

BIOLOGICAL EVALUATION OF MEDICAL DEVICES IN THE FORM OF SUPPOSITORIES FOR RECTAL AND VAGINAL USE

O.V. Dmytrenko^{1*}, M.A. Arkhipova², D.B. Starosyla², S.L. Rybalko², M. Gevorkyan³, A.Yu. Galkin⁴

¹UA "PRO-PHARMA" LLC, Kyiv, Ukraine

²SI "L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases of the NAMS of Ukraine", Kyiv, Ukraine

³Gelingchem GmbH&Co., KG, Hilden, Germany

⁴Igor Sikorsky Kyiv Polytechnic Institute, Kyiv, Ukraine

*Corresponding author: regulatory@pro-pharma.com.ua

Received 26 November 2021; Accepted 22 December 2021

Background. Programs of preclinical safety studies of the health care products depend on the regulatory status of the investigated products. The classification of such products, in particular suppositories for rectal and vaginal use, is a critical step of developing tactics for their biological evaluation. Adaptation of biological evaluation methods for the medical devices based on the combination of biologically active substances, as well as evaluation of the results of such studies is urgent task of biomedicine.

Objective. To substantiate the regulatory status and to carry out a biological evaluation of medical devices in the form of vaginal suppositories based on octenidine dihydrochloride ("Prodexyn") and in the form of rectal suppositories based on *Saw palmetto*, *Levisticum officinale* and *Calendula officinalis* extracts ("Pravenor").

Methods. Biological evaluation was conducted according to the requirements of ISO 10993 standards using *in vitro* and *in vivo* biological test systems (cytotoxicity in cell culture and the MTT test, sensitizing and irritating effect in guinea pigs).

Results. The cytotoxicity (CC₅₀) of the medical device "Prodexyn" extract in Vero cell culture was 8.35 µg/ml calculated as octenidine dihydrochloride and 416.65 µg/ml calculated as dexpanthenol. "Pravenor" medical device was found to be non-toxic in Vero cell culture. According to the results of MMT assay CC₅₀ for octenidine dihydrochloride was 1.67 µg/ml, and 83.33 µg/ml – for dexpanthenol. CC₅₀ indicators calculated for the different active ingredients of the medical device "Pravenor" were the following: 50 mg/ml for the dwarf palm berries extract (*Saw palmetto*), 16.67 mg/ml for the lovage roots extract (*Levisticum officinale*), and 16.67 mg/ml for the calendula flowers extract (*Calendula officinalis*). No sensitizing or skin irritating effects were observed in guinea pigs.

Conclusions. Biological evaluation of medical devices in the form of rectal suppositories "Pravenor" and vaginal suppositories "Prodexyn" performed using *in vitro* and *in vivo* biological systems. It was demonstrated an acceptable level of safety of the products. The MTT test was 5 times more sensitive than the Vero cell culture method in determination of cytotoxicity.

Keywords: medical devices; rectal suppositories; vaginal suppositories; antibacterial suppositories; cytotoxicity; sensitizing effects; irritating effects.

Introduction

Today it is impossible to imagine the creation of a modern innovative health care system without the development and implementation of advanced medical technologies in all medical spheres – both preventive and clinical. The wide use of medical devices (MD) allows efficient solving of the issues in medical diagnostics, prevention, and therapy. High-tech and innovative MD became an indispensable mechanism in providing replicability, mass scale and foreseeability of clinical and diagnostic results [1].

Specific features of drugs as a type of products in healthcare system are expressed mostly by their extreme variability of design, origins, methods of manufacture, and use [2, 3]. Such circumstances

considerably restrict the development of general (universal) rules for MD standardization, in particular, from the point of view of their quality and safety. Unlike medicinal products with detailed directives on quality and safety (Pharmacopoeias, the Guidelines of the International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, and national regulatory authorities), similar detailed international and national normative documents are absent for medical devices. The mentioned characteristic of MD is also reflected in the systems of MD access for various national markets, which mostly envisage the procedures of compliance assessment, which involve wide range of authorized bodies [4, 5]. The regulatory bodies of developed countries, industry associations, and

international institutions make significant contributions to the international MD standardization system. The Guidelines for Biological Assessment of Medical Devices are developed by International Organization for Standardization, and currently are implemented in the majority of countries, including Ukraine. The series of ISO 10993 standards (Biological evaluation of medical devices) allowed unification of the requirements for various types of MD depending on their route of administration, type of contact with organism, and the contact's duration. The assessment concept, included in standard ISO 10993-1:2018, forms the basis for the development of the medical devices' assessment programs; however, it is not binding, namely due to the extremely wide range of MD peculiarities. MD diversity is supported by the fact that for many MDs the form of manufacture is very similar to medicinal products (for example, eye drops, vaginal and rectal suppositories, skin solution, patches, etc.) In such cases, the primary problem is to meticulously classify the product as a certain medical product's class [6]. The results of class's potential risk determination and MD biological assessment are important incoming information for MD assessment and risk management, which are an integral part of general quality assurance system of any medical devices manufacturer [7–9].

The aim of this article is to carry out a scientific and medical justification of the regulatory status (classification) of the medical devices in the form of vaginal and rectal suppositories, as well as their biological evaluation according to ISO 10993 standards.

Materials and methods

Medical devices. We used the following samples for testing: medical device "Pravenor" (rectal suppositories, further referred to as Pravenor), and medical device "Prodexyn" (vaginal suppositories, further referred to as Prodexyn). Pravenor has the following composition: extract of dwarf palm berries (*Saw palmetto*) – 150 mg, extract of lovage roots (*Levisticum officinale*) – 50 mg, extract of calendula flowers (*Calendula officinalis*) – 50 mg, excipients: hard fat. Prodexyn has the following composition: octenidine dihydrochloride – 2 mg, dexpanthenol – 100 mg, excipients: Macrogol 4000, Macrogol 400.

To dilute the suppositories we used centrifuge tubes and mix of ether (3 ml) and physiological solution (3 ml) in ratio 1:1 (v/v). The suppository was kept in a stoppered tube for 1 h till complete dissolution. At the next stage, the lower phase was

collected into another tube, which was left open for 30 min for ether evaporation. The obtained extract (pH 6.8–7.2) was used for the device's biological evaluation. The calculated concentration of active components in the obtained extract was the following: for medical device Prodexyn – 0.67 mg/ml of octenidine dihydrochloride and 33.33 mg/ml of dexpanthenol; for medical device Pravenor – 50 mg/ml of dwarf palm berries extract (*Saw palmetto*), 16.67 mg/ml of lovage root extract (*Levisticum officinale*), and 16.67 mg/ml of calendula flowers extract (*Calendula officinalis*).

Cell culture and its culturing. Vero cell culture (passaged green monkey kidney cell culture), obtained from cell bank of D.I. Ivanovsky Institute of Virology of RAMS (Moscow, Russian Federation) and maintained at the research facility "L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases of the NAMS of Ukraine" (Kyiv, Ukraine) was used.

The cells were maintained in culture via the common method, using complex medium, consisting of 90% RPMI-1640 medium (Sigma, USA) with the addition of 10% inactivated fetal bovine serum (FBS) (Sigma, USA), and antibiotic Kanamycin (50 IU/ml).

The cells were cultured in 50–100 ml glass or plastic vials (Nunc, Denmark) at 37 °C in the 5% CO₂ atmosphere. Every 3–4 days live cells were counted by staining the cells with trypan blue and seeded in the initial cell concentration per 1 ml.

The passaged cells were extracted from vial surface with Gibco® Versene Solution (0.2 g EDTA per litre of phosphate-buffered saline) (Thermo Fisher Scientific, USA), centrifuged and added into 1 ml of medium for precipitation, pipetted and counted in Goryaev's hemocytometer. The cells were seeded into well plates for cell culture (Sigma, CIIIA) with estimated number of 200,000 cells in 1 ml of the medium.

Determination of the medical devices' cytotoxic concentration (CC₅₀). To determine CC₅₀ of the medical devices, we used at least ten rows of wells in plates with cell culture for each product's dilution. The plates with cell culture were incubated at 37 °C in the 5% CO₂ atmosphere for 5 days. Every day the test and control cultures were monitored for presence or absence of cytopathogenic effect (CPE). CPE degree was determined by changes in cells morphology (rounding, wrinkling of cells, detachment from well surface) and degenerative changes via the following system: "–" – complete absence of cell degeneration; "+" – NMT 25% of cell monolayer is affected; "++" – NMT 50% of

cell monolayer is affected; "+++" – NMT 75% of cell monolayer is affected; "++++" – complete degeneration of cell monolayer. CC_{50} of medical device was defined as the maximal rate, which did not cause degeneration of 50% of cells. Control was defined as a cell monolayer without addition of medical devices' extracts.

MTT assay. The method is based on functioning of the dehydrogenase system in intact cells' mitochondria that convert 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into formazan. The reaction product may be determined quantitatively by spectrophotometer. The MTT conversion into formazan decreases after death of cells, affected by toxic substances. The cells in concentration $5 \times 10^5 \text{ ml}^{-1}$ were cultured in 96-well plates in RPMI-1640 medium with addition of 10% FBS, which contained test substances in various concentrations. Controls were cells that not treated with the studied product. Each concentration was tested in 3 replicates. The plates with cells were incubated at 37 °C in the 5% CO_2 atmosphere for 48 h. MTT substrate (Sigma, USA) was dissolved in sterile phosphate-buffered saline solution (PBS) (0.2 M NaCl, 0.2 M K_2HPO_4 , 0.2 M $\text{Na}_2\text{H}_2\text{PO}_4$, 0.2 M KCl), pH 7.2, at room temperature in a concentration of 5 mg/ml. The filtered MTT solution in a volume of 25 μl per 100 μl of cell suspension was put into wells and incubated for 3 h at 37 °C in the 5% CO_2 atmosphere. After incubation the plates with cells were centrifuged for cell precipitation at 1500 rpm for 10 min and rejected the supernatant. 100 μl of 96% ethanol were added to precipitation; it further dissolved crystalline formazin. After 10 min of thorough shaking at 37 °C the optical density of solutions was measured by spectrophotometer at wavelength 540 nm. The percentage of inhibition of cell viability when subjected to the test products was determined by the measuring of optical density of the test samples in comparison with the control cells (CC), which was taken as 100%. For convenience of assessment of the obtained results of cytotoxicity CC_{50} on the basis of MTT assay on the corresponding plot indicated the value corresponding to half of the CC (CC 50%).

Animals. In the study we used random-bred laboratory Guinea pigs, aged 3–4 months, weighing 300–400 g. The animals care was complied with the requirements of international standard ISO 10993-2:2006 "Biological evaluation of medical devices – Part 2: Animal welfare requirements".

Sensitization and skin irritation effects study. The hair was cut from the zones of 2×3 cm on the animals' backs; melted suppositories were applied

directly on the skin, fixated with gauze bandage, and left for 4 h. Further monitoring for erythema and oedema was performed 12, 24, 48 and 72 h after the bandage's removal. Photo fixation of results was carried out. Each test group included 6 animals.

Bioethics norms. All work with animals, described in this article, was performed according to the Law of Ukraine "On protection of animals from abuse", European Convention for the Protection of Vertebrate Animals, as well as the Guide for the Care and Use of Laboratory Animals (8th edition). Upon completion of the study the animals were sacrificed via a humane method, aimed at minimal physical and psychological suffering.

Results

Classification of medical devices and justification of biological testing program

Functional characteristics of the medical device Pravenor. To understand the product's functional characteristics, the product's content should be analyzed along with roles of its ingredients in the functioning of male urogenital system should be assessed. Berries of dwarf palm (*Saw palmetto*) contain the following biologically active substances that are necessary for normal functioning of prostate: phytosteroles (hormone precursors, synthesized in a human body), fatty acids (palmitic, linoleic, linolenic acids), lipase (promotes the digestion of fats, fatty acids, fat-soluble vitamins A, D, E) [10]. Lovage roots (*Levisticum officinale*) contain many essential oils (terpineol, cineole, acetic, isovaleric and benzoic acids), which promote urination [11]. Calendula flowers (*Calendula officinalis*) contain essential oils, carotenoids and flavonoids that support regeneration processes of body tissues [12]. Therefore, phytocomponents of this medical device represent a complex of biologically active substances that are necessary for and promote normal functioning of urogenital system in males, mainly – prostate. It is worth mentioning that the above phytocomponents are also included in several medicinal products and dietary supplements (in oral forms). The majority of medicinal products, containing extracts or tinctures of dwarf palm, lovage and calendula, are the so-called traditional medicinal products, which have been developed and marketed in Ukraine for more than 20 years. At present such new oral products are placed on the Ukrainian market as dietary supplements, and this reflects their functional principle of action better, i.e., provision of nutrients for better functioning of

the body's system. The preventive effect of such products is implemented in such a way along with their help in mitigation of respective diseases' course. Therefore, the effect of medical device Pravenor (rectal suppositories) on human body cannot be described as pharmacological or immunological, as well as the one modifying metabolism. Thus, this product may be considered as a medical device in the terms of Technical Regulations for Medical Devices, approved by the Decree No. 753 by the Cabinet of Ministers of Ukraine, dated October, 2, 2013, and Regulation (EU) 2017/745 of the European Parliament and of the Council as of April, 5, 2017 on medical devices.

The medical device Pravenor is classified as a short-term device because its continuous use is envisaged for less than 30 days. Considering rectal route of administration and the device partial absorption, the Rule 21 (Regulation (EU) 2017/745 of the European Parliament and of the Council of April, 5, 2017 on medical devices) may be applied to this medical device, namely: the devices that are composed of substances or substance combinations that are intended to be introduced into in human body via a body orifice or skin application, and that are absorbed or locally dispersed in a human body, and classified as class III, and which achieve their intended purpose in the stomach or lower gastrointestinal tract and which or whose products of metabolism are systemically absorbed by the human body.

Functional characteristics of medical device Prodexyn. To understand the product's functional characteristics, the product's content should be analyzed along with its ingredients's roles in the functioning of female urogenital system. Octenidine dihydrochloride is an antiseptic agent for mucus membranes, skin and wounds. It reacts with microbial cell's wall components and microbial membranes, causing disorders in cell functions. Its antimicrobial mechanism of action also involves the increase of cell membrane's permeability for potassium ions. Octenidine dihydrochloride is not absorbed by the gastrointestinal tract or skin and mucus membranes. Its penetration through placenta may be excluded. Octenidine dihydrochloride is not absorbed by vaginal mucous membrane or by wounds [13, 14]. Dexpanthenol, which is also a component of the product, is converted in tissues into a pantothenic acid, which is an integral part of coenzyme A, and plays important role in epithelium's normal functioning and acceleration of skin and mucous membranes' regeneration. Dexpanthenol is rapidly absorbed by skin and is immediately

converted into pantothenic acid, which is widely distributed in organism tissues, mostly in the form of coenzyme A. Pantothenic acid is eliminated predominantly in unchanged form with urine and to a lesser extent – with faeces [15, 16].

Octenidine dihydrochloride in various pharmaceutical forms and methods of use is included also in medicinal and disinfecting products. Dexpanthenol is widely used both by the pharmaceutical and cosmetic industries due to its positive effect on skin and mucous membranes' regeneration. Thus, the medical device Prodexyn contains the antiseptic agent octenidine dihydrochloride, which does not have systemic effects on human organism, and which medical effect lies in inhibition of the development of extraneous microbiota in the vagina and prevention of sexually transmitted diseases. Another device's component, dexpanthenol, plays auxiliary role, aiming at prevention of potential adverse effects of octenidine dihydrochloride, namely – its irritating action. Therefore, the effect of medical device Prodexyn (vaginal suppositories) on human body cannot be described as pharmacological or immunological, as well as the one modifying metabolism. Thus, this product may be considered as medical device in the terms of Technical Regulations for Medical Devices, approved by the Decree No. 753 by the Cabinet of Ministers of Ukraine, dated October, 2, 2013, and Regulation (EU) 2017/745 of the European Parliament, and of the Council as of April, 5, 2017 on medical devices.

The medical device Prodexyn is classified as a short-term device because its continuous use is envisaged for less than 30 days. Considering vaginal route of administration, absence of absorption and systemic effect of octenidine dihydrochloride, the Rule 21 (Regulation (EU) 2017/745 of the European Parliament and of the Council as of April, 5, 2017 on medical devices) may be applied to this medical device, namely: the devices that are composed of substances or of combinations of substances, that are intended to be introduced into the human body via a body orifice or applied to skin, and that are absorbed by or locally dispersed in the human body, and classified as class IIb in all other cases. Taking into account the vaginal route of administration (invasive device) and its intended purpose (prevention of sexually transmitted diseases), the Rule 15 (Regulation (EU) 2017/745 of the European Parliament and of the Council as of April, 5, 2017 on medical devices) may be also applied to this medical device, namely: all devices, used for contraception or prevention of sexually transmitted diseases are classified as class IIb, un-

less they are implantable or long-term invasive devices, and in this case, they are classified as class III.

Program of biological testing. Considering the recommendations of standard ISO 10993-1:2018 "Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process" (device group: surface device; type of contact: mucosal membrane; contact duration: 24 h – 30 days), as well as well-established safety profile of the substances in the device content, it was considered sufficient to include three following parameters into the biological testing program: cytotoxicity, sensitizing, and skin-irritating effects.

Biological evaluation of medical devices

Determination of the device cytotoxic concentration (CC_{50}). Vero cells culture (Fig. 1) was used to determine the CC_{50} of the products. At least ten rows of wells in plates with cell culture were used in the experiments for each device dilution in medium. The test and control cultures were monitored every day to determine the presence or absence of CPE. The study results are summarized in the Table and Fig. 1.

The maximum level of dilution of the extract of the product Prodexyn, which led to the CPE of Vero cell culture, was 1:80, which corresponds to the concentration of octenidine dihydrochloride – 8.35 $\mu\text{g/ml}$, for dexpanthenol – 416.65 $\mu\text{g/ml}$. At the same time, Pravenor was found to be completely non-toxic when tested in Vero cells culture.

According to MTT assay results (Fig. 2), it was shown that cytotoxic effect CC_{50} was registered for extract dilution 1:400 for medical device Prodexyn (line intersection "CC 50%" and "No. 1 Prodexyn" on Fig. 2). This dilution corresponds to the following values of CC_{50} : for octenidine dihydrochloride – 1.67 $\mu\text{g/ml}$, for dexpanthenol – 83.33 $\mu\text{g/ml}$.

According to MTT assay results, medical device Pravenor did not demonstrate a toxic effect,

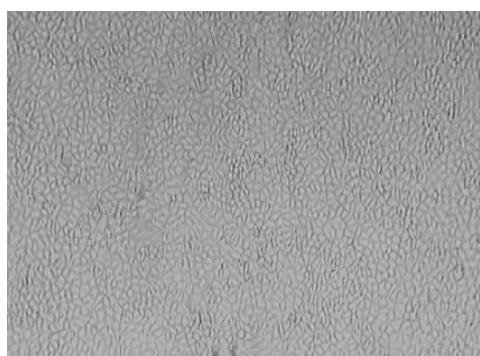


Figure 1: Vero cell culture, used in the experiment (magnification $\times 10$)

which was confirmed by the obtained ratio of optical density for test product versus optical density for control cell culture (see Fig. 2). Based on the obtained results, it can be concluded that CC_{50} for this product exceeds 50 mg/ml for dwarf palm berried extract (*Saw palmetto*), 16.67 mg/ml for lovage root extract (*Levisticum officinale*), and 16.67 mg/ml for calendula flowers extract (*Calendula officinalis*).

Studies of sensitization and skin irritation effects

Monitoring of experimental animals have shown that both devices do not cause any skin irritation and are safe to use. The observations were recorded in photographs (Fig. 3).

Discussion

Medical device regulatory status has crucial meaning for the strategy of a scientific research project. There is a considerable difference in studies of the safety and efficiency of medicines and medical devices. Some health care products may have similar or even equivalent forms (solutions for injection, skin solution, nasal sprays, vaginal and rectal suppositories, etc.), however, they may be classified into different regulatory groups. In addition to the aforementioned aspects of preclinical and clinical studies of medical products their regulatory status sometimes influences pharmaco-economic parameters of the respective projects [17]. In the terms of access of the developed medical products, in particular – medical devices to various segments of global pharmaceutical market the problem of unification of regulatory requirements in various states and supranational structures is very important (for example, EU) for managing quality, safety, and efficiency of such products [18, 19]. Manufacturers of medical devices and other medical products constantly discuss the issues of interconnection between scientific-technical issues in this sphere in terms of regulatory requirements [20–23].

Table: The study results of the device cytotoxic concentration in Vero cell culture

Dilution	Device	
	Prodexyn	Pravenor
1:10	10/10*	0/10
1:20	10/10	0/10
1:40	10/10	0/10
1:80	5/10	0/10
1:160	0/10	0/10
1:320	0/10	0/10
1:640	0/10	0/10
1:1280	0/10	0/10
CC_{50}	1:80	Non-toxic

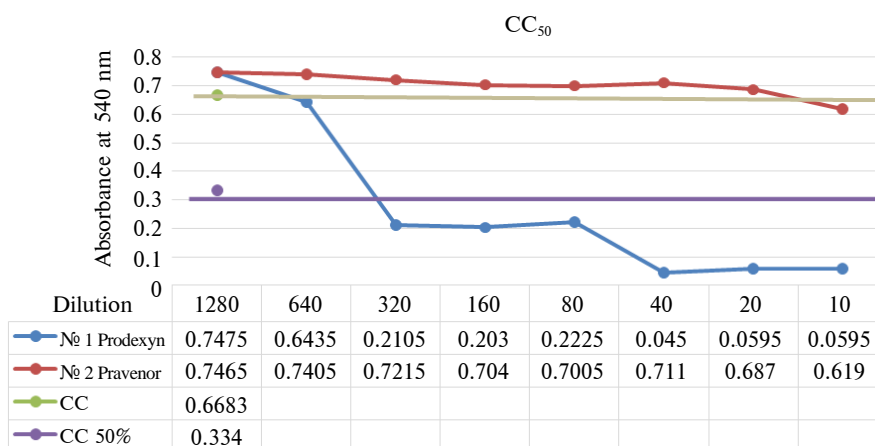


Figure 2: Results of cytotoxicity assessment by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

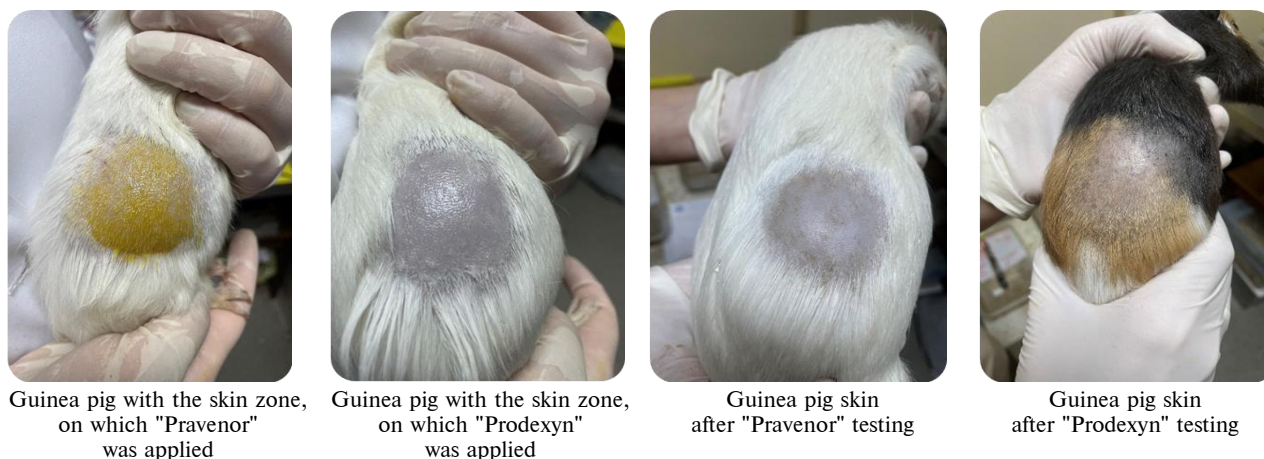


Figure 3: Results of determining of the devices' sensitizing and skin-irritating effects

There are many products for vaginal and rectal use, which are sold on the developed countries markets, and which are classified as medical devices. Usually intra-vaginal medical devices in traditional pharmaceutical forms (suppositories, tablets, capsules, etc.) are developed for correction (restoration) of vaginal microbiota [24–26]. Other indications for such devices include promotion of fertilization [27], delivery of anti-HIV drugs [28], etc. Medical devices in the form of rectal suppositories, creams, and gels are used for treatment and prevention of proctological and urological diseases [29, 30]. In view of this, our medical-scientific justification of classification of the studied products as medical devices is harmonized with regulatory bodies practice (Conformity Assessment Body) of the European Union countries. It is worth noting that at present the current Ukrainian Technical Regulations on Medical Devices and Medical Device Regulations, adopted in the European Union [23, 31], do not match in the part of establishing risk

classes for several medical devices, including invasive devices. However, such regulatory disparities between Ukrainian and EU markets do not affect significantly the determination of biological evaluation strategy for such devices.

The obtained data on cytotoxicity of vaginal suppositories (Prodexyn), containing octenidine dihydrochloride and dexpanthenol, correspond to the data of other scientists. Thus, in the study [32] cytotoxic effect of octenidine solution was assessed on fibroblast cell cultures and MCF7 cells (epithelial-like cell line, obtained from invasive adenocarcinoma of human mammary ducts). Octenidine solution in the concentration of 0.5 mg/l significantly inhibited cell growth in 24 h, however, with the concentration of 0.012 mg/l the cytotoxic effect was not observed. Such results allowed authors to recommend octenidine solution for cutaneous use in the treatment of purulent wounds. Similar results were obtained while studying several substances with antiseptic properties as the candidates

for gingivitis treatment: octenidine showed the lowest cytotoxic effect on fibroblasts and epithelial cells [33] among the studied five agents. The modern toxicological studies of dexpanthenol-containing combinations are aimed at determination of the decrease of toxic (in particular, cytotoxic) effects after introduction of dexpanthenol into combination. This approach is justified by dexpanthenol's biological properties, namely – its transformation into pantothenic acid in a body. Pantothenic acid is an integral part of coenzyme A and participates in acetylation, carbohydrate and fat metabolism, synthesis of acetylcholine, corticosteroids, porphyrins; it stimulates regeneration of skin and mucous membranes, normalizes cell metabolism, accelerates mitosis and increases strength of collagen fibres [15, 34]. Most often dexpanthenol is included in drugs for nasal use to improve their safety profile. Thus, the study [35] showed that dexpanthenol significantly decreased the toxic effect of xylometazoline, used on amniotic epithelial cells (human amniotic cell line). Another study [36] proved that dexpanthenol (5%) decreased the toxic effects of xylometazoline and benzalkonium chloride used on amniotic epithelial cell growth. These data suggest the adequacy of the chosen combination in medical device Prodexyn (octenidine + dexpanthenol) both from the point of efficacy and safety profile. The obtained data are absolutely new on the proposed combination and its method of administration.

Phytocombination of medicinal herbal extracts in the medical device Pravenor is original, therefore the study of the cytotoxic effect of this product on cell culture was relevant both from scientific and regulatory point of view. The available literature data [37–42] on cytotoxic effects of extracts from *Saw palmetto*, *Levisticum officinale*, *Calendula officinalis* of biologically active substances, obtained from these medicinal herbs, are, on one hand, addressed by their individual studies (as monopreparations), and, on the other hand, are focused on assessment of anti-cancer activities. Therefore, the obtained data on the absence of cytotoxic effect of the medical device Pravenor are an important prerequisite for safe use of this product as a prostate protector.

Conclusions

Scientific-medical and regulatory justification of classifying Prodexyn and Pravenor as invasive medical devices for prolonged use was performed. The cytotoxic, sensitization and skin irritation stu-

dies are sufficient for biological evaluation of such medical devices.

The maximum cytotoxic concentration of the medical device's Prodexyn active ingredients, which led to the CEP of Vero cell culture, was for octenidine dihydrochloride – 8.35 µg/ml and dexpanthenol – 416.65 µg/ml. According to the MTT assay results for the medical device Prodexyn the cytotoxic effect CC_{50} was recorded for dilution that corresponds to the values of CC_{50} : octenidine dihydrochloride – 1.67 µg/ml and dexpanthenol – 83.33 µg/ml.

The medical device Pravenor was completely non-toxic in the study in Vero cell culture. Similar results were obtained in the MTT assay: it was established that the CC_{50} for this product exceeds 50 mg/ml for the extract of dwarf palm berries (*Saw palmetto*), 16.67 mg/ml for lovage root extract (*Levisticum officinale*), and 16.67 mg/ml for calendula flowers extract (*Calendula officinalis*).

The method of cytotoxicity evaluation, which is based on the assessment of the condition of dehydrogenase system of mitochondrial cells (MTT assay), was 5 times more sensitive if compared to the approach of detecting cytopathic effects on Vero cell culture. Thus, the MTT assay can be considered as a more informative method for the medical devices' cytotoxicity evaluation.

Both medical devices did not demonstrate sensitizing and skin-irritating effects after application on the skin.

The obtained data on safety profile of medical devices Prodexyn and Pravenor allow recommending the products for use in the claimed medical field.

Funding

This work was funded by "UA "PRO-PHARMA" LLC (Ukraine), whose support is gratefully acknowledged.

Disclosure of Interest

Oleksandra Dmytrenko reports being employee of "UA "PRO-PHARMA" LLC; the author declare that she has no competing interests. Alexander Galkin is the Editor-in-Chief of *Innovative Biosystems and Bioengineering* and was not involved in the editorial evaluation or decision to accept this article for publication. The remaining authors have no conflicts of interest to declare.

References

- [1] Tretiakova A.M. The role and place of medical technologies in modern healthcare systems. *Med Technol Assess Choice*. 2011;4:48-52.
- [2] Peter L, Hajek L, Maresova P, Augustynek M, Penhaker M. Medical devices: regulation, risk classification, and open innovation. *J Open Innov Technol Market Complex*. 2020;6(2):42. DOI: 10.3390/joitmc6020042
- [3] Green J. Medical device legislation for custom-made devices after the UK has left the EU: answers to ten important questions. *Br Dent J*. 2021 Oct;231(8):513-521. DOI: 10.1038/s41415-021-3530-x
- [4] Onkar B, Krishan P, Singh G. Regulatory requirements for medical devices: an insight. *Appl Clin Res Clin Trials Regul Affairs*. 2017;4(1). DOI: 10.2174/2213476X03666160804153513
- [5] Parvizi N, Woods K. Regulation of medicines and medical devices: contrasts and similarities. *Clin Med (Lond)*. 2014 Feb;14(1):6-12. DOI: 10.7861/clinmedicine.14-1-6
- [6] Godlee F. Why aren't medical devices regulated like drugs? *BMJ*. 2018;363:k5032. DOI: 10.1136/bmj.k5032
- [7] Kramer DB, Tan YT, Sato C, Kesselheim AS. Ensuring medical device effectiveness and safety: a cross-national comparison of approaches to regulation. *Food Drug Law J*. 2014;69(1):1-i.
- [8] Miclăuş T, Valla V, Koukoura A, Nielsen AA, Dahlerup B, Tsianos GI, et al. Impact of design on medical device safety. *Ther Innov Regul Sci*. 2020 Jul;54(4):839-49. DOI: 10.1007/s43441-019-00022-4
- [9] Palojoki S, Saranto K, Lehtonen L. Reporting medical device safety incidents to regulatory authorities: An analysis and classification of technology-induced errors. *Health Informatics J*. 2019 Sep;25(3):731-40. DOI: 10.1177/1460458217720400
- [10] Geavlete P, Multescu R, Geavlete B. *Serenoa repens* extract in the treatment of benign prostatic hyperplasia. *Ther Adv Urol*. 2011;3(4):193-8. DOI: 10.1177/1756287211418725
- [11] Spréa RM, Fernandes Â, Finimundy TC, Pereira C, Alves MJ, Calhelha RC, et al. Lovage (*Levisticum officinale* W.D.J. Koch) roots: a source of bioactive compounds towards a circular economy. *Resources*. 2020;9(7):81. DOI: 10.3390/resources9070081
- [12] Ashwlayan VD, Kumar A, Verma M, Garg VK, Gupta SK. Therapeutic potential of *Calendula officinalis*. *Pharm Pharmacol Int J*. 2018;6(2):149-55. DOI: 10.15406/ppij.2018.06.00171
- [13] Stahl J, Braun M, Siebert J, Kietzmann M. The effect of a combination of 0.1% octenidine dihydrochloride and 2% 2-phenoxyethanol (octenisept) on wound healing in pigs in vivo and its in vitro percutaneous permeation through intact and barrier disrupted porcine skin. *Int Wound J*. 2010 Feb;7(1):62-9. DOI: 10.1111/j.1742-481X.2009.00648.x
- [14] Stahl J, Braun M, Siebert J, Kietzmann M. The percutaneous permeation of a combination of 0.1% octenidine dihydrochloride and 2% 2-phenoxyethanol (octenisept®) through skin of different species in vitro. *BMC Vet Res*. 2011 Aug 11;7(1):44. DOI: 10.1186/1746-6148-7-44
- [15] Proksch E, de Bony R, Trapp S, Boudon S. Topical use of dexpanthenol: a 70th anniversary article. *J Dermatolog Treat*. 2017 Dec;28(8):766-773. DOI: 10.1080/09546634.2017.1325310
- [16] Gorski J, Proksch E, Baron JM, Schmid D, Zhang L. Dexpanthenol in wound healing after medical and cosmetic interventions (postprocedure wound healing). *Pharmaceuticals (Basel)*. 2020 Jun 29;13(7):138. DOI: 10.3390/ph13070138
- [17] Stern AD. Innovation under regulatory uncertainty: evidence from medical technology. *J Public Econ*. 2017 Jan;145:181-200. DOI: 10.1016/j.jpubeco.2016.11.010
- [18] Kramer DB, Xu S, Kesselheim AS. How does medical device regulation perform in the united states and the european union? A systematic review. *PLoS Med*. 2012;9(7):e1001276. DOI: 10.1371/journal.pmed.1001276
- [19] Van Norman GA. Drugs and devices: comparison of European and U.S. Approval processes. *JACC Basic Transl Sci*. 2016 Aug 29;1(5):399-412. DOI: 10.1016/j.jacbts.2016.06.003
- [20] Van Norman GA. Drugs, devices, and the FDA: Part 1: An overview of approval processes for drugs. *JACC Basic Transl Sci*. 2016 Apr 25;1(3):170-9. DOI: 10.1016/j.jacbts.2016.03.002
- [21] Van Norman GA. Drugs, devices, and the FDA: Part 2: An overview of approval processes: FDA approval of medical devices. *JACC Basic Transl Sci*. 2016 Jun 27;1(4):277-87. DOI: 10.1016/j.jacbts.2016.03.009
- [22] Tian J, Song X, Wang Y, Cheng M, Lu S, Xu W, et al. Regulatory perspectives of combination products. *Bioact Mater*. 2022;10:492-503. DOI: 10.1016/j.bioactmat.2021.09.002
- [23] Contardi M. Changes in the medical device's regulatory framework and its impact on the medical device's industry: from the medical device directives to the medical device regulations. *Erasmus Law Rev*. 2019;12(2):166-77. DOI: 10.5553/ELR.000139
- [24] Di Piero F, Di Paola G, Risso P. Role of a medical device for intra-vaginal use in improving the quality of the colposcopic examination and the anatomical/pathological reading of the cytological test and biopsy. *Acta Biomed*. 2014 Aug 20;85(2):121-6.
- [25] Plummer EL, Bradshaw CS, Doyle M, Fairley CK, Murray GL, Bateson D, et al. Lactic acid-containing products for bacterial vaginosis and their impact on the vaginal microbiota: A systematic review. *PLoS One*. 2021 Feb 11;16(2):e0246953. DOI: 10.1371/journal.pone.0246953

- [26] Papa R, Troncone M, Altruda F, Rullo V, Saponati G. Clinical evaluation of the efficacy and safety of a medical vaginal device containing rigenase® for the treatment of vaginosis: a randomized study. *J Clin Gynecol Obstetr.* 2017;6(1):6-11. DOI: 10.14740/jcgo424w
- [27] Montanino Oliva M, Poverini R, Lisi R, Carra MC, Lisi F. Treating woman with myo-inositol vaginal suppositories improves partner's sperm motility and fertility. *Int J Endocrinol.* 2016;2016:7621942. DOI: 10.1155/2016/7621942
- [28] Ham AS, Buckheit RW Jr. Designing and developing suppository formulations for anti-HIV drug delivery. *Ther Deliv.* 2017;8(9):805-17. DOI: 10.4155/tde-2017-0056
- [29] Sibona M, Destefanis P, Agnello M, Lillaz B, Giuliano M, Cai T, et al. The association of Boswellia resin extract and propolis derived polyphenols can improve quality of life in patients affected by prostatitis-like symptoms. *Arch Ital Urol Androl.* 2020 Jan 14;91(4):251-5. DOI: 10.4081/aiua.2019.4.251
- [30] Montrone S, Gonnelli A, Cantarella M, Sainato A. Use of Proktis-M suppositories in patients undergoing neoadjuvant radio-chemotherapy for adenocarcinoma of the rectum. *Minerva Gastroenterol Dietol.* 2015;61(4):293-7.
- [31] Maresova P, Rezny L, Peter L, Hajek L and Lefley F. Do regulatory changes seriously affect the medical devices industry? Evidence from the Czech Republic. *Front Public Health.* 2021 Apr;9:666453. DOI: 10.3389/fpubh.2021.666453
- [32] Jenull S, Hojdar K, Laggner H, Velimirov B, Zemann N, Huettinger M. Cell growth and migration under octenidine-antiseptic treatment. *J Wound Care.* 2015;24(6):280-8. DOI: 10.12968/jowc.2015.24.6.280
- [33] Schmidt J, Zyba V, Jung K, Rinke S, Haak R, Mausberg RF, et al. Cytotoxic effects of octenidine mouth rinse on human fibroblasts and epithelial cells - an in vitro study. *Drug Chem Toxicol.* 2016;39(3):322-30. DOI: 10.3109/01480545.2015.1121274
- [34] Proksch E, Berardesca E, Misery L, Engblom J, Bouwstra J. Dry skin management: practical approach in light of latest research on skin structure and function. *J Dermatolog Treat.* 2020 Nov;31(7):716-22. DOI: 10.1080/09546634.2019.1607024
- [35] Klöcker N, Rudolph P, Verse T. Evaluation of protective and therapeutic effects of dexpanthenol on nasal decongestants and preservatives: results of cytotoxic studies in vitro. *Am J Rhinol.* 2004;18(5):315-20.
- [36] Klücker N, Verse T, Rudolph P. The protective effect of dexpanthenol in nasal sprays. First results of cytotoxic and ciliary-toxic studies in vitro. *Laryngorhinootologie.* 2003;82(3):177-82. DOI: 10.1055/s-2003-38406
- [37] Iguchi K, Okumura N, Usui S, Sajiki H, Hirota K, Hirano K. Myristoleic acid, a cytotoxic component in the extract from *Serenoa repens*, induces apoptosis and necrosis in human prostatic LNCaP cells. *Prostate.* 2001;47(1):59-65. DOI: 10.1002/pros.1047
- [38] Shimada H, Tyler VE, McLaughlin JL. Biologically active acylglycerides from the berries of saw-palmetto (*Serenoa repens*). *J Nat Prod.* 1997;60(4):417-8. DOI: 10.1021/np960552o
- [39] Sertel S, Eichhorn T, Plinkert PK, Efferth T. Chemical composition and antiproliferative activity of essential oil from the leaves of a medicinal herb, *Levisticum officinale*, against UMSSC1 head and neck squamous carcinoma cells. *Anticancer Res.* 2011;31(1):185-91.
- [40] Sargazi S, Saravani R, Galavi H, Mollashahee-Kohkan F. Effect of *Levisticum officinale* hydroalcoholic extract on DU-145 and PC-3 prostate cancer cell lines, gene cell tissue. 2017; 4(4):e66094. DOI: 10.5812/gct.66094
- [41] Cruceriu D, Diaconeasa Z, Socaci S, Socaciu C, Rakosy-Tican E, Balacescu O. Biochemical profile, selective cytotoxicity and molecular effects of *Calendula officinalis* extracts on breast cancer cell lines. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca.* 2020;48(1):24-39. DOI: 10.15835/nbha48111778
- [42] Jiménez-Medina E, Garcia-Lora A, Paco L, Algarra I, Collado A, Garrido F. A new extract of the plant *calendula officinalis* produces a dual in vitro effect: cytotoxic anti-tumor activity and lymphocyte activation. *BMC Cancer.* 2006 May 5;6(1):119. DOI: 10.1186/1471-2407-6-119

О.В. Дмитренко¹, М.А. Архипова², Д.Б. Старосила², С.Л. Рибалко², М. Геворкян³, О.Ю. Галкін⁴

¹ТОВ "УА "ПРО-ФАРМА", Київ, Україна

²ДУ "Інститут епідеміології та інфекційних хвороб ім. Л.В. Громашевського НАМН України", Київ, Україна

³Gelingchem GmbH&Co., KG, Гільден, Німеччина

⁴КПІ ім. Ігоря Сікорського, Київ, Україна

БІОЛОГІЧНЕ ОЦІНЮВАННЯ МЕДИЧНИХ ВИРОБІВ У ФОРМІ СУПОЗИТОРІЇВ ДЛЯ РЕКТАЛЬНОГО ТА ВАГІНАЛЬНОГО ЗАСТОСУВАННЯ

Проблематика. Програми доклінічного вивчення безпеки продуктів у системі охорони здоров'я залежать від регуляторного статусу досліджуваних продуктів. Класифікування таких продуктів, зокрема супозиторіїв для ректального та вагінального застосування, є критичним етапом для розробки тактики їх біологічного оцінювання. Актуальними для біомедицини залишаються питання адаптації методів біологічного оцінювання медичних виробів на основі комбінації біологічно активних речовин, а також оцінки результатів такого оцінювання.

Мета. Здійснити обґрунтування регуляторного статусу та біологічне оцінювання медичних виробів у формі вагінальних супозиторіїв на основі октенідину дигідрохлориду ("Продексин") та у формі ректальних супозиторіїв на основі екстрактів рослин *Saw palmetto*, *Levisticum officinale* і *Calendula officinalis* ("Правенор").

Методика реалізації. Біологічне оцінювання проводили згідно з вимогами стандартів серії ISO 10993 за допомогою біологічних тест-систем *in vitro* та *in vivo* (цитотоксичність у культурі клітин та у МТТ-тесті, сенсibiliзуюча та подразнювальна дія на мурчаків).

Результати. Цитотоксичність (CC₅₀) екстракту медичного виробу "Продексин" на культурі клітин Vero становила 8,35 мкг/мл у перерахунку на октенідину дигідрохлорид та 416,65 мкг/мл у перерахунку на декспантенол. Медичний виріб "Правенор" виявився нетоксичним на культурі клітин Vero. За результатами МТТ-тесту CC₅₀ для октенідину дигідрохлориду становила 1,67 мкг/мл, декспантенолу – 83,33 мкг/мл. CC₅₀ у перерахунку на активні інгредієнти медичного виробу "Правенор" становила 50 мг/мл для екстракту ягід карликової пальми (*Saw palmetto*), 16,67 мг/мл для екстракту коренів любистку лікарського (*Levisticum officinale*), 16,67 мг/мл для екстракту квіток нагідок лікарських (*Calendula officinalis*). Для медичних виробів не було виявлено сенсibiliзуючої та шкіроподразнювальної дії на мурчаків.

Висновки. Біологічне оцінювання медичних виробів у формі ректальних супозиторіїв "Правенор" і вагінальних супозиторіїв "Продексин", проведене із використанням біологічних систем *in vitro* та *in vivo*, засвідчило прийнятний рівень безпечності цієї продукції. При визначенні цитотоксичності МТТ-тест виявився у 5 разів чутливішим порівняно з методом на основі культури клітин Vero.

Ключові слова: медичні вироби; ректальні супозиторії; вагінальні супозиторії; антибактеріальні супозиторії; цитотоксичність; сенсibiliзуюча дія; шкіроподразнювальна дія.

А.В. Дмитренко¹, М.А. Архипова², Д.Б. Старосила², С.Л. Рыбалко², М. Геворкян³, А.Ю. Галкин⁴

¹ООО "УА "ПРО-ФАРМА", Киев, Украина

²ГУ "Институт эпидемиологии и инфекционных болезней им. Л.В. Громашевского НАМН Украины", Киев, Украина

³Gelingchem GmbH&Co., KG, Гильден, Германия

⁴КПИ им. Игоя Сикроского, Киев, Украина

БИОЛОГИЧЕСКОЕ ОЦЕНИВАНИЕ МЕДИЦИНСКИХ ИЗДЕЛИЙ В ФОРМЕ СУППОЗИТОРИЕВ ДЛЯ РЕКТАЛЬНОГО И ВАГИНАЛЬНОГО ИСПОЛЬЗОВАНИЯ

Проблематика. Программы доклинического исследования безопасности продуктов в системе здравоохранения зависят от регуляторного статуса исследуемых продуктов. Классифицирование таких продуктов, в частности суппозитории для ректального и вагинального применения, является критическим этапом разработки тактики их биологического оценивания. Актуальными остаются вопросы адаптации методов биологического оценивания медицинских изделий на основе комбинации биологически активных веществ, а также оценки результатов такого оценивания.

Цель. Произвести обоснование регуляторного статуса и биологическое оценивание медицинских изделий в форме вагинальных суппозитории на основе октенидина дигидрохлорида ("Продексин"), а также в форме ректальных суппозитории на основе растительных экстрактов *Saw palmetto*, *Levisticum officinale* и *Calendula officinalis* ("Правенор").

Методика реализации. Биологическое оценивание проводилось согласно требованиям стандартов серии ISO 10993 с помощью биологических тест-систем *in vitro* и *in vivo* (цитотоксичность в культуре клеток и в МТТ-тесте, сенсibiliзирующее и раздражающее действие на морских свинок).

Результаты. Цитотоксичность (CC₅₀) экстракта медицинского изделия "Продексин" на культуре клеток Vero составила 8,35 мкг/мл в пересчете на октенидина дигидрохлорид и 416,65 мкг/мл в пересчете на декспантенол. Медицинское изделие "Правенор" оказалось нетоксичным на культуре клеток Vero. По результатам МТТ-теста CC₅₀ для октенидина дигидрохлорида составила 1,67 мкг/мл, декспантенола – 83,33 мкг/мл. CC₅₀ в пересчете на активные ингредиенты медицинского изделия "Правенор" составила 50 мг/мл для экстракта ягод карликовой пальмы (*Saw palmetto*), 16,67 мг/мл для экстракта корней любистока лекарственного (*Levisticum officinale*), 16,6 мг/мл для экстракта цветков календулы лекарственной (*Calendula officinalis*). Для медицинских изделий не было обнаружено сенсibiliзирующего и кожераздражающего действия на морских свинок.

Выводы. Биологическое оценивание медицинских изделий в форме ректальных суппозитории "Правенор" и вагинальных суппозитории "Продексин", проведенное с использованием биологических тест-систем *in vitro* и *in vivo*, удостоверяло приемлемый уровень безопасности данной продукции. При определении цитотоксичности МТТ-тест оказался в 5 раз более чувствительным по сравнению с методом на основе культуры клеток Vero.

Ключевые слова: медицинские изделия; ректальные суппозитории; вагинальные суппозитории; антибактериальные суппозитории; цитотоксичность; сенсibiliзирующее действие; кожераздражающее действие.