SIMULATING THE PHARMACOKINETICS OF TABLETIZED AMBROXOL USING THE DYNAMICS OF DRUG DISTRIBUTION IN SALIVA

S. N. Kondratenko,[1] A. K. Starodubtsev,[1] I. V. Zolkina,[1] and G. A. Belyakova[1]

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The pharmacokinetics of ambrogexal (generic) and ambroxol (INN) tablets were investigated in 18 healthy volunteers after peroral administration in a single dose of 60 mg. The levels of ambroxol hydrochloride in saliva were significantly higher (statistically reliable) than those in the blood plasma for the first 1.5 hours after administration due to features of the drug absorption in the gastrointestinal tract. There are statistically reliable differences between the pharmacokinetic parameters (C_{max}, T_{max}, $T_{1/2}$, V_z) calculated from the dynamics of ambroxol hydrochloride concentration in the saliva and plasma. Using the proposed method of extrapolation of the drug concentration, it is possible to use saliva for studying the pharmacokinetics of ambroxol administered in tabletized drug forms.

Key words: ambroxol, pharmacokinetics, concentration, plasma, saliva, biomodeling

The majority of pharmacokinetic investigations is based on quantitative determination of a drug in blood plasma. However, saliva is interesting as a specimen for pharmacokinetics due to the simple and non-invasive sampling for analysis [1 – 3].

A reliable correlation between blood plasma concentrations (serum) and saliva has been established for several drugs such as acetaminophen [4 – 6], moxifloxacin [7 – 9], ofloxacin [10, 11], and theophylline [12, 13]. This enables saliva to be used in pharmacokinetic investigations. Furthermore, actual trends in the distribution of many drugs (including ambroxol hydrochloride) in saliva of patients are still practically unstudied and represent an exceedingly critical problem.

EXPERIMENTAL PART

The pharmacokinetic investigation included 18 healthy volunteers, 9 men and 9 women. The average age of the volunteers was 29.8 ± 7.1 y with a range from 20 to 42 y. The body mass varied from 57 to 98 kg and averaged 76.3 ± 12.2 kg; the average height of patients, 169 ± 4.9 cm with individual variations from 152 to 187 cm.

In order to avoid periodic effects and disregard interspecies variability, the investigation was conducted by an open randomized and cross-checked scheme. For this, 18 healthy volunteers were divided by simple randomization into two equal groups. Volunteers from the first group in random order took first two tablets (60 mg) of the reference drug ambrogexal (Gexal AG, Germany) and then, after 7 d, two tablets (60 mg) of the manufactured drug ambroxol (OOO Ozon, Russia). The other group of volunteers took the drugs in the reverse order.

Drugs were administered once on an empty stomach at 8 – 9 am. Volunteers received a standard breakfast 4 h after taking the drugs. Blood and saliva were taken in order to study the drug content before and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, and 12 h after administration. Ambroxol hydrochloride concentration in blood plasma and saliva of volunteers was determined by reversed-phase HPLC with UV spectrophotometric detection [14]. A total of 720 biological samples was analyzed (360 blood plasma and 360 saliva samples).

The distribution of ambroxol hydrochloride in blood plasma and saliva of volunteers was analyzed using the M-IND program [15] for PC, calculating model-independent parameters such as maximum concentration C_{max}, time to reach it T_{max}, area under the curve of concentration-time AUC_{0-t} and $AUC_{0-\infty}$, total clearance Cl_t, mean retention time of drug in the organism *MRT*, elimination half-life $T_{1/2}$, dis-

[1] Sechenov Medical Academy, Moscow, Russia.

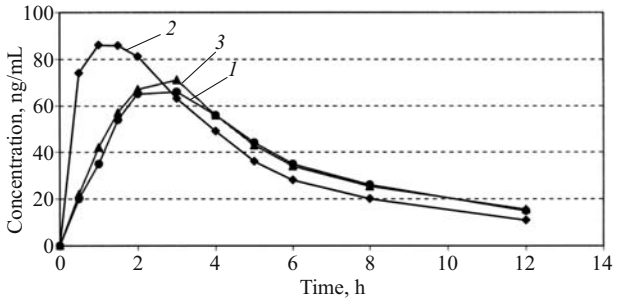

Fig. 1. Average pharmacokinetic curves of ambrogexal: plamsa (*1*), saliva (*2*), extrapolation (*3*).

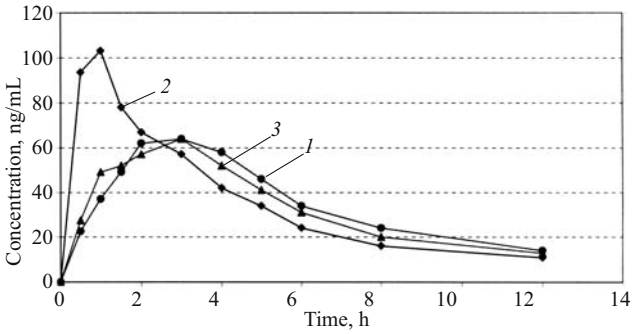

Fig. 2. Average pharmacokinetic curves of ambroxol: plasma (*1*), saliva (*2*), extrapolation (*3*).

tribution volume V_z, and ratio of maximum concentration to area under the pharmacokinetic curve $C_{max}/AUC_{0-\infty}$ (as a characteristic of absorption rate).

The results were treated statistically using the InStat program for PC. Differences were considered reliable for $p < 0.05$. Individual values of the ratio of ambroxol hydrochloride concentration in saliva to those in blood plasma were calculated in order to evaluate the possibility of using saliva samples in pharmacokinetic investigations of ambrogexal and ambroxol. A correlation analysis of drug concentration in blood plasma and saliva and pharmacokinetic parameters calculated from the data in plasma and saliva was conducted. For this, the correlation coefficient *r* and reliability criteria (probability of error) *p* were calculated.

RESULTS AND DISCUSSION

Figure 1 shows the dynamics of ambroxol hydrochloride concentration in blood plasma and saliva of healthy volunteers after a single administration of ambrogexal at a dose of 60 mg. It was found that the concentration of ambroxol hydrochloride in saliva of volunteers in the time period 0.5 – 1.5 h was significantly greater (by 4.56 ± 1.01 and 1.64 ± 0.14 times, respectively) than in plasma; after 4 – 12 h after administration, lower by an average of 1.1 – 1.3 times. The differences were statistically reliable at time points 0.5, 1, 1.5, and 6 h. Comparison of individual ambroxol hydrochloride concentrations in saliva and plasma showed that the average of these ratios was 1.53 ± 0.22.

The significantly greater drug concentration in saliva than in plasma may be due to the fact that its concentration in arterial blood was greater than in venous blood at the time of absorption. The extent of elevated saliva concentration relative to plasma concentration is proportional to the degree of absorption; the duration, to the duration of absorption [6, 16]. We performed a correlation analysis of the $AUC_{0-1.5}$ to the saliva concentration of ambroxol hydrochloride at time point 1.5 h in order to comfirm this hypothesis. A high correlation ($r = 0.68$, $p = 0.02$, $n = 18$) was found and indicated that the elevated ambroxol hydrochloride concentration in saliva relative to that in plasma was due to continuous absorption into the arterial system for 1.5 h after administration.

A weak linear correlation ($r = 0.32$, $p = 0.0001$, $n = 180$) was found from a correlation analysis of individual ambroxol hydrochloride concentrations in plasma and saliva of volunteers. We found a relationship between the ambroxol hydrochloride content in plasma and saliva that is expressed by the following empirical formula:

$$C_p = \frac{C_s}{a \times t^b},$$

where C_p is the ambroxol hydrochloride concentration in plasma (from 7 to 124 ng/mL); C_s, the ambroxol hydrochloride concentration in saliva (from 50 to 290 ng/mL); *t*, the time after administration of the drug (h); and *a* and *b*, regression coefficients, the values of which depend on *t* (for $t = 0.5 – 3$, $a = 2.044203$, $b = –0.696082$; for $t = 3 – 12$, $a = 1.097498$, $b = –0.107021$).

The reliability of the approximation (in the time range 0.5 – 3 h, $R^2 = 0.9681$; in the range 3 – 12 h, $R^2 = 0.9207$) enables the dependence to be used for extrapolation of concentrations in plasma from the individual concentrations of ambroxol hydrochloride in saliva.

Figure 1 shows the dynamics of averaged extrapolated values of ambroxol hydrochloride in plasma after administration of ambrogexal. It can be seen that the extrapolated values at all time points practically coincide and are statistically equivalent to the averaged ambroxol hydrochloride concentrations in blood plasma (measured by HPLC). An analysis of the individual pharmacokinetic curves showed that the pharmacokinetic curves for 6 volunteers (33.33%) that were constructed using plasma concentrations and extrapolation were identical; for eight volunteers (44.44%), analogous. However, differences that exceeded the relative error of the results for a separate determination of the applied HPLC method (19%) were observed at time points 1 – 3 (19%). The curves did not differ significantly for four volunteers (22.22%). It must also be noted that individual concentrations at time point 3 h were extrapolated by two methods, i.e., for $a = 2.044203$ and $b = –0.696082$ and for

a = 1.097498 and b = –0.107021. The resulting values were practically the same and did not differ statistically.

A correlation analysis of individual ambroxol hydrochloride concentrations in plasma of volunteers and the extrapolated values revealed a strong linear correlation that was statistically reliable (r = 0.66, p = 0.0000001, n = 180).

TABLE 1 lists the calculated pharmacokinetic parameters for ambrogexal from its distribution dynamics in plasma, saliva, and extrapolated concentrations (as average ± standard error of the mean). It was found that parameters C_{max}, T_{max}, and C_{max}/AUC_{∞} that were calculated from the drug distribution dynamics in saliva differed statistically reliably from those calculated for plasma. A reliable correlation between individual values of the pharmacokinetic parameters of ambrogexal that were calculated using data in plasma and saliva was not found.

Statistically reliable differences were not found for the parameters of ambrogexal calculated from the distribution dynamics in plasma and from extrapolated concentrations. Correlation analysis of individual values of pharmacokinetic parameters that were calculated from data in plasma and extrapolated concentrations revealed a highly reliable linear correlation for AUC_t (r = 0.79, p = 0.0001, n = 18), AUC_{∞} (r = 0.73, p = 0.0006, n = 18), Cl_t (r = 0.67, p = 0.002, n = 18) and a linearly correlated relationship of the average sizes for the MRT (r = 0.47, p = 0.05, n = 18).

Figure 2 shows the dynamics of average ambroxol hydrochloride concentrations in blood plasma and saliva of healthy volunteers after single peroral administration of ambroxol at a dose of 60 mg. It was found that the drug concentration in saliva in the time range 0.5 – 1.5 h was significantly greater than in plasma (by 5.32 ± 1.17 and 1.67 ± 0.11 times, respectively); in the range 4 – 12 h, substantially less (by 1.3 – 1.5 times). The found differences were statistically reliable at time points 0.5, 1, 1.5, 4, 5, 6, and 8 h. The average value of ratios of individual ambroxol hydrochloride concentrations in saliva and plasma was 1.60 ± 0.21. A reliable correlation between individual ambroxol hydrochloride concentrations in plasma and saliva of volunteers after administration of ambroxol was not found.

Individual concentrations calculated using the empirical equation did not statistically reliably differ from ambroxol hydrochloride concentrations in blood plasma of volunteers

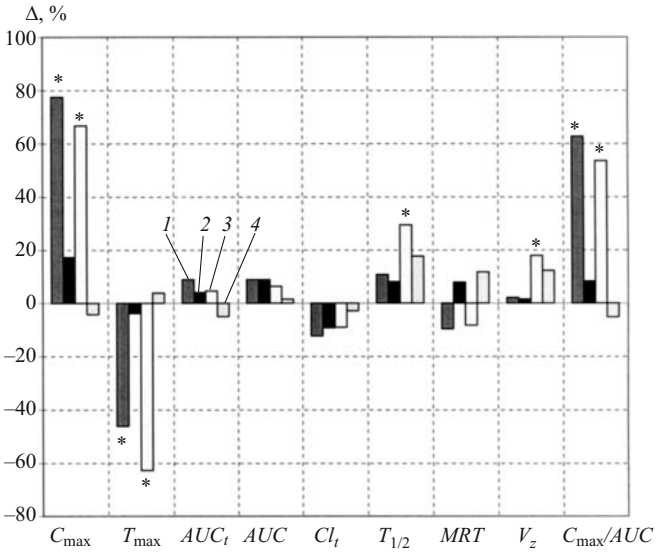

Fig. 3. Change dynamics of pharmacokinetic parameters of ambrogexal and ambroxol calculated by distribution dynamics in saliva and by extrapolated concentrations. * Statistically reliable differences compared with parameters calculated by plasma concentration. ambrogexal-saliva (*1*), ambrogexal-extrapolation (*2*), ambroxol-saliva (*3*), ambroxol-extrapolation (*4*).

TABLE 1. Pharmacokinetic Parameters of Ambrogexal

Parameter	Method for calculating pharmacokinetic parameters		
	by plasma concentration (n = 18)	by saliva concentration (n = 18)	by extrapolation (n = 18)
C_{max}, ng/mL	72.9 ± 4.2	129.5 ± 14.1*	85.5 ± 8.4
T_{max}, h	2.6 ± 0.2	1.4 ± 0.2*	2.5 ± 0.2
AUC_{0-t}, ng · h/mL	416.9 ± 21.6	453.5 ± 17.7	433.5 ± 20.5
$AUC_{0-\infty}$, ng · h/mL	478.1 ± 28.0	520.8 ± 21.4	520.4 ± 32.7
Cl_t, L/h	134.1 ± 9.0	117.7 ± 4.3	121.9 ± 6.6
$T_1/_2$, h	3.7 ± 0.3	4.1 ± 0.2	4.0 ± 0.3
MRT, h	6.3 ± 0.3	5.7 ± 0.4	6.8 ± 0.4
V_z, L	676.6 ± 33.3	690.7 ± 44.5	686.7 ± 33.9
$C_{max}/AUC_{0-\infty}$, 1/h	0.156 ± 0.004	0.254 ± 0.030*	0.169 ± 0.017

* Statistically reliable differences compared with parameters calculated by plasma concentration.

TABLE 2. Pharmacokinetic Parameters of Ambroxol

Parameter	Method for calculating pharmacokinetic parameters		
	by plasma concentration (n = 18)	by saliva concentration (n = 18)	by extrapolation (n = 18)
C_{max}, ng/mL	71.1 ± 3.1	118.6 ± 8.9*	68.1 ± 4.7
T_{max}, h	2.6 ± 0.2	0.97 ± 0.1*	2.7 ± 0.2
AUC_{0-t}, ng · h/mL	406.4 ± 20.8	425.2 ± 17.3	386.3 ± 15.6
$AUC_{0-\infty}$, ng · h/mL	461.6 ± 28.1	491.2 ± 19.7	469.2 ± 23.2
Cl_t, L/h	138.0 ± 8.2	125.6 ± 5.7	134.0 ± 7.9
$T_1/_2$, h	3.4 ± 0.2	4.4 ± 0.3*	4.0 ± 0.3
MRT, h	6.0 ± 0.2	5.5 ± 0.3	6.7 ± 0.5
V_z, L	644.2 ± 29.3	759.4 ± 40.3*	723.2 ± 40.5
$C_{max}/AUC_{0-\infty}$, 1/h	0.157 ± 0.005	0.241 ± 0.020*	0.149 ± 0.010

* Statistically reliable differences compared with parameters calculated by plasma concentration.

whereas the average values at all time points were similar. An analysis of individual ambroxol hydrochloride concentrations in plasma of volunteers and extrapolated values revealed a highly linear correlation that was statistically reliable ($r = 0.67$, $p = 0.0000001$, $n = 180$).

TABLE 2 gives pharmacokinetic parameters for ambroxol that show that C_{max}, T_{max}, $T_{1/2}$, V_z, and C_{max}/AUC_{∞} calculated using data in saliva differed statistically reliably from those calculated from concentration dynamics in plasma. However, statistically reliable differences were not found for parameters calculated from extrapolated concentrations.

A reliable correlation between individual values of pharmacokinetic parameters of ambroxol calculated using data in plasma and saliva was not found. A reliable linear correlation of average values for C_{max} ($r = 0.60$, p 0.009, $n = 18$), AUC_t ($r = 0.51$, $p = 0.03$, $n = 18$), and V_z ($r = 0.42$, $p = 0.08$, $n = 18$) was found for individual values of parameters calculated using data in plasma and extrapolated concentrations.

Figure 3 shows the change dynamics of pharmacokinetic parameters for ambrogexal and ambroxol that were calculated from the distribution dynamics in saliva and extrapolated concentrations compared with those calculated from plasma data. Obviously the use of the proposed method for extrapolating concentration can even out significant (up to 78 Δ%) statistically reliable differences due to the use of saliva to study the drug pharmacokinetics. Differences between pharmacokinetic parameters calculated using the developed method and those calculated by the traditional method (from concentration dynamics in plasma) were less than 18 Δ% and are statistically unreliable.

Thus, the investigation of the pharmacokinetics of ambrogexal and ambroxol at a dose of 60 mg in healthy volunteers showed that concentration curves and pharmacokinetic parameters calculated from the distribution dynamics of ambroxol hydrochloride in plasma and saliva of volunteers differ significantly and statistically reliably. This is due to features of drug absorption. The proposed method for extrapolation using individual drug concentrations in saliva can even out differences and can use saliva instead of blood plasma for pharmacokinetic investigations. Statistically reliable differences between ambroxol hydrochloride concentrations in plasma of volunteers (measured by HPLC) and extrapolated values (using concentrations in saliva of volunteers) and between pharmacokinetic parameters calculated from drug distribution data in plasma and from extraplated concentrations were not found. Therefore, it can be concluded that use of the proposed method enables the pharmacokinetics of ambroxol hydrochloride tablets to be studied in healthy volunteers from its distribution dynamics in saliva. Additional research is needed to confrim that the developed method can be used for pharmacokinetic investigations of other ambroxol hydrochloride forms (e.g., time-release capsules) or in other patients (e.g., patients with gastrointestinal tract diseases).

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