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Research article / Оригинальная статья

An Open-label Randomized Crossover Study with Adaptive Design of the Pharmacokinetics and Bioequivalence of GP30121 and Ceraxon® Corrected for Endogenous Analyte Level

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Abstract

Introduction. Citicoline is an endogenous nucleoside consisting of cytidine and choline linked by a diphosphate bridge that is involved in the synthesis of membrane phospholipids. Drugs containing citicoline have neuroprotective and neurometabolic effects and are used for the treatment of a wide range of neurological disorders. In its turn, bioequivalence study is a pathway to register a generic citicoline drug in Russian Federation.

Aim. The aim of the study was to investigate the comparative pharmacokinetics (PK) and bioequivalence of two citicoline-containing drugs GP30121 and Ceraxon® in healthy male volunteers when taken on an empty stomach.

Materials and methods. We evaluated the pharmacokinetics of citicoline-containing drugs corrected for endogenous analyte (uridine) level using an adaptive design. We determined uridine concentration by high-performance liquid chromatography with mass spectrometric detection. We used R Project software, version 3.6.3. for performing statistical analysis for the study.

Results and discussion. GP30121 and Ceraxon® exhibited similar PK profiles. It was shown that the values of 94.12 % confidence interval (CI) at $\alpha = 0.0294$ for the geometric mean ratios for the primary PK parameters of the main metabolite of the active ingredient of the investigated drugs were fully contained within the predefined equivalence limits of 80.00–125.00 %.

Conclusion. The study demonstrates bioequivalence of GP30121 and Ceraxon® proving the approach with the correction for endogenous analyte could be considered in studies of other drugs.

Keywords: citicoline, uridine, bioavailability, adaptive design, safety

Conflict of interest. The study was sponsored by LLC "GEROPHARM". The authors Roman V. Dry, Igor E. Makarenko, Artem R. Dorotenko, Anna N. Arefieva are employees of CJSC "Pharm Holding", which is a division of LLC "GEROPHARM". The authors Igor E. Shokhin, Sergey M. Noskov, Timofey N. Komarov, Olga A. Archakova, Natalya S. Bagaeva are employees of institutions that performed this contract study for LLC "GEROPHARM".

Contribution of the authors. Roman V. Drai, Igor E. Makarenko, Artem R. Dorotenko, Anna N. Arefeva participated in the planning and coordination of all stages of the bioequivalence study. Sergey M. Noskov supervised the clinical stage of the study. Igor E. Shokhin supervised the analytical part of the study. Timofey N. Komarov and Olga A. Archakova performed the analytical part of the study, participated in the development and validation of the analytical methodology. Natalya S. Bagaeva performed the statistical and pharmacokinetic part of the study. All authors participated in the discussion of the results and preparation of the manuscript.

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Compliance with the principles of ethics. The condition for conducting the study was the Permit of the Ministry of Health of the Russian Federation No. 173 dated March 31, 2021 and the approval of the study by the Ethics Council (extract from the minutes of the meeting of the Ethics Council No. 269 dated March 23, 2021). The study protocol GP30121-P4-11, version 4.0 dated February 5, 2021, and all the main study documents were approved by the Independent Ethics Committee of the State Budgetary Health Institution of the Yaroslavl Region "Clinical Hospital No. 3" (extract from the protocol No. 142 of the meeting of the Independent Ethics Committee dated January 28, 2022). The study was carried out in accordance with the ethical standards developed in accordance with the Declaration of Helsinki of the World Medical Association "Ethical principles for conducting scientific medical research involving humans" as amended in 2000 and "Rules of Clinical Practice in the Russian Federation", approved by the Order of the Ministry of Health of the Russian Federation dated 19.06.2003 No. 266. All subjects provided written informed consent before any screening procedures were carried out.

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Сравнительное адаптивное исследование фармакокинетики и биоэквивалентности препаратов GP30121 и Цераксон® с использованием коррекции на уровень эндогенного аналита

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Резюме

Введение. Цитиколин – естественный эндогенный нуклеозид, состоящий из цитидина и холина, связанных дифосфатным мостиком, участвующий в синтезе мембранных фосфолипидов в качестве промежуточного звена. Препараты цитиколина обладают нейропротекторным и нейрометаболическим действием, используются для лечения широкого спектра неврологической патологии. Для регистрации воспроизведенного препарата цитиколина необходимо проведение исследования биоэквивалентности.

Цель. Целью данного исследования являлось изучение сравнительной фармакокинетики (ФК) и биоэквивалентности препаратов, содержащих цитиколин – GP30121 и Цераксон®, у здоровых добровольцев при приеме натощак.

Материалы и методы. В настоящей статье описана оценка фармакокинетики препаратов, содержащих цитиколин, с коррекцией на фоновое значение аналита (уридина). Для проведения исследования был выбран адаптивный дизайн. Определение концентрации уридина было выполнено методом высокоэффективной жидкостной хроматографии с масс-спектрометрическим детектированием. Статистический анализ проводили при помощи программного обеспечения R Project, версия 3.6.3.

Результаты и обсуждение. Было показано, что значения 94.12%-х ДИ при α = 0,0294 для отношений геометрических средних основных ФК параметров основного метаболита действующего вещества исследуемых препаратов укладываются в допустимые пределы 80.00–125.00 %.

Заключение. Была доказана биоэквивалентность препаратов, из чего можно сделать вывод, что данный подход может быть рассмотрен в исследованиях других лекарственных средств.

Ключевые слова: цитиколин, уридин, адаптивный дизайн, биодоступность, безопасность

Конфликт интересов. Исследование спонсировалось ООО «ГЕРОФАРМ». Авторы Р. В. Драй, И. Е. Макаренко, А. Р. Доротенко, А. Н. Арефьева являются сотрудниками ЗАО «Фарм-Холдинг», который является подразделением ООО «ГЕРОФАРМ». Авторы И. Е. Шохин, С. М. Носков, Т. Н. Комаров, О. А Арчакова, Н. С. Багаева являются сотрудниками учреждений, выполнявших данное контрактное исследование для ООО «ГЕРОФАРМ».

Вклад авторов. Р. В. Драй, И. Е. Макаренко, А. Р. Доротенко, А. Н. Арефьева участвовали в планировании и координации всех этапов исследования биоэквивалентности. С. М. Носков руководил клиническим этапом исследования. И. Е. Шохин руководил аналитической частью исследования. Т. Н. Комаров и О. А. Арчакова выполняли аналитическую часть исследования, участвовали в разработке и валидации аналитической методики. Н. С. Багаева выполняла статистическую и фармакокинетическую часть исследования. Все авторы принимали участие в обсуждении результатов и подготовке рукописи.

Финансирование. Исследование финансировалось ООО «ГЕРОФАРМ».

Соответствие принципам этики. Условием для проведения исследования являлись Разрешение МЗ РФ № 173 от 31.03.2021 и одобрение исследования Советом по Этике (выписка из протокола заседания Совета по Этике № 269 от 23.03.2021). Протокол исследования GP30121-P4-11, версия 4.0 от 5 февраля 2021 г., и все основные документы исследования были одобрены Независимым этическим комитетом Государственного бюджетного учреждения Здравоохранения Ярославской области «Клиническая больница № 3» (выписка из протокола № 142 заседания Независимого Этического Комитета от 28 января 2022 г.). Проведение исследования соответствовало этическим стандартам, разработанным в соответствии с Хельсинкской декларацией Всемирной медицинской ассоциации «Этические принципы проведения научных медицинских исследований с участием человека» с поправками 2000 г. и «Правилами клинической практики в Российской Федерации», утвержденными Приказом Минздрава РФ от 19.06.2003 г. № 266. Все лица, участвующие в исследовании, подписали информированное согласие на участие в исследовании.

Для цитирования: Арефьева А. Н., Доротенко А. Р., Носков С. М., Макаренко И. Е., Драй Р. В., Комаров Т. Н., Арчакова О. А., Багаева Н. С., Шохин И. Е. Сравнительное адаптивное исследование фармакокинетики и биоэквивалентности препаратов GP30121 и Цераксон® с использованием коррекции на уровень эндогенного аналита. *Разработка и регистрация лекарственных средств*. 2023;12(3):218–227. https://doi.org/10.33380/2305-2066-2023-12-3-218-227

INTRODUCTION

Over the past two decades, there has been a promising decline in mortality rates from cerebrovascular accidents (CVA) and related secondary diseases across the globe. However, despite this positive trend, the number of individuals affected by CVA continues to grow, making it the second leading cause of death worldwide [1]. Russian Federation faces a significant burden of CVA, with an alarming 21.4 % share of total mortality attributed to this condition. Furthermore, mortality rates from CVA among working-age individuals have surged by over 30 % in the last decade, resulting in approximately 41 deaths per 100,000 people; and the early 30-day mortality after stroke rate is approaching 35 %[1].

Citicoline (CDP-Choline or cytidine 5'-diphosphocholine), a naturally occurring nucleoside, has gained recognition as a potent neuroprotector and neurometabolic agent, particularly in the treatment of ischemic stroke and cognitive impairment associated with dyscirculatory encephalopathy. As suggested by national clinical guidelines for neurology, patients who have experienced an ischemic stroke are typically prescribed citicoline medication. Intravenous administration of the drug for 10 days, followed by a shift to oral administration, significantly enhances patient outcomes, reducing both disability and mortality rates[2]. At the moment, citicoline is the only neuroprotector included in European guidelines for stroke treatment[3].

Citicoline's mechanism of action involves its role in the synthesis of membrane phospholipids, as well as its ability to be metabolized into choline and cytidine when taken orally, ultimately leading to the production of uridine, a key metabolite of exogenous citicoline and precursor of endogenous citicoline [4]. Additionally, the drug can oxidize to betaine, acting as a methyl group donor, which, on par with participating in the synthesis of acetylcholine, further contributes to its neuroprotective and neurometabolic effects.

Currently, there is only one citicoline drug available in the Russian Federation, in the form of film-coated tablets. This dosage form offers several advantages, including convenience and ease of use, as it eliminates the need for qualified personnel (comparing to parenteral forms) and avoids difficulties associated with liquid oral forms, such as difficulty swallowing (especially relevant for patients suffering from the consequences of the CVA) or allergies to excipients. Given these benefits, creating a generic version of citicoline in tablet form is a pressing matter, which requires establishment of bioequivalence between the reproduced and original drugs.

The purpose of this study was to assess the comparative pharmacokinetics (CPK) and bioequivalence of two drugs containing citicoline – GP30121 and Ceraxon® – in healthy volunteers under fasting conditions. In addition to evaluating the pharmacokinetic parameters (PK parameters) of the primary metabolite of citicoline, a comparative analysis of the data on the tolerability of the studied products (SD) was performed.

MATERIALS AND METHODS

Study Medication

The comparator product (CP) is Ceraxon®, oral tablets, film-coated, 500 mg (Ferrer Internacional S.A., Spain). The test drug (TD) is GP30121, oral tablets, film-coated, 500 mg (GEROPHARM LLC, Russia).

Design and clinical part of the study

This clinical study was conducted in compliance with the relevant regulations, including the Helsinki Declaration, ICH GCP, and local laws in the Russian Federation and EAEU. The study protocol was approved by the Ministry of Health of the Russian Federation (Permission No. 173 dated 03/31/2021) and the Ethics Council (extract from the minutes of the meeting of the Ethics Council No. 269 dated 03/23/2021). All participants signed an informed consent form before the study began.

An adaptive design (Potvin C) was employed for this study, allowing for the possibility of conducting a second part if the results of the first part failed to demonstrate 80% power and non-confirmation of bioequivalence, with consideration given to the correction of the error of the first kind ($\alpha = 0{,}0294$) [2]. The study aimed to recruit 42 volunteers, with a minimum of 38 volunteers required to complete the study accor-

[1]Neurology. National leadership. Brief edition. Available at: https://www.rosmedlib.ru/book/ISBN9785970428900.html. Accessed: 14.08.2023.

[2] Neurology. National leadership. Brief edition. Available at: https://www.rosmedlib.ru/book/ISBN9785970428900.html. Accessed: 14.08.2023.

[3] Guidelines for Management of Ischaemic Stroke and Transient Ischaemic Attack 2008. Available at: http://www.congrex-switzerland.com/fileadmin/files/2013/esostroke/pdf/ESO08_Guidelines_Original_english.pdf. Accessed: 01.09.2021.

ding to the protocol, given the CV_{intra} for the most variable index (AUC) did not exceed 29 %[1].

Inclusions and exclusions criteria were established to ensure the participation of healthy volunteers aged 18 to 45 years, men and women, non-smokers, and those without a history of alcohol or drug addiction. Volunteers with positive serological tests for infections, a burdensome allergic history, difficult-to-access veins in the upper extremities, a history of significant blood loss in the previous three months, unusual dietary habits or lifestyle, current use of medications, or consumption of choline and/or citicoline-containing supplements within 14 days of screening were excluded from the study. Pregnant and lactating women were also ineligible.

The clinical phase of the study comprised screening, two stages of drug administration, a wash-out period, and a final telephone contact. Each stage lasted 40 hours, with a seven-day wash-out period between stages (>5 $t_{1/2}$). A telephone contact was made on the eighth day after the second stage. The total duration of the study for each participant did not exceed 31 days.

Uridine, an endogenous substance with pronounced circadian fluctuations, was chosen as the analyte for evaluating the pharmacokinetics of the test drug. The lowest levels of endogenous uridine in blood plasma are reached in the afternoon, by about 15 o'clock, and its peak concentration is at midnight [3]. Therefore, at each stage of this clinical trial, on the day preceding the day of admission of the studied product, blood sampling was performed to measure basal levels of uridine according to the same rules and at the same time points as the sampling of biological samples on the day of admission of the studied product, but without direct administration of drugs. This approach aligns with modern EAEU regulatory requirements and allows for accurate assessment of the increase in analyte concentrations post-drug administration.

Volunteers were hospitalized at the research center 12 hours before the initial sampling of biological samples.

They received the test drug or a comparator product at a dose of 1000 mg (two 500 mg tablets), accompanied by 200 mL of still water at room temperature, on an empty stomach (last meal consumed at least 12 hours before drug intake).

Blood samples were collected at predetermined intervals: before (–10 min) and after 15 min, 30 min, 45 min, 1 h, 1 h 15 min, 1 h 30 min, 1 h 45 min, 2 h, 2 h 30 min, 3 h, 3 h 30 min, 4 h, 5 h, 6 h, 8 h, 12 h, and 16 h relative to the scheduled drug administration time (for example, if the drug was taken at 6:00, then blood sampling within the first day at the point "–10 minutes" was carried out at 5:50).

To minimize potential impacts on pharmacokinetics, volunteers were instructed to refrain from lying down or consuming liquids during the first four hours post-drug administration. Following this period, they were provided with food and allowed to drink fluids as needed.

Throughout each hospitalization, the researcher conducted dynamic monitoring of the volunteers, including physical examinations, measurements of body temperature, blood pressure, respiratory rate, and heart rate. Additional clinical and biochemical blood tests, as well as urine analyses, were performed at the end of stage 2 to assess drug safety.

Finally, a telephone call was made on the eighth day after drug administration to check on the volunteer's well-being.

Analytical part of the study

Blood samples were collected into 5 ml vacutainer tubes containing sodium fluoride (NaF) and potassium salt EDTA (K_2EDTA or K_3EDTA) during each study period. After 30 minutes, the samples were centrifuged under cooling conditions, and the resulting plasma was divided into two aliquots (A and B), placed into polypropylene tubes, sealed and frozen on dry ice. The plasma volume in the analyzed aliquot was at least 1.0 ml, and all samples were stored at a temperature of ≤ –70 °C.

The concentration of uridine in the blood plasma of the study participants was determined using high-performance liquid chromatography with mass spectrometric detection. The methodology was developed considering the experiences described in the available literature[2] [4].

[1] 6th World Congress on Bioavailability & Bioequivalence: BA/BE Studies Summit, August 17-19, 2015 Chicago, USA. The bioequivalence of Citicoline 500 mg film tablet. Available at: https://d2cax41o7ahm5l.cloudfront.net/cs/speaker-pdfs/onursal-saglam-novagenix-biyoanalitik-ilac-turkey.pdf]. Accessed: 05.2019.

[2] 6th World Congress on Bioavailability & Bioequivalence: BA/BE Studies Summit, August 17–19, 2015 Chicago, USA. The bioequivalence of Citicoline 500 mg film tablet. Available at: https://d2cax41o7ahm5l.cloudfront.net/cs/speaker-pdfs/onursal-saglam-novagenix-biyoanalitik-ilac-turkey.pdf. Accessed: 05.2019.

Protein precipitation with acetonitrile was used for sample preparation, followed by quantitative determination via HPLC using a triple quadrupole mass spectrometer. The methodology was validated according to the Rules for Conducting Bioequivalence Studies of Pharmaceuticals of the Eurasian Economic Union (approved by decision No. 85 of the Council of the Eurasian Economic Commission dated 11/03/2016), fulfilling the validation parameters such as selectivity, specificity, suitability of the reference standard, matrix effect, calibration curve, accuracy, precision, degree of extraction, lower limit of quantification, carryover effect, short-term stability, stability during triple freezing and defrosting, stability of initial and working standard solutions, and long-term stability of the analyte in the matrix. The validated calibration range of the procedure was 60.00–6000.00 ng/ml in blood plasma, making the procedure suitable for the analytical study of the pharmacokinetics of citicoline drugs by their active metabolite uridine.

Statistical analysis

After the bioanalytical procedures were completed, statistical analysis was conducted in a single step for each part of the clinical trials. This involved calculating pharmacokinetic parameters and performing an analysis of variance using the R Project software, version 3.5.1 (Package "bear" 2.8.3-2) and the MS Excel package. Additionally, the R Project software, version 3.6.3, was utilized for statistical analysis of the initial subject data and safety assessments.

RESULTS AND DISCUSSION

In this study, 46 volunteers were screened, as well as 4 additional subjects; 42 subjects (21 women and 21 men) were randomly assigned to one of two groups in a 1:1 ratio. Descriptive statistics for the age of volunteers and initial anthropometric characteristics are presented in Table 1.

The volunteers were either given the studied product GP30121 or the comparator product Ceraxon®. No adverse events were reported during the study, and the individual values of safety profiles for all volunteers were within the physiological norm.

The analytical part of the study found that the changes in the concentration of uridine in the blood plasma of volunteers before taking the studied product approximately coincided with those described in the literature (Figure 1). The minimum concentrations of endogenous uridine were documented at 12:00, which is earlier than in the literature data (about 15:00), however, it is necessary to take into account the early awakening of volunteers on the day of sampling of biological samples (about 05:00). When constructing the uridine concentration profile after taking the studied products without correcting for the basal level of the analyte, the contribution of endogenous uridine to this profile was obvious, and the concentration varied according to the time of day. This made it impossible to calculate pharmacokinetic parameters to assess bioequivalence. In particular, higher concentrations of uridine were observed at the moment before taking the drug compared to the time point "15 minutes" after taking the drug, which corresponded to the shape of the curve of endogenous uridine and was leveled when its concentration was accounted for (the minimum concentration of uridine was observed at the point "–10 minutes") (Figure 1).

Table 1. Demographic and anthropometric information about subjects randomized to the study. Descriptive statistics (*N* = 42)

	Age (years)	Body weight, kg	Height, sm	BMI, kg/m²
Mean	33.7	71.9	173.0	23.9
SD	6.8	9.3	6.2	2.0
Median	33.5	73.7	172.0	24.1
Min Max	18.0 45.0	54.5 89.2	160.0 189.0	20.4 27.5

After correcting for the basal level of the analyte, the concentration of uridine in the blood plasma of volunteers did not exceed 3368.19 ng/ml.

One subject had an AUC_{0-t} uridine index after taking the comparator product that was less than 5 % of the geometric mean AUC_{0-t} uridine after taking the comparator product (it was 259.39 ng/ml, with the 5 % of Geom Mean = 278.96 ng/ml). Therefore, this subject was excluded from the analysis of pharmacokinetics and bioequivalence assessment.

The following pharmacokinetic parameters were calculated for each volunteer based on the dependence of uridine concentrations in blood plasma on time: AUC_{0-t}, C_{max}, t_{max}, $t_{1/2}$, K_{el} и $AUC_{0-\infty}$ (Table 2).

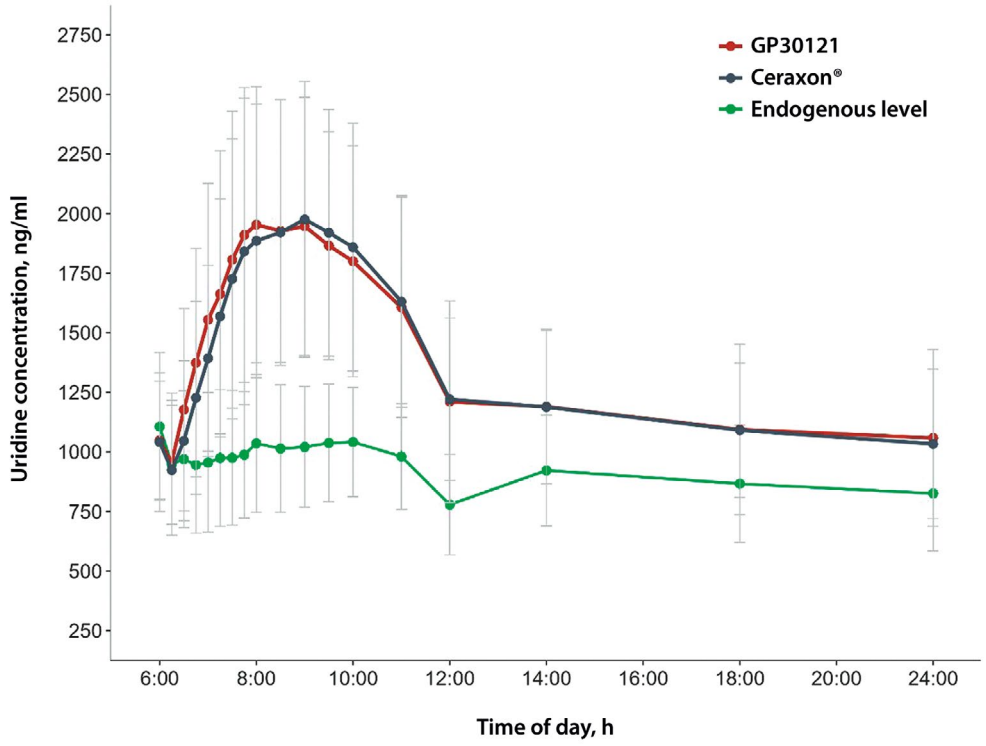

Figure 1. Uridine plasma concentration during both periods after administration of GP30121 and Ceraxon® in comparison with the level of endogenous uridine depending on the time of the day. Blue – PK profile of the main metabolite of Ceraxon®, red – PK profile of the main metabolite of GP30121, green – concentration of endogenous uridine

Table 2. Descriptive statistics for pharmacokinetic parameters of study drugs (*N* = 41)

Parameter									
	C_{max}, ng/mL	AUC_{0-t}, ng · h/mL	$AUC_{0-\infty}$, ng · h/mL	$ln(C_{max})$	$ln(AUC_{0-t})$	$ln(AUC_{0-\infty})$	K_{el}, h^{-1}	t_{max}, h	$t_{1/2}$, h
Ceraxon®									
Mean	1198.78	6745.04	7950.27	6.99	8.68	8.83	0.347	2.41	3.63
Geom Mean	1088.77	5891.42	6844.79	6.98	8.66	8.81	0.259	2.14	2.68
Median	1194.02	6161.66	6856.96	7.09	8.73	8.83	0.241	2	2.88
SD	511.30	3278.29	4018.43	0.46	0.58	0.62	0.271	1.30	3.05
CV, %	42.65	48.60	50.54	6.61	6.65	7.03	78.04	53.86	84.23
Min	323.31	740.19	741.56	5.78	6.61	6.61	0.054	1	0.63
Max	2599.65	15026.56	19683.34	7.86	9.62	9.89	1.110	6	12.78
GP30121									
Mean	1198.78	6745.04	7950.27	6.99	8.68	8.83	0.347	2.41	3.63
Geom Mean	1088.77	5891.42	6844.79	6.98	8.66	8.81	0.259	2.14	2.68
Median	1194.02	6161.66	6856.96	7.09	8.73	8.83	0.241	2	2.88
SD	511.30	3278.29	4018.43	0.46	0.58	0.62	0.271	1.30	3.05
CV, %	42.65	48.60	50.54	6.61	6.65	7.03	78.04	53.86	84.23
Min	323.31	740.19	741.56	5.78	6.61	6.61	0.054	1	0.63
Max	2599.65	15026.56	19683.34	7.86	9.62	9.89	1.110	6	12.78

Figure 2 shows the average pharmacokinetic profiles of uridine after correction for its endogenous level in the blood plasma of volunteers after taking the comparator product and the studied product. The profiles have the same shapes.

Due to the fact that the study used an adaptive design with a consistent approach according to Potvin, type C, based on data obtained from 41 subjects, bioequivalence was assessed at α = 0,05 and standard 90 % confidence intervals (CI), and power calculation was performed. The values of the calculated 90 % CI for the ratio of geometric averages of the main pharmacokynetic parameters of the primary metabolite of the studied product's active substance fell within the acceptance window of 80.00–125.00 % (Table 3).

However, when evaluating the power of the study, taking into account the coefficients of intraindividual variation of the pharmacokinetic parameters C_{max} (CV_{intra} = 31,21 %) and AUC_{0-t} (CV_{intra} = 31,27 %), it was found that the power of the study for the pharmacokinetic parameters C_{max} and AUC_{0-t} was less than 80 % (C_{max} = 79.64 %, AUC_{0-t} – 79.51 %). Therefore, further bioequivalence analysis and hypothesis evaluation were performed at a significance level of α = 0.0294 and a CI value of 94.12 % for AUC_{0-t} and C_{max} uridine.

The results showed that the values of 94.12 % CI at α = 0,0294 for the ratio of geometric averages of the main pharmacokynetic parameters of the primary metabolite of the studied product's active substance fell within the acceptance window of 80.00–125.00 % (Table 3).

Table 3. **Calculated confidence intervals for uridine AUC_{0-t} and C_{max}**

Parameter	Ratio of geometric means, %	Lower limit, %	Upper limit, %	Valid values, %
90 % confidence interval				
AUC_{0-t}	105.41	94.08	118.10	80–125
C_{max}	106.83	95.37	119.67	80–125
94.12 % confidence interval				
AUC_{0-t}	105.41	92.43	120.20	80–125
C_{max}	106.83	93.70	121.79	80–125

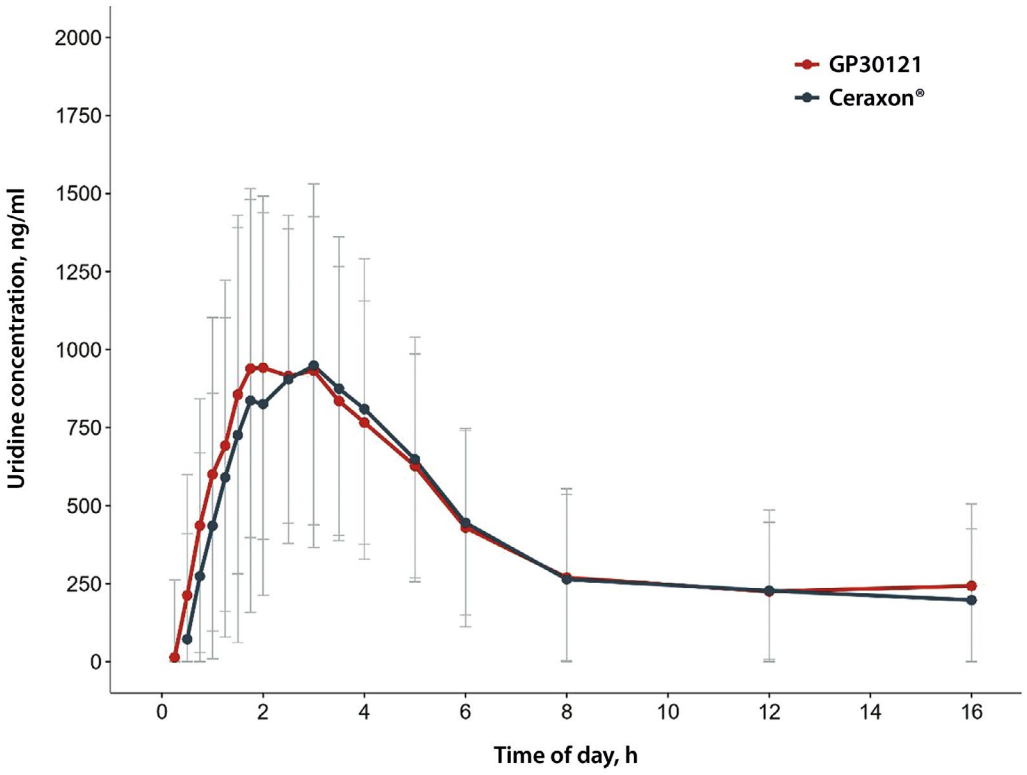

Figure 2. **Arithmetic mean pharmacokinetic profile on linear scale of uridine. Mean ± SD (*N* = 41). *Y*-axis – uridine plasma concentration (ng/mL), *X*-axis – time after administration of either GP30121 or Ceraxon®. Blue – PK profile of the main metabolite of Ceraxon®, red – PK profile of the main metabolite of GP30121**

GP30121 (GEROPHARM LLC, Russia) and Ceraxon® (Ferrer Internacional S.A., Spain), both film-coated tablets containing 500mg of citicoline, have been found to be bioequivalent.

Citicoline, a naturally occurring substance, plays a vital role in various physiological processes, including cell membrane synthesis and acetylcholine production. Its biological value is primarily attributed to its constituent choline, which serves as an intermediate in the synthesis of structural phospholipids and acetylcholine. Cytidine, another metabolite of citicoline, is converted to uridine and participates in the synthesis of DNA, RNA, and phospholipids. However, assessing the pharmacokinetics of citicoline can be challenging due to its rapid metabolism and the presence of endogenous compounds. When ingested orally, citicoline is quickly metabolized into choline and cytidine, followed by the rapid deamination of cytidine to uridine. This process introduces difficulties in evaluating drug concentrations and calculating pharmacokinetic parameters, as the baseline levels of choline and uridine exhibit daily fluctuations in accordance with the circadian rhythm of substance secretion. These fluctuations can significantly impact the accuracy of drug concentration assessments.

To address these challenges, various scientific approaches have been developed for bioequivalence studies of drugs with endogenous metabolites. These approaches aim to account for the presence of endogenous compounds and ensure accurate assessments of drug concentrations [5].

In the Russian Federation, there are no rigid requirements for bioequivalence studies of endogenous compound analogues. However, guidelines provided in the Examination of Medicines manual align with European recommendations. Researchers are advised to measure analogue concentrations in a way so that the background level of the endogenous analyte is taken into consideration. This allows pharmacokinetic parameters to reflect additional concentrations achieved through drug intake. Standard subtraction methods, such as deducting the average pre-dose endogenous substance concentration or average AUC, are preferred.

When the drug-induced increase in endogenous substance concentration surpasses the baseline substantially, correcting for the background content may become unnecessary [6]. Researchers can utilize supratherapeutic doses of the studied drugs if needed to reliably measure concentrations exceeding the baseline [6]. To confirm the absence of differences in analyte concentrations at varying doses, a pilot study or a specific stage of the bioequivalence study can be conducted.

In foreign studies, it is recommended to determine the endogenous concentration of the analyte in the blood before each stage of the study and subtract it from the total concentration of the measured compound. If the subtraction yields a negative value, it should be considered 0 when calculating the adjusted AUC. Additionally, statistical analysis of the obtained data is recommended for both adjusted and non-adjusted concentrations of the studied compound, taking into account the background value[1].

Numerous studies have investigated the bioequivalence of endogenous compound analogues, employing diverse approaches to study design and data processing. According to both Russian [5] and foreign researchers [7], no single standard approach exists for studying endogenous compounds with a specific baseline concentration. Nevertheless, it can be assumed that research on drugs like citicoline should at least incorporate the following elements:

a) volunteers should be standardized as much as possible according to demographic, anthropometric, and dietary factors;
b) the duration and sampling points for biological samples to determine baseline concentrations should match those taken after ingestion of the studied drugs.
c) correction methods for baseline concentrations of compounds should be employed if the drug-induced increase in concentration is minor and does not significantly exceed the baseline level.

While adjusting to the basal level of the analyte is not universal in citicoline bioequivalence studies [4], this approach was adopted in this study, as it is grounded in Russian and international recommendations.

Studies have reported changes in choline and cytidine concentrations after administering 2000 mg of citicoline [8]. Following drug intake, the average choline concentration increased by 48 %, while cytidine levels rose by 136 % after two hours. Another study revealed that taking 500, 2000, and 4000 mg of citicoline resulted in uridine concentrations of 101, 136, and 134 % compared with basal levels, respectively, and

[1] Bioequivalence Studies With Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA Guidance for Industry, FDA, 2021. Available at: https://www.fda.gov/media/87219/download. Accessed: 10.2022.

choline concentrations of 23, 32, and 43 %, respectively [9]. Uridine has also been employed as an analyte in bioequivalence studies[1] [4], supporting its selection as an indicator metabolite for assessing the pharmacokinetics of the studied drug.

Previous research findings were considered when selecting the drug dose. For instance, taking 2000 mg of citicoline yielded the highest uridine concentrations relative to basal levels [13]. Currently, 2000 mg is considered to be the maximum therapeutic dose. Notably, a study using a similar approach showed that adjusting for basal uridine levels after administering 500 mg of citicoline resulted in peak uridine concentrations of 600–800 ng/ml[2], implying the need for an analytical method with a lower limit of quantitation (LLOQ) around 30 ng/ml (5 % of C_{max}). Considering these factors, a dose of 1000 mg was chosen for the study, as it provided an optimal balance between maximizing the difference between post-drug and basal uridine levels while minimizing potential adverse effects.

Only one study[3] provided literature data on the variations of key indicators (AUC and C_{max}) required for calculating the number of subjects needed for bioequivalence studies. β-type errors may occur when an insufficient number of subjects are included in the study, leading to the failure to confirm bioequivalence even when it exists. Performing two studies, including a pilot study, can address this issue but would increase the time and cost of the research process. Therefore, an adaptive design was chosen for this study, specifically the Potvin C algorithm, which allows for a more efficient and accurate evaluation of bioequivalence. This approach involves a two-stage study design. The first stage includes a small group of volunteers, the number of whom is calculated based on available literature data or assumptions of low drug variability (less than 30 %) can be made . After the first stage, an interim analysis is conducted to determine the necessary additional number of volunteers. Finally, a combined sample analysis is performed to conclude on bioequivalence. This approach applies to a wide range of group sequential methods with the possibility of early termination of the study, both in terms of demonstrating effectiveness and uselessness [10]. Adaptive designs like Potvin C offer several advantages, including reduced sample sizes and shorter study durations, which can accelerate the approval of safe and effective treatments[4].

However, one limitation of adaptive designs is the potential introduction of statistical errors due to inadequate control of the first-kind error rate. As the null hypothesis is tested twice, the likelihood of accidental refutation increases. To mitigate this issue, the S. Pocock approach can be employed, which reduces the α level from 0.05 to 0.0294 and narrows the scope of denial of the null hypothesis [11]. This approach is considered in various design algorithms, including Potvin C [2].

In summary, the Potvin C algorithm was selected for this study as it balances the need to reduce the α level with the requirement to test bioequivalence using standard 90 % confidence intervals [2]. While the study did not encounter issues related to completing the study prematurely, larger sample sizes may be necessary for drugs with higher variability [12].

CONCLUSION

This study successfully demonstrated the bioequivalence of drugs whose metabolites are produced within the human body, despite the lack of research in this area and limited literature data to guide sample size estimation. This achievement paves the way for the registration of the reproduced drug, as bioequivalence is a crucial prerequisite.

Notably, the novel approach utilized in this study enabled the establishment of comparable bioavailability between the studied drug and the comparator product, thereby meeting the stringent requirements set forth by regulatory agencies. This methodology can now be applied to the study of other precursor drugs of endogenous compounds.

[1] 6th World Congress on Bioavailability & Bioequivalence: BA/BE Studies Summit, August 17-19, 2015 Chicago, USA. The bioequivalence of Citicoline 500 mg film tablet. Available at: https://d2cax41o7ahm5l.cloudfront.net/cs/speaker-pdfs/onursal-saglam-novagenix-biyoanalitik-ilac-turkey.pdf. Accessed: 05.2019.

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