BUTOCONAZOLE NITRATE PHARMACOKINETICS STUDIED BY CAPILLARY ELECTROPHORESIS

S. P. Senchenko,[1] K. S. Checheneva,[1] M. V. Gavrilin,[1] and L. S. Ushakova[1]

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In view of the wide use of butoconazole nitrate in practical gynecology, a method for determining this drug in the blood of experimental animals has been developed based on capillary electrophoresis. Samples were prepared using sedimentation of plasma proteins by acetonitrile. The separation was carried out at 27°C in a quartz capillary (75 μm diameter, 65 cm working length) at 20 kV. The leading electrolyte was a phosphate buffer solution at pH 3.6. Detection was performed by spectrophotometry at 210 nm. The proposed technique was used to study the pharmacokinetics of butoconazole nitrate in rats upon a single intraperitoneal injection at a dose of 80 mg/kg. The kinetic curves of butoconazole nitrate concentration in the blood were constructed and were typical of drug removal with bile.

Key words: capillary electrophoresis, butoconazole nitrate, pharmacokinetics.

Recent publications reveal a persistent tendency toward an increase in the patient population with vaginal infections [1, 2].

One of the most common gynecological fungal diseases is vulvovaginal candidosis (VVC). This disease is one of the leading causes of vaginal and vulval infections. The occurrence of VVC has doubled in the last 10 years and is responsible for 30 – 45% of such infections [3].

Preparations used to treat VVC are designed for both topical and systemic application. Systemic antifungal therapy is indicated for pronounced clinical VVC and chronic disease that is resistant to topical therapy. The most effective preparations for systemic treatment are azoles such as fluconazole and itraconazole [4].

Topical preparations are used for mild VVC. Vaginal suppositories, tablets, and special vaginal creams are currently used as topical antifungal agents. Existing compounds include, as a rule, azole antimycotics such as miconazole, clotrimazole, and ketoconazole. Imidazoles remain the first-line preparations for treating VVC. One of the most effective preparations today is butoconazole nitrate, which exhibits funcidal activity and is active against the fungus genera *Candida*, *Trychophyton*, *Microsporum*, and *Epidermaphyton* and several Gram-positive bacteria [4]. It increases the permeability of membranes by blocking formation of ergosterol from lanosterol in the fungal cell wall. This causes lysis of the fungal cell [5].

Many VVC treatment courses cover from 7 to 14 days. However, it is recognized that up to 50% of patients stop treatment after the symptoms subside. Therefore, a reduction of the drug treatment course through optimization of the dosing pattern was proposed in order to increase observance of the routine. As a result, the treatment courses of miconazole nitrate and clotrimazole were shortened from an initial 14 days to 7 and 3, respectively. Eventually a treatment course was developed that consisted of a single dosage of the preparation. This became possible because of the creation of a novel bioadhesive matrix based on a cream with prolonged action for intravaginal application with 2% butoconazole nitrate (Gynofort, vaginal cream produced by the Hungarian company Gedeon Richter in collaboration with KB Pharmaceutical Co., USA) [6].

This preparation is at present one of the most effective for treating vaginal candidosis. The domestic chemical and pharmaceutical industry does not produce an analogous preparation that is as effective. Therefore, it became necessary to develop a domestic analog of similar activity. It was necessary to select a composition of excipients such that it would provide prolonged residence of the preparation at the administration site that, in turn, would require modern analytical equipment. The literature describes an HPLC method for determining butaconazole nitrate [7]. In addition to this, it seemed advantageous to develop a method for determining

[1] Pyatigorsk State Pharmaceutical Academy, Pyatigorsk, Stavropol Region, 357500, Russia.

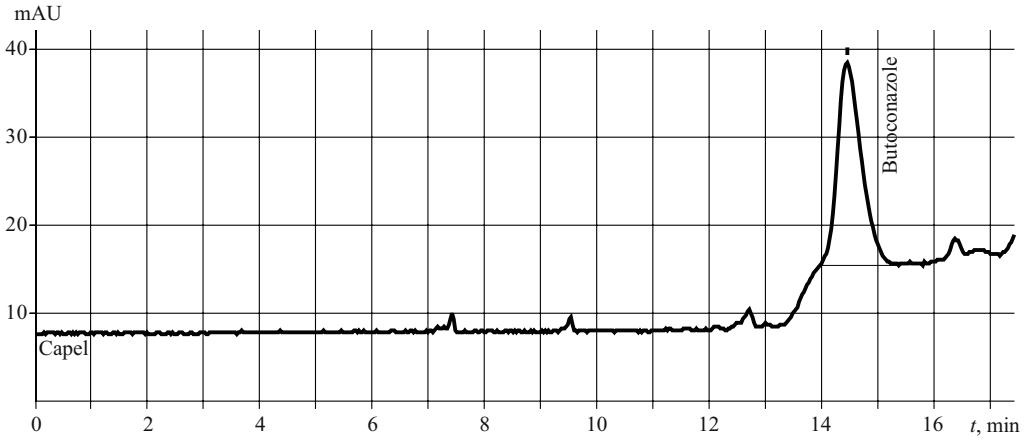

Fig. 1. Electrophoregram of standard butoconazole nitrate solution.

butoconazole nitrate using capillary electrophoresis because this method has several advantages over HPLC. These include a highly effective separation (hundreds of thousands of theoretical plates); a small volume of analyte and buffers; the avoidance of highly pure costly solvents; the lack of columns and sorbents and problems with their aging; a simple and inexpensive apparatus; and a rapid and intrinsically inexpensive single analysis [8].

Our goal was to develop a unified method for quantitative determination of butoconazole nitrate using capillary electrophoresis and to apply it to a study of the pharmacokinetics of the compound in rat blood after i.p. administration.

EXPERIMENTAL PART

Basic electrolytes, for example sodium tetraborate solutions (5 – 20 mg/mL), are frequently used for electrophoretic separation of drugs in pharmaceutical analysis. Therefore, the research started with a study of the stability of drug solutions in the electrolyte in order to develop the analytical method.

For this, we prepared several solutions of various concentrations, from 0.5 μg/mL to 20 mg/mL. It was shown that butoconazole nitrate precipitated in basic solution. Adding organic solvents, for example acetonitrile (CH_3CN) (up to 30%), to the buffers increased the solubility of the drug. However, increasing its concentration up to 100 μg/mL and higher did not solve the problem. Increasing the CH_3CN concentration in the electrolyte (up to 50%) caused sodium tetraborate to precipitate with time and to adsorb on the capillary walls.

The next step in developing the method was to study the behavior of the drug in acidic buffers.

For this, we used acetate buffer (0.2 M, pH 4), which was checked beforehand for stability of drug solutions with various concentrations of drug and organic solvent (CH_3CN). It was found that increasing the concentration of CH_3CN did not cause the components of the buffer to precipitate. Furthermore, CH_3CN increased substantially the solubility of butoconazole nitrate. We used a solution of butoconazole nitrate in this electrolyte with 50% CH_3CN for the analysis.

Spectra of the analyzed solutions were measured in order to select the optimum detection conditions. The optimal wavelength for detection of butoconazole nitrate in this solvent mixture was 210 nm.

The analytical conditions were as follows: capillary of diameter 75 μm and working length 65 cm, potential 20 kV, analysis temperature 27°C. Detection was performed in the cathodic region of the capillary at wavelength 210 nm.

However, butoconazole gave a double peak in the electrophoregram for analysis under these conditions. This was possible due to differences in the degree and nature of butoconazole ionization under these conditions.

Next, the leading electrolyte was selected taking into account the stability of butoconazole nitrate solutions of various concentrations, pH, buffer composition, and maximum possible addition of organic solvent. It was found that symmetric single peaks could be produced using phosphate buffer with pH less than 3.6 as the leading electrolyte. Figure 1 shows an electrophoregram of the test solution.

The next step was to use this method to study the kinetics of butoconazole nitrate in blood after i.p. administration of a solution of the preparation.

Experiments were performed on white female Wistar rats. Animals were administered i.p. a solution of butoconazole nitrate in DMSO at a dose of 80 mg/kg. Animals were decapitated after 15 and 30 min and 1, 2, 4, 8, 12, 16, and 20 h. Blood was collected for analysis. Eight animals were used for each time point. The controls were blood samples from untreated animals.

Blood samples were centrifuged for 15 min at 3,000 rpm. Serum (1000 μL) was treated with glacial acetic acid (50 μL) and CH_3CN (950 μL), stirred, and centrifuged for 15 min at 5,000 rpm.

The capillary was prepared and its surface was regenerated by treatment successively with water, NaOH solution

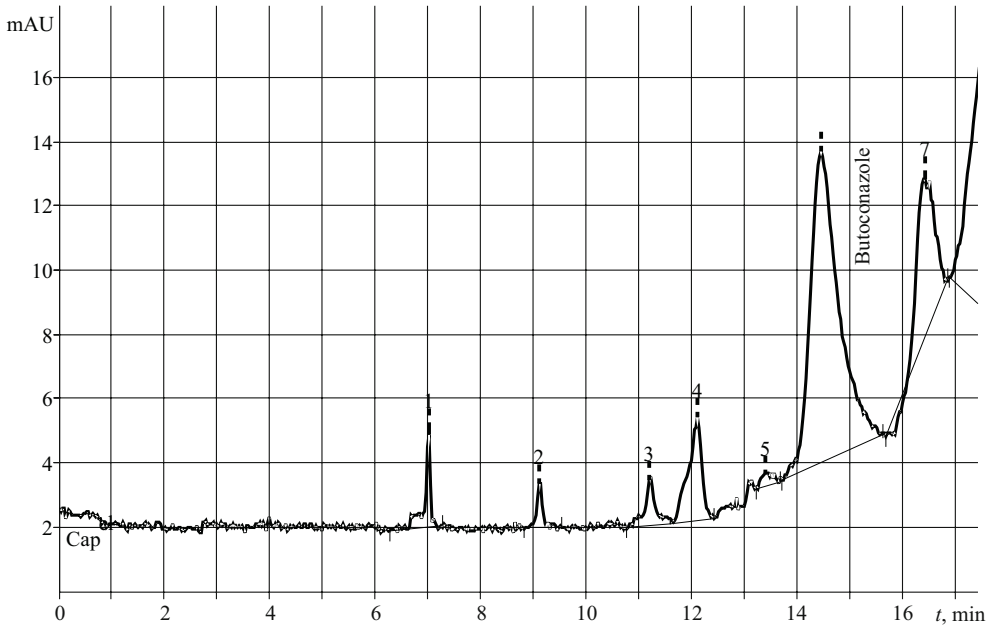

Fig. 2. Electrophoregram of blood serum after i.p. injection of butoconazole nitrate solution at a dose of 80 mg/kg (30 min after injection).

(1 M), water, HCl solution (1 M), water, and leading electrolyte.

Measurements were made in a Capel 105 instrument (ZAO Lyumeks, St. Petersburg). For this, supernatant (500 μL) was placed in a tube. A sample was injected using elevated pressure (30 mBar) for 5 sec. Then, a potential (20 kV) was imposed on the electrodes to carry out the separation in phosphate buffer at pH 3.6. The electrophoresis time was 20 min. Electrophoregrams were processed using the Multikhrom program. Electrophoresis of a series of standard butoconazole nitrate solutions of various concentrations was carried out in parallel under these conditions.

Figure 2 shows the resulting electrophoregram of blood serum after injection of a butoconazole nitrate solution.

The results were used to construct a kinetic curve for butoconazole nitrate after a single i.p. administration of the drug solution at a dose of 80 mg/kg.

Figure 3 shows the kinetic curve constructed from the experimental results.

The resulting curve was a typical example for elimination of a substance with bile (in addition to the principle elimination pathway with urine) [9].

According to the literature, drugs are eliminated with bile if their molecular weight exceeds 300 D. Drugs and their metabolites that are eliminated with bile into the intestine can be extracted and absorbed again into blood or metabolized.

In this particular case, the most probable metabolic process is enzymatic hydrolysis of conjugates in which the released compound is absorbed again into the bloodstream, which produces a second peak.

Therefore, the curve can be broken into several fragments.

The first fragment is the time during which the preparation reaches the minimum concentration in blood and before it is absorbed again.

The second fragment is a valley (minimum on the curve) corresponding with food intake, when bile is released and reabsorption of the drug into the bloodstream begins.

The third fragment is the time from the start of reabsorption of the drug into blood.

Thus, a method for determining butoconazole nitrate by capillary electrophoresis is proposed. The method can be used to detect the compound in blood plasma of tested animals and to study its pharmacokinetics.

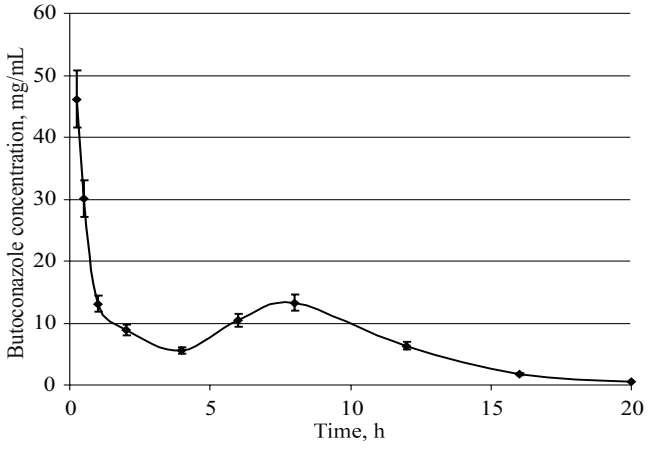

Fig. 3. Butoconazole nitrate accumulation in rat blood after i.p. injection of butoconazole nitrate solution at a dose of 80 mg/kg.

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