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Pharmacokinetic Study of the Pharmaceutical Composition Based on Diindolylmethane in Female Patients with Endometrial Hyperplasia without Atypia

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Abstract

We assessed the pharmacokinetics of the new pharmaceutical composition Cineton based on the active substance 3,3′-diindolylmethane (DIM), containing pluronic, on five female patients with endometrial hyperplasia without atypia. DIM concentration in plasma was determined by high-performance liquid chromatography with UV detection after a single oral administration of Cineton at a dose of 300 mg of DIM. The average value of the area under the pharmacokinetic curve (AUC) was 343.9 ± 112.9 ng·h/ml, the average maximum concentration (C_{max}) of DIM in the blood plasma was 121.5 ± 38.7 ng/ml, and the average time to reach C_{max} was 2.2 ± 0.45 h. It has been established that the DIM substance is determined in the blood plasma of patients during 10 h after a single administration of the drug. The calculated average retention time of the drug in the systemic circulation (MRT_{0inf}) was 4.4 ± 1.35 h. The half-life ($T_{1/2}$) was 1.9 ± 1.6 h. The obtained results have confirmed the high bioavailability of the drug Cineton.

Keywords

3,3'-Diindolylmethane (DIM); Clinical study; Pharmacokinetics; Bioavailability; Endometrial hyperplasia; Pluronic

Introduction

Endometrial cancer (EC) is one of the most common gynecological oncological diseases in developed countries (14.7 cases per 100,000 of the female population) occurring mainly in premenopausal and perimenopausal women [1]. In the structure of cancer incidence among women, EC ranks fifth in the world, amounting to about 320,000 cases per year, while the incidence and mortality from EC have been steadily growing [2]. In the treatment of early stages of EC, a surgical method is mainly applied, comprising hysterectomy or bilateral oophorectomy (surgical removal of both ovaries) in combination with pelvic lymphadenectomy (pelvic lymph node dissection) [3]. For female patients, who passed the surgical treatment, the prognosis for living is relatively favorable, but there is still the likelihood of recurrence and/or adverse side events [4]. To reduce the risk of the EC postoperative recurrence, the adjuvant chemotherapy and radiotherapy are used in various combinations, but the question about the need for adjuvant therapy for ER remains debatable [5].

It is known that there are several precancerous conditions predisposing and triggering carcinogenic processes in the endometrium. According to modern ideas, the endometrial hyperplasia (EH), in particular its complicated clinical and morphological forms, can be regarded as a precursor to EC [6]. In 1994, the WHO adopted a classification of EH, according to which simple and complex hyperplasia without cellular atypia as well as simple and complex hyperplasia with cellular atypia can be distinguished. It has been established that there is a correlation between the severity of atypical cytological changes in the endometrium and the risk of EC [7-9].

In recent decades, there has been increased interest in the development of new therapeutic approaches to the treatment of EH in order to prevent malignancy neoplastic processes in the endometrial tissue. To date, a considerable amount of experimental and clinical data have been accumulated on the molecular processes underlying the pathogenesis of hyperplastic processes in organs and tissues of the female reproductive system. Searching, development, and the use of pharmaceutical agents influencing the key molecular targets can allow an effective prevention of EC.

3,3'-Diindolylmethane (DIM) is a stable *in vivo* metabolite of indole-3-carbinol (I3C) [10]. DIM provides a powerful antitumor cell protection, due to a broad spectrum of its biological activities [11] and is a promising therapeutic agent with respect to EH and EC [12,13].

It has been proven that estrogens play a key role in stimulating abnormal proliferation of transformed cells of the endometrium – a typical hormone-dependent tissue [14]. The clinical studies have demonstrated the ability of I3C and DIM to restore the disturbed estrogen balance by stimulating the production of 2-hydroxyestrone (2-OHE1)—the antiproliferative metabolite of estrogen [15,16].

Numerous *in vivo* experiments have demonstrated the ability of DIM to inhibit the development of malignant tumors of the reproductive system organs and tissues [17], including the EC [18], due to its antiproliferative [19], proapoptotic [20], antiangiogenic [21], anti-inflammatory [22], and other basic antitumor effects. A newly discovered DNA-demethylating activity of DIM, which leads to the restoration of the tumor-suppressor genes activity, is of great

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significance [23]. The selective activity of DIM against special pool of cancer cells, the aggressive cancer stem cells, which, according to modern ideas, are the main cause of cancer recurrence and metastasis, was also shown recently [24].

It is known that the therapeutic activity of the drug substance is a function of its concentration in the blood of the patient. DIM typically exhibits low bioavailability in the target tissues due to its low solubility and its limited ability to cross the barrier membranes [25]. Therefore, the actual problem is the development of innovative pharmaceutical compositions with improved DIM biodistribution.

The drug Cineton, developed by us, includes highly bioavailable DIM, placed in pluronic nanocontainers (a block of copolymer of oxyethylene and oxypropylene), which can significantly increase the active substance bioavailability after its oral administration. Safety and efficacy of Cineton have been confirmed in previous preclinical studies [26].

The purpose of this study was to investigate the pharmacokinetic parameters of the new pharmaceutical composition Cineton based on DIM after its single administration in female patients with EH without atypia.

Materials and Methods

Test formulations

Capsules (DIM) (100 mg of DIM per capsule, MiraxBioPharma, Closed Joint Stock Company, Russia) contain Kolliphor 407 (pluronic), lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, and magnesium stearate as excipients.

Patients and treatment

Five female patients with histologically verified diagnosis of EH without atypia in the age range of 28–49 years were included in the study.

Trial subjects passed screening examination, including an anamnesis collection, physical examination, PayPal-endometrial biopsy with histological examination of the material, pelvic ultrasound, gynecological smear on flora, tests for infectious diseases, clinical and biochemical blood tests, and an electrocardiogram, 15 days prior to the beginning of active treatment. Premenopausal and menopausal women, women with uterine submucosal myoma, endometriosis III level, polycystic ovary syndrome, as well as the female patients having EC or malignant neoplasms of any other localization, arterial or venous thromboembolic disorders, lactose intolerance, diabetes, diseases of cardiovascular and nervous systems, as well as renal or hepatic impairment have been excluded from the study. Pregnancy and lactation, a positive test for Reaction Wasserman and/or HIV, alcohol abuse, drug, or medicinal dependence have also been taken as criteria for exclusion from the study. The use of other medications 30 days prior to the first dose of study drug was not allowed.

The study was reviewed and approved by the ethics board of the Russian Ministry of Health.

The pharmacokinetic study was conducted on the day of the first dose of the drug Cineton after the morning dose intake (300 mg DIM). At the same time, on the day of the study, and until the final patient's blood sampling, the drug Cineton was no longer taken. An intravenous catheter was introduced into the cubital vein of female patients. Prior to taking Cineton, the initial blood sample (5-7 ml) was collected. The female patients received the study drug Cineton at a dose of 300 mg DIM (three capsules of 100 mg DIM) on an empty stomach. The female patients swallowed the medication without chewing and washed it down with 200 ml of boiled water at room temperature in the presence of a physician-researcher. The first meal was permitted in 4 h after the drug administration. The repeated blood sampling was performed in plastic tubes (containing 2-3 drops of heparin) in 1, 2, 3, 4, 5, 6, 7, 8, 12, and 24 h after drug administration. The blood samplings were carried out with the help of a cubital catheter, which was removed from a vein within 24 h after installation. The blood plasma was separated by centrifugation and stored at 20°C until the analysis.

Preparation of blood plasma samples for subsequent analysis

One thousand microliters of acetonitrile was added to 500 μl of plasma and mixed by Vortex for 10 s. Then, it was centrifuged at 14,000 rpm for 10 min. The supernatant was separated and evaporated by a rotary vacuum concentrator. The rest was dissolved in 100 ml of acetonitrile. Then, the aliquots of 50 μl were injected into high-performance liquid chromatography (HPLC) for analysis.

Determination of the DIM concentration in the blood plasma

The DIM concentration in the blood plasma was determined by HPLC. Quantitative determination of the DIM in the plasma samples was carried out on a liquid chromatograph with UV-spectrometric detection using "Agilent 1200" (Agilent Technologies, Santa Clara, CA, USA). The separation was performed on a Eclipse XDB-C18 column (150 × 2.1 mm; 5 μl) (Agilent Technologies) at 20°C. The mixture of acetonitrile and 0.1% formic acid solution in deionized water was used as the mobile phase. The flow rate was 1 ml/min. The wavelenght of UV detector is 280 nm. The retention time of DIM is 8.0 min. The detection limit of DIM concentration is 10 ng/ml.

The calculation of standard pharmacokinetic parameters has been performed on the basis of the following concentration indicators: the area under the pharmacokinetic curve (AUC); the maximum blood plasma concentration (C_{max}); the time to reach maximum plasma concentration (T_{max}); the half-life of DIM from the blood plasma ($T_{1/2}$); the volume of distribution normalized to a bioavailability (Vd/F); the systemic clearance normalized to a bioavailability (CL/F); and the retention time of the drug in the body (MRT_{0inf}).

Statistical analysis

The statistical analysis of data and presentation of results were carried out using the packages SPSS Statistics 19.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2007. The following statistical parameters were calculated: the average value and the standard deviation. The pharmacokinetic parameters were calculated using the model-independent method of statistical moments using the program WinNonlin (Pharsight Corporation, Mountain View, California USA) for Personal computer.

Results

In order to evaluate the bioavailability of the test drug, the DIM concentrations were determined in the samples of blood plasma of patients with EH without atypia after a single oral administration of the drug Cineton (at a dose of 300 mg of DIM). Based on the data obtained, the main pharmacokinetic parameters have been calculated, allowing qualitative characterization of the bioavailability parameters (CL/F; Vd/F), the value of the area under the pharmacokinetic curve of the concentration–time (AUC_{0-t}), the time to reach maximum plasma

Parameters	C_{max} (ng/ml)	AUC_{0-t} (ng·h/ml)	AUC_{0-inf} (ng·h/ml)	CL/*F* (l/h)	T_{max} (h)	$T_{1/2}$ (h)	Vd/F (l/h)	MRT_{0inf} (h)
Average value	121.5	304.1	343.9	0.01	2.2	1.9	0.03	4.4
Standard deviation	38.7	107.6	112.9	0.01	0.45	1.6	0.02	1.35

Table 1: Pharmacokinetic parameters of 3,3'-diindolylmethane (DIM) in the blood plasma of female patients with EH without atypia after a single dose of the drug Cineton (at a dose of 300 mg of DIM)

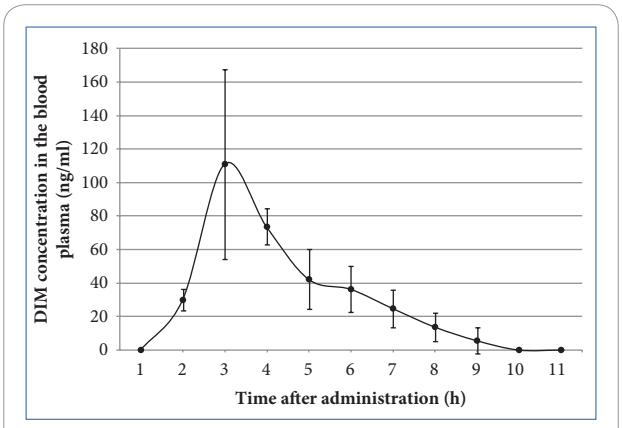

Figure 1: The dependence of concentrations of 3,3'-diindolylmethane (DIM) in the blood plasma of female patient with EH without atypia on the time after a single dose of the drug Cineton (at a dose of 300 mg of DIM) (the average value and the standard deviation are shown for each moment of time)

concentration (T_{max}), and the maximum blood plasma concentration (C_{max}).

The pharmacokinetic study has allowed us to reveal that the drug Cineton possesses a good bioavailability; the mean AUC was 343.9 ± 112.9 ng·h/ml. The average maximum concentration (C_{max}) of DIM in plasma reached 121.5 ± 38.7 ng/ml in T_{max} 2.2 ± 0.45 h, and the half-life of DIM from the blood plasma ($T_{1/2}$) was 1.9 ± 1.6 h. More detailed pharmacokinetic parameters are shown in Table 1.

The pharmacokinetics curve (the average pharmacokinetic profile of DIM) after a single administration of the drug Cineton (at a dose of 300 mg of DIM) is shown in Figure 1.

Discussion

In general, the nature of the pharmacokinetics curve of DIM, obtained from female patients with EH without atypia in the present study, appeared similar to the pharmacokinetic profiles of DIM obtained previously in healthy patients [27] and animal studies [28]. According to the results of preclinical pharmacokinetic studies of the substance DIM [29], the dependence of DIM concentrations in the blood plasma on the time (AUC) had a form, characteristic of oral administration.

The pharmacokinetic curve of DIM, as shown in Figure 1, indicates that the test compound rapidly enters the systemic circulation from the gastrointestinal tract and is determined in blood plasma samples over 10 h after a single administration of the study drug Cineton to female patients. The calculated value of MRT_{0inf}, corresponding to an average retention time of the active substance in the body, was 4.4 ± 1.35 h (Table 1). This period of time is more than sufficient for the maximum manifestation of the therapeutic activity of the active substance [30].

Conclusion

The obtained results have confirmed the high bioavailability of the drug Cineton – the new pharmaceutical composition based on DIM—containing pluronic. Cineton may be considered as the perspective approach to preventing hyperplasia from becoming cancerous.

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