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Research Article

PHARMACOKINETIC ALTERATIONS OF WARFARIN IN ITS CLATHRATE WITH ARABINOGALACTAN

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ABSTRACT

Pharmacokinetic parameters of warfarin (WF) in its clathrate with arabinogalactan (AG) were investigated after oral administration at a dose of 20 mg/kg. The concentrations of warfarin in rat plasma were analyzed by the MS/MS method. Noncompartmental analysis was used for calculation of pharmacokinetic parameters: clearance (CL), mean residence time (MRT), $T_{1/2}$ and AUC. It was found that C_{max} of WF was achieved for 7 hours faster (T_{max}) then such of WF in the clathrate with AG and clearance value of clathrated WF is greater than for blank WF (1.52±0.03 vs 1.93±0.18 ml/h).

Keywords: arabinogalactan, clathrate, pharmacokinetics, rats, warfarin.

INTRODUCTION

The clathration of drugs or in other words the incorporation of drugs into a supramolecular complex with carbohydrate-containing plant metabolites has the aim similar in use with cyclodextrins, chitosan etc [1,2]. With the application of this approach it is possible to increase the solubility and bioavailability in water thereby reducing both toxic effects and the necessary effective dose of clathrated compounds [3].

Arabinogalactan is a polysaccharide that is contained in the woody tissue of the Siberian Larch (*Larix sibirica*) and the Dahurian Larch (*Larix Gmelinii*), amounting to no less than 10% [4-7]. Clathrates of AG with the following drugs were synthesized and their pharmacological properties were studied: Indometacin, Sibazon, Mezapam, Azaleptin, Nifedipine[3].

Previously we studied the pharmacological effects of the clathrate of AG with Warfarin (WF), an indirect oral anticoagulant for long-term administration. The main disadvantage of WF therapy is the development of hemorrhages, particularly during the induction period, as well as the difficulties connected with dose control[8,9]. It was found that clathration increased water solubility of WF by an order of 5.3. The pharmacological data we have obtained were as follows: a 20 mg/kg (WF 2 mg/kg) dose of WF:AG 1:10 leads to an significant increase in prothrombin time (PT) in 24 hours but it was 28,5% less than warfarins (30 vs 42 sec). However, in 48 hours after a single administration the PT value was equal (21 sec) for both agents [10]. The PT value for blank animals is 11 sec. Here PT is a measure of the extrinsic pathway of coagulation. It is used to determine the clotting tendency of blood [11].

The aim of the present work is to investigate the pharmacokinetic parameters of clathrate WF:AG 1:10 using an experimental design similar to the one described above. Moreover, at the time of writing there exists no pharmacokinetic data for drug clathrates with AG, thus this study is the first of its kind in this area.

MATERIALS AND METHODS

Chemicals. WF was synthesized in the NIOCH SB RAS (Prof. A. Y. Tikhonov). AG was obtained at the Laboratory of Wood Chemistry (Prof. V. A. Babkin) of the A. E. Favorsky Institute of Chemistry SB RAS. The clathrate WF:AG in the ratio 1:10 by mass were synthesized using the mechanochemical method at the Institute of Solid State Chemistry and Mechanochemistry SB RAS [12,13].

Animals. The study involved female rats of the Wistar line (190-210 g). The animals were obtained from the laboratory of experimental animals at the Institute of Cytology and Genetics (SB RAS, Novosibirsk). All manipulations were performed in full accordance with the rules and principles of humane animal treatment.

The animals (6 in group) were without food for 24 hours before the doses were administered to them. All the compounds were dissolved in distilled water. The compounds were administered intragastrically at a dose of 2 mg/kg for WF and 20 mg/kg for the clathrate (WF dose 2 mg/kg). TWEEN 80 was used additionally to dissolve WF. Blood was collected from the neck vessels of the sacrificed animals and administered into tubes containing 3,8% sodium citrate (9:1) 1, 8, 10, 12, 24, 48 and 72 hours after single WF or WF:AG administration.

Pharmacokinetic analysis

Preparation of standard stock solutions

The stock standard solution of racemic warfarin (1 mg/mL) was prepared by dissolving the compound within 50% acetonitrile in water. A series of calibration solutions of racemic warfarin were prepared via the addition of the appropriate volumes of the stock standard solution to drug-free rat plasma. Plasma standards were aliquoted, stored and treated the same way as rat plasma samples. Calibration plasma solutions contain 0.2, 0.5, 1, 2, 3, 6, 8, 10, 15, 20 mg/ml of warfarin. Calibration curves were constructed via linear regression analysis of peak-area ratios versus the respective concentration of the calibration plasma solutions.

Samples preparation

Whole blood samples (5 mL) at 1, 8, 10, 12, 24, 48 and 72 hours after WF or clathrate WF:AG were centrifuged for 10 min at 3000 rpm to separate plasma fraction. The 1 mL of the 1 M H_2SO_4 was added to 1 mL of sample plasma in the 15 mL tubes. The tubes were vortex-mixed for 10 second and 5 mL of diethyl ether was added as extraction solvent. The solutions were shaken for 60 min at 37 °C at 250 cycles per min in a vertical position using Shaker-Incubator ES-20/60 (Biosan, Latvia). The diethyl ether layer was moved into a clean tube and was evaporated to dryness under air flow. For samples, the reconstitution tubes were washed consequently by 150 mL of acetonitrile, then by 150 mL of 50% acetonitrile in water with a total sample volume – 300 mL of 75% acetonitrile in water. 250 mL of 50% acetonitrile in water was added to 50 mL aliquot of sample solutions and 50 mL aliquot of the resulted solution was injected for MS/MS analysis.

MS/MS analysis of warfarin

The concentrations of warfarin in the all plasma samples were analyzed via the MS method using the mass- spectrometer Agilent 6410 QQQ (Agilent Technologies, USA). Mass spectrometric analysis was performed in the positive-ion detection mode (ESI) and set up in the full product ion scan (MS2) mode. A deprotonated precursor ion [M-H]- at m/z 307.0 of warfarin was analyzed. Nitrogen was used as a desolvation and nebuliser gas with gas flow 3.5 L/m. The source and desolvation temperatures were set at 200 and 350 °C,

respectively. The capillary voltage was 2.5 kV. The water acetonitrile mixture (1:1) was used as the mobile phase with 1mL/min flow. Each sample was analyzed three times.

Calibration curves were prepared and assayed in quadruplicate for warfarin concentrations up to 20 mg/ml, in order to evaluate the linearity. The linear assay range was 0.2-8 mg/ml for warfarin (r^2>0.98). No interference in the blank sample was noted.

Pharmacokinetic data analysis

The maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were determined directly from the individual concentration-time data. A non-compartmental analysis (NCOMP 3.1 software) was used for the calculation of pharmacokinetic parameters: clearance (Cl), mean residence time (MRT), apparent elimination half-time ($T_{1/2}$), the area under the plasma concentration–time curve (AUC).

RESULTS AND DISCUSSION

The mean plasma concentration–time profile of WF:AG 1:10 and WF after single oral administration to female rats are shown in Figure 1, and the pharmacokinetic parameters of agents are listed in Table 1.

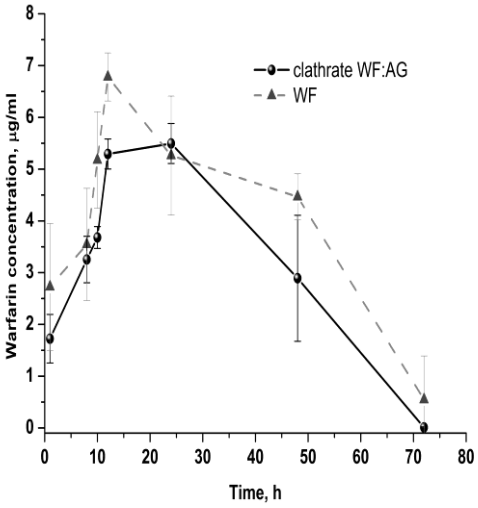

Figure 1: It shows the mean plasma concentration–time profile of WF:AG 1:10 and blank WF after single oral administration at a dose of 20 mg/kg (dose of WF is equal to 2 mg/kg) and 2 mg/kg, respectively

Table 1: It shows the pharmacokinetic parameters of WF and WF: AG 1:10

	WF, 2 mg/kg	WF:AG 1:10, 20 mg/kg
CL, ml/h	1.52±0.03	1.93±0.18*
MRT, h	31.39±1.82	21.81±2.38*
Terminal half life, $T_{1/2}$, h	5.11±0.24	6.38±2.55
T_{max}, h	11,00±1.41	18,00±8.49
C_{max}, µg/ml	6.47±0.91	5.64±0.19
AUC, µg h/ml	263.01±0.02	08.34±20.03*

***p<0,05 against WF**

The plasma concentrations of the agents under study increased in a similar way but the C_{max} of WF was achieved 7 hours faster (T_{max}) then that of the WF in the clathrate. After 24 hours their plasma concentrations equalize. An inverse relationship was observed during the elimination period, i.e. blank WF eliminates slower than WF in the clathrate. It is confirmed by clearance value (CL) of clathrated WF that is 27 % greater than for blank WF. Thereby, the plasma concentration of clathrated WF increases more smoothly than the concentration of blank WF. It could result in the reduction of the risk of bleeding caused by a rapid increase of the WFs plasma concentration that in turn rapidly increases the PT during the induction period of WF treatment. In addition, the smaller Mean Residence Time (MRT) of clathrated WF could secure repeat dosing and accelerate the WFs elimination in the case of drug withdrawal.

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CONCLUSION

The determined differences in the pharmacokinetic parameters of blank Warfarin and WF:AG 1:10 clathrate after a single dosing helps us to understand their effects upon the previously observed prothrombin time[10]. Thereby, the use of WF:AG 1:10 instead of blank WF could reduce the risk of bleeding during anticoagulant therapy.

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