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Elimination kinetics of synthetic interferon inducer tilorone in experimental animals

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Abstract

Objective. A comparative investigation was carried out on the kinetics of [^{3}H]-tilorone ([^{3}H]-I) excretion in rats and mice.
Materials and method. Kinetics of urinary and biliary excretion of [^{3}H]-I in rats and mice was studied following a single oral and intravenous administration. The excretion mass balance was monitored in the faeces and urine of rats and mice for 5 and 10 days, respectively. Radioactivity was determined in the samples of excreta using a liquid scintillation counter.
Results. In rats, [^{3}H]-I were nearly fully (~80%) eliminated with excreta in 5 days, indicating a lack of any significant accumulation of the drug in the body. Faecal excretion of tilorone predominated (69.0% ± 2.8%), while the elimination in urine was less significant (9.8%8 ± 1.2%). A different pattern of elimination kinetics was observed in mice as excretion proceeded with equal efficiency in urine (27.85–28.9%) and faeces (26.45– 24.4 %), regardless of the route of administration of the substance and at a substantially slower rate compared to rats: only 57.35–52.68% of total radioactivity was eliminated over 10 days following oral and intravenous administration of tilorone, respectively. MRT of tilorone in rats was 36 h and in mice 120–150 h.
Conclusion. Parameters for the excretion of tilorone showed significant differences between the 2 groups of animals. Using the example of tilorone excretion kinetics validates the presented novel modified approach to determine the mean residence time (MRT) and amount of drug eliminated from the body during an infinitely long experimental period. This approach can be generally applied for interpretation of nonlinear elimination kinetics of xenobiotics.

Key words

pharmacokinetics, excretion, tilorone, modeling

INTRODUCTION

Tilorone (trade name Amixin IC) is a broad-spectrum antiviral drug with a polymodal mechanism of action that includes direct inhibition of viral replication in infected cells and stimulation of interferon production [1–6]. It is one of the first effective low molecular weight interferon inducers with anticancer activity [7–9]. Numerous studies have demonstrated the efficacy of tilorone in treating many viral infections, as well as conditions accompanied by weakened immune system defences [10–12]. Tilorone pharmacokinetics have not been extensively studied. The first such study that addressed the distribution of tilorone in mice at a single time point following oral administration was published in 1973 [1,13]. The authors of the presented study have recently published a more detailed study of the metabolism and distribution of tilorone after intravenous and oral administration in mice, which demonstrated that tilorone does not undergo significant biotransformation in the body [14,15]. The absolute bioavailability of tilorone amounted to circa 70% following oral administration, as 21% of the administered drug underwent pre-systemic elimination in the liver. The pulmonary first-pass effect was observed after intravenous injection of tilorone [15]. The equilibrium distribution ratio of substance between a tissue and blood was higher than unity, indicating the rapid distribution of tilorone into organ tissues. Irreversible binding of 1–2% of the total administered drug by the spleen was also noted [15]. Golovenko et al. studied the distribution of the total radioactivity after multiple oral doses of [^{3}H]-tilorone [16]. A high concentration of the drug in mice over 10 days of the experiment and a slow rate of elimination was shown.

The aim of this investigation was to study [^{3}H]-tilorone excretion in mice and rats following a single administration of the drug.

MATERIALS AND METHOD

This study used labeled 2,7-bis-[2-N,N-diethylamino-[^{3}H]-ethoxy]-fluorene-9-one dihydrochloride (tilirone, I) (JSC Interkhim, Odessa, Ukraine). The high radiochemical purity of [^{3}H] – I (99.6%) was inferred from radiochromatographic analysis and the specific radioactivity was 2.3Ci/mol.

The animals were obtained from the breeding facility of the Odessa State Medical University (Odessa, Ukraine). The experiments were performed on female outbred mice (n=8, per group) weighing 18–24 g and female Wistar rats (n=6) 9–11 weeks old, weighing 200–250 g. All animals received a standard laboratory diet and water *ad libitum*. The experimental animals were kept under a continuous 12 h light-dark cycle at room temperature.

Experimental protocols were approved by the Ethics Committee of the Pharmacological Committee of Ukraine, carried out in strict accordance with the Ethics Committee regulations for the use of experimental animals; experimental

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protocols were approved by the Ethics Committee of the Department of Biology, Odessa National University.

Two routes of administration of the examined compounds, i.e., oral and intravenous, were used in the study of tilorone excretion in mice. An isotonic solution of [^{3}H]-tilorone was administered to the eighth mice intravenously ***via*** the tail vein (with a 28-G needle) at a dose of 50 mg/kg.

Six rats and 8 mice were administered with [^{3}H]-I at a dose of 50 mg/kg by oral gavage. The dosing volume was 10 ml/kg for the mouse and 20 ml/kg for the rat. The oral dose was dissolved in an isotonic solution. Following gavage, the rat and mice were housed individually in metabolism cages (Tecniplast, Varese, Italy) with free access to food and water throughout the experiments. Urine and faeces samples were collected daily from rats and mice for 5 and 10 days post-dosing, respectively (this time period was chosen because previous studies have shown a slow speed of the process of elimination of tilorones in the mice) [15]. Urine volume was measured, and samples were stored at –18 °C until analysis. Faeces were air-dried, weighed, and ground to a fine powder before dissolving. About 100 mg nanopure was added to the sample of formic acid (2 ml), and the mixture was left in the shaker for 24 h until thorough dissolution.

Radioactivity was determined in the samples of excreta using a Tri Carb 2700 liquid scintillation counter (Canberra Packard, USA).

Pharmacokinetic analysis. The following pharmacokinetic parameters were calculated for tilorone: elimination rate constant (k_{el}), half-life in elimination phase ($t_{1/2}$), MRT, and the amount of drug eliminated from the body over an infinitely long experimental period ($A_{0-\infty}$); in order to determine such fractional elimination parameters, regression analysis by a modified "rate-quantity" method was utilized, described in earlier studies [17], and using validated software PKSolver [18]. This was based on the use of the integrated analog of the basic equation of the method and its further transformation to adapt to reproof poly exponential kinetics of xenobiotics excretion. This enabled determination of the total quantity of the drug eliminated from the body in conditions of infinite exposure ($A_{0-\infty}$) and the constant elimination (k_{el}) using regression analysis on the basis of the following equations:

$$\frac{A_{0-t}}{t} \approx A_{0-\infty} \cdot k_{el} - k_{el} \left(\frac{\int_0^t A_{0-t} \cdot dt}{t} \right), \quad (1)$$

and for *MRT*

$$\left(1-\left(A_{0-t}/A_{0-\infty}\right)\right)/k_{el} \approx MRT - \left(t - \left(\int_0^t A_{0-t} \cdot dt \Big/ A_{0-\infty}\right)\right) \quad (2)$$

Equation (2) suggests that at the same time interval, the values of $\left(1-\left(A_{0-t}/A_{0-\infty}\right)\right)/k_{el}$ are distributed linearly concerning values of $\left(t-\left(\int_0^t A_{0-t} \Big/ A_{0-\infty}\right)\right)$. Then, the regression curve has a tangent angle that equals one and crosses the ordinate and the abscissa in the point equal to $\left(1-\left(A_{0-t}/A_{0-\infty}\right)\right)/k_{el}$ and $\left(t-\left(\int_0^t A_{0-t} \Big/ A_{0-\infty}\right)\right)$, equal to the value of MRT.

Statistical analysis. All average values are given as mean ± SD. **V**alues of total radioactivity in excretes of mice and rats were compiled with the Student's *t*-test for unpaired data. The acceptable probability for a significant difference was $p<0.05$.

RESULTS

Elimination of [^{3}H]-tilorone in rats. In rats, almost 80% of [^{3}H] total radioactivity was excreted in faeces and urine over the course of 120 h postdose. The principal route of tilorone elimination in rats was faecal, as it mediated excretion of about 69% of the total administered dose during the first 5 days after administration of the drug.

Urinary excretion in rats was a steady-state process characterized by a slow elimination rate. In the first 120 h postdose, 10% of the total administered dose was eliminated via the renal route. The efficiency of [^{3}H]-tilorone excretion in faeces was 7-fold higher than in urine in rats (Fig. 1); thus, overall elimination is mainly determined by the parameters of faecal excretion. The maximum radioactivity elimination rate from the body was observed in the first 72 h post-dose.

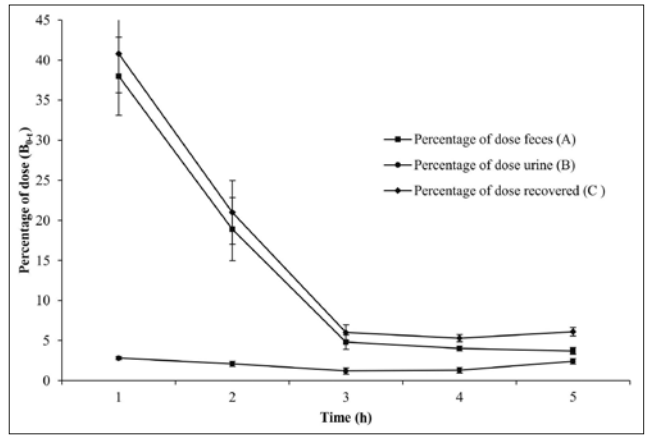

Figure 1. Elimination of the total radioactive material (% of the administered dose) in excretes of rats after a single administration of [^{3}H]-tilorone at a dose of 50 mg/kg. The data represent mean±SD, n=6

Overall elimination and faecal excretion in particular were biphasic with a rapid phase (0–72 h) responsible for elimination of 62–68% of the drug and slow phase (72–120 h) mediating excretion of 8–11% of the total administered dose. The urinary elimination rate was slow and mono-exponential throughout the whole period of the study.

The kinetics of the overall elimination and faecal excretion in rats could be described by a classic 2-compartment model. Corresponding kinetic parameters are given in Tab. 1. Elimination half-time was 21 h post-dose in the rapid phase, while the remaining radioactivity (~4–7% of the total administered dose) had a longer elimination half-life of about 150 h.

Elimination of [^{3}H]-tilorone in mice. A characteristic feature of tilorone metabolism in mice was a slower elimination rate compared to rats, as only 57.35% of the oral administered dose was eliminated in total within 10 days. Also, in contrast to observations in rats, the efficacy of excretion in urine and faeces was similar: 27.85% and 26.45% of the administered oral dose, respectively (Tab. 2). Elimination kinetics in urine

Table 1. Kinetic parameters for elimination of total radioactive material (% of dose) in excretes of rats after a single administration of [^{3}H] -I (50mg/kg) in the fast (24–72 h) and slow phases (72–120 h) of the experiment

Parameters	Percentage of dose: faeces		Percentage of dose recovered	
	fast phase (24–72 h)	*slow phase* (72–120 h)	*fast phase* (24–72 h)	*slow phase* (72–120 h)
k_{el} (h^{-1})	-0.035±0.009	-0.0052±0.0002	-0.0328±0.004	-0.0048±0.0002
$T_{0,5}$ (h)	19.7±3.67	133.3±12.43	21.1±5.78	144.4±14.09
C_0 (% of dose)	74.4 ± 2.45	6.7 ± 0.98	81.3 ± 7.12	3.4 ± 0.78

Table 2. Elimination of total radioactive material (% of administered dose) in excretes of mice after a single oral administration of [^{3}H] -tilorone (50 mg/kg).

Time (h)	Percentage of dose faeces (A)	Percentage of dose urine (B)	Percentage of dose recovered[1] (C)
24	5.02 ± 1.04	5.68 ± 1.38	11.0 ± 1.73
48	2.95 ± 0.44	4.13 ± 0.69	8.08 ± 0.82
72	2.81 ± 0.31	4.17 ± 0.24	7.07 ± 0.39
96	2.77 ± 0.69	3.91 ± 0.47	6.88 ± 1.83
120	3.33 ± 0.64	3.22 ± 0.57	6.85 ± 1.86
144	2.18 ± 0.59	2.19 ± 0.23	4.67 ± 0.63
168	2.08 ± 0.32	1.87 ± 0.19	4.05 ± 0.37
192	1.85 ± 0.41	1.43 ± 0.15	3.58 ± 0.44
216	1.79 ± 0.42	0.78 ± 0.19	2.77 ± 0.46
240	1.67 ± 0.45	0.47 ± 0.09	2. 40 ± 0.46

[1] Percentage of dose also includes cage washes

and faeces were not parallel as the elimination via the renal route was almost 2-fold higher than in faeces. Regardless of the route of administration of the examined substance, the process of excretion in the urine and faeces in mice is almost equally effective. Average excretion of [^{3}H]-I for a 10-day period in case of intravenous administration was 28.9±0.24% in urine, and in faeces – 24.5±0.48% of the administered dose. After 240 h of substance application, only 52.68±0.39% of the administered dose was excreted in urine and faeces (Tab. 3).

As equation (1) displays, irrespective of the species (mouse or rat), experimental data are strictly linear in these coordinates (Fig. 2); therefore, parameters of elimination can be determined without decomposition of the kinetic model.

Table 3. Elimination of total radioactive material (% of administered dose) in excreta of mice after a single intravenous administration of [^{3}H] -tilorone (50 mg/kg).

Time (h)	Percentage of dose faeces (A)	Percentage of dose urine (B)	Percentage of dose recovered[1] (C)
24	5.50 ± 2.32	6.30 ± 1.17	11.90 ± 2.60
48	3.10 ± 0.31	4.20 ± 0.54	7.38 ± 0.59
72	2.61 ± 0.44	5.31 ± 0.49	6.17 ± 0.66
96	2.51 ± 0.33	3.60 ± 0.56	6.22± 0.54
120	3.03 ± 0.51	2.62 ± 0.32	5.78 ± 0.43
144	1.81 ± 0.24	2.32 ± 0.31	4.27 ± 0.53
168	1.63 ± 0.22	1.80 ± 0.07	3.45 ± 0.24
192	1.45 ± 0.23	1.23 ± 0.14	2.78 ± 0.27
216	1.40 ± 0.25	1.02 ± 0.23	2.47 ± 0.48
240	1.51 ± 0.17	0.59 ± 0.08	2.26 ± 0.25

[1] Percentage of dose also includes cage washes

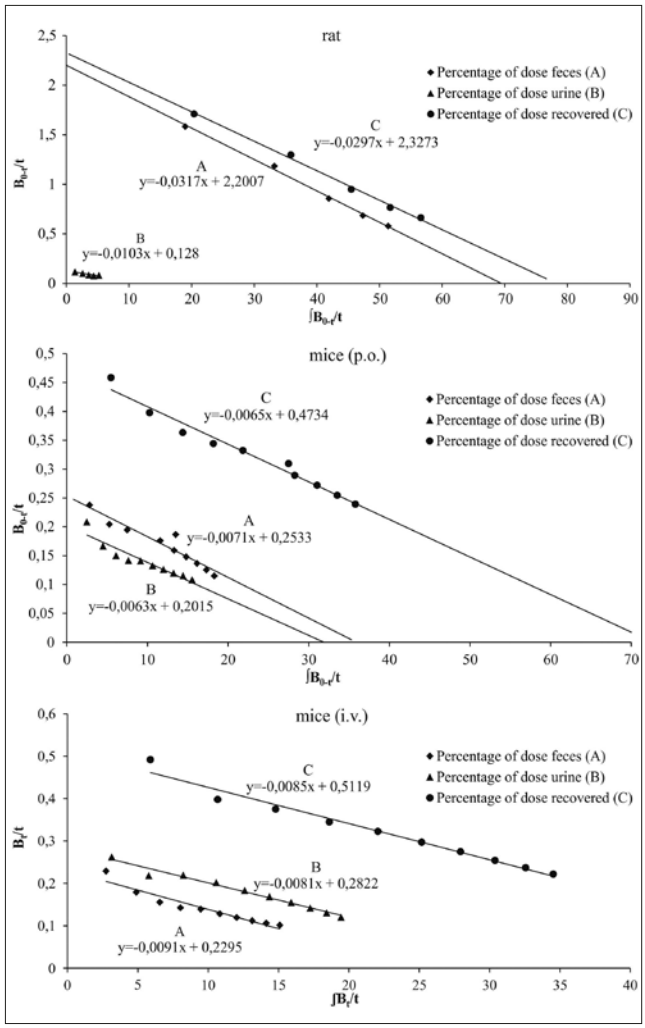

Figure 2. Graphic interpretation of equation (1) based on data on the excretion kinetics of tilorone and metabolites in rat and mice (determination of values k_{el} and $B_{0-\infty}$ using the relationship between B_{0-t}/t and B_{o-t}/t)

The elimination rate of tilorone and its metabolites in rats is significantly dependent on the excretion route: renal k_{el} value, as determined from the tangent of the slope angle, is 3 times lower than the faecal k_{el} value. Elimination half-time equaled 22 and 67 h post-dose, respectively. A similar pattern was observed for the total value of eliminated tilorone and its metabolites under infinite exposure ($A_{0-\infty}$ = 69% with faeces and 12% with urine).

In contrast, the elimination of tilorone in mice under oral administration was nearly identical via the renal and faecal routes, and overall fairly slow (Fig. 2). The half-life of time of elimination ($t_{1/2}$) in urine was 110 ± 5.02 h after oral administration, while after intravenous administration, it was significantly lower $t_{1/2}$= 85.57 ± 1.88 h ($p \leq 0.05$). $t_{1/2}$ in the case of intestinal and hepatic excretion – 97.62 ± 7.85 and 85.57 ± 3.65 h, respectively. In infinite exposure, the amount of total radioactivity excreted was 32–36% in the case of oral administration, and 25–35% in the case of intravenous dosing.

Having determined the values of k_{el} and $A_{0-\infty}$ by linear regression, it is possible to use them for the calculation of MRT. MRT value can be determined from the crossing point of the plot line with ordinate and abscissa in accordance with Equation 2 (linear anamorphosis using the value of

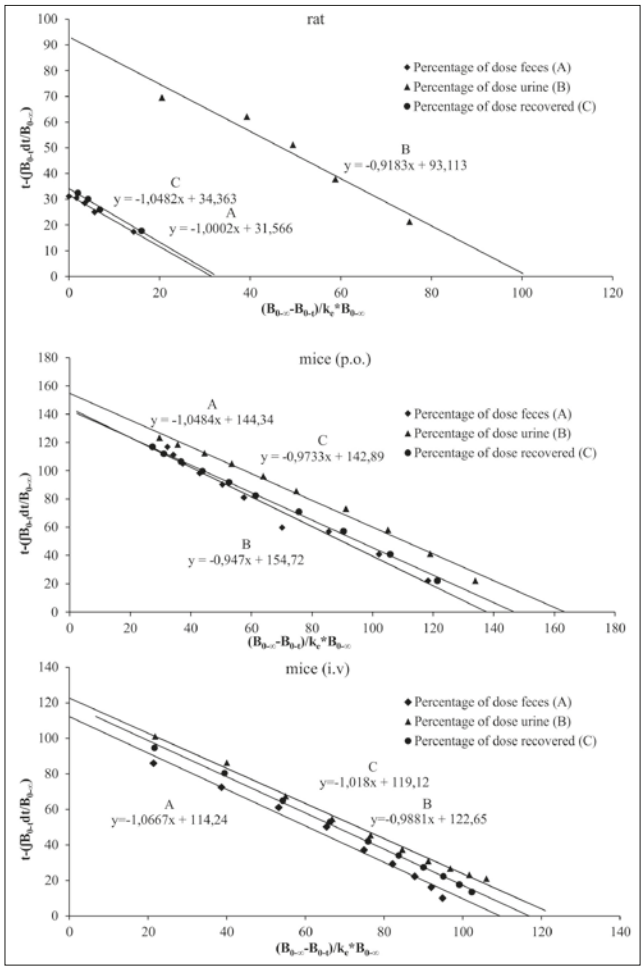

Figure 3. Graphic interpretation of equation (2) – the relationship between t-($\int B_{0-t}dt/B_{0-\infty}$) and ($B_{0-\infty}-B_{0-t}$)/($k_e B_{0-\infty}$)

t-($\int A_{0-t}dt/A_{0-\infty}$) as ordinate and ($A_{0-\infty}-A_{0-t}$)/($k_e A_{0-\infty}$) as abscissa (Fig. 3).

According to this method, the calculated values are presented in Table 4. The method proposed by the authors allowed highly linearized sets of experimental data to be obtained, as evidenced by the tangent of the slope angle equal to unity in Figure 3. MRT values were 140–150 h in mice, reflecting a slower elimination rate. Only 66% of the oral administered drug was excreted in conditions of infinite exposure. Intravenous [^{3}H]-I administration to mice caused an insignificant but a reliable ($p \leq 0.05$) change in the MRT value, which was 110–124 h, $A_{0-\infty}$» 61%.

Table 4. Kinetic parameters for elimination of total radioactive material in excreta of rats and mice after a single administration of [^{3}H]-tilorone

Parameters	$B_{0-\infty}$ (% of doses)	MRT (h)
	Figure 2	***Figure 3***
Rats		
Percentage of dose: urine	12.8±2,89	93.1±4,98
Percentage of dose: feces	69.1±4,32	31.6±2,78
Percentage of dose recovered	78.4±3,68	34.3±2,58
Mice (p.o)		
Percentage of dose: urine	32.9±1.79	154.7±9.76
Percentage of dose: feces	34.5±2.54	144.3±6.94
Percentage of dose recovered	66.8±5.09	142.9±8.32
Mice (i.v.)		
Percentage of dose: urine	34.53±2.68	122.65±6.22
Percentage of dose: feces	26.21±2.23	114.24±5.05
Percentage of dose recovered	60.98±4.98	119.12±7.86

DISCUSSION

This paper presents the comparative examination of the process of tilorone excretion in rats and mice after a single administration (oral and intravenous). Significant differences were demonstrated in the effectiveness of the excretion processes in urine and faeces in the experimental animals. Faecal elimination in rats is of a 2-phase character constituted 69% of the administered dose, whereas the process of excretion in urine (about 10%) was stationary during the whole period of examination. During 5 days of the experiment, almost the entire administered dose was excreted from the rat's organism (~90%) (Tab. 1, 4). In the case of mice, irrespective of the route of administration of [^{3}H]-I, a slow elimination process was observed in the organism, and mathematical calculations demonstrated that only 60% of the administered dose in an infinite exposition was excreted from the organism of the examined animals. On examination of the distribution processes, it was inferred that the process of irreversible binding to the spleen may explain the extremely slow excretion in urine and faeces and that only 70% of the administered dose is eliminated in case of infinite exposure. It is interesting to note that the route of administration of [^{3}H]-I to mice has an insignificant effect on the kinetic parameters of excretion – the amount of excreted drug was the same, and the elimination process rate after intravenous administration insignificantly higher. Previous studies on tilorone metabolism by the authors of this study showed that this drug undergoes little biotransformation in rodents [13,15,19,]. Around 89% of the drug is eliminated in its original, parent compound form in the faeces of rats and mice. Two minor metabolites resulting from oxidation of aliphatic ends of the tilorone molecule were observed in urine [14,19].

The presented data show no significant differences in metabolic processes of tilorone in rats and mice, and the data give no grounds for explaining the differences in the process of its excretion.

Currently, the literature does not provide any research data concerning the processes of tilorone distribution in rats, leading to no possibility of an evaluation of the relationship between these processes and the processes of the excretion of the examined substance. Perhaps the results of future studies of tilorone distribution in rats will help explain the differences in the kinetics of tilorone elimination from the rats and mice.

MRT calculation using multi-compartmental models or models in which processes with various constant rates may be distinguished is very complex; therefore, a new mathematical model based on non-compartmental modeling and regression analysis was applied that allowed the precise calculation of the examined parameters. MRT calculations in accordance with the software PKSolver showed comparable values with the parameters calculated using the authors' proposed modification of the 'rate-quantity' method (Tab. 4).

Therefore, for rats, MRT is equal to 47.6 h (dose recovered), and MRT for mice is equal to 106.34 h and 151.34 h under *i.v.* and *p.o.* administration, respectively. Novel mathematical approaches that allowed efficient calculation of elimination parameters in this study may, therefore, be useful for the analysis of the poly-exponential kinetics of drug.

Declaration of Interest

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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