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Short communication

Determination and pharmacokinetic study of ferrocene-A in blood serum of rabbits

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Abstract

Ferrocene-A (ferrocenyl-1-phenyl-1-dioxy-1,4-butin-2) has significant antitumour and antibacterial properties. This study was designed to estimate main pharmacokinetic parameters of ferrocene-A (FC-A). These parameters are very important from the standpoint to elucidate the mechanism of pharmacological action of ferrocene-A. Examined substance was administrated intramuscularly and orally to rabbits. For determination of small quantities of ferrocene-A in a blood plasma liquid–liquid extraction and reversed-phase high performance liquid chromatography (HPLC) method was applied. Kinetic parameters were estimated using the one-compartment linear pharmacokinetic model. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Pharmacokinetics; Microcolumn HPLC; Ferrocene-containing dihydric acetylenic alcohols; Antitumour and antibacterial action

1. Introduction

Ferrocene-A (ferrocenyl-1-phenyl-1-dioxy-1,4-butin-2) pertains to ferrocene-containing dihydric acetylenic alcohols (Fig. 1). In experiments in vivo ferrocene-A (FC-A) can successfully be applied to preventive maintenance of malignant tumours, caused by different chemical carcinogens [1,2]. Administration of FC-A with chemical carcinogens (both simultaneous and preliminary) prevents the process of malignisation in experimental animals. FC-A has shown also certain antibacterial properties [2,3].

The molecular weight of FC-A is 346. It is a light brown crystalline substance that is insoluble in water and soluble in chloroform, acetone, acetonitrile, ether, hexane. Hydrophobic nature of FC-A molecule is stipulated by cyclopentadiene rings and phenyl group (Fig. 1). This compound

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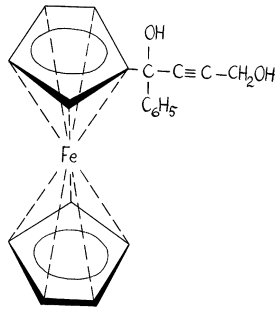

Fig. 1. Structure of ferrocene-A (FC-A).

is characterised by low toxicity and does not show any adverse effect on the functioning of the liver, kidneys, cardiovascular and central nervous systems [4]. In all above-mentioned studies, less attention was paid to the metabolism profiling and pharmacokinetics of FC-A. The latter problem was the aim of present investigation.

2. Experimental section

2.1. Equipment and materials

Chromatographic analyses were carried out with a Milikhrom series Nauchpribor, Russia) microcolumn high-performance liquid chromatograph (HPLC) equipped with a diode matrix-type UV detector set in the wavelength range of 190 to 360 nm. The stainless steel columns (62×2.1 mm i.d.) were packed with the commercially available stationary phase separon-C_{18} (Lachema, Brno, Czech Republic), with particle size 5 pm. Phenotiazine (PTZ) was used as an internal standard.

FC-A was synthesised in Laboratory of Elementorganic Compounds (Tbilisi State University, Tbilisi, Georgia) [2]. Sodium dodecylsulfate (SDS) was obtained from Ekross (St. Petersburg, Russia).

2.2. Sample preparation

To prepare the samples for HPLC analysis a liquid–liquid extraction method was used. Samples of serum (0.2 ml) were added to 10 ml glass centrifuge test-tube. The samples were treated with 0.2 ml of internal standard (solution of PTZ in hexane–isoamyl alcohol mixture (98:2) at the concentration 0.4 μg ml^{-1}). Sodium hydroxide (0.5 ml, 1.0 M) was added and vortexed briefly. The analytes were extracted with 3-ml hexane–isoamyl alcohol mixture with ratio 98:2 (v/v).

The extraction mixture was agitated by hand for 1 min and centrifuged for 10 min at $3000 \times g$. Then the upper organic layer was filtered, transferred into a clean test-tube and evaporated at room temperature with a gentle current of air. The residue was dissolved in 50 μl of mobile phase and 25–30 μl was injected for HPLC analysis.

2.3. Chromatographic conditions

The mobile phase was ethanol–0.01 M potassium dihydrogen phosphate with ratio 60:40 (v/v). The mobile phase pH was native (pH 6). The flow-rate of mobile phase was 50 μl min^{-1}. Detection wavelength was 210 nm. Chromatographic analyses were performed at a room temperature.

2.4. Animal studies

Experiments were carried out in buck rabbits with body weight about 2 kg and age not less than 6 months. The solution of FC-A in alcohol was administrated intramuscularly (i.m. dose 100 mg kg^{-1}) or orally (oral dose 200 mg kg^{-1}). Blood samples were collected from the auricle at various times.

2.5. Statistical analysis

The results were processed with help of program 'Statistica' (Version 4.3, ©Statsoft inc. 1993) and 'PHAKIN', written earlier [4,5]. 'Statistica' was used for non-linear regression of pharmacokinetic data by one and two compartment models and their consequent comparison by F-criterion; it has been established that there is no significant difference between these models for kinetic data of FC-A. Because of this reason one compartment model was chosen for calculation of pharmacokinetic parameters.

3. Model

The program PHAKIN uses one-compartment kinetic model and is applied to study kinetics both i.m. and orally administrated drugs [4,5]. It is supposed that absorption in gastrointestinal tract and elimination in blood are described with following equation system.

$$\frac{dD}{dt} = -K_1 D$$

$$\frac{dc}{dt} = \frac{K_1 D}{V} = K_2 c \quad (1)$$

where K_1 is constant of absorption; K_2 is constant of elimination; V denotes volume of distribution; D stands for dose of drug in gastrointestinal tract. The solution of (Eq. (1)) system looks like

$$D = D_0 e^{-k_1 t} \quad (2)$$

$$c = \frac{K_1 D_0}{V(K_2 - K_1)}(e^{-k_1 t} - e^{-k_2 t}) \quad (3)$$

PHAKIN uses Eq. (3) as a regression equation for kinetic data $c(t)$; K_1, K_2 and V are unknown parameters. Their values computer calculates during nonlinear regression.

For i.m. administration the system (Eq. (1)) simplifies. At an assumption, that the time of distribution of FC-A in the body is possible to neglect in comparison to elimination time, there, one equation

$$\frac{dc}{dt} = -k_2 c \quad (1')$$

The solution of Eq. (1′) is

$$c = \frac{D_0}{V} e^{-k_2 t} \quad (3')$$

Eq. (3′) is applied as a regression equation at i.m. administration. Body clearance

$$CL = K_2 V$$

Absorption and elimination half-times

$$T_{1/2\ abs} = \frac{\ln 2}{K_1}$$

$$T_{1/2\ el} = \frac{\ln 2}{K_2}$$

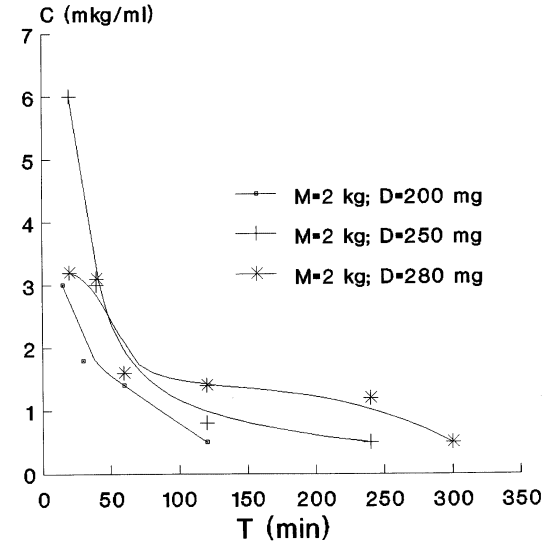

Fig. 2. Plasma concentration–time profiles following i.m. administration; M, body weight; D, initial dose.

For known therapeutic range (maximal allowable and minimal necessary concentration of drug in a blood plasma), PHAKIN program can predict a doze and interval between administrations for a drug, if its kinetics can be described by one-compartment model.

4. Results and discussion

During experiments with i.m. administration of FC-A, we observed an exponential decrease of concentration in blood (Fig. 2). Eq. (3′) was used as an equation of regression. Correlation between experimental and predicted values of concentration is 0.97. Calculated values of pharmacokinetic parameters is given in Table 1. FC-A is characterised with high distribution volume (57–88 l) and medium speed of elimination (elimination half-time 45–120 mm).

The HPLC analysis has shown, that at oral administration at least part of a FC-A does not endure any chemical transformation during absorption in gastrointestinal tract. Chromatographic peaks of FC-A coincides in time both at i.m. and at oral administration (Fig. 3). Their UV spectra in eluent aro also identical. Following the

oral administration, the absorption of FC-A was prolonged with a peak serum concentration, occurring at 3–4 h after dosing. Typical $C-t$ dependencies are shown in Fig. 4. Eq. (1) could not describe these curves (1). On the other hand, these curves could not be described with two-compartment model either. The presence of several peaks on a kinetic curve cannot be explained by coinciding of FC-A and its possible metabolites since extract of blood plasma with FC-A was tested in different chromatographic systems. On our view absorption of FC-A in intestine during oral administration needs a special mathematical model, but its creation was not our aim now.

Therefore, usage of one or two compartment linear models (for oral administration) for the given substance is inadmissible. In these models absorption in gastrointestinal tract is described with one kinetic equation. The presence of several statistically authentic maximums on a curve c can be explained by different speed of FC-A's absorption from various parts of intestine, which is presumably stipulated by poor solubility of FC-A. Absorption ends in 6–7 h, after their exponential elimination occurs with the same time constant, as at i.m. administration.

That is why, calculation of absorption constant with help of one-compartment model has relative character. The calculated value of K_1 is possible to be named as an average or effective constant of absorption (Table 1).

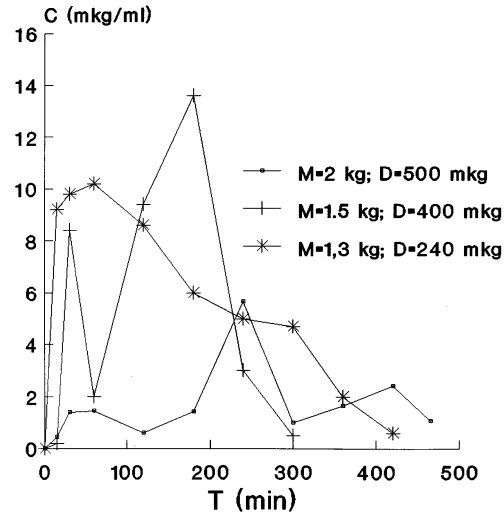

Fig. 4. Plasma concentration–time profiles following oral administration; M, body weight; D, initial dose.

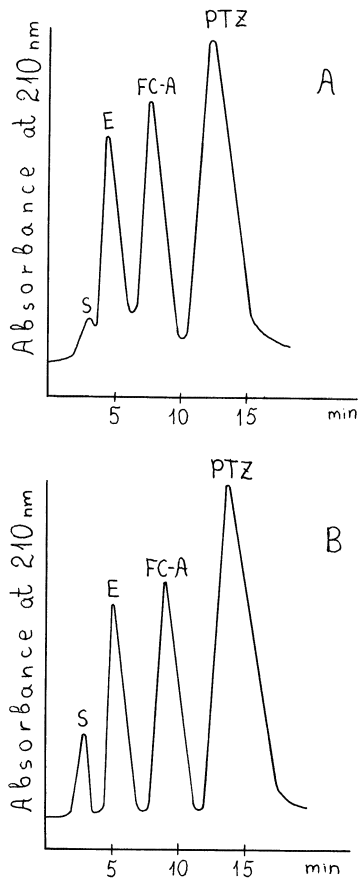

Fig. 3. Chromatograms of FC-A; (A) oral administration; (B) i.m. administration; PTZ, internal standard; FC-A, ferrocene-A; E, endogenous compound; S, solvent peak.

Table 1
Values and ranges of main pharmacokinetic parameters

Parameter	Mean	Range
K_1 (min^{-1})	0.07	0.05–0.11
$T_{1/2\ abs}$ (min)	9.9	6.3–13.8
K_2 (min^{-1})	0.011	0.0057–0.015
$T_{1/2\ el}$ (min)	63	46.2–121.6
V (l)	61.2	57.8–87.5
CL (ml min^{-1})	670	498–923

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