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Review Article

Review of bioequivalence studies of cholecalciferol drugs

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Abstract

Introduction: The general requirements for assessing bioequivalence of endogenous drugs are described in the relevant guidelines, but they do not provide a complete picture of how to adequately develop a design of such a study. The aim of this article is to offer recommendations on the development of a design for bioequivalence studies of endogenous drugs, using cholecalciferol as an example.

Materials and methods: A systematic review of our database on the results of bioequivalence studies of generic drugs revealed one study of cholecalciferol drugs, which was performed using a simple cross-over design. The study involved 24 healthy adult subjects. The data of 24 volunteers were retrospectively analyzed to identify endogenous cholecalciferol concentrations and intraindividual variability (CV_{intra}) for C_{max} and AUC_{0-t}. As part of a retrospective analysis, we also assessed gender differences of pharmacokinetics.

Results and discussion: Assessment of the bioequivalence of cholecalciferol drugs was complicated by the presence of endogenous concentrations of cholecalciferol for the tested drug – 1.27 (±0.55) ng/ml and for the reference drug – 0.98 (±0.55) ng/ml. The results of the analysis of the intraindividual variability of C_{max} and AUC_{0-72} of the tested and reference drugs showed the following CV_{intra} values – 22.80% and 21.58%, respectively. A comparative analysis of pharmacokinetic parameters did not reveal statistically significant gender differences. The article presents approaches to the planning of future bioequivalence studies of cholecalciferol drugs.

Conclusion: Cholecalciferol is not a highly variable drug; however, it relates to drugs – analogues of endogenous compounds, which requires determining the endogenous concentrations.

Keywords

bioequivalence, endogenous concentrations, cholecalciferol.

Introduction

At present, in Europe, in the USA, as in the Russian Federation, there are special guidelines for the selection of a design, evaluation and interpretation of the results of comparative pharmacokinetic bioequivalence studies (CHMP 2010; U.S. Food and Drug Administration 2013; Mironov 2013).

Assessment of bioequivalence of some generic and reference drugs is complicated by the presence of basic endogenous concentrations of these compounds (for example, ions, vitamins, hormones, etc.) in the body; in some cases, endogenous concentrations can be more or less constant, in other cases – significantly variable (for example, due to various endogenous processes, circadian rhythms, etc.); in some situations, endogenous concen-

trations may remain unchanged when administering the test drug in blood, but the concentration of the compound increases in another compartment of the body, such as urine (Schindel 2000; Sanjeeva 2010). The general principles for conducting and evaluating the results of such studies are described in the relevant guidelines (CHMP 2010; Food and Drug Administration 2013; Mironov 2013), but they do not provide a complete picture of how to adequately develop a design of such a study. In particular, such general recommendations do not take into account the nature of each individual endogenous substance; therefore, it is relevant not only to create general recommendations, but also particular ones, based on the existing scientific experience.

The aim of this article is to offer recommendations on the development of a design for bioequivalence studies of drugs active substances of which are present in the body as endogenous compounds, using cholecalciferol as an example.

Material and methods

Material for retrospective analysis

The systematic analysis of the databases of Scientific Centre for Expert Evaluation of Medicinal Products and Yaroslavl State Medical University on the results of clinical trials of cholecalciferol drugs, one bioequivalence study was revealed. The study was performed with a simple crossover design in two periods and two sequences with a single administration of the test and reference drugs in fasting condition. The study involved 24 healthy Russian adult subjects (16 males and 8 females). The dosage of 5000 IU (125 μg) test and reference drugs was assessed in the study in fasting condition. Blood samples were taken within 72 hours after drug administration, and plasmatic concentrations were determined using a validated high-performance liquid chromatography with tandem mass spectrometric detection (HPLC MS/MS) of analytes. The wash-out period in the study was 14 days; the schedule of sample collection included the blood sampling -24; -10; -2; 0 hours before and 3; 6; 8; 9; 11; 12; 13; 4; 15; 16; 18; 20; 24; 30; 36; 48; 72 hours after drug administration in each period. The lower limit of quantification (LLOQ) was 0.5 ng/ml. The test and reference formulations of cholecalciferol were bioequivalent since the 90% of CIs for the geometric mean test/reference ratios were within the predetermined range from 80.00% to 125.00%.

Material for the review

The literature sources and data obtained by searching the Internet (PubMed, Google, ResearchGate) were also analyzed to evaluate the intra-individual variance and endogenous concentrations of cholecalciferol drugs. The search terms were bioequivalence and cholecalciferol.

Methods of statistical processing for retrospective analysis

The data for 24 subjects were retrospectively analyzed, i.e. the analysis included the datasets for C_{max} and AUC_{0-t}. The value $AUC_{0-t}$was computed by the trapezoidal method. The pharmacokinetic parameters were transformed into logarithms and analyzed using ANOVA. The factors contributing to the observed variation that were included in the ANOVA were the sequence, subjects, period, and drug. The mean-square errors (MSEs) were used to compute coefficient CV_{intra} for C_{max} and AUC_{0-t}.

Methods of statistical processing for the review

The pooled CV_{intra} of the 5studies was computed. As part of a retrospective analysis, we also calculated the main pharmacokinetic parameters C_{max} and AUC_{0-t} separately for male and female subjects and performed a statistical comparison.

Pharmacokinetic parameters, CV_{intra} and statistical tests were calculated using SSPS Statistics v. 25 and Microsoft Office Excel 2016 software.

Results and discussion

The bioequivalence study of cholecalciferol, revealed through the systematic analysis of databases of The Scientific Centre for Expert Evaluation of Medicinal Products and Yaroslavl State Medical University", was performed with a simple cross-over design.

As a result of a retrospective analysis of the cholecalciferol concentrations corrected for the endogenous concentration, the pharmacokinetic parameters C_{max}, AUC_{0-t}, and t_{max} were calculated. Table 1 presents the average pharmacokinetic parameters after correction for endogenous concentration.

Figure 1 presents the endogenous levels of cholecalciferol in two groups of subjects: before administration of the test and reference drugs (within 24 hours), and pharmacokinetic profiles after administration of the test and reference drugs (within 72 hours) without correction for the endogenous level.

In the study, prior to taking the drugs, some volunteers showed an endogenous concentration of cholecalciferol above LLOQ (0.5 ng/ml). For the remaining volunteers, the values of endogenous concentrations did not exceed LLOQ and were equated to 0. The average endogenous concentration over 24 hours in the tested drug group (n=3) was 1.27 (±0.55) ng/ml and in the reference drug group (n=5) – 0.98 (±0.55) ng/ml. Within 24 hours, the concentrations of cholecalciferol did not undergo significant fluctuations (Fig. 1). Taking into account the uncorrected mean values obtained in the study after taking cholecalciferol in a dosage of 125 μg, C_{max} for the study drug was 6.96 (±2.79) ng/ml and for the reference drug – 7.29 (±3.28) ng/ml. The endogenous level of cholecalciferol revealed in some volunteers was more than 5% of C_{max}.

Table 1. Averaged pharmacokinetic parameters of cholecalciferol.

№	C_{max} T, ng/ml, (SD)	C_{max} R, ng/ml, (SD)	AUC_{0-t} T, ng*h/ml, (SD)	AUC_{0-t} R, ng*h/ml, (SD)	t_{max} T, h, (SD)	t_{max} R, h, (SD)
1	6.80 (2.70)	7.09 (3.19)	198.29 (95.14)	220.22 (119.47)	13.17 (3.66)	12.36 (2.20)

Note: C_{max} – maximum plasma concentration; AUC_{0-t} – area under the plasma concentration-time curve between 0 to time of the last blood sampling; t_{max} – time to reach maximum plasma concentration; T – test drug; R – reference drug; SD – standard deviation.

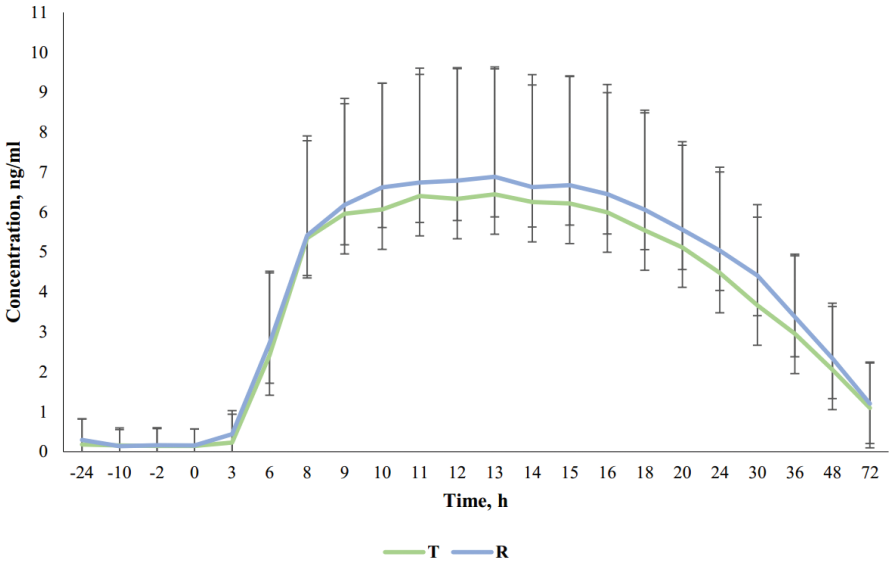

Figure 1. Pharmacokinetic profiles of cholecalciferol. **Note:** T – test drug; R – reference drug.

According to the literature data, the values of endogenous concentrations of cholecalciferol were about 1 ng/ml, after taking 70 μg of cholecalciferol, C_{max} was about 4 ng/ml, t_{max} – 12–24 hours. The method for determination of cholecalciferol was HPLC MS/MS, LLOQ 0.5 ng/ml (Barger-Lux et al. 1998; Xie et al. 2011; Marzo et al. 2013).

According to Hamish Wright et al. (2015), endogenous cholecalciferol concentrations were about 1–1.5 ng/ml, after taking 5600 IU of cholecalciferol (140 μg), C_{max} was about 11 ng/ml, t_{max} – 12.5–13.5 h. The method for determination of cholecalciferol was HPLC MS/MS, LLOQ 0.5 ng/ml (Wright et al. 2015).

According to the open access data, in 5 bioequivalence studies with determination of cholecalciferol concentrations, the following pharmacokinetic parameters were obtained (Table 2) (Denker et al. 2011; Public assessment report 2017; Public assessment report 2018).

Thus, there were basic endogenous concentrations of cholecalciferol at a level of 1–1.5 ng/ml and the comparability of pharmacokinetics after administration of cholecalciferol drugs (Chen et al. 1990a; Chen et al. 1990b; Takeuchi et al. 1995; Porras et al. 1999; Raimundo et al. 2015). When taking a dosage of 70 μg, the maximum concentrations reach 4–6 ng/ml, when taking 140 μg – 8–13 ng/ml, t_{max} – 9–14 hours (Francis et al. 1996; Heaney et al. 2003; Ilahi et al. 2008; Bouillon et al. 2013; Fort et al. 2016; Imga et al. 2018).

The results of a retrospective analysis of the intraindividual variability of C_{max} and AUC_{0-72} of the tested and reference drugs showed the following CV_{intra} values – 22.80% and 21.58%, respectively. Thus, in this study, a low value of the coefficient of intraindividual variability of cholecalciferol was obtained for both parameters.

In the literature, the data on CV_{intra} of the parameter C_{max} also indicate its low variability (11–26%), with respect to the parameter AUC, the data are contradictory (12–42%) (Table 3).

The pooling data of CV_{intra} of 3 standard design studies (2×2×2) described in the literature and the results of our retrospective study showed the following values: for C_{max} – 18.84% (upper limit is 80% of the confidence interval of 19.95%), for AUC – 17.87% (the upper limit is 80% of the confidence interval of 18.92%).

The pooling data of CV_{intra} of 2 studies with replicate design (2×4×4) described in the literature showed the following values: for C_{max} – 18.79% (upper limit is 80% of the confidence interval – of 19.52%), for AUC – 28.89% (upper limit is 80 % of the confidence interval of 30.00%).

Thus, it can be assumed that cholecalciferol most likely does not have high intraindividual variability (Matsuoka et al. 1992; Van Der Klis et al. 1996; Trang et al. 1998; Lips et al. 1999; Vieth 1999; Jafri et al. 2011). However, when calculating the sample size, it is worth focusing on the variability values of about 20–30%.

Table 2. Averaged pharmacokinetic parameters of cholecalciferol according to literature.

№	Dose, μg	C_{max} T, ng / ml, (SD)	C_{max} R, ng / ml, (SD)	AUC_{0-t} T, ng *h/ ml, (SD)	AUC_{0-t} R, ng *h/ ml, (SD)	t_{max} T, h	t_{max} R, h	$t_{1/2}$ T, h, (SD)	$t_{1/2}$ R, h, (SD)
1	140	12.77 (3.99)†	13.13 (3.41)†	455.92 (142.97)†	475.93 (133.64)†	12.00	12.00	21.03 (5.71)	21.34 (5.71)
2	70‡	4.19 (1.18)	4.29 (1.29)	116.40 (44.86)	117.79 (49.32)	10.00	10.00	17.58 (5.51)	16.55 (4.29)
		4.17 (0.93)	4.29 (1.14)	124.75 (35.98)	127.28 (40.34)	12.00	12.00	18.31 (4.78)	17.53 (4.31)
3	140	8.50 (1.80)	8.20 (1.60)	303.00 (71.00)	295.00 (58.00)	14.00	14.00	–	–
4	70	5.90 (3.30)†	6.60 (3.10)†	296.40 (375.50)†	337.90 (344.20)†	12.00	9.00	–	–
5	140	12.20 (5.60)†	13.00 (5.90)†	490.20 (259.60)†	518.70 (269.80)†	10.00	9.00	–	–

Note: references to studies ##1, 2 – Public assessment report (2018); references to study #3 – Public assessment report (2017); references for studies ##4, 5 – Denker AE et al. (2011); C_{max} – maximum plasma concentration; AUC_{0-t} – area under the plasma concentration-time curve between 0 to t of the last blood sampling; t_{max} – time to reach maximum plasma concentration; $t_{1/2}$ – period of half-life; T – test drug; R – reference drug; SD – standard deviation; † – values without correction for the endogenous level (endogenous concentration was not determined); ‡ – study with a replicate design (pharmacokinetic parameters are given for each period); – no data available.

Table 3. Results of analysis of studies of bioequivalence of cholecalciferol drugs in fasting condition.

№	Dose, μg	Design	N	$CV_{intra}C_{max,\%}$	$CV_{intra}AUC_{0-t,\%}$
1	140	2×2×4	55	11†	14†
2	70	2×2×4	41	26†	42†
3	140	2×2×2	26	14	12
4	70	2×2×2	28	13	17
5	140	2×2×2	60	21	19

Note: references to studies ##1, 2 – Public assessment report (2018); references to study #3 – Public assessment report (2017); references to studies ##4, 5 – Denker AE et al. (2011); C_{max} – maximum plasma concentration; AUC_{0-t} – area under the plasma concentration-time curve between 0 to t of the last blood sampling; CV_{intra} – coefficient of intraindividual variability; T – test drug; R – reference drug, † – values of intraindividual variability of the reference drug; 2×2×2 – simple cross-over design (2 treatments, 2 sequences, 2 periods); 2×2×4 – replicate design (2 treatments, 2 sequences, 4 periods).

As part of a retrospective analysis of cholecalciferol bioequivalence studies, C_{max} and AUC were also calculated for the populations of male and female subjects. A comparative analysis of pharmacokinetic parameters did not reveal statistically significant differences.

As a result the systematic review, the following approaches to the planning of future bioequivalence studies of cholecalciferol drugs were developed:

1. According to a retrospective analysis of bioequivalence studies of cholecalciferol, the weighted average intraindividual variability of C_{max} was at the level of 20% and AUC – at the level of 20–30%. Accordingly, the number of subjects for bioequivalence studies with a standard design and a point estimate of 0.95, type I error of 5%, type II error of 20%, should be approximately 20–40.
   It is recommended that subjects of both sexes be included in the studies in equal proportions to assess possible gender differences in the pharmacokinetics of the test and reference drugs.
2. The half-life of cholecalciferol is long and ranges from 18 hours to several days, according to various studies (Denker et al. 2011; Public assessment report 2017; Public assessment report 2018). Thus, it can be assumed that it is acceptable to take blood samples for the determination of cholecalciferol within 72 hours. Based on the above data, the washout period should be at least 14 days.
3. The blood sampling schedule for the pharmacokinetic analysis of cholecalciferol must include predose samples, taking into account the presence of an endogenous concentration. According to the retrospective analysis and literature data, endogenous concentrations were 1–1.5 ng/ml and did not undergo significant daily fluctuations. Therefore, it is advisable to determine the average endogenous concentration before each dosing period. It is recommended to determine the endogenous level 24, 16, 8 hours and immediately before administration of the test and reference drugs (point "0"). The average endogenous concentration should be subtracted from the concentrations for each time point after taking the studied drugs. If, after correction, a negative plasma concentration occurs, it should be set at 0 before calculating the adjusted AUC_{0-72}.

   To describe the curve "concentration-time" in the ascending part of the curve and the time to reach C_{max} 9–14 hours after taking the studied drugs, the following time points can be recommended: 1; 2; 4; 6; 8; 9; 10; 10.5; 11; 11.5; 12; 12.5; 13; 13.5; 14 hours; to describe the descending part of the curve – 15; 16; 20; 24; 36; 48; and 72 hours.
   Thus, the following blood sampling schedule can be recommended:
   - to determine an endogenous concentration: - 24, -16, -8; and 0 hours;
   - to determine the concentration of cholecalciferol after administration of the test drugs - 1; 2; 4; 6; 8; 9; 10; 10.5; 11; 11.5; 12; 12.5; 13; 13.5; 14; 15; 16; 18; 20; 24; 36; 48; and 72 hours.
4. To determine cholecalciferol, it is recommended to use the most sensitive determination method, for example, analytical HPLC-based methods with mass spectrometric detection or tandem mass spectrometric detection. According to the retrospective study, after taking 70–140 μg of cholecalciferol, maximum concentrations were observed at a level of 4–13 ng/ml. Therefore, the analytical method should allow achieving an adequate LLOQ, for example, at least 0.2 ng/ml

(5% of 4 ng/ml) when studying a dosage of 70 µg, or 0.65 ng/ml when studying a dosage of 140 µg.

5. In a study with a standard design, it is necessary that 90% of the confidence intervals for the ratios of the geometric means of the parameters C_{max} and AUC_{0-72} of the test and reference drug were in the range of 80.00–125.00%.

Conclusion

1. Cholecalciferol is not a highly variable drug; however, it relates to drugs – analogues of endogenous compounds, which requires to determine endogenous concentrations.
2. The analysis of the pharmacokinetics of the subpopulations of men and women did not reveal any statistically significant differences.
3. When planning and evaluating the results of bioequivalence studies, it is possible to be guided by the above approaches.

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Conflict of interest

The authors declare no conflict of interests to be disclosed in this article.

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