PHARMACOKINETICS OF CALCIUM FROM CALCIUM SUPPLEMENTS IN HEALTHY VOLUNTEERS

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ABSTRACT
Calcium supplementation is widely used in deficiency status and as an adjuvant in the treatment of osteoporosis. The objective of this study was to compare the oral bioavailability of calcium from tablets containing calcium fumarate to that of calcium gluconate. Twelve healthy volunteers participated in the study. Single-dose, two-treatment, two-sequence-crossover, randomized design test methodology was applied.
The tablets were prepared by direct compression and were subjected to tests: drug content, hardness, friability, disintegration time and in vitro dissolution studies.
The preparations were compared using pharmacokinetic parameters such as the area under the plasma concentration – time curve $AUC_{(0-11)}$, peak plasma concentration C_{max}, time to reach maximum plasma concentration T_{max}. No statistically significant difference was observed for any of the parameters, and the 90% confidence intervals calculated for the ratio of the logarithmically transformed $AUC_{(0-11)}$ values of both formulations were within the bioequivalence limit of 0.80-1.25. It can be concluded that the two tablet preparations of calcium are likely to be bioequivalent.

Keywords: Calcium fumarate, calcium gluconate, bioavailability, healthy volunteers.

INTRODUCTION

The important role played by calcium in the mammalian organism, is now well recognized. Calcium is the major cation of bone mineral. It also plays an important role as intracellular messenger in many organs and cells (e.g., cardiac, renal, etc.). Chronic calcium deficiency resulting from inadequate intake or poor absorption is one of several important causes of reduced bone mass and osteoporosis (Cashman, 2002).

Numerous calcium preparations are available on the market and differ only in regard to their bioavailability. However, this difference has little clinical significance except in patients with achlorydia or elderly persons with low gastric secretion (Blanch and Pros, 1999; Fujita *et al*., 1995; Recker, 1985). Soluble calcium sources such as calcium citrate, calcium lactate and calcium gluconate are absorbed normally in elderly subjects with atrophic gastritis. However, calcium carbonate, a relatively insoluble calcium salt, when ingested alone, is a poor source of calcium (Heller *et al*., 2000; Sakhaee *et al*., 1999).

The researches on working out increased bioavailability calcium preparation are still being conducted. One of them is calcium fumarate.

The effectiveness and tolerance of calcium fumarate in fighting the calcium-phosphate balance disorders in patients dialyzed because of chronic renal insufficiency has been determined in comparison to calcium carbonate therapy. It has been found that in order to get a similar metabolic effect of calcaemia and phosphataemia in dialyzed patients it is enough to use on average 30% less calcium in the form of fumarate than carbonate and that the preparation does not have any side effects (Kokot *et al*., 1999).

An *in vitro* method through the rat's ileum showed highest penetration rate of calcium for the calcium fumarate solutions, a lower one for calcium gluconate and the lowest – for calcium chloride solutions (Gadomska-Nowak *et al*., 2003).

The computed QSAR parameters and molecular structure of the organic calcium salts (calcium gluconate and calcium fumarate) suggest that calcium ions in organic salts are better protected from exterior influences than in case of calcium carbonate. Better availability of calcium from organic salts may be explained by the formation of a complex between calcium ions and organic ligands (Szulc *et al*., 2001).

The aim of present study is to compare the bioequivalence of two tablet formulations of calcium: calcium fumarate tablets and calcium gluconate tablets after oral administration to volunteers.

EXPERIMENTAL

MATERIALS AND METHODS

Materials

Avicel PH-101 and potato starch were purchased from Sigma-Aldrich (Germany); magnesium stearate from Merck (Darmstadt; Germany).Calcium gluconate was a

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gift from PPH "POCH" Gliwice (Poland). Calcium fumarate was a gift from Nitrogen Plant "Kędzierzyn" Kędzierzyn-Koźle (Poland). All studied calcium salts have met standard and Polish Pharmacopoeal requirements (Polish Pharmacopoeia VI, 2002).

The calcium fumarate tablets and calcium gluconate tablets have been developed in the Department of Applied Pharmacy and Drug Technology, Silesian Medical University in Katowice. All other reagents are of pharmaceutical grade.

Methods

Preparation of Tablets

Tablets prepared by direct compression. The ingredients were manually screened through 0.5 mm screen and next they were blended for 10 minutes. Finally, 2% magnesium stearate as a lubricant was added and the mixture was blended for 5 minutes. The formulations were compressed in a single–punch Erweka tablet press (Erweka AR400, Germany), equipped with concavity-faced punches of 12mm diameter. The content of elementary calcium in tablets was 75mg. The ingredients of the formulations are summarized in table 1.

Table 1: Composition of calcium tablets

Ingredient	Formulation 1	Formulation 2
	Amount (mg)	
Calcium fumarate	0.379	-
Calcium gluconate	-	0.805
Avicel PH-101	0.05	0.05
Potato starch	0.05	0.05

Characterization of Tablets

The tablet’s physical parameters, including weight (Sortorius, Germany), hardness (Pleizer, Hardness Tester, Germany), friability (MRT, obrm Polfa, Poland), were evaluated using the official Polish Pharmacopoeia methods. The disintegration time was determined for 6 tablets with the USP disintegration apparatus (F2, ZDM Polfa, Poland) at 37^{o}C±0.5^{o}C in water.

The release of calcium from the tablet was accomplished in 0.1N HCl with a USP dissolution paddle assembly at 100 rpm and temperature of 37^{o}C±0.5^{o}C. Aliquot samples were collected at predetermined intervals, and replaced with the same volume of fresh dissolution medium. The calcium content in the sample was determined by atomic absorption spectrometry. Cumulative percentage drug release was calculated using an equation obtained from a standard curve.

Bioavailability Study

The study was approved by an Ethics Committee of the Silesian Medical University and performed according to international guidelines and recommendations. A total number of 12 healthy, volunteers were included into the study after having been informed about the purpose of the study and after they had given written informed consent. All volunteers underwent physical and clinical laboratory tests (hematology and biochemical). Among the 12 volunteers there were 4 women and 8 men aged between 34 and 48. Their body weight ranged from 66 to 73 kg. The subjects were asked not to take any medications for 7 days before the study and during the study.

Table 2: Physical properties for calcium tablets

Properties	Calcium fumarate	Calcium gluconate
Friability (%)*	0.45	0.51
Hardness (kp)**	8.3±0.58	8.0±0.43
Disintegration Time (seconds)**	290±23	310±29
Content uniformity (%)*	99.2±4.0	98.4±3.1
% Dissolved After 45minutes	63.96±2.04	59.51±1.69

*All values are expressed as mean ±SD, n=20; ** All values are expressed as mean ±SD, n=6.

Table 3: Comparison of some pharmacokinetic parameters of calcium in volunteers serum after administration of calcium preparations

Parameters	Calcium gluconate	Calcium fumarate	Statistical results
k_{el} [h^{-1}]	0.036±0.003	0.041±0.005	NS
t_{50}% [h]	19.25±2.0	16.9±1.82	NS
K_a [h^{-1}]	1.72±0.21	1.82±0.26	NS
C_{max} [mmol/l]	2.94±0.32	3.26±0.40	NS
t_{max} [h]	2.19±0.26	2.11±0.31	NS
$AUC_{(0\text{-}11)}$ [mmol·h/l]	27.74±3.12	29.43±4.12	NS
$AUC_{(0\text{-}\infty)}$ [mmol·h/l]	28.29±3.16	30.16±4.22	NS

EBA = (30.16: 28.29)x100=106.61%

Table 4: 90% confidence intervals for the mean pharmacokinetic of calcium formulations

Pharmacokinetic parameter	T/R ratio	Confidence limits
$AUC_{(0\text{-}t)}$	1.018	0.95-1.10
$AUC_{(0\text{-}\infty)}$	1.019	0.95-1.10
C_{max}	1.074	0.98-1.19
T_{max}	0.949	0.87-1.04

t-test formulation calcium fumarate; R – reference formulation calcium gluconate

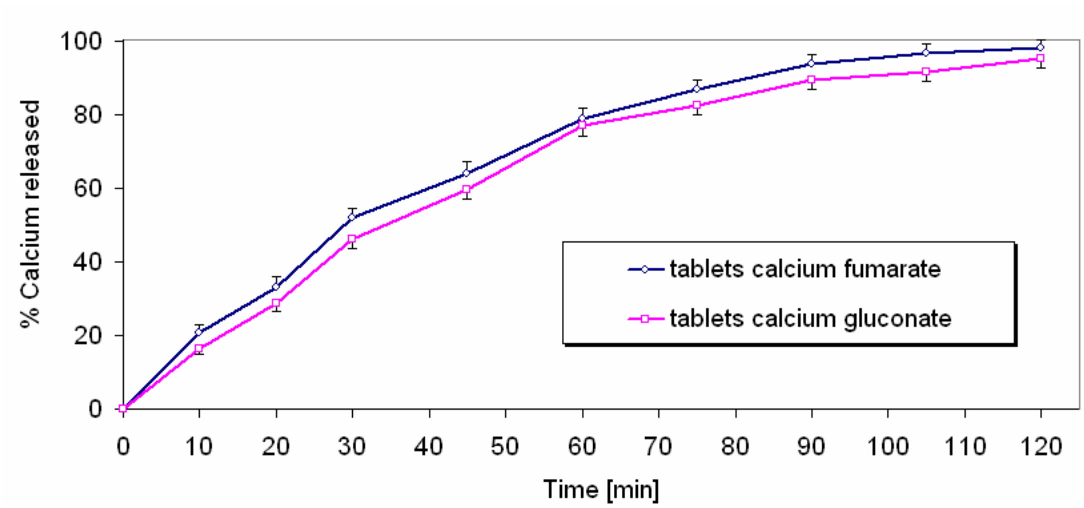

Fig. 1: Dissolution profiles of release of calcium from calcium preparations.

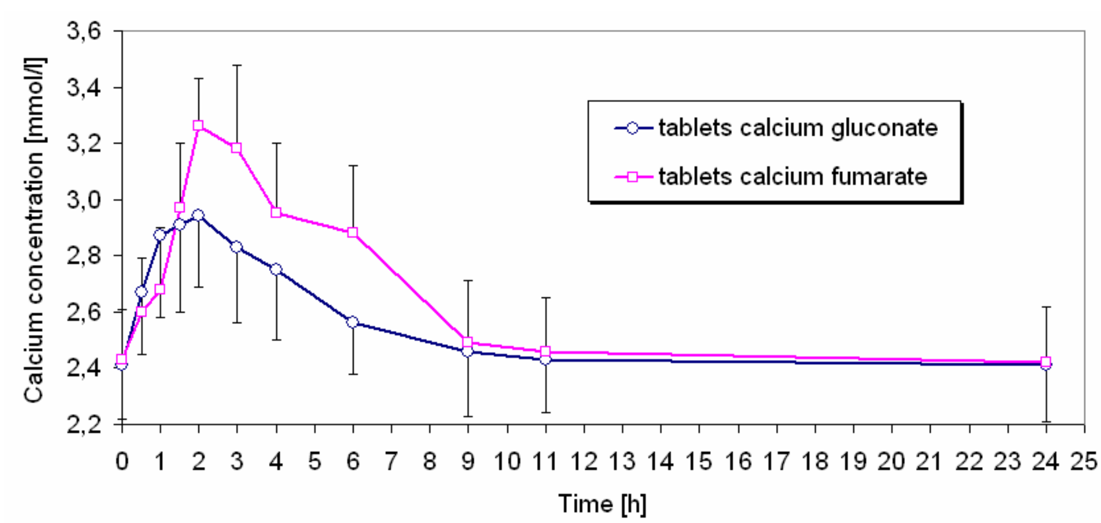

Fig. 2: Serum concentration of calcium observed at the indicated time intervals after administration of calcium preparations.

The study consisted of two randomized cross-over periods separated by one week. According to the treatment schedule, after a 12 hours overnight fast, each subject was given tablets containing 675mg calcium in total either in the form of calcium gluconate or calcium fumarate orally with a glass of water. Standard breakfast was served after the 4th hour.

Blood samples were taken from a forearm vein immediately prior to the dose (time zero) and 0.5, 1, 1.5, 2, 3, 4, 6, 9, 11 and 24 hrs post dose. Serum was obtained by centrifugation and stored at 20^0C until analyzed for calcium content by the atomic absorption spectrometry at the wavelength of 422.7nm.

Analysis of data

The coefficients (A and B) and the exponents (k_{el} and k_a) of biexponential equations describing one compartment open models for post administration were obtained graphically (Notari, 1975). The maximum plasma concentration (C_{max}) and the time to the peak (T_{max}) for each drug were directly obtained from the experimental data. Areas under the plasma drug concentration-time curve $AUC_{(0-\infty)}$ was calculated by adding the area from zero to the last sampling time t (AUC_{0-t}) and the area from time t to infinity $AUC_{(t-\infty)}$. The former was calculated by using the trapezoidal rule and latter by dividing the last measurable plasma drug concentration with elimination rate constant (k_{el}). The elimination half-life ($t_{0.5}$) in h was estimated by dividing 0.693 by k_{el}. EBA (extent of bioavailability) was estimated according to the pattern: EBA = ($AUC_{calcium\ fumarate}$: $AUC_{calcium\ gluconate}$) x 100%.

The data were expressed as mean ± standard deviation (n=12) One-way analysis of variance (ANOVA) tests were applied to analyze the difference among the formulations. Statistical significance was defined as $p<0.05$.

RESULTS AND DISCUSSION

The obtained tablets met the quality requirements specified by the Polish Pharmacopoeia VI. The tablets showed acceptable mechanical properties and their disintegration time in water was comparable (table 2). The release pattern of calcium from formulated tablets is illustrated in fig. 1. The following release rate constant values were obtained for the studied formulations: 0.02828h^{-1} for calcium fumarate tablets, and 0.03054 h^{-1} for calcium gluconate tablets.

The mean plasma concentration – time profile curve of the two formulations of calcium is shown in Figure 2. The curve has the shape characteristic for the one-compartment model. The simplest equation describing the observed calcium concentration changes has the following formula:

in the case of calcium fumarate: $Cp=3.6\cdot e^{-0.041t} - 4.1\cdot e^{-1.821t}$

in the case of calcium gluconate: $Cp=3.24\cdot e^{-0.036t} - 3.3\cdot e^{-1.72t}$

Resulting pharmacokinetic parameters are summed up in Table 3. Mean maximum plasma concentrations of 3.26±0.40 mmol/l and 2.94±0.32 mmol/l were achieved for calcium fumarate and calcium gluconate. Time to reach maximum plasma concentration was 2.11h and 2.19 h respectively.

The mean AUC_{0-t} and total AUC of both tablet formulations were similar; no statistically significant differences were observed.

Table 4 shows the parametric 90% confidence intervals of mean values after logarithmic transformation of pharmacokinetic characteristics (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}) as well as the point estimated for T/R ratio. The confidence limits for the mean of pharmacokinetic parameters indicate that these values are entirely within the bioequivalence acceptable range of 0.8-1.25.

In this comparative study of the two tablet formulations of calcium salts no significant differences were found for the parameters of the bioavailability: AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, and additionally for all others pharmacokinetic parameters. Thus, calcium fumarate tablets and calcium gluconate tablets used in the study are likely to be bioequivalent.

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