the Commission of the Institute of Medico-Biological Problems, Russian Academy of Sciences, for biomedical ethics. Informed consent was obtained from each of the volunteers.

The study was carried out in two phases. First, volunteers were administered once on an empty stomach tableted acetaminophen at a dose of 625 mg under ordinary conditions (background study).

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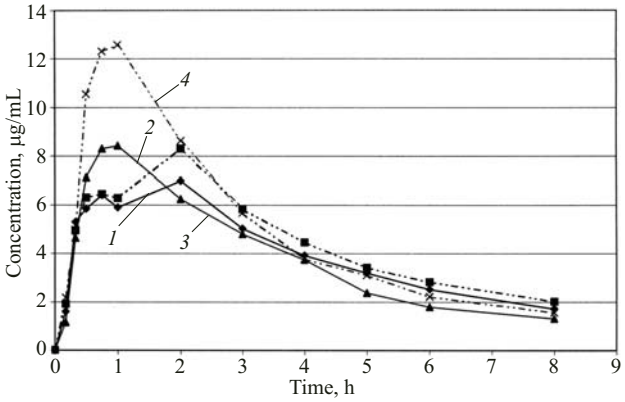

Fig. 1. Average pharmacokinetic curves of acetaminophen: plasma background (*1*), saliva background (*2*), ANOH plasma (*3*), ANOH saliva (*4*).

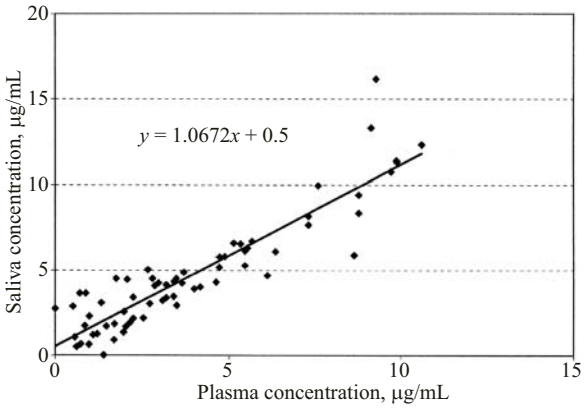

Fig. 2. Regression analysis of acetaminophen concentrations in saliva of volunteers and in blood plasma after a single peroral administration at a dose of 625 mg under ordinary conditions.

After a hiatus of two days volunteers were placed under antiorthostatic hypokinesia (ANOH) conditions with head-down bed rest at a angle of 8° to horizontal.

On the second day of ANOH volunteers were again administered on an empty stomach the drug at the same dose. Volunteers thoroughly rinsed their mouths after receiving the drug.

Blood was collected for analysis from a cubital vein into heparinized tubes before drug administration and 10, 20, 30, and 45 min and 1, 2, 3, 4, 5, 6, and 8 h after administration. Blood plasma was separated by centrifugation and preserved at –20°C until analysis. Saliva was collected at the same time points and preserved at –20°C until analysis.

The acetaminophen concentrations in blood plasma and saliva of volunteers were determined by HPLC with UV-spectrophotometric detection at 254 nm [2]. The drug detection limit was 0.2 μg/mL of biological fluid with regression coefficient $r^2 = 0.9969$.

Data were analyzed using the M-IND program [3] for PC by calculating model-independent parameters such as maximum concentration C_{max}, time to reach it T_{max}, area under the time-concentration curve $AUC_{0-\infty}$, total clearance Cl_t, mean retention time of the drug in the organism *MRT*, period of half-elimination $T_1/_2$, and distribution volume V_Z.

Furthermore, the relative bioavailability f and ratio of maximum concentrations f^{II} of acetaminophen under ANOH and ordinary conditions were calculated (using individual values of $AUC_{0-\infty}$ and C_{max}, respectively) [4].

Results were treated statistically using the InStat program for PC. Differences were considered significant for $p < 0.05$.

Ratios of acetaminophen concentrations in saliva and blood plasma were calculated in order to study acetaminophen distribution in saliva of volunteers. Correlations of the drug concentrations in blood plasma and saliva and the pharmacokinetic parameters calculated from data in plasma and saliva were analyzed. For this, we calculated the correlation coefficient r, significance criterion (error probability p), and coefficients of a linear regression of the form $y = ax + b$, where x are data points obtained from the acetaminophen distribution dynamics in blood plasma of

TABLE 1. Pharmacokinetic Parameters of Acetaminophen after Single Peroral Administration at a Dose of 625 mg

Parameter	Blood plasma concentration		Saliva concentration	
	Bkgd. (n = 7)	ANOG (n = 7)	Bkgd. (n = 7)	ANOG (n = 7)
C_{max}, μg/mL	9.79 ± 0.80	10.12 ± 1.46	10.52 ± 1.55	14.61 ± 2.54
T_{max}, h	1.19 ± 0.29	0.86 ± 0.07	1.33 ± 0.30	0.86 ± 0.07
$AUC_{0-\infty}$, μg · h/mL	42.12 ± 4.99	36.02 ± 4.80	46.89 ± 9.06	47.18 ± 6.73
Cl_t, L/h	16.22 ± 2.01	18.68 ± 1.71	15.76 ± 2.68	14.52 ± 1.55
$T_{1/2}$, h	3.90 ± 0.71	2.90 ± 0.24	3.86 ± 0.48	2.98 ± 0.33
MRT, h	5.57 ± 0.78	4.14 ± 0.13	5.45 ± 0.51	4.02 ± 0.33
V_Z, L	86.16 ± 14.54	78.00 ± 10.0	85.72 ± 17.19	62.85 ± 10.94
f, %	–	82.37 ± 6.06	–	–
f^{II}, %	–	109.37 ± 17.08	–	–

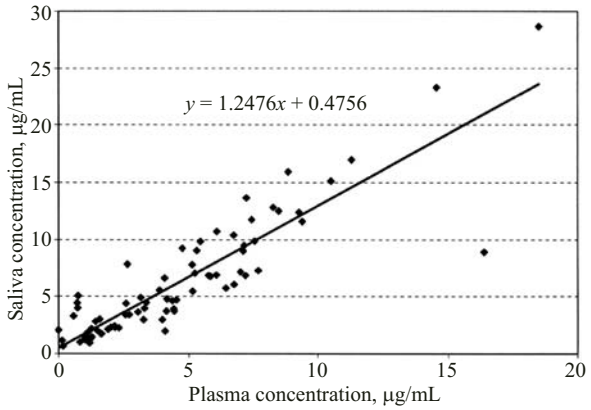

Fig. 3. Regression analysis of acetaminophen concentrations in saliva of volunteers and in blood plasma after a single peroral administration at a dose of 625 mg under antiorthostatic hypokinesia conditions.

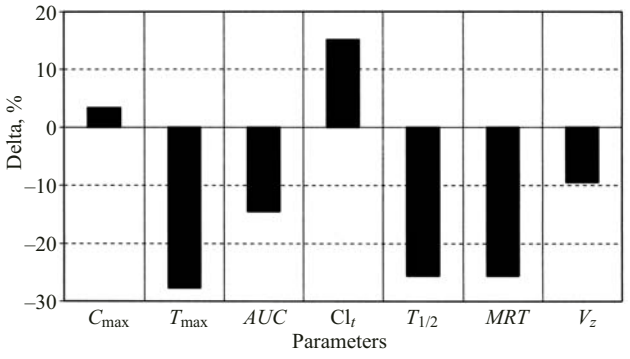

Fig. 4. Dynamics of change of pharmacokinetic parameters of acetaminophen under antiorthostatic hypokinesia conditions.

volunteers; *y*, data obtained from acetaminophen distribution dynamics in saliva of volunteers.

RESULTS AND DISCUSSION

Figure 1 shows the dynamics of mean concentrations of acetaminophen in blood plasma and saliva of volunteers after administration of tebleted drug at a dose of 625 mg under ordinary (background) and ANOH conditions. It can be seen that acetaminophen concentrations in blood plasma and saliva of volunteers are parctically the same at all time points under ordinary conditions. Two peaks of acetaminophen concentration are recorded in both plasma and saliva 0.75 h (6.40 ± 1.57 and 6.45 ± 1.09 μg/mL, respectively) and 2 h (7.0 ± 0.87 and 8.29 ± 1.87 μg/mL) after administration of the drug. One peak of concentration, the time to attain which in plasma varies from 0.33 to 2 h and in saliva from 0.5 to 2 h, is observed in the individual pharmacokinetic curves. A comparison of the individual acetaminophen concentrations in saliva and plasma shows that concentrations in saliva are slightly higher than those in plasma, the mean value of these ratios is 1.1036. The maximum differences were observed at time points 0.17 and 2 h (1.21 – 1.18 times). The individual acetaminophen concentrations in blood plasma and saliva of volunteers after administration of the drug under ordinary conditions ($r = 0.91$, $p < 0.001$, $n = 77$) were highly linearly correlated. Figure 2 plots the data.

The average pharmacokinetic curves under ANOH conditions are identical. One peak in concentration is recorded (1 h after administration). However, acetaminophen levels in saliva are higher than in plasma (12.56 ± 1.23 and 8.43 ± 0.78 μg/mL, respectively). An analysis of the individual pharmacokinetic curves showed that the time to attain the maximum concentration in plasma and saliva varies over much narrower limits, from 0.5 to 1 h, than for the background. A comparison of the acetaminophen distribution dynamics in plasma and saliva of volunteers under ANOH conditions shows that the saliva concentrations are greater than in blood plasma. These differences are much greater than for the background although they are not statistically significant. The greatest differences in the concentrations were observed at time points 0.17 h (1.89 times), 0.5 – 2 h (1.49 – 1.38 times), and 5 h (1.31 times). The mean ratios of acetaminophen concentrations in saliva and plasma are 1.3382. The correlation between individual acetaminophen concentrations in blood plasma and saliva of healthy volunteers after administration of the drug under ANOH conditions is highly linear ($r = 0.89$, $p < 0.001$, $n = 77$). Figure 3 plots the data.

TABLE 1 gives the calculated pharmacokinetic parameters of acetaminophen (as mean values ± standard error of the mean). The data suggest that the acetaminophen pharmacokinetic parameters under ordinary conditions that were calculated from the concentrations in blood plasma and saliva were practically the same. A correlation analysis of individual pharmacokinetic parameters calculated from the data for plasma and saliva showed a highly significant linear correlation for AUC ($r = 0.91$, $p < 0.01$); T_{max} ($r = 1.0$), Cl_t ($r = 0.97$, $p < 0.001$), and V_Z ($r = 0.75$, $p = 0.05$). The mean values for C_{max} ($r = 0.60$, $p < 0.1$), $T_{1/2}$ ($r = 0.68$, $p < 0.1$), and MRT ($r = 0.61$, $p < 0.1$) are linearly correlated.

Under ANOH conditions, the values C_{max} and AUC calculated for the saliva concentration are much greater than for blood plasma (by 44.4 and 31.0 Δ%, respectively); Cl_t and V_Z, slightly less (by 22.3 and 19.4 Δ%, respectively); and T_{max}, $T_1/_2$, and MRT, practically the same. These differences were not statistically significant. A highly linear correplation is seen for C_{max} ($r = 0.94$, $p < 0.002$), AUC ($r = 0.94$, $p < 0.01$), T_{max} ($r = 1.0$), Cl_t ($r = 0.80$, $p < 0.05$), MRT ($r = 0.80$, $p < 0.05$), and V_Z ($r = 0.74$, $p = 0.05$). The mean values for $T_{1/2}$ ($r = 0.60$, $p < 0.1$) are linearly correlated. The linear regression coefficients for C_{max} were $a = 1.6312$, $b = –1.9001$; for AUC, $a = 1.3111$, $b = –0.0367$; for Cl_t, $a = 0.726$, $b = 0.959$; for V_Z, $a = 0.8118$, $b = –0.4707$.

Figure 4 shows the dynamics of change of acetaminophen pharmacokinetic parameters (from blood

plasma) under ANOH conditions expressed in Δ% compared to ordinary conditions. A tendency toward increased absorption rate and total clearance of acetaminophen and decreased relative bioavailability, half-elimination period, and mean retention time of the drug in the organism is observed under hypokinesia conditions. However, these differences are not statistically significant. Apparently these changes during absorption and elimination of acetoaminophen under ANOH conditions cause the acetaminophen concentration in saliva of volunteers to be substantially greater than that in blood plasma.

It should be noted that our results do not agree completely with those in the literature. Thus, studies of the effect of motor activity on acetaminophen pharmacokinetics [5] found that the pharmacokinetic parameters of the drug changed insignificantly during sleep and bed rest with the execption of *MRT*, which decreased. Apparently these effects are due to not only a limitation on motor activity but also changes of hemodynamics under ANOH conditions and the neutralizing effect of acetaminophen on glomerular filtration under ANOH conditions.

Thus, the pharmacokinetic study showed that acetaminophen concentrations in blood plasma and saliva of volunteers and pharmacokinetic parameters calculated from the dynamics of its concentrations in blood plasma and saliva under both ordinary and ANOH conditions are highly significantly correlated. This enables saliva to be used in pharmacokinetic studies of acetaminophen. However, it should be considered that the drug concentrations in saliva under hypokinesia conditions are substantially greater than those in blood plasma.

A tendency toward decreased time to attain the maximum concentration; decreased relative bioavailability, half-elimination period, and mean retention time in the organism; and increased total clearance of the drug is observed under ANOH conditions.

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