Open Questions on Bioequivalence

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Introduction

In bioequivalence trials the Investigator is often faced with specific problems not considered in operating guidelines.

These problems are defined in the literature as „Open questions on Bioequivalence“ and require particular attention from the Investigator in preparing the study protocol, and defining some specific procedures for the investigation.

The main „open questions“ are discussed in the present lecture.

Role of the Titre

In most cases the titre of drugs must be comprised within the 95-105% interval. In certain cases a wider range is allowed, e.g. 90-110 % or 90-115 %.

A relevant difference of the titre of test and reference can lead to a bioequivalence conclusion of bioinequivalent formulations or to bioinequivalence conclusion of two bioequivalent formulations.

The titre normalization of $C_{max}$ and AUC should solve this problem.

US FDA guidelines state that in BE trials, titre difference between test and reference must be $\leq 5\%$.

This precaution is a good alternative solution to the titre normalization.
High data variability

- This is a frequent problem in BE trials.
- High data dispersion can be:
  - intrinsic to the molecule,
  - the result of fist-pass effect,
  - the result of the presence of a multiple peak phenomenon or
  - congregated with endogenous substances.
- Data dispersion is managed by increasing the pool size of volunteers.
- $C_{\text{max}}$ and AUC congregate in most cases with lower variability when investigated in repeated dose regimen in steady state.
Poor sensitivity in the bioassay method or very low plasma concentrations

Examples are: bisphosphonates (enteral absorption <0.7%) diosmin (not appreciable plasma concentr.)

In the case of bisphosphonates urine concentrations are higher than plasma levels and urinary excretion can mirror enteral absorption. The high variability makes necessary even more than 100 subjects for statistical computation.

Drugs cleared solely or mainly via urine can be managed like bisphosphonates.
The puzzle of endogenous substances

This is a very complex problem, neglected by EU guidelines and in some specific cases considered in FDA guidelines (e.g. potassium and thyroxine). A slow absorption rate, conjugated with an excretion rate faster in certain cases (e.g. renal threshold), the homeostatic equilibria, specific storage organs, reversible interchangeable pools, physiologic rhythms and the dilution of the exogenous part (poor) with the endogenous part (relevant), all operate leading to flat profiles of plasma concentration.

An example of physiologic rhythm: Melatonin

A. Marzo, A. Rescigno

A. Marzo, D. Vuksic, F. Crivelli
A pharmacodynamic support to pharmacokinetics: calcium absorption and PTH

In some specific cases pharmacodynamics can support bioavailability conclusions, like parathyroid hormone (PTH) which mirrors enteric absorption of calcium better than calcium concentrations.

% Variation Ca$^{++}$ levels

PTH serum levels

variation %

serum concentration (ng/ml)
The complex problem of active metabolites (1)

Several drugs produce one or more active metabolite(s) in the body.

Three cases can occur:
1) Bioassay and assess bioequivalence only on parent drug.
2) Bioassay all active moiety, but assess bioequivalence only on parent drug; alternatively assess bioequivalence only on the main active metabolite.
3) Bioassay and assess bioequivalence on all the active moiety.

What is reported in guidelines?
US FDA and EMA guidelines and most specific updated literature would suggest the option 1).

Bioequivalence behind the scenes. A. Marzo
The complex problem of active metabolites (2)

Some drugs produce active metabolites which are largely prevalent on parent drug, such as

- Enalaprilat/Enalapril = 2.5 ratio
- Zofenoprilat/Zofenopril = 6.7 ratio
- Glyceryldinitrate/Nitroglycerin = 10.0 ratio
- Oxypurinol/Allopurinol = 44 ratio
- Hydroxyflutamide/Flutamide = 60 ratio
- Acid metabolite/Terfenadine = 100 ratio

With a largely prevalent metabolite, the bioequivalence should be assessed on the active metabolite.
An example of pharmacokinetic/pharmacodynamic relationship with an ACE inhibitor: Zofenopril

**Pharmacodynamics** can be a good support to pharmacokinetics in bioavailability and bioequivalence trials not only with endogenous substances, but also with xenobiotics, like glucose/insulin levels with antidiabetic drugs and angiotensin converting enzyme (ACE) with ACE-inhibitors.
Some drugs are cleared with long half-lives, like:
amiodarone 50 days;
chloroquine 41 days;
tamoxifen 8 days;
tamoxifen active metabolite 11 days.
Since blood should be drawn over a period lasting 3 times half-life and wash-out should last 7-10 times half-life, in certain cases the crossover design should be replaced by the two-parallel-group design.
The borderline case is tamoxifen:

For drugs with a long half-life, relative bioavailability and bioequivalence can be adequately estimated using truncated AUC, for instance over a 72 h period. In this case the sample collection time should be adequate to ensure comparison of the absorption process.

Experimental, extrapolated and truncated AUC in bioequivalence trials
Reversible metabolism

Reversible metabolism is very common with endogenous substances, but it is also present in some xenobiotics, like:
- canrenone/spironolactone and metabolites (acid/lactone forms)
- atorvastatine and metabolites (acid/lactone forms)
- sulindac: sulfoxide/sulfide forms
- encainide
- haloperidol
- cyclophosphamide
- warfarin

In these cases protocols should be tailored on case-by-case basis.
The multiple peak phenomenon (1)

A second peak is observed with some drugs, like piroxicam, **GLIBENCLAMIDE**, bile acids, and is related to enterohepatic circulation.

Second peak
8-12 h post-dose

Glibenclamide plasma concentrations
The multiple peak phenomenon (2)

**DICLOFENAC** produces a second peak 1.5 – 2.5 h after dosing. Fast absorbing (fast acting) formulations do not produce the second peak, which appears with medium absorption rate formulations.
The multiple peak phenomenon (3)

This was attributed to the competition of two kinetics: the absorption and the hydration of the drug to tetrahydrodiclofenac, which is less water-soluble than diclofenac.

Not all subjects present two peaks, and the two peaks have different height in individual subjects. This fact poses the problem of how to manage $C_{\text{max}}$ comparison between test and reference in bioequivalence trials.

Marzo and Reiner have suggested to consider primary parameter AUC and a secondary parameter $C_{\text{max}}$, which however must not affect activity and safety.

Open questions on bioequivalence: the case of multiple peak phenomenon
In some cases volunteers are not confined in the clinical facility during the night time. This can cause the following complications in bioequivalence trials:

1) An incorrect evaluation of half-life
2) The real fasting situation of the subjects is not under control; they may consume breakfast, smoke, drink alcohol, even if the protocol requests fasting since the previous evening, no smoking, no drinking.

The disrespect of the above procedures invalidates some critical aspects of the clinical trial.
Half-life and blood sampling schedule

In some bioequivalence trials, blood sampling times have long gaps, namely from 12 to 24 hours or from 24 to 48 hours post-dosing. This simplifies the study organization because volunteers are not kept confined in the clinical facility during the night. This long gap produces poorly defined half-life values, especially for drugs with $t_{1/2}$ in the range of 5-12 hours. An inappropriate determination of $t_{1/2}$ leads to an inappropriate calculation of $AUC_{\infty}$, a primary parameter in bioequivalence assessment:

$$AUC_{\infty} = AUC_{t} + \frac{C_{\text{last}}}{0.693 \cdot t_{1/2}}$$
Ethical issues

Some drugs, like cytostatic, can’t be given to healthy volunteers. There are various other drugs for which the administration to healthy individuals is questionable as well, mainly in repeated dose at steady-state.

Examples:
- Cyclosporine (affects renal clearance)
- Carbamazepine (requires 30 days for steady-state)
- Morphine (vomiting, cardiorespiratory problems, tolerance)
- Warfarin (requires a long treatment period to steady-state)
- Clozapine (syncope and serious cardiovascular severe side effects)
- Most drugs acting on CNS
- Nitroglicerin transdermal patches
Additional open questions (1)

- What to do with stereogenic drugs present on the market as racemates? Use enantioselective or non-enantioselective bioassay? Operating guidelines suggest the use of enantioselective bioassay, with only a few exceptions; in most cases, however, replicators use the less expensive non-enantioselective bioassay, and resulting reports are accepted by agencies.

- Polimorphic metabolism

- How to manage poor metabolizers (PM)? In single dose crossover trial this is not a problem. In parallel group design this could be a statistical problem; in steady state trials this could be a safety problem.
Additional open questions (2)

- How to manage drop-outs? Replace them or not? The general view is to avoid their replacement.

- Extended-release formulations producing flip-flop concentrations in the excretion phase: avoid to evaluate $t_{1/2}$ and $AUC_\infty$, use only $AUC_t$.

- Food effect must be checked also with enteric coated formulations.

- Bioassay in blood some drugs with a high blood/plasma concentration ratio, e.g. cyclosporin, chlorthalidone.
Conclusions (1)

BE, BinE and B(in)E

Clinical trials can produce results of bioequivalence (BE) or bioinequivalence (BinE). In several cases however these trials can produce results of bio(in)equivalence [B(in)E], which would mean that test and reference would seem to be bioequivalent, but this cannot be demonstrated in compliance with operating guidelines. This is often the result of a protocol which has not considered some specific issues. In most cases situations which would lead to B(in)E can be avoided leading to BE simply tailoring appropriately the study protocol.
Conclusions (2)

BE, BinE and B(in)E

This precaution has allowed several trials to produce positive results for the ANDA procedure.

The strategy selected must not conflict with operating guidelines and should be clearly and extensively discussed in the study protocol, as well as in the research and expert reports.