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Черных И.В. – к.б.н., ассистент кафедры общей и фармацевтической химии ФГБОУ ВО РязГМУ Минздрава России, г. Рязань, Российская Федерация. SPIN 5238-6165, ORCID ID 0000-0002-5618-7607, Researcher ID R-1389-2017.
E-mail: ivchernykh88@mail.ru

Щулькин А.В. – к.м.н., доцент кафедры фармакологии с курсом фармации ФДПО ФГБОУ ВО РязГМУ Минздрава России, г. Рязань, Российская Федерация. SPIN 2754-1702, ORCID ID 0000-0003-1688-0017, Researcher ID N-9143-2016.

Якушева Е.Н. – д.м.н., профессор, заведующая кафедрой фармакологии с курсом фармации ФДПО ФГБОУ ВО РязГМУ Минздрава России, г. Рязань, Российская Федерация. SPIN 2865-3080, ORCID ID 0000-0001-6887-4888, Researcher ID T-6343-2017.
E-mail: e.yakusheva@rzgmu.ru

Гацанога М.В. – аспирант кафедры фармакологии с курсом фармации ФДПО ФГБОУ ВО РязГМУ Минздрава России, г. Рязань, Российская Федерация. SPIN 9645-5079, ORCID ID 0000-0002-1116-6271, Researcher T-3803-2017.

Попова Н.М. – к.м.н., старший преподаватель кафедры фармакологии с курсом фармации ФДПО ФГБОУ ВО РязГМУ Минздрава России, г. Рязань, Российская Федерация. SPIN 7553-9852, ORCID ID 0000-0002-5166-8372, Researcher ID B-1130-2016.

STUDY OF FABOMOTIZOLE BELONGING TO P-GLYCOPROTEIN SUBSTRATES

I.V. Chernykh, A.V. Shchulkin, E.N. Yakusheva, M.V. Gatsanoga, N.M. Popova

Ryazan State Medical University,
Vysokovoltnaya str., 9, 390026, Ryazan, Russian Federation

P-glycoprotein (Pgp) is a membrane efflux protein transporter with numerous drug-substrates. In addition, a lot of drugs alter the activity of the transporter. It can lead to drug-drug interactions during polypharmacy. Fabomotizole (afobazol) is a Russian anxiolytic drug with neuroprotective activity, applied over a wide range of indications. The drug belongs to a potential substrate of Pgp according to its chemical structure. *Aim*. The aim of the study was to assess belonging of fabomotizole to Pgp substrates. *Materials and Methods.* The work was performed on 12 male Chinchilla rabbits. The belonging of fabomotizole to Pgp substrates

was evaluated by comparing pharmacokinetic parameters of the test-substance after course administration of known transporter inducers and inhibitors – rifampicin and verapamil respectively. Fabomotizole was administered orally as a single dose of 3.8 mg/kg b.w. and blood was taken from the ear vein after 5, 10, 15, 20, 30, 60, 90, 120 and 240 min followed by it's pharmacokinetic analysis by HPLC. Pharmacokinetic parameters of fabomotizole were manually calculated by a model-independent method. The animals were then divided into 2 groups of 6 rabbits each: the 1st group received verapamil at a dose 20 mg/kg b.w. 3 times a day for 14 days, the 2nd – rifampicin in a similar course and dose. After the administration of Pgp modulators the pharmacokinetics of fabomotizole were re-analyzed. *Results.* It was found that only the absorption coefficient of fabomotizole in the rifampicin series was significantly reduced by 1.27 times as compared to the parameter of intact animals (90% CI 0.66-0.94, p=0.04322). However, this change was not clinically significant, because 90% CI overlapped the range of 0.80-1.25, noted by FDA. The remaining pharmacokinetic parameters of Pgp marker substrate were not significantly changed in any series. This is evidence that fabomotizole is not a Pgp substrate. The insignificant participation of Pgp in fabomotizole pharmacokinetics testifies that the drug can be administered together with drug-modulators of transporter activity without dose correction. *Conclusion.* In vivo experiment on Chinchilla rabbits showed that fabomotizole is not a substrate of P-glycoprotein.

***Keywords**: P-glycoprotein, fabomotizole, pharmacokinetics, rifampicin, verapamil, substrate.*

P-glycoprotein (Pgp) is a membrane protein-transporter which uses the energy of ATP for efflux of endo- and xenobiotic with different chemical structure (among them a large number of modern drugs) from cells. The activity of the transporter varies under the influence of external and internal factors, including consumption of some medications. Thus, the transporter has a significant role in the development of drug-drug interactions [1].

Fabomotizole (afobazol) is an original Russian selective anxiolytic with neuroprotective activity and a wide range of indications for use and OTC release from pharmacies [2]. The research complex has shown that Pgp substrates are predominantly lipophilic aromatic compounds with a molecular weight in the 300-500 Da range, which include hydrogen bonds in the amino group or a nitrogen atom protonated at physiological pH [3-5]. Similar properties are characteristic for fabomotizole (Fig. 1), which suggests that it belongs to the transporter protein substrates.

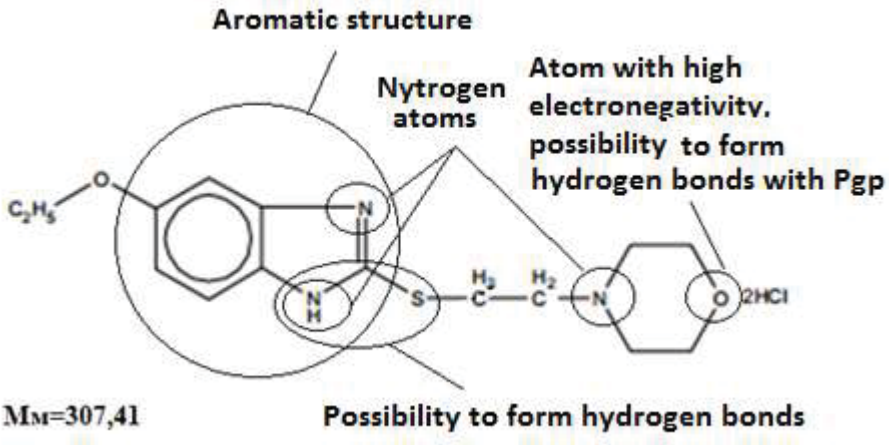

Fig. 1. Chemical structure of fabomotizole with indication of it's characteristic groups

Studies devoted to evaluation of the fabomotizole belonging to substrates, inducers and inhibitors of Pgp have not been found in the scientific literature.

Thus the aim of this work was to assess the belonging of fabomotizole to Pgp substrates in order to predict possible drug-drug interactions and use effective and safe pharmacotherapy.

Materials and Methods

The work was performed on 12 male mature Chinchilla rabbits with an average mass of 3500-4300 g in compliance with the rules of laboratory practice (Order of the Ministry of Health of the Russian Federation №199n (April 1, 2016 «On Approval of the Rules for good laboratory practice») [6]. The belonging of fabomotizole to Pgp substrates was evaluated by analysis of the test-substance pharmacokinetic parameters during course administration of known transporter protein inducers and inhibitors − rifampicin and verapamil respectively.

In case of Pgp's participation in the pharmacokinetics of the test-substrate it's plasma conentration is expected after a course of verapamil administration and reverse changes after the introduction of rifampicin [7].

The animals were orally administered fabomotizole (Afobazol tablets, 10 mg, Pharmstandard-Leksredstva, Russia) at a dose 3.8 mg/kg [8,9] in the form of a suspension in purified water. Blood samples were then collected from the ear vein after 5, 10, 15, 20, 30, 60, 90, 120 and 240 min in a volume of 5 ml, followed by analysis of its pharmacokinetics by HPLC.

The quantitative analysis of the test substance in the blood plasma was carried out by the method of absolute calibration by the area of peaks. Calibration solutions were prepared by addition to the intact blood plasma certain amount of standard solution with concentration of 10 μg/ml. A matrix solution (1 mg/ml) was prepared on methanol and stored at 4°C. The standard was supplied by developers.

The research was carried out in isocratic mode. As a mobile phase, a mixture of acetonitrile-water-methanol-acetic acid ice-triethylamine was used in the ratio 100-240-100-0.3-0.25 (pH 6.10). The retention time of fabomotizole was 10.00±0.11 min. The lowest limit of detection and detection limit were 3.4 and 7.8 ng/ml respectively.

The calibration dependence of the peak area on the fabomotizole concentration was determined in a concentration range of 50-1000 ng/ml at 6 points, 5 measurements were performed for each point. In the indicated concentration range, the correlation «concentration of fabomotizole – peak area» was linear. The Pearson correlation coefficient was 0.99935. The regression equation was: $y = 1.9994*x+10.536$, where x is peak area, and y is the concentration of fabomotizole.

To extract the target substance from the blood plasma and prepare the mobile phase the following reagents were used: methanol, acetonitrile «HPLC» (Merck, Germany), acetic acid (Ecos-1, Russia), triethylamine «HPLC» (Lab-Skan, Poland).

Extraction of fabomotizole from blood plasma was carried out with diethyl ether (1.5 ml of plasma and 6 ml of extragent) by shaking on a Shaker device at 400 rpm for 10 min, centrifugation at 3500 rpm for 10 min and evaporation of the supernatant at 40°C. The extraction degree was 87%.

«Statistica 7.0» and «Microsoft Excel» were used for calculation of the metrological characteristics and the main validation parameters of the developed method, as well as the Manual for Industry «Verification of the bioanalytical method» (2013).

Fabomotizole pharmacokinetic parameters were calculated by a model-independent method by hand.

After analyzing the basal fabomotizole pharmacokinetics the animals were divided into 2 groups of 6 rabbits each: the 1st group received Pgp inhibitor verapamil (coated tablets, 80 mg, Valenza Pharmaceuticals, Russia) at a dose 20 mg/kg 3 times a day for 14 days [10] as suspension on purified water, the second – the inductor of the transporter – rifampicin (capsules Rifampicin, 150 mg, Pharmasintez, Russia) at a dose of 20 mg/kg mass for 14 days [11] as suspension on a starch paste. After the Pgp modulators administration, fabomotizole was administered repeatedly and it's pharmacokinetics were reanalyzed in the animals. It should be noted that the last verapamil administration was carried out in the morning - before the fabomotizole introduction, and rifampicin - in the evening of the previous day.

The results were processed using StatSoft Statistica 7.0 program. The presence

of significant differences between the T_{max} values of fabomotizole was assessed using Wilcoxon test, and the results of the studies are presented as median, lower and upper quartiles (Med, lq, uq). The statistical significance of the differences between other pharmacokinetic parameters was estimated from the notion of a log-normal distribution of data. Comparison of the studied pharmacokinetic parameters was implemented using ANOVA test after their logarithm. Differences were considered statistically significant at $p<0.05$. In addition, a two-sided 90% confidence interval was calculated. According to the recommendations of the US Food and Drug Administration, the Center for Drug Evaluation and Research, significant differences considered between pharmacokinetic parameters a two-sided 90% confidence interval of their ratios is outside the range of 0.8-1.25 (80-125%). The results are presented in tables as a geometric mean and 95% confidence interval.

Results and Discussion

A 14-day administration of Pgp modulators verapamil and rifampicin did not lead to changes in fabomotizole pharmacokinetic parameters (Tab. 1, 2).

The averaged fabomotizole pharmacokinetic curves of intact animals and rabbits after the course administration of Pgp inhibitor and inducer are presented respectively in figures 2 and 3.

Table 1

Fabomotizole pharmacokinetic parameters before and after the course rifampicin administration

Pharmaco-kinetic parameter	Basal values, n=6	Rifampicin 14 days, n=6	p
C_{max}, ng/ml	381.81 (203.00; 718.11)	235.26 (158.13; 350.01)	0.1738
T_{max}, min	5.0 (5.0; 10.0)	5.0 (5.0; 5.0)	1.0000
$T_{½}$, min	88.26 (27.49; 283.38)	72.32 (47.35; 110.44)	0.59620
AUC_{0-t}, (ng/ml)×h	11568.47 (6037.34; 22166.98)	9005.91 (5979.30; 13564.52)	0.3352
$AUC_{0-\infty}$, (ng/ml)×h	15768.75 (10440.89; 23815.35)	10625.97 (7556.48;14942.30)	0.1064
Cl, l/min	0.24 (0.16; 0.36)	0.36 (0.25; 0.50)	0.1064
C_{max}/AUC_{0-t}, 1/min	0.033 (0.027; 0.040)	0.026 (0.019; 0.035)*	0.04322
MRT, min	127.35 (39.66; 408.92)	104.35 (68.33; 159.36)	0.59620
K_{el}, 1/min	0.008 (0.002; 0.025)	0.010 (0.006; 0.015)	0.59620
Vd, 1/min	30.69 (8.66; 108.72)	37.32 (18.76; 74.21)	0.6671

Note: * – significant differences compared with intact animals (basal level). The data is presented as the geometric mean and its 95% confidence interval. The values of T_{max} are presented in the form of the median and lower and upper quartiles

Table 2

Fabomotizole pharmacokinetic parameters before and after the course verapamil administration

Pharmaco-kinetic parameter	Basal values, n=6	Verapamil 14 days, n=6	p
C_{max}, ng/ml	413.02 (345.39; 493.90)	291.10 (168.23; 503.70)	0.11680
T_{max}, min	5.0 (5.0; 5.0)	10.0 (5.0; 17.5)	0.4795
$T_{½}$, min	62.59 (20.53; 190.85)	82.14 (26.53; 254.34)	0.5951
AUC_{0-t}, (ng/ml)×h	11067.60 (8638.72; 14179.41)	9741.60 (4929.07; 19252.89)	0.5242
$AUC_{0-\infty}$, (ng/ml)×h	12539.24 (11246.22; 13980.92)	13313.00 (7139.04; 24826.31)	0.7449
Cl, l/min	0.30 (0.27; 0.34)	0.29 (0.15; 0.53)	0.7449
C_{max}/AUC_{0-t}, 1/min	0.037 (0.026; 0.053)	0.030 (0.020; 0.044)	0.2636
MRT, min	90.32 (29.62; 275.40)	118.52 (38.28; 367.01)	0.5951
K_{el}, 1/min	0.011 (0.004; 0.034)	0.008 (0.003; 0.026)	0.5951
Vd, 1/min	27.37 (8.31; 90.21)	33.83 (7.80; 146.71)	0.7092

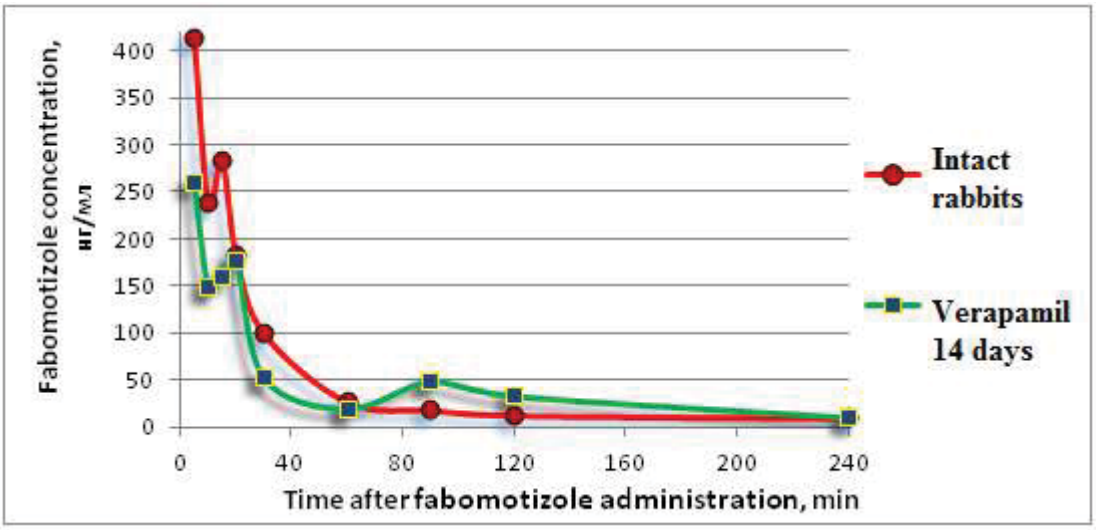

Fig. 2. Averaged fabomotizole pharmacokinetic curves of intact animals and rabbits after the course verapamil administration

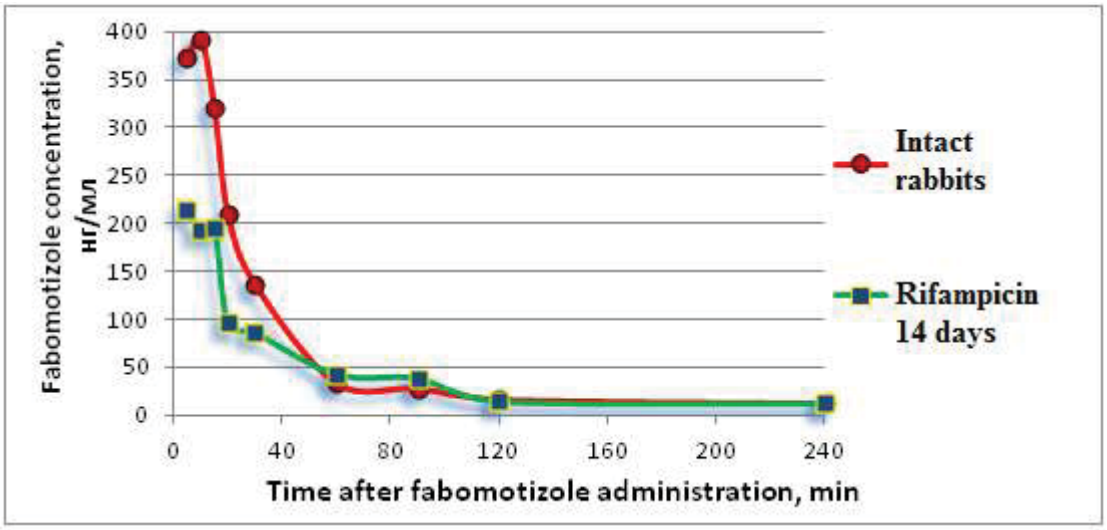

Fig. 3. Averaged fabomotizole pharmacokinetic curves of intact animals and rabbits after the course rifampicin administration

From the data given, it can be seen that only the fabomotizole absorption coefficient (C_{max}/AUC_{0-t}) in the rifampicin series significantly decreased by 1.27 times as compared to the similar parameter of intact animals (90% CI 0.66-0.94, p=0.04322). However, this change was not clinically significant, because the 90% confidence interval significantly overlapped the range of 0.80-1.25, noted by the recommendations of the FDA. A weak tendency to decrease the area under the pharmacokinetic curve ($AUC_{0-\infty}$) and a similar increase in the fabomotizole total clearance in this series is probably due to the induction of microsomal liver enzymes under the rifampicin action and the intensification of the test drug metabolism [8].

In our study no significant changes in fabomotizole pharmacokinetic parameters during the introduction of Pgp modulators were found. However, in multidrug-resistant cell cultures it has been shown that a number of benzimidazole derivatives penetrate them to a much lesser degree than normal cells, indicating that they may belong to Pgp substrates [12]. We have found that the test drug is not among Pgp substrates, and the role of the transporter in its absorption, distribution and excretion is not key even though it's chemical structure has signs of substrate specificity to the transporter.

The insignificant participation of Pgp in the fabomotizole pharmacokinetics shows that the drug can be administered together with medicaments-modulators of the transporter activity without adjusting its dose. In addition, the penetration of fabomotizole through the blood-brain barrier, where this transporter is active, will be sufficient to achieve a minimum therapeutic concentration in brain tissue, even despite its induction in conditions of oxygen deficiency [6,10]. This is confirmed by the results of numerous studies of fabomothiazole efficacy as an anxiolytic and neuroprotective agent [13,14].

Conclusion

In vivo experiment on Chinchilla rabbits it has shown that fabomotizole is not a substrate of P-glycoprotein transporter protein.

Authors have no conflict of interest to declare.

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Chernykh I.V. – PhD in Biological sciences, Assistant of General and Pharmaceutical Chemistry Department, Ryazan State Medical University, Ryazan, Russian Federation. SPIN 5238-6165, ORCID ID 0000-0002-5618-7607, Researcher ID R-1389-2017.
E-mail: ivchernykh88@mail.ru

Shchulkin A.V. – MD, PhD, Assistant of Pharmacology Department with Course of Pharmacy of Continuing Professional Education Faculty, Ryazan State Medical University, Ryazan, Russian Federation. SPIN 2754-1702, ORCID ID 0000-0003-1688-0017, Researcher ID N-9143-2016.

Yakusheva E.N. – MD, Grand PhD, Professor, Head of Pharmacology Department with Course of Pharmacy of Continuing Professional Education Faculty, Ryazan State Medical University, Ryazan, Russian Federation. SPIN 2865-3080, ORCID ID 0000-0001-6887-4888, Researcher ID T-6343-2017.
E-mail: e.yakusheva@rzgmu.ru

Gatsanoga M.V. – Postgraduate Student of Pharmacology Department with Course of Pharmacy of Continuing Professional Education Faculty, Ryazan State Medical University, Ryazan, Russian Federation. SPIN 9645-5079, ORCID ID 0000-0002-1116-6271, Researcher ID T-3803-2017.

Popova N.M. – MD, PhD, Assistant of Pharmacology Department with Course of Pharmacy of Continuing Professional Education Faculty, Ryazan State Medical University, Ryazan, Russian Federation. SPIN 7553-9852, ORCID ID 0000-0002-5166-8372, Researcher ID B-1130-2016.