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BIOEQUIVALENCE STUDIES OF MEDICINAL PRODUCTS OF TRANEXAMIC ACID

This article presents results of complex of research on biowaiver procedure to establish bioequivalence of medicinal products of tranexamic acid in form of tablets 500 mg. As a result of solubility and extent of permeation studies for active substance tranexamic acid is established I class according to BCS. It was established, that the objects of the study belong to highly soluble medicinal products in all recommended dissolution media. The similarity of dissolution profiles demonstrates the equivalence of test and reference medicinal products of tranexamic acid.

Key words: tranexamic acid; tablets; biopharmaceutical classification system; biowaiver; in vitro bioequivalence studies; solubility; dissolution profiles

INTRODUCTION

Today around the world, including in Ukraine, significant part of the pharmaceutical market consists of generic medicinal products (MP) of different pharmacotherapeutic groups and dosage forms, that are much more cheaper on price constituent than innovative products. According to information of various authors for different time periods [1, 7], number of registered generic MPs in Ukraine was within 55-90 %. But along with economic benefits for generic MPs can be outlined also negative aspects, such as side effects and inappropriate effectiveness compared to original drugs. The basis for the prevention of significant pharmacological differences in medical use of generic MPs and main condition for registration of generics according to international regulatory practice is proof of their bioequivalence to reference product, that should be original MP with proven efficacy, safety and quality.

One of methods to prove bioequivalence of generic and reference MPs for solid dosage forms of systemic exposure with immediate-release for oral use is simplified-procedure of registration for generic MP, that is called "biowaiver" and is based on biopharmaceutical classification system (BCS) of active substances (AS) and category of MP regarding dissolution in media of gastrointestinal tract (GIT). This approach allows to evaluate substitution of generic and reference drugs on the basis of their comparison in conditions *in vitro*. In this case it is necessary to consider quantitative and qualitative composition of products in terms of AS and excipients (EX), balance of risks in terms of health of population and individual patient, results of solubility and permeation of AS into

gastrointestinal tract and the results of comparative *in vitro* studies using dissolution profiles of MPs in media, that reflect physiological composition of different media of gastrointestinal tract [6, 11]. So, to be able to use "biowaiver" procedure for registration of solid dosage forms of systemic exposure for oral use with immediate-release in is necessary to conduct complex of studies.

MATERIALS AND METHODS

Analysis of recent studies and publications

Basic requirements for studies regarding "biowaiver" procedure are approved by the majority of world leading regulatory agencies on assessment of medicinal products (FDA, 2000, WHO, 2006, EMA, 2010) [3, 4, 8]. In Ukraine, this approach is governed by guidelines ST-N of MOHU 42-7.1: 2014 "Medicinal products. Bioequivalence Study" [11], that is harmonized with relevant regulations of the European Union [4]. Foreign and domestic literature provides many study results of different ASs and their MPs regarding *in vitro* study and evaluation of bioequivalence of generic and reference drugs. These studies are devoted to main directions of "biowaiver" procedure, such as determination of solubility and degree of permeation of AS and determination on the basis of AS class, according to biopharmaceutical classification system (BCS) [9], determination of MP category and comparison of dissolution profiles of generic and reference MPs [5, 9], methods development for study of dissolution kinetics of AS of their medicinal products [15].

RESULTS AND DISCUSSION

Highlighting of not resolved before parts of general problem

During conducting literature review on matter of studies of bioequivalence of generic and reference MPs

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of tranexamic acid, we have not found any publications highlighting relevant issues, to which this article is devoted, that makes it impossible to assess the results of such studies and to use them in our work.

Formulation of objectives of the article

The aim of this work was to conduct complex of *in vitro* bioequivalence studies of medicinal product Cyclocapron-Zdorovyie film-coated tablets 500 mg, with active substance tranexamic acid, to determine possibility to use biowaiver procedure for state registration of generic MP.

Presentation of the basic material of the study

The objects of the study were:

- tranexamic acid substance, manufacturer Changzhou Yinsheng Pharmaceutical Co Ltd., (China);
- medicinal product "Cyclocapron-Zdorovyie film-coated tablets 500 mg" of experimental-production batch manufactured by "Pharmaceutical company "Zdorovyie", Ltd., Ukraine;
- reference medicinal product "Cyclocapron® film-coated tablets 500 mg", manufactured by "MEDA Manufacturing GmbH", Germany.

All studies were performed according to standardized procedure, that was developed in accordance with recommendations [4, 11] and approved by the company.

Dissolution study was performed using dissolution apparatus PTWS610 manufactured by company "Pharma Test" (Germany) under following conditions: apparatus with basket, volume of dissolution medium – 500 ml, temperature of dissolution medium – $(37 \pm 1)^\circ\text{C}$, stirring speed – 100 rpm, sampling points: 10, 15, 20 and 30 min, buffer solutions with pH 1.2, pH 4.5 and pH 6.8. Buffer solutions were prepared according to requirements of SPU [10] using reagents of Pharmacopoeial grade, such as: hydrochloric acid, sodium acetate, glacial acetic acid, potassium dihydrogen phosphate, sodium hydroxide and sodium chloride. For each study were used 12 units of MP for statistical evaluation. In accordance with the requirements [4, 11] was conducted control of pH values of buffer solutions before and after dissolution of MP of tranexamic acid to confirm absence of impact of AS on pH of buffer solutions.

Assay of tranexamic acid in all studies was conducted according to developed and validated method by liquid chromatography. For this purpose was used reference standard of tranexamic acid USP RS, catalog number 1672745, batch number R021KO.

In the studies following analytical equipment was used: electronic laboratory balance AG 204, manufacturer "Mettler Toledo" (Switzerland); liquid chromatograph Agilent 1100 3D LC System, manufacturer "Agilent Technologies" (USA), column Hypersil ODS, Agilent, dimensions 250×4.6 mm with a particle size of 5.0 microns; pH meter Seven Easy pH equipped with electrodes "Mettler Toledo" (China); measuring glassware class A.

To solve task set at the beginning of the study was analyzed performance of general conditions regarding possibility of use of "biowaiver" procedure to objects being

Table 1

COMPARATIVE EVALUATION OF OBJECTS OF THE STUDY

Composition of MP			
study		reference	
trans-4 (aminomethyl) cyclo-hexanecarboxylic acid	500 mg	trans-4 (aminomethyl) cyclo-hexanecarboxylic acid	500 mg
Excipients			
Microcrystalline cellulose		Microcrystalline cellulose	
Povidone		Hypromellose	
Crospovidone		Povidone K25	
Magnesium stearate		Magnesium stearate (EP) (plant)	
Colloidal anhydrous silica dioxide		Finely dispersed silica dioxide	
Talc		Talc	
Dry mixture of Opadry white (contains titanium dioxide, hypromellose, triacetin)		Basic butyl methacrylate-copolymer	
		Titanium dioxide	
		Macrogol 8000	
		Vanillin	

studied. It was established, that studied and reference MPs had solid dosage forms (tablets) with systemic exposure, immediate-release for oral use, containing AS tranexamic acid in same amount of 500 mg and don't have narrow therapeutic index. EXs, that were used in composition of test MP were well-studied, met requirements [4, 11] and performed functions that were similar to that of EXs of reference MP, namely were qualitatively similar (Tab. 1). So, objects of the study for all criteria met the requirements for use of "biowaiver" procedure.

The next stage of the studies was to establish the BCS class for tranexamic acid. One of the criteria for classification AS in accordance with BCS [4, 11] is the solubility of the highest single dose in a volume ≤ 250 ml of each of three buffer solutions pH 1.2, pH 4.5 and pH 6.8 at $(37 \pm 1)^\circ\text{C}$. This volume is calculated as the ratio of the highest single dose to the equilibrium solubility of AS (HSD/S_{av} , ml).

According to world leading pharmacopoeias [2, 12], tranexamic acid is trans-4 (aminomethyl) cyclo-hexanecarboxylic acid, that belongs to freely soluble in water substances. Solubility and acid-base properties of AS are determined by its chemical structure (Fig. 1), that has groups of a dual nature: acidic part in form of a carboxyl group and main part in the form of amino group. It describes tranexamic acid as highly polar substance that is responsible for good solubility in water and evidences of its presence in solution as zwitter-ions depending on pH of medium. Based on these considerations it can be suggested, that this AS is soluble in recommended dissolution media within pH range 1.2-6.8.

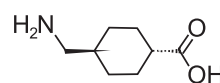


Fig. 1. Structural formula of tranexamic acid

Table 2

RELEASE RATE OF TRANEXAMIC ACID FROM MPS DEPENDING ON PH OF THE MEDIA

Release rate	pH	Reference MP				Test MP			
		Sampling points, min							
		10	15	20	30	10	15	20	30
Average value, in %	1.2	65.46	88.79	98.52	98.87	64.07	87.34	98.05	98.80
RSD, in %		4.34	2.24	2.13	2.09	2.93	2.05	2.07	2.05
Average value, in %	4.5	62.86	87.20	96.83	100.47	62.63	86.70	93.57	96.92
RSD, in %		2.31	2.19	2.10	2.13	2.29	2.11	2.08	2.02
Average value, in %	6.8	60.34	86.92	100.48	102.05	59.04	86.74	97.21	98.49
RSD, in %		2.98	2.05	2.09	2.07	3.45	2.30	2.07	2.10

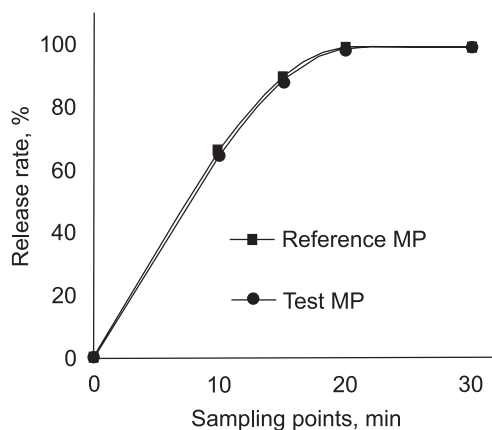


Fig. 2. Dissolution profiles of reference and test MPs in dissolution medium with pH 1.2

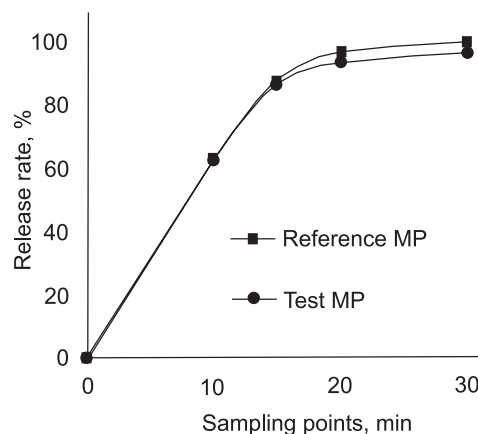


Fig. 3. Dissolution profiles of reference and test MPs in dissolution medium with pH 4.5

In order to establish BCS class and to confirm this assumption was carried out complex of studies on solubility of tranexamic acid according to the requirements of regulatory documents [11], that included study of profile of pH-dependent equilibrium solubility of tranexamic acid and calculation of volume, in which highest single dose dissolves in studied media. According to results of conducted studies [13] it was established, that tranexamic acid refers to substances with high solubility in all studied buffer solutions with pH 1.2; 4.5; 6.8.

Extent of permeation of AS tranexamic acid was established in SI "Institute of Pharmacology and Toxicology of NAMS of Ukraine" using method of measurement of permeation rate through monolayer of cell culture Caco-2. Average permeation rate for tranexamic acid was $(1.24 \pm 0.11) \times 10^{-5}$ cm/s, that corresponds to the criterion of "high extend of permeation".

Thus, based on obtained results of conducted studies for the substance (solubility parameters and extend of permeation) it was found that tranexamic acid belongs to BCS class I, meaning that it shows high solubility and extend of permeation.

Next stage of complex studies is devoted to the study of release kinetics of tranexamic acid from test and reference MPs and comparison of their *in vitro* dissolution profiles. Average values of the AS release rate and relative standard deviation (RSD), obtained in the dissolution

study of generic and reference products of tranexamic acid are given in Tab. 2. Dissolution profiles of the objects of the study at pH 1.2, pH 4.5 and pH 6.8 are shown in Fig. 2-4.

According to the results in Tab. 2 it is shown, that release of tranexamic acid from tablets of generic and reference products in all 3 recommended dissolution media having pH 1.2, pH 4.5 and pH 6.8 after 15 min is more than 85 % of labeled amount of active substance, indicating that the objects of the study belong to very quickly soluble MPs in all recommended dissolution media.

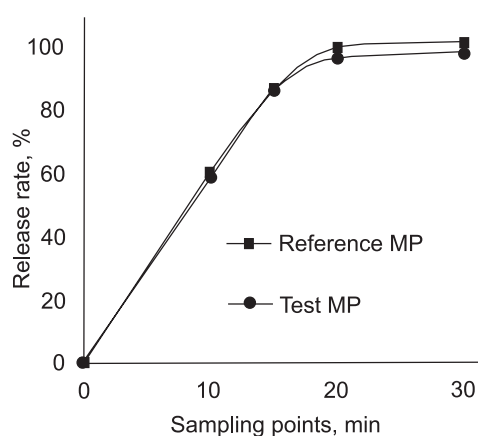


Fig. 4. Dissolution profiles of reference and test MPs in dissolution medium with pH 6.8

On the basis of established dissolution profiles of test and reference MPs and reliability of the results, as indicated by relative standard deviation (RSD) for generic and reference MPs (Tab. 2), similarity of the studied tablets of tranexamic acid can be accepted without calculation of similarity factor.

CONCLUSIONS

1. Was conducted complex of studies to determine bioequivalence between test and reference MPs of tranexamic acid, that included analysis of fulfillment of general conditions regarding possibility to use "bio-waiver" procedure, study of solubility and permeation rate of tranexamic acid for determination of BCS class, determination and comparison of dissolution profiles of test and reference drug of tranexamic acid.
2. According to results of the study was established following:
 - affiliation of tranexamic acid to class I according to BCS;
 - affiliation of test and reference MPs of tranexamic acid to very quickly soluble MPs in all recommended dissolution media.
 - similarity of dissolution profiles, that evidences on equivalence of generic and reference MPs of tranexamic acid in form of tablets.
3. Based on the obtained results it was concluded on registration of generic MP "Cyclocapron-Zdorovyie film-coated tablets 500 mg", manufactured by "FC Zdorovyie", Ukraine, on biowaiver procedure without conducting *in vivo* bioequivalence studies.

REFERENCES

1. Brands and generics: Myths and Reality // Pharmacy. – 2014. – Vol. 967 (46). – Available at: <http://www.apteka.ua/article/313948>
2. European Pharmacopoeia. – 8-th ed. – Strasbourg: Council of Europe, 2013. – 3639 p.
3. Guidance for industry: Waiver of *in vivo* bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a Biopharmaceutics Classification System [Electronic resource]. – U.S. Department of Health and Human Services, Food and Drug Administration (HHS-FDA), Centre for Drug Evaluation and Research (CDER). – 2000. – Available at: <http://www.fda.gov/cder/guidance/index.htm>
4. Guidance on the Investigation of Bioequivalence. European Medicines Agency (EMA). Committee for Medicinal Products of Human Use (CHMP), 2010.
5. Nazarova O. S. Study of dissolution kinetics of medicinal products with Ornidazole / O. S. Nazarova, L. O. Bobrytska // Management, economics and quality assurance in pharmacy. – 2012. – № 4. – P. 13-18.
6. Order of MOH of Ukraine number 460 from 23.07.2015 "On Amendments to the Procedure for the examination of registration materials for medicinal products submitted for state registration (re-registration) as well as expertise of amending registration materials during the registration certificate validity period and improvement of Procedure of verification of materials added to amendment to the state registration of certain medicinal products regarding their circulation. – Available at: <http://zakon.rada.gov.ua/laws/show/z1210-15>
7. Podpruzhnikov Yu. V. Original drugs and generics: pro and contra // Health of Ukraine INFORMEDIA. – 27.03.2015. – Available at: <http://health-ua.com/articles/2582>
8. Proposal to waive *in vivo* bioequivalence requirements for WHO Model List of Essential Medicines immediate-release, solid oral dosage forms. Technical Report Series, № 937, 40-th Report, Annex 8 of WHO Expert Committee on Specifications for Pharmaceutical Preparations. – World Health Organization (WHO), 2006.
9. Setkina S. B. Influence of biopharmaceutical factors on *in vitro* equivalence of generic drugs based on Clopidogrel [Vliyanie biofarmaceuticheskikh faktorov na ekvivalentnost' *in vitro* vosproizvedennykh lekarstvennykh sredstv na osnove klopidogetrelja] / S. B. Setkina, O. M. Hishova, L. V. Zubkevich, A. V. Kaplina // Bulletin of Pharmacy (Belarus). – 2014. – № 1 (63). – P. 33-38.
10. State Pharmacopoeia of Ukraine: in 3 Vol. / State Enterprise "Ukrainian Scientific Pharmacopoeial Center on Quality of Medicinal Products". – 2-nd ed. – Kharkiv: State Enterprise "Ukrainian Scientific Pharmacopoeial Center on Quality of Medicinal Products", 2015. – Vol. 1. – P. 1128.
11. ST-H MOHU 42-7.1: 2014. Medicinal products. Bioequivalence study / SE "State Expert Center of MoH of Ukraine", MOH of Ukraine. – Enter. 2014.06.30. – K.: Ministry of Health of Ukraine, 2014. – P. 59.
12. The United States Pharmacopoeia / The National Formulary. USP 29.
13. Visych S. Yu. Solubility study of tranexamic acid in establishing the class according to the BCS / S. Yu. Visych, A. V. Dorovskoy, E. G. Fetisova, L. N. Andryukova // Pharmaceutical education, science and practice: Development Horizons: collection of scientific articles All Russ. scien.-pract. conf. with int. particip. (Kursk, October 20-21, 2016). – Kursk, 2016. – P. 324-331.
14. Yarushok T. A. Determination of equilibrium biopharmaceutical solubility of Flutamide substance [Opredelenie ravnovesnoj biofarmaceuticheskoy rastvorimosti substancii flutamida] / T. A. Yarushok, I. E. Shokhin, A. Y. Savchenko, Y. S. Kartieva // Development and Registration of Medicinal Products. – 2012. – № 1. – P. 30-36.
15. Yarushok T. A. Development of methods for determination of the equilibrium biopharmaceutical solubility of slightly soluble substance on example of Aripiprazole substance [Razrabotka metodiki opredeleniya ravnovesnoj biofarmaceuticheskoy rastvorimosti malorastvorimogo veshhestva na primere substancii aripiprazola] / T. A. Yarushok, I. E. Shokhin, A. Yu. Savchenko // Development and Registration of Medicinal Products. – 2012. – № 1. – P. 14-18.

УДК 615.07:615.453.6:001.891**С. Ю. Висыч, А. В. Доровской, Л. Н. Андрюкова, Е. Г. Фетисова****ИССЛЕДОВАНИЕ БИОЭКВИВАЛЕНТНОСТИ ЛЕКАРСТВЕННЫХ СРЕДСТВ ТРАНЕКСАМОВОЙ КИСЛОТЫ**

Приведены результаты комплекса исследований по процедуре «Биовейвер» по установлению биоэквивалентности лекарственных средств транексамовой кислоты в форме таблеток по 500 мг. По результатам изучения растворимости и степени проникновения установлен I класс по БСК для действующего вещества транексамовая кислота. Определено, что объекты исследования относятся к очень быстрорастворимым лекарственным средствам во всех рекомендованных средах растворения. Подобность профилей растворения свидетельствует об эквивалентности испытуемых и референтных лекарственных средств транексамовой кислоты.

Ключевые слова: транексамовая кислота; таблетки; биофармацевтическая система классификации; биовейвер; исследования биоэквивалентности *in vitro*; растворимость; профили растворения

УДК 615.07:615.453.6:001.891**С. Ю. Вісич, О. В. Доровський, Л. М. Андрюкова, О. Г. Фетісова****ДОСЛІДЖЕННЯ БІОЕКВІВАЛЕНТНОСТІ ЛІКАРСЬКИХ ЗАСОБІВ ТРАНЕКСАМОВОЇ КИСЛОТИ**

Наведені результати комплексу досліджень за процедурою «Біовейвер» щодо встановлення біоеквівалентності лікарських засобів транексамової кислоти у формі таблеток по 500 мг. За результатами вивчення розчинності і ступеня проникнення встановлено I клас за БСК для діючої речовини транексамова кислота. Визначено, що об'єкти дослідження належать до дуже швидко розчинних лікарських засобів у всіх рекомендованих середовищах розчинення. Подібність профілів розчинення свідчить про еквівалентність досліджуваних та референтних лікарських засобів транексамової кислоти.

Ключові слова: транексамова кислота; таблетки; біофармацевтична система класифікації; біовейвер; дослідження біоеквівалентності *in vitro*; розчинність; профілі розчинення

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