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MOLECULAR BIOLOGY PROBLEMS OF DRUG DESIGN AND MECHANISM OF DRUG ACTION

PHARMACOKINETIC STUDIES OF THE INNOVATIVE ANTITUBERCULOSIS DRUG THIOZONIDE IN PLASMA

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We present here our results from studies of the pharmacokinetic parameters of the original antituberculosis agent thiozonide, 100-mg capsules (ZAO Farm-Sintez, Moscow) using single increasing doses in different groups of healthy volunteers in a phase I clinical trial.

Keywords: thiozonide, pharmacokinetics, clinical trials, phase I, plasma

Tuberculosis is a global problem in contemporary healthcare. About a third of the total world population is infected with *M. tuberculosis*, with 8 – 10 million new cases being recorded annually [1, 2]. Tuberculosis remains the leading cause of death among the treatable infectious diseases.

Over the last 20 – 25 years, there have been significant changes in the levels of recorded disease in Russia. A gradual decrease in the 1970s and 1980s to 34.1 per 100,000 of the population changed in 1991 – 2000 to a significant increase, by a factor of 2.7 (to 90.7 in 2000), with subsequent stabilization in the first years of the new century at a level of 82 – 85 (85.1 in 2008). Finally, the recorded morbidity of tuberculosis started to decline over the last two years, reaching 77.44 per 100,000 of the population in 2010 (82.6 in 2009) [1].

According to the World Health Organization (WHO) "Global Tuberculosis Control 2012" report, 8.7 million cases of tuberculosis were recorded in 2011, with 1.4 million deaths among people not infected with HIV, with a further 430,000 deaths from HIV-associated tuberculosis [2]. Multidrug antituberculosis chemotherapy occupies the major place in the treatment of patients with tuberculosis. The growing frequency of cases with multiple drug resistant (MDR) *Mycobacterium tuberculosis* (MTB) has played a significant role in the development of the tuberculosis (TB) epidemic, as has infection with human immunodeficiency virus (HIV) [3 – 5].

According to the WHO definition, multiple drug resistant tuberculosis is a case of pulmonary tuberculosis from which MTB resistant to at least two of the most active antituberculosis drugs – isoniazid and rifampicin – is isolated.

In this light, the problem of treating TB patients with MDR has gained high importance [6, 7]. Antituberculosis drugs (ATD) of the reserve group are used for this purpose: ethionamide, kanamycin (amikacin), capriomycin, cycloserine, para-aminosalicylic acid (PAS), fluoroquinolones (ciprofloxacin, ofloxacin), rifabutin, and thioacetazone, as well as alternative remedies [3, 4, 6]. However, various serious side effects of these agents hinder the course of treatment and limit the potential for effective antituberculosis treatment.

Thus, the development of new classes of antituberculosis drugs effective in the treatment of tuberculosis, including MDR tuberculosis, is a current task in contemporary tuberculosis medicine. Thiozonide – {1R,2S + 1S,2R}-1-(6-bromo-2-chloroquinolin-3-yl)-4-(dimethylamino)-2-(naphthalin-1-yl)-1-phenylbutan-2-ol – compound I (Russian patent No. 2404971) is among the potential innovative drugs for

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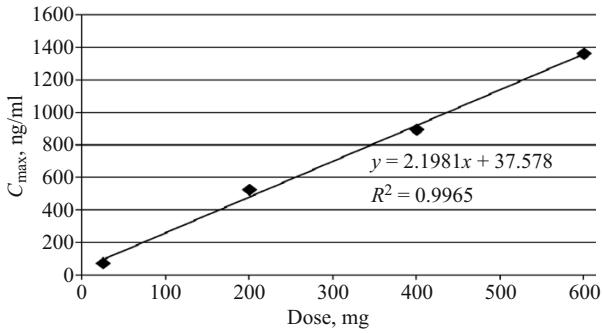

Fig. 1. Linearity of the pharmacokinetics of thiozonide in terms of C_{max}.

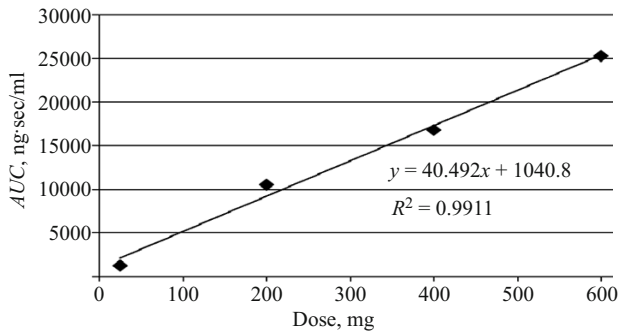

Fig. 2. Linearity of the pharmacokinetics of thiozonide in terms of AIC.

this purpose. Compound I is a synthetic antimicrobial drug which acts against MTB, both sensitive strains such as H37Rv and strains resistant to first-line drugs such as CN-37, CN-40, and MS-115. *In vitro* studies have shown it to have bacteriostatic actions against the atypical mycobacteria *M. smegmatis* NCTC 8159 and *M. fortuitum* NCTC 389. It has been suggested that thiozonide interferes with ATP synthesis in *M. tuberculosis* cells [8].

EXPERIMENTAL SECTION

During bioanalytical studies, a method for assay of compound I in plasma was developed and validated [9]. Sample preparation included precipitation of proteins with acetonitrile. Analyses were performed on an Agilent 1200 liquid chromatograph with an Agilent MS 6120 mass spectrometric detector (Agilent Technologies, USA). Data were processed using the program ChemStation (version A.01.04.122) (Agilent Technologies, USA).

The method developed was validated in terms of the following validation characteristics: selectivity, linearity, correctness, precision, quantitative detection limit, sample transfer, and solution stability. The analytical range of the method was 1 – 1000 ng/ml of I in plasma. This analytical range allowed the method to be used for investigations of the pharmacokinetics of thiozonide. Detailed information in this study has been presented in [9].

The method was used to study the pharmacokinetics of thiozonide, 100-mg capsules (ZAO Farm-Sintez), given as increasing single oral doses to different groups of healthy volunteers as part of a phase I clinical trial.

The study was performed in compliance with legislative requirements and the ethical principles laid out in Federal Law "The Circulation of Medicines" (No. 61-FZ of April 12, 2010) [10], the Helsinki Declaration of the World Medical Association (1964, with subsequent supplements), and current legislation.

Study design: single-center prospective study with sequential recruitment of volunteers with dose escalation to evaluate the safety and tolerance of the drug, first use in humans. Four groups of volunteers took part in the study (each of 10 volunteers) and received oral drug at doses of 25 mg (capsules specifically prepared for the study) and 200, 400, and 600 mg (two, four, and six 100-mg capsules respectively). The main requirements for volunteers were: gender, age 18 – 45 years, weight 60 – 100 kg inclusive, body mass index 18 – 32 kg/m^2, confirmed "healthy." Blood sample collection times for pharmacokinetic studies were as follows: 0 (before dosage), 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 24, 36, 48, 72, 96, 120, 144, and 168 h after dosage. Blood for measurement of pharmacokinetic parameters was collected in Vacutainer tubes treated with sodium heparin, with at least 4 ml collected for each time point. Blood was then centrifuged for 10 min at 3000 rpm. Plasma was frozen at –35°C and transferred to the laboratory for pharmacokinetic analysis.

Studies of the pharmacokinetics of I included estimation of the plasma concentration of active substance I in each volunteer given study formulation. Data were recorded and summarized in tables with stratification by dose. These tables included the numbers of observations, the mean, the standard deviation, the median, and the minimum and maximum values.

The following pharmacokinetic parameters were calculated for each volunteer:

1. Area under the concentration-time curve ($AUC_{0\text{-}t}$);
2. The maximum plasma concentration (C_{max});
3. The time taken to reach the maximum concentration (t_{max});
4. The total area under the curve $AUC_{0\text{-}\infty}$;
5. The half elimination time ($T_{½}$);
6. The $AUC_{0\text{-}t}/AUC_{0\text{-}\infty}$ ratio;
7. The rate of absorption ($C_{max}/AUC_{0\text{-}t}$);
8. The constant of elimination (k_{el}).

Plasma thiozonide assay results and pharmacokinetic parameters were analyzed statistically in terms of the arithmetic mean (Mean) and geometric mean (GMean), the standard deviation (SD), the coefficient of variation (CV, %), the median (Median), and their integral evaluation (significant interval, L-90%, U-90%).

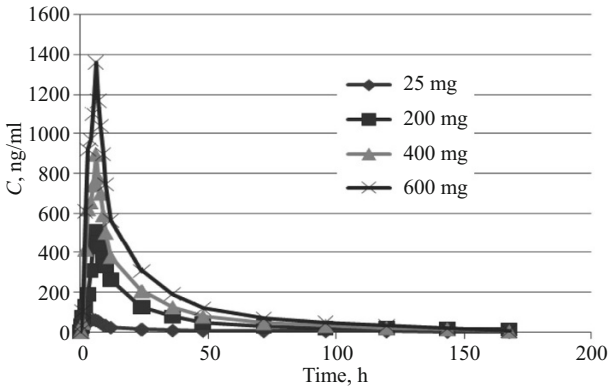

Fig. 3. Averaged pharmacokinetic profiles of thiozonide (after single oral doses of 25, 200, 400, and 600 mg).

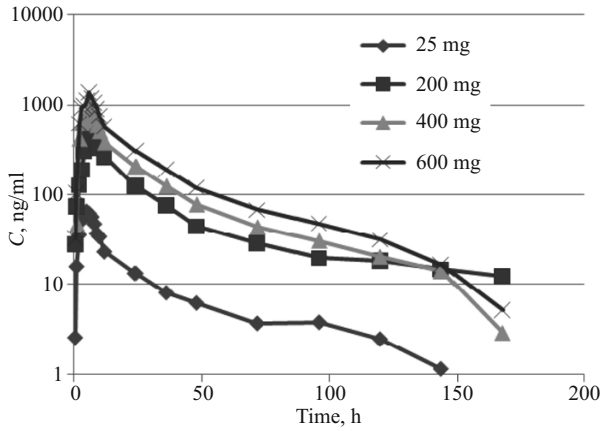

Fig. 4. Averaged pharmacokinetic profiles of thiozonide (after single oral doses of 25, 200, 400, and 600 mg), semilogarithmic scale.

Descriptive statistics and calculations of pharmacokinetic parameters were performed using Microsoft Excel with the Boomer extension for pharmacokinetic analysis (developed by Joel L. Usansky, Ph.D., Atul Desai, M.S., and Diane Tang-Liu, Ph.D., Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA 92606, USA) [MIG1]. Interval values of pharmacokinetic parameters were calculated as: L-90 = Mean – ΔX and U-90 = Mean + ΔX, where ΔX is the significant interval. The coefficient of variation was calculated as CV = (SD.100)/Mean.

The main pharmacokinetic parameters in the program used here were calculated using the following equations:

$$AUC_{0-t} = \sum_{i=0}^{n-1} (t_{i+1} - t_i) \cdot (C_i + C_{i+1}) \,/\, 2,$$

where AUC_{0-t} is the area under the concentration-time curve from zero to the moment at which the last blood sample was collected (168 h), C_i is the concentration of I at time point t, t is the time at which the sample was collected, n is the total

TABLE 1. Individual Pharmacokinetic Parameters after Administration of Thiozonide, 25-mg Capsules

Volunteer No.	C_{max}, ng/ml	T_{max}, h	AUC_{0-168}, ng.h/ml	k_{el}, h^{-1}	$AUC_{0-\infty}$, ng.h/ml	$t_{1/2}$, h	C_{max}/AUC, h^{-1}	$AUC_{0-168}/AUC_{0-\infty}$ ratio
1	54	4.00	599	0.038	599	18.36	0.090	1.00
2	53	5.00	557	0.037	557	18.90	0.095	1.00
3	79	4.00	929	0.031	929	22.38	0.085	1.00
4	96	5.00	1221	0.032	1221	21.91	0.079	1.00
5	77	6.00	2167	0.020	2167	34.24	0.036	1.00
6	72	6.00	1310	0.025	1310	27.28	0.055	1.00
7	70	3.00	1789	0.020	1789	34.57	0.039	1.00
8	82	4.00	1221	0.048	1221	14.38	0.067	1.00
9	60	5.00	1687	0.030	1687	22.78	0.036	1.00
10	53	3.00	1189	0.025	1189	27.58	0.044	1.00
Mean	70	4.50	1267	0.031	1267	24.24	0.063	1.00
GMean	68	4.38	1168	0.030	1168	23.42	0.058	1.00
SD	15	1.08	509	0.009	509	6.65	0.023	0.00
CV	21	24.00	40	28.245	40	27.45	37.567	0.00
Median	71	4.50	1221	0.031	1221	22.58	0.061	1.00
Sig. int.	8	0.56	265	0.005	265	3.46	0.012	0.00
L-90 %	62	3.94	1002	0.026	1002	20.78	0.050	1.00
U-90 %	77	5.06	1532	0.035	1532	27.70	0.075	1.00

number of sample collection time points, and i is the sequence number of the sample collection time point.

$$AUC_{0\text{-}\infty} = AUC_{0\text{-}t} + C_t/k_{el},$$

where C_t is the concentration at the last sample collection time point and $T_{1/2} = \ln 2/k_{el}$.

The constant of elimination was calculated taking cognizance of the whole of the descending part of the pharmacokinetic curve with nonzero concentrations.

Values for individual pharmacokinetic parameters for thiozonide, 25 mg capsules (as an example) after administration of formulation are shown in Table 1.

RESULTS AND DISCUSSION

During the study, individual plasma I concentrations (C) were determined over time, along with individual and averaged pharmacokinetic parameters of I.

The half-elimination time of I was about 25 h. Compound I was virtually undetectable in plasma at 168 h after single doses of 25 mg but could be detected at low levels at doses of 200, 400, and 600 mg.

C_{max} values of compound I were 70 ± 8, 522 ± 94, 892 ± 131, and 1359 ± 193 ng/ml for doses of 25, 200, 400, and 600 mg respectively.

AUC_{0-168} values for compound I were 1267 ± 265, $10{,}508 \pm 2108$, $16{,}760 \pm 1713$, and $25{,}231 \pm 1862$ ng · h/ml for doses of 25, 200, 400, and 600 mg respectively.

Comparison of the AUC_{0-168} values with the total $AUC_{0\text{-}\infty}$ value (the ratio was significantly greater than 80%) provided evidence that the pharmacokinetic study method used here provided the necessary reliability for evaluation of the pharmacokinetic parameters of I.

Pharmacokinetics over the dose range 25 – 600 mg were linear in terms of C_{max} and AUC_{0-168} ($r^2 > 0.99$); linearity plots are shown in Figs. 1 and 2. Mean pharmacokinetic profiles of thiozonide are shown in Figs. 3 and 4.

Thus, pharmacokinetic studies of the original antituberculosis drug thiozonide, 100-mg capsules, demonstrated that the pharmacokinetics of this drug were linear over the dose range 25 – 600 mg. Thiozonide was shown to be absorbed gradually into the systemic circulation after oral ingestion (T_{max} about 4.5 h). The half-elimination time was 25 h, indicating slow clearance of the agent from the plasma. These data show a possible regime for administration of the agent – 1 – 2 times daily – for investigation of the drug in subsequent clinical trials.

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