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Full Length Original Research Paper

Study of Kinetic rates of Medical product with Antimicrobic action by *In Vitro* method

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Abstract: Kinetic parameters of medical product under study with contents of metronidazole and quinosol designed for medical treatment of patients of gynaecological profile are uncovered in the given article. Degree of active pharmaceutical ingredients release from dosage forms is determined in *in vitro* experiments.
It was found out that kinetic processes of release run according to first order equation, and velocity of release process decreases when increasing the half-value period, which attests process slowing-down. According to determined periods of metronidazole and quinosol half-value from processed gel and cream, the degree of prolongation of developed products action was established. According to the results of kinetic research it was proved that the highest prolongation in the series of developed medical products was shown by cream and the lowest – by gel.

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Introduction

Preservation of nation reproductive health depends upon attitude of every person to their family planning [1]. At the moment rational choice of spermicidal agents is the basis for family planning [2], [24].

Induced termination of pregnancy is one of the most considerable cause of female health disorder, and complications caused by it lead to almost every tenth loss in death rate connected with pregnancy and childbirth [3], [4, [5].

An open secret is that the use of modern contraceptive methods has considerable advantages over abortions. According to data of the Centre of Health Statistics of MOH of Ukraine [6], 185 million of pregnancies arise annually in the world, 75 million of which are unintended and 45 million end up with abortion [7]. Annually about 70 thousand of women on our planet die for reasons connected with abortions [6]. 10% of women are diagnosed with inflammatory processes of genital organs within one year after abortion and menstrual disorders and subfertility within 4-5 years. So this problem acquires not only of social, but also medical importance.

According to expert opinion, the main reason of abortions is ignorance and reluctance of women to use modern methods of contraception, hormonal tablets in particular [8], [9]. At the moment the most widespread method of contraception is peroral hormonal contraception, though it has a number of contraindications. At the same time,

sexually transmitted infections are wide spread, they can't be protected from by peroral hormonal contraception means. The key moment in this issue is insufficient awareness about wide choice and possibility of individual selection of the effective contraceptive for every woman. At the same time it is possible to consider not only phenotype and age peculiarities, but also choose easy to administrate formulation with certain administration schedule [10], [11, [12].

During last years significant success was achieved in medicine and pharmaceutics as for improvement and supplementation of contraceptives range. They developed the schemes, which enable to take into account peculiarities of every woman, in particular: state of health, existence of bad habits, conditions of work, individual needs, fertility phase, age, etc. In connection with this, urgent issue is the use of local contraception (LC), which has such advantages as safety, absence of contraindications and also protective action against sexually transmitted infections (STIs). That's why it's urgent to make LC means, which combine spermicidal and antibacterial action and can not only to avoid unintended pregnancy, but also be means of STIs prophylaxis. In this aspect we've carried out marketing analysis [13], [14], [16]of medical products (MP) of G-group (medications that influence genitourinary system and sex hormones) according to Anatomical Therapeutic Chemical Classification System (ATC) as of December 2010. It is shown that in the middle of G-group of MP for female disorders (group G01), medications of group G01 AA02 (Natamycin) comprise 6%; G01 AA10 (Clindamycin) – 8%; G01 AA05 (Chloramphenicol) – 2%, G01 AA05 (Combination medications) - 6%; G01 AF01 (Metronidazole) – 12%; G01 AF02 (Clotrimazole) – 14%; G01 AF04 (Miconazole) – 5%; G01 AX (Combination medications) - 2%, etc.

In group of G01 MP of different commercial names the quantity of metronidazole (G01 AF01) made up 8 names; clotrimazole (G01 AF02) – 10; miconazole (G01 AF04) – 3; sertaconazole 9 G01 AF19) – 2 medications, etc. The most frequently used medications (TOP-medications) were found out and united into separate groups according to active factor, particularly: G01 AF01 – metronidazole, G01 AF02 – clotrimazole.

According to dosage forms, medications of group G01 are divided into solid dosage forms (SDF) – 26%, of which 23% are pills and 3% are capsules, soft MP – 64%, of which 15% are creams, 41% are suppositories, 3% are gels and 5% are ointments. And only 10% are comprised by aerosols, coats, etc.

Medical products of group G02BB, which are included into pharmacotherapeutic group "Contraceptives for local use", include 10 medications, moreover, four of them represent the series of different dosage forms under one commercial name "Pharmatex" (France, Lab. Innothera): cream, suppositories, pills, vaginal tampons.

The most frequently used substance in spermicidal medications is benzalkonium chloride (82%), more rarely – quinosol (9%) and nonoxynol 9 (9%).

According to forms of presentation on pharmaceutical market of Ukraine spermicidal MP are 50% represented in the form of suppositories, the rest 50% fall to other dosage forms – vaginal creams, vaginal tablets and capsules, solutions for external use.

Some other medications may be used as spermicides as well, but in this research they were not considered because of their other code in

ATC classification that is contraceptive effect is not essential for them.

On the assumption of urgency of the subjects, we developed composition and technology of combined SMP in the form of gel and cream, which contain metronidazole and quinosol as active pharmaceutical ingredients (API).

In obstetric-gynecologic practice soft medical products (SMP) – ointments, creams, gels, have wide use [17], [18], [19], [20]. They are used with the aim of inflammatory diseases treatment as well as with the aim of contraception. MP for local contraception (LC) are to have certain specific character – they are to have good penetrating power and meet medico biologic requirements: to have high adhesive property to vaginal tissues, to ensure accuracy of dosing and stability, not to show adverse reaction, not to irritate vagina mucosa, not to influence vaginal microflora, not to penetrate into blood, to keep physicochemical parameters during application time, to be comfortable on application, easy for transportation and storage.

Contraceptive SMP which have certain rheological properties, are easily distributed along mucosa, are mixed with natural vagina secretion, thus they simultaneously show lubricating effect, which may be necessary during coitus in case of vagina dryness. So, in the presence of other dosage forms (aerosols, coats, suppositories), which are used with the aim of contraception, creams, ointments and gels keep their actuality.

Considering all positive characteristics of creams and gels for vaginal use, we may draw a conclusion that making new contraceptives exactly in the form of gel and cream is actual as they increase supplying of population with accessible contraceptives and increase of awareness for carrying out of contraception method selection, which is a necessary step for preservation of nation reproductive health and its reproduction. That is why it is reasonable to develop optimal composition and technology of spermicidal medical products with antimicrobial property.

The aim of our research is the study of pharmacokinetic parameters of worked up SMP (gel, cream) by *in vitro* method for ascertainment of degree of metronidazole and quinosol release from dosage form.

Methods of research

Estimation of SMP kinetic parameters was conducted according to the method of dialysis through semipermeable membrane [21, 22, 23, 25]. Dialyzer consists of dialyzation chamber and inner cylinder, the bottom of which is semipermeable membrane – cellophane film (thickness of swollen film is 45 ±0,4 mcm, degree of swelling is 125 ±2,2, range of porosity is 6,25 g/ml).

Inner cylinder with sample of 5g of gel/crème was placed into camera with certain amount of treated water (100 ml) under the temperature 36 ± 1°C. After sampling (10 ml) periodically the amount of water in dialyzation chamber was carried to initial level (100 ml). Sampling was made in 30, 60, 180, 360, 720 and 1440 minutes.

Quantitation of API in dialyzate was carried out by method of high performance liquid chromatography (HPLC). The research was made on basis of the chair of clinical chemistry, forensic medical toxicology and pharmacy of Kharkiv Medical Academy of postgraduate education under the direction of Prof. G. N. Petyunin.

Velocity of release reaction of metronidazole and quinosol was determined by formula (1):

$$K_B = \frac{\lg C_{(1)} - \lg C_{(2)}}{t_2 - t_1}, \quad (1)$$

Where: K_B – velocity of release reaction of MS, sec^{-1};

$C_{(1)}$; $C_{(2)}$ –concentration of released substance for an hour t_1, t_2 і t_2, t_3

t_1, t_2 –sec.

Parameters of velocity of reaction constant in time were calculated by the formula (2):

$$k = \frac{2{,}303}{t} \lg \frac{C_0}{C}, \quad (2)$$

where: k - velocity of reaction constant;

t -sec;

C_0 – initial concentration of MS (%);

C – concentration (%)of released MS in time t.

Half-value period of medication was determined by formula (3):

$$t_{1/2} = \frac{0{,}693}{k}, \quad (3)$$

where: $t_{1/2}$ - half-value period, sec;

k –velocity constant.

Results and their consideration: Results of experimental study of gel kinetic parameters in logarithmic scale (lg %) are shown on fig. 1.

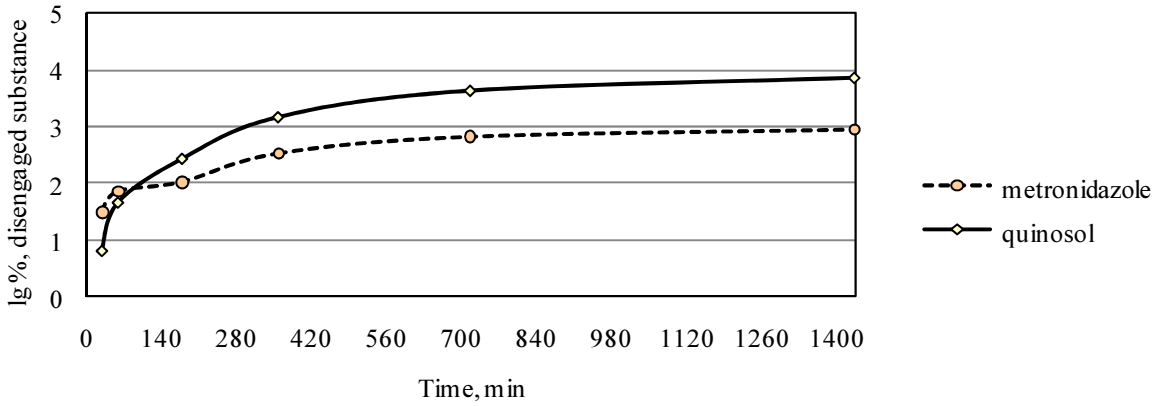

Fig.1: Kinetic dependence of metronidazole and quinosol release out of gel vs time.

By slope of lines on Fig.1 velocity of reaction of metronidazole and quinosol release was calculated, which is comes to determination of release velocity constant. Velocity of reaction of MS release was determined by formula (1)

For metronidazole velocity of reaction of release out of gel equals to:

$K_{B1} = 9{,}4 \cdot 10^{-5}$ sec^{-1};

$K_{B2} = 9{,}7 \cdot 10^{-6}$ sec^{-1};

$K_{B3} = 2{,}0 \cdot 10^{-5}$ sec^{-1};

$K_{B4} = 6{,}0 \cdot 10^{-6}$ sec^{-1};

$K_{B5} = 1{,}4 \cdot 10^{-6}$ sec^{-1}.

And for quinosolvelocity of reaction of release out of gel was:

$K_{B1} = 2{,}1 \cdot 10^{-4}$ sec^{-1};

$K_{B2} = 4{,}7 \cdot 10^{-5}$ sec^{-1};

$K_{B3} = 2{,}9 \cdot 10^{-5}$ sec^{-1};

$K_{B4} = 9{,}3 \cdot 10^{-6}$ sec^{-1};

$K_{B5} = 2{,}5 \cdot 10^{-6}$ sec^{-1}.

Parameters of reaction velocity constant in time was estimated by formula (2).

The second characteristic of substances release velocity is time, which is necessary for concentration of diffusing substance to halve from initial value – half-value period $t_{1/2}$. Medication half-value period was estimated by formula (3).

Investigated kinetic parameters, which describe API release processes out of gel, are shown in table 1.

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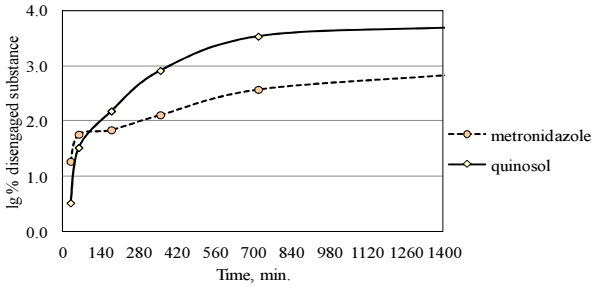
Table 1: Kinetic parameters of gel in experiments in vitro

Kinetic parameters	Metronidazole release, sec					
	1800	3600	10800	21600	43200	86400
k- constant of release process velocity, sec^{-1}	$2,6 \cdot 10^{-3}$	$1,6 \cdot 10^{-3}$	$5,8 \cdot 10^{-4}$	$3,4 \cdot 10^{-4}$	$1,9 \cdot 10^{-4}$	$9,7 \cdot 10^{-5}$
$t_{1/2}$ - half-value period, sec	266,53	433,12	1194,82	2038,23	3647,36	7144,33
	Quinosol release, sec					
k- constant of release process velocity, sec^{-1}	$4,5 \cdot 10^{-3}$	$2,9 \cdot 10^{-3}$	$1,3 \cdot 10^{-3}$	$6,6 \cdot 10^{-4}$	$3,5 \cdot 10^{-4}$	$1,83 \cdot 10^{-4}$
$t_{1/2}$ - half-value period, sec	154	238,96	533,07	1050	1980	3786,88

On the basis of calculations it can be said that constant of release velocity under the temperature of 36°C for metronidazole decreases from $2,6\ 10^{-3}$ sec^{-1} to $9,7 \cdot 10^{-5}$ sec^{-1}, and for quinosol from $4,5 \cdot 10^{-3}$ sec^{-1} to $1,83 \cdot 10^{-4}$ sec^{-1}. It is connected with type of dosage from, particularly with hydrophilicity of the basis, which actively diffuse into liquid. Metronidazole released more actively at the beginning, then the process slows down. For quinosol it's vice versa: first release runs slowly and then release accelerates. This moment is important from the point of view of pathogenesis of gynecological inflammatory diseases.

Estimation of cream kinetic parameters was carried out analogously to gel.



Metronidazole and quinosol release out of cream vs time in logarithmic scale (lg %) is shown on fig.2.

Fig. 2 Kinetic dependence of metronidazole and quinosol release out of cream vs time. Kinetic parameters of cream in *in vitro* experiments are shown in table 2.

Kinetic parameters of cream in experiments in vitro

Kinetic parameters	Metronidazole release, sec					
	1800	3600	10800	21600	43200	86400
Metronidazole						
k- constant of release process velocity, sec^{-1}	$2,3 \cdot 10^{-3}$	$1,510^{-3}$	$5,8 \cdot 10^{-4}$	$2,9 \cdot 10^{-4}$	$8,7 \cdot 10^{-5}$	$9,2 \cdot 10^{-5}$
$t_{1/2}$ - half-value period, sec	301,3	464	1194,8	2389,6	7965,5	7532,6
Quinosol						
k- constant of release process velocity, sec^{-1}	$3,810^{-3}$	$2,6 \cdot 10^{-3}$	$1,0 \cdot 10^{-3}$	$5,9 \cdot 10^{-4}$	$3,3 \cdot 10^{-4}$	$1,7 \cdot 10^{-4}$
$t_{1/2}$ - half-value period, sec	182,4	266,5	693	1174,6	2100	4076,5

On the basis of calculations it can be said that release velocity under the temperature of 310 K for metronidazole decreases from $2,3\ 10^{-3}$ sec^{-1} to $9,2 \cdot 10^{-5}$ sec^{-1}, and for quinosol from$3,8 \cdot 10^{-3}$ sec^{-1} to $1,7 \cdot 10^{-4}$ sec^{-1}.

Comparative analysis of kinetic values of metronidazole and quinosol of gel and cream showed that kinetic processes of metronidazole and quinosol release out of cream and gel run according to first order equation; release of MS out of gel and cream decreases with time;

metronidazole released more actively at the beginning, then the process slows down. For quinosol it's vice versa: first release runs slowly and then release accelerates. Velocity of release process decreases if the period of half-value is increased, which attests process slowing down.

Conclusion: The degree of prolongation reduction of developed medications was determined by established half-value periods of API of worked up SDF. Thus, according to the results of kinetic experiments, the highest prolongation in the series of developed MP is shown by cream and the lowest – by gel.

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