ОЦЕНКА ЭКВИВАЛЕНТНОСТИ БИОАНАЛОГА ИНСУЛИН ЛИЗПРО ДВУХФАЗНЫЙ 25 (ОАО «ГЕРОФАРМ-БИО», РОССИЯ) И ХУМАЛОГ® МИКС 25 («ЛИЛЛИ ФРАНС», ФРАНЦИЯ) С ИСПОЛЬЗОВАНИЕМ МЕТОДА ЭУГЛИКЕМИЧЕСКОГО ГИПЕРИНСУЛИНЕМИЧЕСКОГО КЛЭМПА НА ЗДОРОВЫХ ДОБРОВОЛЬЦАХ

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ОБОСНОВАНИЕ. Современная медицина требует использования эффективных противодиабетических препаратов, способных имитировать естественный профиль инсулина в организме больных сахарным диабетом. К подобным препаратам относится двухфазный инсулин лизпро – смесь инсулина лизпро ультракороткого действия с суспензией инсулина лизпро протамина, обладающей пролонгированным эффектом. В программу клинических исследований (КИ) биоаналогов инсулина входят исследования фармакологии: фармакокинетики (ФК), фармакодинамики (ФД), а также исследование клинической безопасности.

ЦЕЛЬ. Продемонстрировать, что Инсулин Лизпро двухфазный 25, суспензия для подкожного введения, 100 МЕ/мл (ОАО «Герофарм-Био», Россия) и Хумалог® Микс 25, суспензия для подкожного введения, 100 МЕ/мл («Лилли Франс», Франция) имеют сопоставимые фармакокинетические профили в условиях эугликемического гиперинсулинемического клэмпа (ГЭК) на здоровых добровольцах.

МЕТОДЫ. Исследование было проведено на 48 здоровых мужчинах в возрасте от 18 до 50 лет. В качестве дизайна исследования выбрано двойное слепое рандомизированное перекрестное исследование сравнительной ФК препаратов. Исследуемые препараты (ИП) вводили перед ГЭК в дозе 0,4 МЕ/кг однократно подкожно в область подкожно-жировой клетчатки передней брюшной стенки живота. В течение исследования проводили регулярный забор крови, в образцах определяли количество инсулина методом иммуноферментного анализа (ИФА). Результаты определения использованы для расчета ФК-параметров и построения кривых «концентрация–время». На основании измерения гликемии корректировали скорость инфузии глюкозы (СИГ). Эти данные использованы для расчета ФД-параметров.

РЕЗУЛЬТАТЫ. В ходе проведенного исследования было выявлено, что в условиях ГЭК на здоровых добровольцах препараты Инсулин Лизпро двухфазный 25 и Хумалог® Микс 25 имеют сопоставимые ФК- и ФД-профили. Доверительный интервал для логарифмически преобразованного отношения значений параметра $C_{ins.max}$ 87,75–99,90%, а $AUC_{ins.0–12}$ – 83,76–96,98%, которые попадают в заданные границы сопоставимости 80–125%.

ЗАКЛЮЧЕНИЕ. На основании проведенного клинического исследования ИП с использованием метода ГЭК на здоровых добровольцах препараты Инсулин Лизпро двухфазный 25 и Хумалог® Микс 25 являются эквивалентными.

КЛЮЧЕВЫЕ СЛОВА: инсулин лизпро; биосимиляр; клиническое исследование; фармакокинетика; фармакодинамика; сопоставимость; эугликемический гиперинсулинемический клэмп

ASSESSMENT THE EQUIVALENCE OF THE BIOANALOGUE INSULIN LIZPRO BIPHASIC 25 (GEROPHARM-BIO, RUSSIA) AND HUMALOG® MIX 25 (LILLY FRANCE, FRANCE) USING THE EUGLYCEMIC HYPERINSULINUM CLAMP METHOD ON HEALTHY VOLONTERS

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BACKGROUND: Modern medicine requires use of effective antidiabetic drugs that can imitate the natural profile of insulin in the body of patients with diabetes mellitus. Examples of such preparations include biphasic insulin lispro, which is a mixture of insulin lispro ultra-short action and insulin lispro protamine suspension with prolonged effect. The clinical trials (CT) program for biosimilar insulins contains pharmacology studies: pharmacokinetics (PK), pharmacodynamics (PD) and clinical safety studies.

AIMS: To demonstrate Biphasic Insulin Lispro 25, suspension for subcutaneous administration, 100 U/ml (GEROPHARM-Bio, Russia) and Humalog® Mix 25, suspension for subcutaneous administration, 100 U/ml (Lilly France, France) have comparable pharmacokinetic profiles under conditions of hyperinsulinemic euglycemic clamp (HEC) in healthy volunteers.

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MATERIALS AND METHODS: The study was conducted on 48 healthy men aged between 18 to 50 years. This was a double-blind, randomized, crossover study of comparative pharmacokinetics of drugs. The investigational products (IP) were administered before the clamp in a single dose of 0.4 U/kg subcutaneously in the abdominal wall. Regular blood sampling was performed during the study. The insulin concentrations in the samples were determined using an ELISA method. The results of the determination were used to calculate the PK parameters and construct the concentration-time curves. Adjust glucose infusion rates were based on blood glucose measurements. These data were used to calculate the PD parameters.

RESULTS: Our results demonstrated that Biphasic Insulin Lispro 25 and Humalog® Mix 25 have comparable PK and PD profiles under conditions of HEC in healthy volunteers. The confidence intervals for the ratio of the geometric mean for $C_{ins.max}$ and $AUC_{ins.0–12}$ were 87.75–99.90% and 83.76–96.98% respectively, which were well within 80–125% limits for establishing comparability.

CONCLUSIONS: Biphasic Insulin Lispro 25 and Humalog® Mix 25 are equivalent based on this CT applying the HEC technique in healthy volunteers.

KEYWORDS: Insulin lispro; biosimilar; clinical trials; pharmacokinetics; pharmacodynamics; comparability; hyperinsulinemic euglycemic clamp

Natural and recombinant human insulin types are no longer able to meet the needs of modern medicine to the full extent, which requires the use of up-to-date antidiabetic drugs, such as analogues of human short-acting and long-acting insulin [1–3]. This is due to the fact that in healthy people, the peaks of insulin release are related directly to food intake, while between meals the endogenous insulin decreases to the basal level. In diabetic patients, this profile can best be imitated by the use of long-acting insulin combined with rapid- or short-acting insulin [1, 4]. Such mixtures include biphasic insulin lispro or lispro mix (a mixture of rapid-acting insulin lispro with a suspension of insulin protamine lispro, which represents the prolonged action).

Insulin lispro is the first developed and produced insulin analogue [5]. Its molecular structure is identical to that of human insulin, except for positions 28 and 29 of the β-chain of the molecule where lysine and proline are arranged in reverse order. Due to this reversed arrangement, the lispro molecule dissociates twice as fast. As a result, the transition to the active form occurs two times faster than a similar process in recombinant insulins.

Using a mixture of short- and long-acting insulin results in a two-phase activity curve of the drug, which is maximum proximate to the natural insulin, simulating postprandial activity peaks and basal activity of insulin [6]. Biphasic insulin lispro is used in two basic versions, namely 25% insulin lispro + 75% insulin protamine lispro suspension (Humalog® Mix 25; Lilly France, Neuilly-sur-Seine, France) and 50% insulin lispro + 50% insulin protamine lispro suspension (Humalog® Mix 50).

The development of biotechnology has led not only to the emergence of new biological products able to reduce significantly some of the previously incurable diseases, but also their bioanalogues or biosimilars. A bioanalogue is a biological drug similar in terms of quality, efficacy and safety to a reference biological drug in the same dosage form and has an identical administration route [7].

Over the past few years, clinical studies have been performed, and other insulin bioanalogues have been introduced to the market [8]. The first insulin biosimilar registered by the European Medical Agency in September 2014, was ABASAGLAR®, which contains insulin glargine as an active pharmaceutical substance [9]. In January 2017, another bioanalogue of glargine, Lusduna, was approved [10]. Also, biosimilars of insulin analogues were introduced to local markets. In particular, in September 2017, the Ministry of Health of the Russian Federation registered the first insulin analogue glargine manufactured by Gan and Lee Pharmaceuticals (Beijing, China).

Currently, the rules for assessing the quality, compliance with the original (reference) drugs for efficacy and safety are regulated in accordance with the national procedures and guidelines of the Eurasian Economic Union, the requirements of which were considered when conducting this study [11–18].

According to modern requirements, the insulin biosimilars programme is a complex multistage process. At the first stage, the absence of differences in the physicochemical properties of the insulin developed and the original preparation must be proven. The composition; physical properties; primary, secondary and tertiary structure; related compounds and impurities; industrial impurities; N- and C-terminal sequences; free SH-groups and disulfide bridges and so forth are studied. In the second stage, pharmacodynamic studies are conducted in vitro, including binding to insulin receptors (including on-off kinetics) and biological activity, namely receptor autophosphorylation and metabolic activity. Methods of glycogen formation, lipogenesis, inhibition of stimulated lipolysis, glucose transport and so forth are used to study metabolic activity. At stage 3, insulin biosimilars in humans are studied in clinical trials. Only those drugs demonstrating their identity in the previous stages are used in clinical studies, as was shown for the biosimilar biphasic insulin lispro 25 (Geropharm-bio, St. Petersburg, Russia).

The clinical studies programme of insulin bioanalogues includes pharmacology studies, such as a double-blind study of pharmacokinetics (PK) and pharmacodynamics (PD), and a clinical safety study with an emphasis on immunogenicity. The clinical safety of the study drug is currently underway, and therefore, it is not specified in this report.

To study the pharmacologic properties of insulin, PK and PD, in accordance with these recommendations [11, 18, 19], the glucose clamp technique (GCT) is used. GCT is the best available method to determine the action

of insulin and is the 'gold standard' for studying the pharmacodynamic properties of antidiabetic drugs [20–22]. From the viewpoint of studying insulin biosimilars, GCT has a core role in the clinical studies programme due to the fact that a comparative study of PK/PD properties using GCT is highly sensitive to detect differences between the original drug and its biosimilars [11, 12].

According to the Guide, this study is performed on healthy volunteers or patients with type 1 diabetes mellitus [17]. During GCT, PK and PD are studied simultaneously, which enables the study of concentration–time dependence (area under the curve [AUC]) and glucose infusion rate (GIR) time (effect-time, AUCGIR) on the same population in the same study. Additionally, the maximum concentration (Cmax) and the speed of its achievement (tmax), as well as the time of onset of insulin action (tGIRlag), time to achieve maximum effect (GIRmax) and time of its manifestation (tGIRmax) are assessed. The specified PK and PD indicators enable researchers to comprehensively characterise the metabolism, degree and rate of hypoglycaemic action and to make a conclusion about the similarity or difference between the two types of insulin under study.

The obtained data on the comparative pharmacology of the test (TD) and reference (RD) drugs can lend evidence of their clinical comparability due to the fact that GIR is an accepted surrogate marker that measures directly the effect of insulin, which consists in the disposal of glucose administered exogenously [21]. It correlates with the outcome in patients to such an extent that the confirmation of a similar effect on the PD marker will provide a similar effect on the clinical outcome [11, 12, 17]. This means that there is no need to conduct separate efficacy studies when investigating insulin biosimilarity, since the endpoints analysed in these studies (usually haemoglobin A1c [HbA_{1c}]) are not considered sensitive enough to identify potential clinically significant differences between the two types of insulin [11, 12, 17].

AIM

We demonstrated that biphasic insulin lispro 25, suspension for subcutaneous administration, 100 IU/mL (Geropharm-bio) and Humalog® Mix 25, suspension for subcutaneous administration and 100 IU/mL (Lilly France) have comparable pharmacokinetic profiles under GCT conditions on healthy volunteers.

METHODS

Study design

The study was designed as a double-blind, randomised, crossover study of comparative PK of the TD biphasic insulin lispro 25 and the RD (RD ПС) Humalog® Mix 25

Inclusion criteria

In accordance with regulatory recommendations [11, 17], the study was conducted on male Caucasian race volunteers aged 18 to 50 years (inclusive), with a body mass index (BMI) of 18.5–27 kg/m^2, proved to be healthy according to standard clinical, laboratory and instrumental methods of examination. Main exclusion criteria were a history of hypoglycaemic episodes or a family history of a verified diagnosis of diabetes mellitus (DM) in the next of kin; fasting plasma glucose level exceeding 6.1 mmol/l; HbA_{1c} level >6%; blood glucose level ≥7.8 mmol/l according to oral glucose tolerance test (2 hours after glucose loading).

Conditions of the study

The study was conducted at two clinical centre, the National Medical Research Centre of Endocrinology (Moscow, Russia) and the company BioEc (St. Petersburg, Russia). Healthy volunteers from a database of volunteers of research centres participated in the study.

Duration of the study

The study duration for each volunteer did not exceed 43 days. The total study duration was 5 months (from April 10 to 7 September 2017).

Description of medical intervention

In this study, each volunteer made five visits to the investigational site.

Visit 1 – screening

At this visit, a medical history was taken. Standard clinical and biochemical blood tests, urinalysis and physical examination were performed. BMI and vital signs (blood pressure, heart rate, respiratory rate) were analysed. Based on the results and conformity with criteria, the medical investigator determined the volunteer's ability to take part in the study.

Visits 2 and 4 – GCT

Volunteers who successfully passed the screening (brief screening) were allowed into GCT study periods. In period I (visit 2), volunteers were randomised into one of the two study groups, except for the following: periods I (visit 2) and II (visit 4) proceeded in a similar manner. On the eve of GCT, the volunteers were hospitalised at the clinical centre. The last food intake was no later than 19.00 to ensure that the research procedures were performed on an empty stomach with a fasting period of at least 12 hours before the injection of insulin preparation (IP).

In the morning before the start of the GCT procedures, an examination was conducted as part of the study of IP safety according to protocol. Blood samples also were obtained for PK and basal blood glucose was determined.

Approximately 60 minutes before the planned IP administration, the participants were placed in a horizontal position. Preparations were performed for the GCT procedure with the insertion of intravenous catheters and lines for infusions into the ulnar vein of one hand and wrist vein of the other hand. Plasma glucose concentration was monitored. If the plasma glucose level corresponded to the target range (4.4–5.6 mmol/l) for 1 hour before IP injection, such a volunteer underwent the GCT procedure. If the plasma glucose level was beyond these limits, the investigator could reschedule the GCT for this participant to another day.

To reduce the possible bias of the investigator, the IP was delivered to the clinical centre in identical

packages to an unblinded team whose main responsibility, among other things, was preparing the IP before administration to the study subject. Preparation was performed for a certain time before injection in accordance with the instructions provided. After that, the IP in the insulin syringe was transferred to the blinded team for the injection. IP was administered subcutaneously immediately before the GCT at a dose of 0.4 IU/kg once in the adipose tissue of the anterior abdominal wall.

After IP injection, plasma glucose levels were monitored. The onset of IP action is believed to manifest by a decrease in the blood glucose level by >5% of the initial value. When recording the IP onset, a controlled infusion of glucose solution was started to maintain target plasma glucose levels of 4.4–5.6 mmol/L (80–100 mg/dL). GIR was monitored and corrected every 5 minutes during the first eight hours, and every 15 minutes from eight to 14 hours.

Visit 3 – brief screening

The visit occurred before period II of the GCT (visit 4), to confirm the correspondence of the volunteer to the criteria for continuing the study. The procedures were similar to those of visit 1.

Visit 5 – final safety visit

At this visit, standard clinical and biochemical blood tests, urinalysis, physical examination, BMI assessment and measurement of vital signs (blood pressure, heart rate, respiratory rate) were performed.

Primary study outcome

The primary endpoints were the pharmacokinetic parameters of the studied drugs, namely the total AUC of insulin concentration under study –the time interval from 0–12 hours (AUCins.0–12), and the maximum concentration of blood insulin during the observation period (Cins.max).

Additional study outcomes

The secondary endpoints included the pharmacokinetic parameters of the studied drugs, namely the total AUC of insulin concentration under study –the time interval up to 2, 6 and 14 hours, represented by (1) $AUC_{ins.0–2}$, (2) $AUC_{ins.0–6}$, (3) $AUC_{ins.0–14}$ and (4) $AUC_{ins.0–\infty}$, respectively; time-to-peak concentration of insulin (t_{max}) (5); insulin half-life ($t_{1/2}$) (6); as well as the pharmacodynamic parameters, such as total AUC GIR-time from 0–12 hours ($AUC_{GIR0–12}$) (7); that ≤14 hours ($AUC_{GIR0–14}$) (8); partial AUC ($AUC_{GIR0–2}$) (9); $AUC_{GIR0–6}$ (10); maximum GIR for the study period (GIR_{max}) (11); time-to-peak GIR of glucose ($tGIR_{max}$) (12) and time between IP administration and the start of glucose infusion ($tGIR_{lag}$) (13).

Safety assessment

The safety assessment criteria included: (1) the frequency and severity of adverse events (AEs); (2) abnormalities of vital signs, namely blood pressure, heart rate, respiratory rate and body temperature; (3) frequency of local reactions at the injection site; (4) changes in blood potassium levels and (5) abnormalities in laboratory values and electrocardiography (ECG).

Analysis in subgroups

The results were analysed based on the data obtained immediately after intake of the TD and RD, the groups of biphasic insulin lispro 25 and Humalog® Mix 25, respectively.

Each volunteer received TD and RD (in different GCT periods). To eliminate bias and other factors affecting the data obtained, as well as the formation of homogeneous subgroups, volunteers were randomised in a 1:1 ratio after screening. Randomisation was performed directly at the clinical centres using the envelope method. The first subgroup received TD during the first period and RD during the second period. The second subgroup, on the contrary, received RD during the first period and TD during the second period. The order of the periods was unknown to the volunteer and investigators.

Methods of outcome registration

Pharmacokinetics

To obtain primary data on PK, blood was sampled to determine concentration of endogenous insulin and lispro insulin 30 minutes immediately before and after IP administration according to the following scheme: up to the 6-hour point, sampling was performed every 15 minutes, while up to the 14-hour point sampling was performed every 30 minutes. The total duration of observation was 14 hours, but in accordance with accepted standards [11, 12, 17], the data were analysed within the dosing interval, which was 12 hours for IP.

Quantitative determination of insulin (endogenous and lispro) was conducted at the analytical laboratory of the company KAYAR using the enzyme-linked immunosorbent assay according to a previously validated method. Transportation from the research centre was performed in compliance with the cold chain no higher than –20°C. The analysis was performed on an automated enzyme-immunoassay analyser Personal Lab (Adaltis S.r.l., Rome, Italy).

Pharmacodynamics

Quantitative blood glucose determinations during the GCT period were conducted in samples of whole venous blood using a glucometer StatStrip Glucose and β-Ketone Hospital Meter (Nova Biomedical, Waltham, MA, USA). Glucometers were calibrated by plasma [23, 24].

Ethical considerations

Before the start of the research procedures, each volunteer signed an informed consent. The study was conducted in accordance with the Declaration of Helsinki of the World Medical Association, as well as the principles of good clinical practice and local regulatory requirements. The study protocol was approved by the Ministry of Health of the Russian Federation (resolution No. 556 dated 2 October 2015), as well as by independent ethical committees at clinical centres of the National Medical Research Centre of Endocrinology (extract from protocol No. 08 dated 26 April 2017) and the company

BioEc (extract from the protocol (without number) dated 5 April 2017).

Statistical analysis

Principles of sample size calculation

Because the primary study aim was a comparison of pharmacokinetic properties of biphasic insulin lispro 25 and Humalog® Mix 25 in healthy volunteers, data on the mean value and standard deviation of the primary FK indicators of $AUC_{ins.0-T}$ and $C_{ins.max}$ were used to calculate the sample size [25]. Sample size calculation was performed for a more variable indicator.

Methods of statistical data analysis

Statistical data processing and the presentation of the results were performed using software packages R 3.4.2. The AUC was calculated using the trapezoidal method.

The primary PK parameters $C_{ins.max}$ and $AUC_{ins.0–12}$ were analysed assuming a log-normal distribution of the indicators. After a logarithmic transformation (based on the natural logarithm), these indicators were analysed using an analysis of variance (ANOVA) employing the general linear model. The ANOVA model included factors, such as drug administration sequence, volunteer (included in the sequence), study period and drug as sources of variation. The estimated residual variation obtained was used in calculating the 90% confidence intervals (CI) for the ratio of the geometric mean PK parameters $C_{ins.max}$ and $AUC_{ins.0–12}$ of the TD (biphasic insulin lispro 25) to the RD (Humalog® Mix 25). Comparability was considered proven if 90% of CI were within 80%–125% [12, 17].

Additionally, 95% CI was calculated for the ratio of the geometric mean PD parameters of GIRmax and $AUC_{GIR0–12}$ to search for differences between the IPs. Comparability was considered proven if 95% CI was within 80%–125% [11, 12, 17].

Secondary PK-parameters ($AUC_{ins.0–2}$, $AUC_{ins.0–6}$, $AUC_{ins.0–14}$, $AUC_{ins.0–\infty}$, $t_{1/2}$) and PD-parameters (GIR_{max}, $AUC_{GIR0–12}$, $AUC_{GIR0–14}$, $tGIR_{max}$, $tGIR_{lag}$) were also analysed using ANOVA, while the parameter tmax was analysed using the Wilcoxon nonparametric paired 2-tailed test.

The AE data were analysed using Pearson χ^2 test.

RESULTS

Objects (participants) of the study

After screening, 48 healthy volunteers who met the inclusion/exclusion criteria were enrolled in the study (Table 1): 45 were included in the data analysis, one was withdrawn from the study early due to withdrawal of the informed consent, and another two were excluded, because the insulin concentration before IP injection was >5% of $C_{ins.max}$. This is consistent with the rules for conducting research on the bioequivalence of drugs, which regulate the exclusion from statistical analysis of information obtained from subjects if the concentration before the drug intake is >5% of C_{max} [26].

In this study, endogenous insulin production was not inhibited completely due to the fact that, while maintaining blood glucose level in the target range (4.4–5.6 mmol/l), the peak endogenous insulin production, significant for assessment of PK and PD parameters, did not occur. Also, the insulin was assessed using an insulin-specific lispro method, which enabled to separate it from the concentration of endogenous insulin.

To confirm the absence of endogenous insulin production, its blood dynamics were analysed for each volunteer (Fig. 1). This indicated the absence of peak endogenous insulin production in response to intravenous administration of glucose solution, thereby confirming the satisfactory quality of the GCT.

Primary study outcome

Figure 2 and Table 2 show the averaged concentration–time pharmacokinetic curves of the TD and RD in the blood plasma of the volunteers. The comparability of the main PK characteristics is noted. Thus, C_{max} was 238.75 ± 64.30 and 256.61 ± 69.79 pmol/l, and $AUC_{0–12}$ was 1214.74 ± 375.89 and 1347.28 ± 396.74 (pmol/l) × h, respectively. A statistically significant difference in the $AUC_{0–12}$ indices was revealed. However, due to the fitting of this parameter in bioequivalence intervals of 0.90 [83.76, 96.98] with the allowability of limits of 80%–125%, these statistically significant differences can be considered clinically insignificant. These data, together with the character of the concentration–time curves, indicated the comparability of the IP PK.

Additional results of the study

Figure 3 and Table 3 show the GIR-time averaged pharmacodynamic curves. The comparability of the parameters of action of the test and reference insulins in volunteers was recorded. Thus, the time between administration of IP and the start of glucose infusion (t_{GIRlag}) was 29.59 ± 13.94 and 27.68 ± 13.24 minutes, respectively. Also time-to-peak glucose infusion rate (t_{GIRmax}), that is the time of onset of the maximum effect of insulin under study and maximum GIR (GIR_{max}), which is the maximum effect itself, were comparable. $tGIR_{max}$ was 3.05 ± 1.39 and 3.13 ± 1.28 hours, GIR_{max} was 8.67 ± 3.78 and 8.28 ± 2.97 mg/kg/min, respectively, for TD and RD. The $AUC_{GIR0–12}$ was 49.21 ± 21.68 and 49.78 ± 18.81 (mg/kg) × 60, respectively. No statistically significant differences in the PD parameters described were observed. These data together with the parameters of the GIR-time curves indicated the comparability of the PD effects of IP.

Adverse events

A summary of AEs is presented in Table 4. During the study, no serious AEs were observed. The following AEs were detected: phlebitis in 11 cases (six in the TD and five in the RD groups), nausea (one in the TD group), vomiting

Table 1. Demographic information on all the randomised subjects (mean±standard deviation, n=48)

Index	Values
Age, years	25.54±5.85
Body mass, kg	75.52±9.85
Height, cm	178.00±6.74
BMI, kg/m^2	23.76±2.01

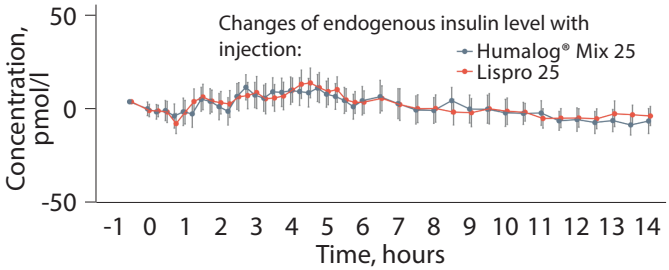

Fig. 1. Averaged curves of the dynamics of changes in the endogenous insulin level before and after subcutaneous injection of the TD biphasic insulin lispro 25 and the RD Humalog® Mix (Mean±SD).

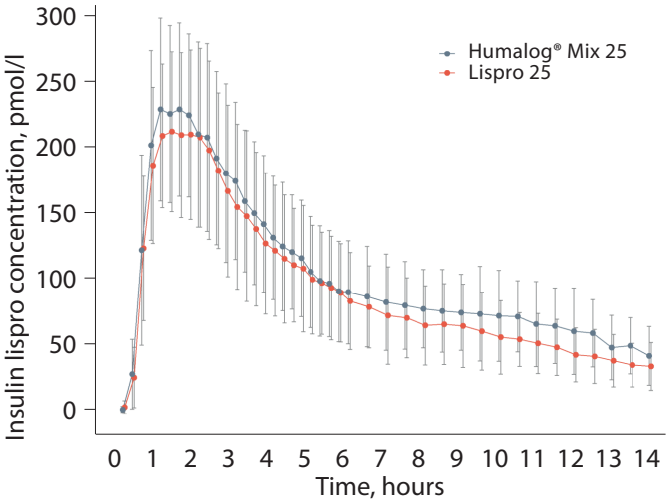

Fig. 2. Averaged pharmacokinetic curves of insulin lispro concentration and time after subcutaneous injection of the TD biphasic insulin lispro 25 and the RD Humalog® Mix.

(one in the RD group), posthemorrhagic anaemia (onein the TD group), syncope (one in the TD group) and skin reaction to the patch (one in the TD group). All AEs were evaluated by the medical investigators as having mild severity. Given the nature of the manipulations and the complexity of the GCT, the AE data did not seem to be related to the IP administration, and were due to the manipulations during the study.

AEs in laboratory findings revealed abnormalities in biochemical indices of blood ('increased bilirubin level' in one volunteer in the RD group and 'increased activity of creatinphosphokinase and transaminases' in one in the TD group) did not manifest clinically and resulted in the normalisation of indices without the use of therapy. They were evaluated by medical investigators as of mild severity. It seems likely that these AEs were not associated with the IP administration, but were due to the GCT.

All vital signs and indices obtained using instrumental methods of research remained within the normal range or standard variants or had clinically insignificant deviations. The blood potassium ion levels remained stable throughout the study. No local reactions to the IP administration were detected.

DISCUSSION

Summary of the primary and additional outcomes of the study

Clinical study of comparative PK and PD of the TD biphasic insulin lispro 25 and RD Humalog® Mix 25 revealed that they have comparable PK and PD profiles under GCT conditions in healthy volunteers. This was confirmed by the similarity of the primary PK/PD-indicators and PK/PD-curves.

According to the results obtained, 90% CI ratios of mean $C_{ins.max}$ and $AUC_{ins.0–12}$ values were created, as well as 95% CI ratios of mean GIR_{max} and $AUC_{GIR.0–12}$ values of TD and RD. The data are provided in Tables 2 and 3. The presence of data within the allowable intervals (80%–125%) indicated the comparability of the PK and PD profiles of the IP.

Discussion of the primary result of the study

In accordance with the regulatory requirements [11, 12, 17], a statistical evaluation of the IP equivalence was performed based on matching the 90% CI of the ratio of primary PK-terminal points of the TD to the RD to predetermined equivalence margins recommended by the European and Russian requirements for the study of bioanalogous (biosimilar) drugs containing recombinant insulin and insulin analogues 80%–125%. We found that CI for the log-transformed ratio of the values of the parameter $C_{ins.max}$ was 87.75%–99.90%, and $AUC_{ins.0–12}$ was

Table 2. Pharmacokinetic parameters of the studied drugs, the results of equivalence evaluation

	N	Biphasic insulin Lispro 25 (T)[a]	N	Humalog® Mix 25 (R)[a]	P-value[c]	T/R ratio [CI 90%][b]
Pharmacokinetics						
$C_{ins.max}$, pmol/l	45	238.75±64.30	45	256.61±69.79	0.116	0.94 [87.75, 99.90]
$AUC_{ins.0–12}$, (pmol/l) × h	45	1214.74±375.89	45	1347.28±396.74	0.027	0.90 [83.76, 96.98]
$AUC_{ins.0–2}$, (pmol/l) × h	45	319.37±88.05	45	340.49±101.62	0.267	
$AUC_{ins.0–6}$, (pmol/l) × h	45	841.89±255.97	45	899.02±265.49	0.156	
$AUC_{ins.0–14}$, (pmol/l) × h	45	1290.23±393.99	45	1450.35±416.77	0.010	
$AUC_{0–\infty}$, (pmol/l) × h	45	1466.92±51.40	45	1680.62±473.56	0.002	
t_{max}, h	45	1.50 (0.50. 3.25)	45	1.25 (0.75. 3.00)	0.052	
$t_{1/2}$, h	45	3.47±0.49	45	3.70±0.42	0.007	

Notes:
[a] – results are presented as mean ± standard deviation, median (min, max); [b] – the ratio of geometric means is presented; 95% CI is given for the ratio for PK parameters; [c] – comparison results using ANOVA; Wilcoxon's paired 2-tailed test was used for tmax; differences between groups were considered significant at $P < 0.05$

83.76%–96.98%. This confirmed the high similarity of the TD biphasic insulin lispro 25 with the original drug.

The detected statistically significant differences of $AUC_{ins.0–12}$ (P = 0.027) were not confirmed by the PD data. Thus, the P-value of $AUC_{GIR0–12}$ was 0.307 when this indicator CI was within the equivalence margins. The combination of these factors with the fact that $AUC_{ins.0–12}$ itself was within the equivalence margins enabled the conclusion that there is no clinical significance of the identified PK differences in $AU_{Cins.0–12}$. These statistical differences may be related to the sample size, which enables to reveal the differences between equivalent drugs [27]. Thus, our study sample was calculated for the most variable parameter AUC_{ins}, and a variability coefficient (CV) of 38% was assumed, while in the study CV was 20.87%. Lower intraindividual variability, when proving equivalence, enables us to reveal statistically significant differences in measured parameters. In the presence of such situations, the decisive role belongs to matching CI within the equivalence margins [28].

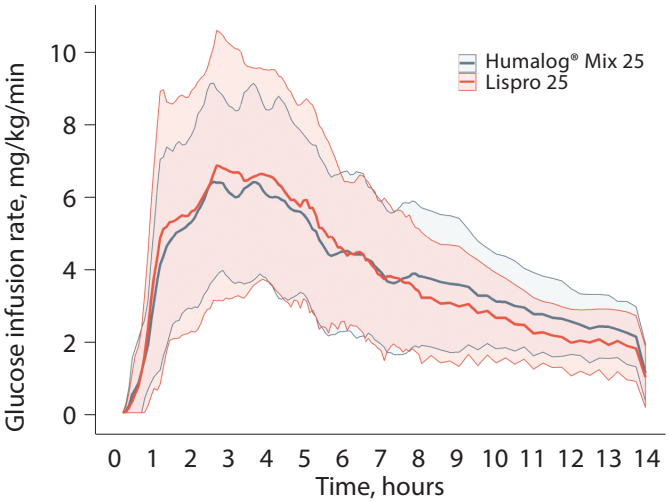

Fig. 3. Averaged pharmacodynamic curves of 'GIR-time' after subcutaneous injection of the TD biphasic insulin lispro 25 and the RD Humalog® Mix

Discussion of the study additional outcomes

Statistical evaluation of the equivalence of PD-indicators of IP was conducted based on adjusting 95% CI of the ratio of primary PD-parameters of the TD to the RD in the predetermined equivalence margins [11, 12, 17] as recommended by the European and Russian requirements for the study of bioanalogous (biosimilar) drugs containing recombinant insulin and insulin analogues 80%–125%. We found that CI for the log-transformed ratio of the values of the parameter GIR_{max} and $AUC_{GIR0–12}$ of TD and RD were 93.33%–111.06% and 83.76%–96.98%, respectively. This confirmed the high similarity of the TD biphasic insulin lispro 25 to the original drug.

For mixtures of average-acting insulin, the initial action indicators also have clinical significance, namely partial $AUC_{GIR0–2}$, $AUC_{GIR0–6}$ as well as $tGIR_{max}$ and $tGIR_{lag}$ (Table 3). The figures obtained did not have statistically significant differences between TD and RD, which also confirmed the high comparability of the effects of biphasic insulin lispro 25 and Humalog® Mix 25.

Study limitations

Using the population of healthy volunteers, the influence of factors of associated diseases on insulin

Table 3. Pharmacodynamic parameters of the studied drugs, the results of equivalence evaluation

	N	Biphasic insulin Lispro 25 (T)[a]	N	Humalog® Mix 25 (R)[a]	P-value[c]	T/R ratio [CI 95%][b]
Pharmacodynamics						
GIR_{max}, mg/kg/min	45	8.67±3.78	45	8.28±2.97	0.684	1.02 [93.33, 111.06]
$AUC_{GIR0–12}$, (mg/kg) × 60	45	49.21±21.68	45	49.78±18.81	0.307	0.95 [85.66, 105.48]
$AUC_{GIR.0–2}$, (pmol/l) × h	45	7.11±4.56	45	6.50±3.09	0.809	
$AUC_{GIR.0–6}$, (pmol/l) × h	45	31.14±15.17	45	29.25±11.44	0.860	
$AUC_{GIR0–14}$, (mg/kg)×60	45	52.83±22.88	45	54.23±19.69	0.186	
$tGIR_{max}$, h	45	3.05±1.39	45	3.13±1.28	0.527	
$tGIR_{lag}$, min	45	29.59±13.94	45	27.68±13.24	0.403	

Notes:
a–results are presented as mean ± standard deviation, median (min, max); b–the ratio of geometric means is presented; 95% CI is given for the ratio for PD parameters; c–comparison results using ANOVA; differences between groups were considered significant at P-value<0.05).

Table 4. Adverse events

	Biphasic insulin Lispro 25 (N = 48)			Humalog® Mix 25 (N = 48)			P-value
	Number of subjects (%)	Number of cases		Number of subjects (%)	Number of cases		
Adverse events	11 (22.92)	Mild	12	7 (14.58)	Mild	7	0.2956a
		Moderate	0		Moderate	0	
		Severe	0		Severe	0	
		Total	12		Total	7	

and plasma glucose concentrations was minimised. Nevertheless, the data obtained on a homogeneous sample without accompanying distortion factors can be extrapolated to the entire population of DM patients.

CONCLUSION

Based on a double-blind, randomised, comparative crossover study of pharmacokinetics of biphasic insulin lispro 25, suspension for subcutaneous administration, 100 IU/mL (Geropharm-Bio) and Humalog® Mix 25, suspension for subcutaneous administration and 100 IU/mL (Lilly France) using the GCT method on healthy volunteers, TD insulin lispro and RD Humalog® Mix 25 are equivalent. Comparability also was confirmed based on the PD data obtained.

The similarity of pharmacologic (PK/PD) characteristics of these types of insulin, together with the obtained data of physicochemical and functional properties, enables the extrapolation of the efficacy of the RD Humalog® Mix 25 on the TD biphasic insulin lispro 25 without conducting full-scale clinical studies of comparative efficacy. Nevertheless, the next stage in the study of biosimiliarity of IPs will be the study of noninferior immunogenicity of the biphasic insulin lispro 25 compared to Humalog® Mix 25.

ADDITIONAL INFORMATION

Source of financing. The sponsor of this clinical study is Geropharm Group of Companies.

Conflict of interest. The authors declare the following conflicts of interest: 1) A.Y. Majorov, E.O. Koksharova and E.E. Mishina are representatives of the clinical centre that conducted the clinical study described, with the financial support of the Geropharm Group of Companies. 2) R.V. Drai, I.E. Makarenko and O.I. Avdeeva are employees of the Geropharm Group of Companies.

Author contributions. A.Y. Majorov - principal investigator, clamp tests conducting, article review; E.O. Koksharova and E.E. Mishina – conducting research, including clamp tests, article review; O.I. Avdeeva – writing reporting documentation, writing an article; R.V. Drai и I.E. Makarenko – analysis of results, article review. All authors contributed equally to the review. All authors have read and approve the final version of the manuscript.

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