BIOEQUIVALENCE OF ENDOGENOUS SUBSTANCES FACING HOMEOSTATIC EQUILIBRIA: AN EXAMPLE WITH POTASSIUM

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Oral administration of endogenous substances in most cases results in negligible net increases in baseline plasma concentrations, associated with high variability. This poses the problem of their bioequivalence. Using the data obtained from a bioequivalence investigation of potassium aspartate (test vs reference formulation), the authors demonstrate the inconsistency of bioequivalence based on plasma concentrations and standard methods.

Potassium aspartate was given orally at a dose of 15.8 mmoles to 12 healthy volunteers as test and reference values according to a two-period, two-formulation, two-sequence design. The individual net values of the area under the curve of plasma concentration (AUC) and cumulative urinary excretion (CUE), both obtained with the test formulation as post-dose minus baseline, were multiplied by 2, 3, 4, 5 and 6 and added to the baseline in order to simulate the administration of increasing single doses of the test, assuming dose–linear kinetics. Data generated with the test formulation were compared with original data of the reference according to 90% confidence intervals.

With AUC, bioequivalence of test and reference formulations was demonstrated with 1 : 1, 2 : 1 and 3 : 1 test to reference dose ratios. With CUE only the 1 : 1 dose ratio comparison produced bioequivalence.

The authors conclude that bioequivalence of endogenous substances conducted with standard procedures in most cases is a useless exercise. With potassium and more generally with drugs cleared via urine, urinary excretion would reflect the extent of absorption more faithfully than AUC.

KEY WORDS: endogenous substances, bioequivalence, plasma concentration, urinary excretion.

INTRODUCTION

Previous papers have reviewed difficulties encountered in the pharmacokinetics of endogenous substances [1–4]. As a result of homeostatic equilibria and dilution of the exogenously absorbed fraction with the endogenous pool, only negligible increases in baseline values associated with very high coefficients of variations (CV%), and constant post-dose values almost overlapping with the baseline are usually encountered when these substances are administered orally. This was ascertained directly, by the authors, with several ions (calcium, magnesium, iron, aluminium), with L-carnitine and its acyl-esters, with glutamine, and with some vitamins (D$_2$, D$_3$, B$_{12}$) in various pivotal trials [4]. These considerations on the one hand prohibit a statistical comparison of net test and reference values, but on the other hand render irrelevant the post-dose comparison in bioequivalence trials.

Data obtained in a bioequivalence trial with potassium aspartate administered in a single dose were used in this paper to demonstrate, through simulation, the inconsistency of bioequivalence trials with standard procedures based on plasma concentration data.

MATERIALS AND METHODS

The investigation was carried out on twelve healthy Caucasian volunteers. Their demographic data were on average ± SD: age 25 ± 6.9 years, height 178 ± 5.8 cm, weight 76 ± 8.6 kg. Volunteers were given orally 15.8 mmoles of potassium aspartate in sachets. The test and reference formulations were administered according to a single-dose, two-formulation, two-period, two-sequence crossover design with a one-week wash-out, under fasting conditions. Both treatments were preceded by a 24 h period of baseline observation, where morning
Table I
Post-dose and net values of AUC (mmol l⁻¹ h) and CUE (mmol) of potassium in healthy volunteers treated with 15.8 mmol of potassium aspartate. Mean values of 12 findings evaluated over a 24-h period; CV% in parentheses

<table>
<thead>
<tr>
<th></th>
<th>Post-dose AUC</th>
<th>Net AUC</th>
<th>Post-dose CUE</th>
<th>Net CUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>87.92 (9)</td>
<td>5.45</td>
<td>53.78 (23)</td>
<td>11.5 (115)</td>
</tr>
<tr>
<td>Reference</td>
<td>87.77 (11)</td>
<td>4.65</td>
<td>54.80 (34)</td>
<td>12.00 (87)</td>
</tr>
</tbody>
</table>

fasting was preserved. The administration of both test and reference was preceded by careful dietary control lasting 3 days. The protocol was approved by the Canton Ticino Ethics Committee and volunteers gave written informed consent before trial initiation. Potassium was measured in timed plasma samples and in cumulatively collected urine samples, both the day before (day −1) and on the day of drug administration (bioday), using a validated photometric bioassay. Plasma was assayed at the following times:

- Day −1: 0, 2, 4, 6, 12, 16 h;
- Bioday: 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16 and 24 h.

Urine was cumulatively collected at the following intervals during both day −1 and bioday: 0–6 h, 6–12 h, 12–24 h. Time 0 was about 8.00 a.m., when the drug was administered. Linear trapezoidal area under the curve of plasma concentrations (AUCs) and cumulative urinary excretion (CUE) were calculated over a 0–24 h period, both on day −1 and on the bioday. Net AUC and CUE values were obtained from each subject as post-dose minus baseline values. In the few cases when the resulting value was negative, it was considered as zero. Net values obtained with the test formulation were multiplied by 2, 3, 4, 5, and 6 and added to the baseline to simulate post-dose AUC and CUE values expected with increasing single doses. Resulting values of the test and original values of the reference were log-transformed and statistically processed to obtain point estimators and 90% confidence intervals for the comparison of test vs reference in the 1 : 1, 2 : 1, 3 : 1, 4 : 1, 5 : 1 and 6 : 1 test to reference dose ratios.

RESULTS

Table I reports AUC and CUE as post-dose and net mean values of test and reference, which overlap as between test and reference. Plasma concentration–time behaviour of potassium observed as mean values of test and reference is reported in Fig. 1. With both test and reference formulations, AUC CV% was ≤10% and CUE CV% ranged between 20% and 40% for baseline and post-dose values, while the CV% of both AUC and CUE was around 100% for the calculated net values.

Table II summarizes point estimators and 90% confidence intervals obtained with AUC and CUE with the 1 : 1 experimental dose ratio and with further simulated ratios. As concerns AUC, the test and reference formulations appear to be bioequivalent, with 90% confidence intervals being within the range of 0.80–1.25 for the 1 : 1, 2 : 1 and 3 : 1 dose ratios, and within the enlarged range of 0.70–1.43 for the 4 : 1 and 5 : 1 dose ratios. The situation is very different, with urine, where bioequivalence is confirmed only with the 1 : 1 dose ratio.

**DISCUSSION**

Data obtained in the simulation with potassium administered in single doses could worsen even more if generated experimentally, due to non-linear kinetics. Non-linearity could be caused by a saturation of enteral absorption, both active and passive diffusion, and, for endogenous substances cleared via urine, including potassium, by a saturation of the tubular reabsorption, which generates the renal threshold [5, 6]. Both these mechanisms would lead to less than proportional increases of AUC, whereas CUE would be influenced mainly by the saturation of enteral absorption.

Data obtained in this trial would suggest the preference of CUE to AUC as a bioavailability mirror. This, however, is possible only with endogenous substances cleared via urine, such as ions, L-carnitine and its esters, but is prohibited with substances cleared through biotransformation or biliary excretion.

Results of this simulation agree with the US FDA guidance on slow-release potassium chloride bioequivalence [7], which bases estimates for potassium bioequivalence only on the CUE of the ion, in well standardized and controlled study situations. Serum estimates were not considered in the above guidance as K⁺ levels are inaccurate because of homeostatic mechanisms that maintain them within a relatively narrow range [7].

With most endogenous substances net values cannot be managed statistically, whereas post-dose values can. However post-dose values in most cases are formed mainly by pre-existing endogenous concentrations and only marginally by the exogenous fraction absorbed. This would lead statistics to demonstrate that individual volunteers are similar to themselves rather than demonstrating the bioequivalence of two formulations.

The process whereby bioequivalence is assessed using post-dose AUC values could be considered adequate in a repeated dose regimen to steady-state studies, where the baseline must be attributed, at least in part, to the drug administered. This procedure was used *inter alia* for levothyroxine in patients suffering from primary hypothyroidism by American and European investigators [8, 9]. In these trials, however, patients were treated with each formulation for 57 days, which allows the baseline to be considered as a result of the treatment.

Pharmacokinetic/pharmacodynamic correlation can lead to useful achievements with some endogenous...
substances. This is the case with the calcium ion, which is absorbed in a way similar to that described above with potassium, thus producing poorly defined shapes of plasma concentration [4]. In contrast, the measurement of parathyroid hormone produces a well defined decrease correlated to the calcium absorbed [10].

However, with most endogenous substances, the measurement of bioavailability and the assessment of bioequivalence remains an unresolved problem. With these substances, single dose bioequivalence trials based on plasma estimates should be considered a useless exercise. Adequate, tailored solutions should be considered on a case-by-case basis.

REFERENCES


