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K. M. Tkachenko
Department of Clinical Pharmacology and Clinical Pharmacy, National University of Pharmacy, Kharkiv, Ukraine.

I. A. Zupanets
Department of Clinical Pharmacology and Clinical Pharmacy, National University of Pharmacy, Kharkiv, Ukraine.

S. K. Shebeko
Department of Clinical Pharmacology and Clinical Pharmacy, National University of Pharmacy, Kharkiv, Ukraine.

I. A. Otrishko
Department of Clinical Pharmacology and Clinical Pharmacy, National University of Pharmacy, Kharkiv, Ukraine.

Ye. F. Grintsov
Department of Clinical Pharmacology and Clinical Pharmacy, National University of Pharmacy, Kharkiv, Ukraine.

Correspondence:
K. M. Tkachenko
Department of Clinical Pharmacology and Clinical Pharmacy, National University of Pharmacy, Kharkiv, Ukraine.

The comparative study of the acute toxicity of Tetracyclines

K. M. Tkachenko, I. A. Zupanets, S. K. Shebeko, I. A. Otrishko, Ye. F. Grintsov

Abstract
The article presents the results of the experimental study of the acute toxicity of tetracyclines. The tasks of this study were to conduct the comparative analysis of safety as well as finding the median lethal dose (LD_{50}) of tetracycline hydrochloride, doxycycline hydrochloride, methacycline hydrochloride and to determine the optimal remedy for the further studies as anti-inflammatory and chondroprotective drug. It has been found that the LD_{50} of tetracycline hydrochloride after single oral administration in rats is 1478.22±201.67 mg/kg, LD_{50} of doxycycline hydrochloride – 1893.03±286.20 mg/kg and LD_{50} of methacycline hydrochloride – 1635.73±199.36 mg/kg. Thus, doxycycline is significantly safer than tetracycline and methacycline.

Keywords: tetracycline hydrochloride, doxycycline hydrochloride, methacycline hydrochloride, acute toxicity.

1. Introduction
Tetracycline antibiotics include a number of drugs related in chemical structure, antimicrobial spectrum and mechanism of action, the features of which still continue to be refined. Among the new applications of tetracyclines is their widely use as a tools for studying the mechanisms of disorders of the various structures of the body, as well as to develop the new approaches to the use of these drugs in the diagnostics and treatment of various human diseases, such as rheumatoid arthritis and other systemic diseases [1, 2, 3, 4, 5]. Currently one of the rapidly developing areas of researches is the study of immunomodulatory and anti-inflammatory effects of antibiotics. While studying the effects of tetracyclines in the therapy of noncommunicable diseases, particularly in the treatment of immune and skin diseases, some authors have been concluded that the therapeutic action of this drugs is realized due to the influence on immune system [6, 7, 8]. Nonantimicrobial effects of tetracyclines also includes pro-apoptotic, anti-tumor, neuroprotective, genotropic and other effects on the macroorganism [9, 10, 11, 12].

Tetracyclines – a relatively low toxic substances, but long-term use leads to the development of side effects of varying severity. One of the possible way to improve the safety of this group is to create combined drugs containing tetracycline antibiotics and biologically active substances that modify their toxic properties.

In order to confirm the feasibility of creating a new complex remedy and selection of the most effective ratio of the active compounds was carried out the study of acute toxicity of tetracycline antibiotics. The tasks of this study were to conduct a comparative analysis of safety, as well as finding the median lethal dose (LD_{50}) of tetracycline hydrochloride, doxycycline hydrochloride, methacycline hydrochloride after single intragastric administration in rats and to choose the most optimal remedy for the further studies.

2. Materials and Methods
A study of acute toxicity of the oral forms of tetracycline hydrochloride, doxycycline hydrochloride, methacycline hydrochloride were conducted according to the V.B. Prozorovsky method in 108 white outbred rats of both genders with the body weight 180,0-200,0 g divided into three series of 6 groups, there were 6 animals in each group. Animals of experimental groups received tetracycline hydrochloride, doxycycline hydrochloride, methacycline hydrochloride in doses ranging from 500 mg/kg to 5000 mg/kg. Drugs were administered intragastrically in appropriate doses dissolving them in the necessary amount of saline solution [13]. For the calculation of the median lethal dose (LD_{50}) was determined the percentage of mortality in each group after 14 days. Using tables and calculations in accordance with the

V.B. Prozorovsky method of probit-analysis of the curves of lethality the value of LD_{50} was determined [14].

According to the standard health and safety regulations the experimental animals were kept on the appropriate food diet in the vivarium at the Central Research Laboratory of the National University of Pharmacy certified by the State Enterprise "Centre for Drug Evaluation and Research at the Ministry of Public Health of Ukraine" as a base for research in experimental pharmacology [15]. The studies were conducted in accordance with EC Directive 86/609 EEC from November, 24, 1986 in compliance with laws, acts and regulations of the EU countries as to protection of animals used for experimentation and other scientific purpose [16, 17].

Evaluation of the toxic effects of drugs was carried out according to the following criteria: overall functional status of the animals, appearance, physical activity, food intake, general signs of intoxication and the level of mortality.

3. Results and Discussion

The observation of the animals has been carried out within two weeks after drug administration. Already on the second day after drug administration in groups of rats used doxycycline in doses 2000 – 5000 mg/kg the first fatal cases were observed. At the end of the first week a certain level of lethality has been observed in all groups, except the group of rats used the drug in the dose 500 mg/kg. Then the average mortality rate reached its maximum at 8-9 days of the experiment (Table 1, 2, 3).

Table 1: The mortality rates of rats during the study of acute toxicity of tetracycline hydrochloride (n = 36)

№ of the group	Dose of tetracyc-line, mg/kg	Num-ber of animals	The number of dead animals					The average of mortality, %
			1 day	4 days	7 days	10 days	14 days	
1	500	6	0	0	0	0	0	0
2	1000	6	0	0	1	3	3	50
3	2000	6	0	2	3	4	4	66.7
4	3000	6	0	1	3	4	5	83.3
5	4000	6	0	3	4	6	6	100
6	5000	6	0	4	6	6	6	100

Table 2: The mortality rates of rats during the study of acute toxicity of doxycycline hydrochloride (n = 36)

№ of the group	Dose of doxycyc-line, mg/kg	Num-ber of animals	The number of dead animals					The average of mortality, %
			1 day	4 days	7 days	10 days	14 days	
1	500	6	0	0	0	0	0	0
2	1000	6	0	0	1	2	3	50
3	2000	6	0	2	3	3	3	50
4	3000	6	0	1	2	3	4	66.7
5	4000	6	0	3	4	5	5	83.3
6	5000	6	0	4	5	5	6	100

Table 3: The mortality rates of rats during the study of acute toxicity of methacycline hydrochloride (n = 36)

№ of the group	Dose of methacy-cline, mg/kg	Num-ber of animals	The number of dead animals					The average of mortality, %
			1 day	4 days	7 days	10 days	14 days	
1	500	6	0	0	0	0	0	0
2	1000	6	0	0	1	2	3	50
3	2000	6	0	2	3	3	3	50
4	3000	6	0	1	2	4	5	83.3
5	4000	6	0	2	3	5	6	100
6	5000	6	0	6	6	6	6	100

The median lethal dose was calculated based on the activity of the drug depending on the applied dose by probit-analysis. Using the tabular data the percentage of mortality in each group were transferred into probits (y) and then the weight coefficients (B) and place of doses (x) were determined carrying out further necessary calculations (Table 4, 5, 6) [14].

Table 4: The dose level value and mortality level to determine the LD_{50} of tetracycline hydrochloride in rats after oral administration according to the V.B. Prozorovsky method

Dose, mg/kg	Morta-lity, %	Place of doses, x	Probit, y	Weight coeffi-cient, B	xB	x^2B	YB	xyB
500	0	1	3.27	1.6	1.6	1.6	5.23	5.23
1000	50.0	2	5.00	5.0	10.0	20.0	25.00	50.00
2000	66.7	4	5.44	4.6	18.4	73.6	25.02	100.10
3000	83.3	6	5.95	3.5	21.0	126.0	20.83	124.95
4000	100	8	7.72	1.2	9.6	76.8	9.26	74.11
5000	100	10	7.72	1.2	12.0	120.0	9.26	92.64
Total				17.1	72.6	418.0	94.61	447.03

Table 5: The dose level value and mortality level to determine the LD_{50} of doxycycline hydrochloride in rats after oral administration according to the V.B. Prozorovsky method

Dose, mg/kg	Morta-lity, %	Place of doses, x	Probit, y	Weight coeffi-cient, B	xB	x^2B	YB	xyB
500	0	1	3.27	1.6	1.6	1.6	5.23	5.23
1000	50.0	2	5.00	5.0	10.0	20.0	25.00	50.00
2000	50.0	4	5.00	5.0	20.0	80.0	25.00	100.00
3000	66.7	6	5.44	4.6	27.6	165.6	25.02	150.14
4000	83.3	8	5.95	3.5	28.0	224.0	20.83	166.60
5000	100	10	7.72	1.2	12.0	120.0	9.26	92.64
Total				20.9	99.2	611.2	110.35	564.62

Table 6: The dose level value and mortality level to determine the LD_{50} of methacycline hydrochloride in rats after oral administration according to the V.B. Prozorovsky method

Dose, mg/kg	Morta-lity, %	Place of doses, x	Probit, y	Weight coeffi-cient, B	xB	x^2B	YB	xyB
500	0	1	3.27	1.6	1.6	1.6	5.23	5.23
1000	50.0	2	5.00	5.0	10.0	20.0	25.00	50.00
2000	50.0	4	5.00	5.0	20.0	80.0	25.00	100.00
3000	83.3	6	5.95	3.5	21.0	126.0	20.83	124.95
4000	100	8	7.72	1.2	9.6	76.8	9.26	74.11
5000	100	10	7.72	1.2	12.0	120.0	9.26	92.64
Total				17.5	74.2	424.4	109.79	446.93

For the further calculations to determine the indicators of LD_{16}, LD_{50} and LD_{84} it was used the equation that shows the relationship between the dose and the probit:

$$y=A_0+A_1x \quad (1)$$

The coefficients A_0 and A_1 were calculated as follows:

$$A_0 = \frac{(\Sigma B) - (\Sigma xB)A_1}{\Sigma B} \quad (2)$$

$$\frac{\Sigma xB}{\Sigma B} \times [\Sigma yB - (\Sigma xB)A_1] + (\Sigma x2B)A_1 = \Sigma xyB \quad (3)$$

After solving these equations the values of A_0 and A_1 were obtained which allowed us to plot the graph of probit analysis of the dependence "dose-mortality" (Fig. 1).

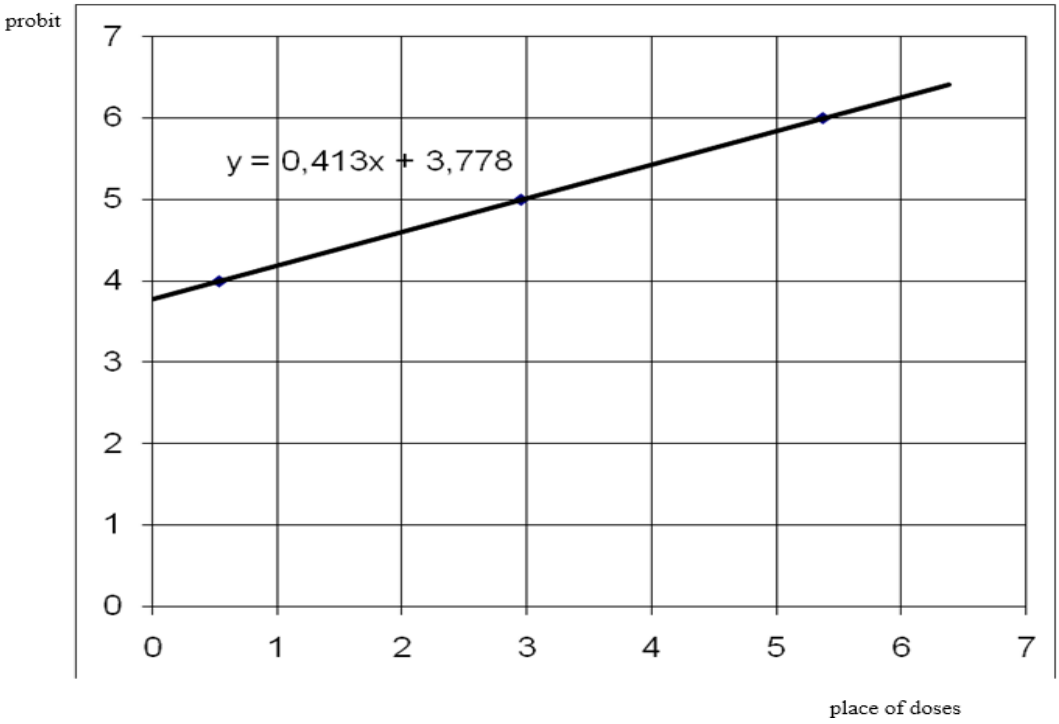

Fig 1: Graph of the probit analysis of dependence "dose-lethality" of the tetracycline

Considering that the value of y is 4 for LD_{16}, LD_{50} – 5 and LD_{84} – 6 it was calculated the value of the place of doses x for LD_{16}, LD_{50} and LD_{84} using the equation: $y = A_0 + A_1x$.

Then we found the standard error s of the LD_{50} values according to the formula:

$$s = \frac{ЛД_{84} - ЛД_{16}}{2\sqrt{n}}, \quad (4)$$

where,

n – the number of observations;

LD_{84} – the dose of the drug at which the level of mortality was 84 %;

LD_{16} – the dose of the drug at which the level of mortality was 16 %.

The results of calculations are presented in the form of Table 7.

Table 7: The results of calculations of the determination of the LD_{50} of the test samples after oral administration in rats according to the V.B. Prozorovsky method

A_1	A_0	The equation of dependence "probit dose"	Place of the dose LD_{50}	Place og the dose LD_{16}	Place of the dose LD_{84}	LD_{50}	s
tetracycline hydrochloride							
0.413	3.78	y = 0.413x + 3.78	2.956	0.536	5.377	1478	201.67
doxycycline hydrochloride							
0.291	3.89	y = 0.291x + 3.89	3.786	0.352	7.220	1893	286.2
methacycline hydrochloride							
0.418	3.63	y = 0.418x + 3.63	3.271	0.879	5.664	1635	199.4

4. Conclusion

1. The results of the conducted investigations and calculations lead to the conclusion that the LD_{50} level of tetracycline hydrochloride after single oral administration in rats is 1478.22±201.67 mg/kg; LD_{50} of doxycycline hydrochloride – 1893.03±286.2 mg/kg; LD_{50} of methacycline hydrochloride – 1635.73±199.36 mg/kg. Thus, doxycycline is significantly safer than tetracycline and methacycline.
2. The obtained results allow to refer the studied drugs to the IV class of toxicity – the low toxic substances according to the standard K. K. Sidorov classification.
3. It is recommended to carry out the further studies of the mechanisms of anti-inflammatory and possible anti-rheumatic effects of tetracyclines and their combinations with potential anti-inflammatory and chondroprotective substances.

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