Review of the EMA guideline on the conduct of bioequivalence studies for veterinary medicinal products: focus on the development of veterinary generics.

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Introduction

In Europe, generics account for 30-40% of the global sales of animal health products. The market is dominated by parasiticides, vaccines and antibiotics, all of them being thereof chosen targets for generic companies. Though many parallels can be drawn between the development of animal and human pharmaceuticals, there are also many differences related to their modes of application (*e.g.* pour-on (PO) formulations), as well as the diversity and the size of the target population. To the author's viewpoint, the specificities of the veterinary healthcare market have only been partly addressed in the EMA guideline on the conduct of bioequivalence studies (EMA_GL$_{BE}$). This manuscript offers a review of these specificities, with emphasis on the development of generic PO and antibiotics.

Problem list

- How to define bioequivalence? Hypotheses and implications

- Testing bioequivalence of pour-on formulations: why is it a challenge?

- Bioequivalence studies in lieu of residues data: is it acceptable?

- A few words of caution on the development of generic antibiotics

How to define bioequivalence? Hypotheses and implications

Bioequivalence refers to the absence of a greater-than-allowable difference between the systemic bioavailability of a test and that of a reference formulation containing the same active substance. The assumption behind bioequivalence is that if two formulations portray similar plasma concentration *vs.* time profiles, they would likewise compare in terms of effectiveness and safety (Toutain & Koritz, 1997). Determination of bioequivalence is supported by the statistical comparison of mean pharmacokinetic parameters (*i.e.* AUC and C_{max}) in a subset of healthy subjects, hence the mention "average bioequivalence" (ABE). The range for AUC/C_{max} of the new formulation is generally set at +/- 20% of the mean AUC/C_{max} of the reference formulation. Nevertheless, a 20% difference in exposure can have a substantial clinical effect, depending on the shape of the exposure-effect relationship, and the width of the therapeutic window (see Figure 1). Thereof, *a priori* bioequivalence limits should be chosen based on clinical, not statistical grounds.

ABE does not guarantee that two formulations are indeed bioequivalent in a patient. In 1997, the Food and Drug Administration proposed additional guidance for the assessment of bioequivalence: population (PBE) and individual (IBE) bioequivalence. PBE and IBE combine the difference between population means and population variances; IBE further accounts for subject switchability from the pioneer to the generic formulation, referred to as subject-by-formulation interaction (Zariffa & Patterson, 2001). Based on available literature, there are only few situations where the comparison of average pharmacokinetic metrics (*i.e.* ABE) would not be suitable for bioequivalence testing: in case of highly variable drugs (HVDs) (*e.g.* PO formulations, see section below), or pharmaceuticals with narrow therapeutic index. In veterinary medicine, the determination of IBE is unrealistic for most of the drugs. However, assessing PBE would be a reasonable strategy in food-producing animals that are frequently medicated on a population basis.

Testing bioequivalence of pour-on formulations: why is it a challenge?

In veterinary medicine, parasiticides sales account for one third of the global healthcare market. The portfolio is headed by topically applied formulations *e.g.* PO. The EMA_GL$_{BE}$ reads: *"for pour-ons (…) the main absorption route is through the skin"*, which is contradicting findings from Laffont *et al.* (2003) who have estimated that only 10% of an ivermectin topical dose would be absorbed percutaneously. More recently, Imperiale *et al.* (2009) have shown that licking had a considerable effect on the availability of moxidectin when applied topically (*i.e.* 12.3 and 4.4 fold increase in AUC $_{(0-5\ days)}$ in plasma and milk, respectively) (see Figure 2). Such variations can result in significant differences in efficacy, as shown in Figure 3 (internal data, undisclosed compound).

This triggers the question of whether licking behavior should be factored into PO bioequivalence trials. From a theoretical viewpoint, showing bioequivalence in both "licking" *vs.* "non-licking" situations seems appealing. In practice though, licking behavior would only be an issue in case of a licking-by-formulation interaction. This situation may occur with non-palatable formulations and results in inconsistent intake compared with the pioneer (more palatable) formulation.

Another feature of PO formulations that makes the demonstration of bioequivalence even more difficult lies in their highly variable disposition[1]. High within-subject variability (WSV) can result in underpowered studies leading to the possible rejection of bioequivalence of a truly bioequivalent formulation. One could repeat the study with a greater number of subjects to meet the bioequivalence criteria, yet with high WSV the needed study size could be cost-prohibitive[2].

Lastly, because cattle lick each other, PO application should not only be viewed as an individual, but also as an oral collective treatment. Hence, there is some level of

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[1] For HVDs the within subject variability of pharmacokinetics parameters (*e.g.* AUC) estimated from the ANOVA equals or exceeds 30%.
[2] Alternative methodologies (not discussed herein) have been proposed *e.g.* widening of bioequivalence limits, or replicate designs for single-dose studies.

intuitive appeal for using PBE instead of ABE to show bioequivalence of PO formulations.

Bioequivalence studies in lieu of residues data: is it acceptable?

With the exception of formulations having a potential to leave local residues (*e.g.* injectables), the EMA_GL$_{BE}$ supports the use of bioequivalence studies for extrapolation of withdrawal times (WTs). Statistics associated with these concepts are however fundamentally different:

- Bioequivalence (ABE) is supported by the statistical comparison of *mean* pharmacokinetic parameters;
- While computation of WTs takes into account *population variability*, by determining tolerance limits (95% percentile in Europe (EU), 99% percentile in the United States (US)).

WTs are determined at a later point in time compared to the time interval used to demonstrate bioequivalence. As presented in Figure 4, two oral formulations could be bioequivalent but have different withdrawal times. As a matter of fact, showing bioequivalence does not guarantee that the upper one-sided 95% (EU)/99% (US) tolerance limit is below the maximum residual limit with 95% confidence for both formulations.

A few words of caution on the development of generic antibiotics

Antibiotics are one of the animal health's best-selling pharmaceuticals. Usage of antibiotics in animal species plays a role in the emergence and spread of resistance, making the development of veterinary generics a topic of great importance.

Several pharmacoeconomic studies have reported a correlation between the introduction of generics and the inflated consumption of certain classes of antibiotics (mostly quinolones), both in human (Monnet *et al.,* 2005) and veterinary (Chauvin *et al.,* 2008) medicine.

Further, the development of generic antibiotics has promoted the use of old antibiotics to the detriment of new (more active) agents. Less active class members are prone to select single-step mutants, thus facilitating the occurrence of other mutations that will make the bacteria resistant to all agents in the class.

As a result, many pathogens have become increasingly resistant to a large variety of antibiotics. Nowadays, about 70% of *Escherichia coli* isolates causing community or hospital-associated infections are resistant to amoxicillin (more than 20% are resistant to trimethoprim) (Finch, 2010).

Concluding thoughts

Some specifics of the veterinary healthcare market have been reviewed in this document, yet additional uncovered topics would be worth a debate (*e.g.* bioequivalence of extended release formulations, intramammary products, or medications intended for use in minor species).

The question of how to assess the bioequivalence of two formulations remains a complex domain that is even more challenging in veterinary medicine. Although ABE does not guarantee that two formulations are bioequivalent in a patient, there is little documented evidence of therapeutic failure following generic substitution. Still, it

would be reasonable to test PBE instead of ABE in food producing animals that are frequently medicated on a population basis (*e.g.* PO formulations).

Additional regulation is needed to lessen the impact of veterinary generics on the spread of antimicrobial resistance. One possibility would be to reevaluate the current dosing regimens and indications of old antibiotics that are being copied, using state of the art methodology[3] (Bousquet-Mélou & Toutain, 2010).

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[3] *e.g.* placebo-controlled trials to assess the value of old antibiotics and justify treatment durations; pharmacokinetics/pharmacodynamic modeling for dose estimation.

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Figure 1: *A priori* bioequivalence limits should be determined based on clinical, not statistical grounds. The decision should be driven by (i) the shape of the exposure-effect relationship [A] and [B], and (ii) the width of the therapeutic window [C] and [D].

The steeper the exposure-effect relationship, the higher the magnitude of effect related to a 20% difference in AUC.
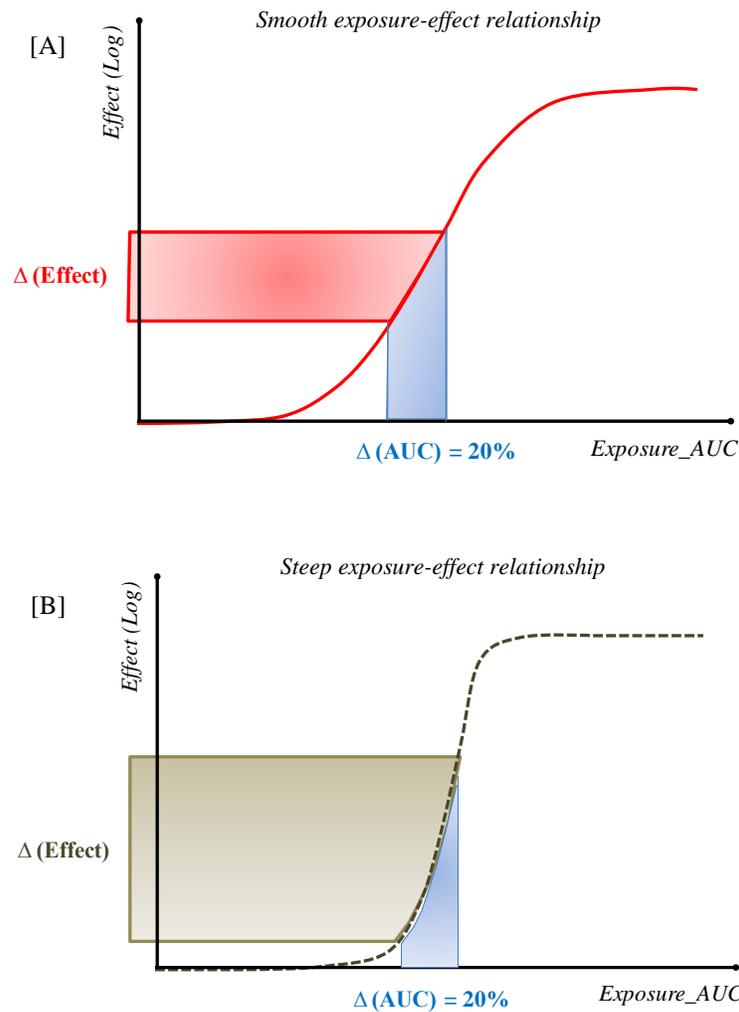
[A]

Smooth exposure-effect relationship

Δ (Effect)

Δ (AUC) = 20%

Exposure_AUC

[B]

Steep exposure-effect relationship

Δ (Effect)

Δ (AUC) = 20%

Exposure_AUC

Figure 1 (cont'd): *A priori* bioequivalence limits should be determined based on clinical, not statistical grounds. The decision should be driven by (i) the shape of the exposure-effect relationship [A] and [B], and (ii) the width of the therapeutic window [C] and [D].

The narrower the therapeutic window, the greater the risk that a 20% difference in AUC leads to sub-therapeutic or toxic exposures.
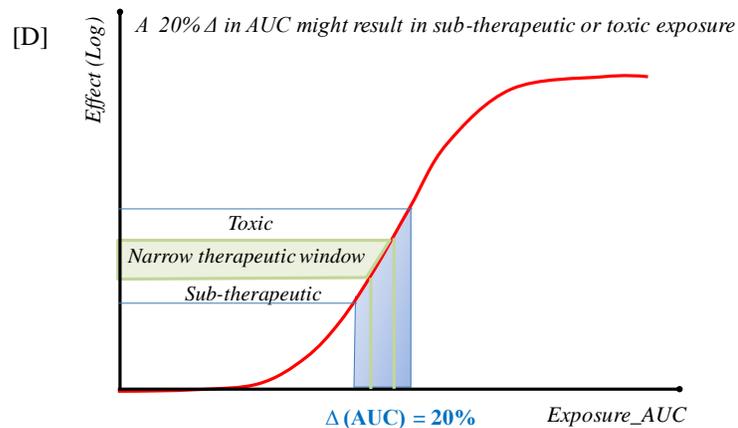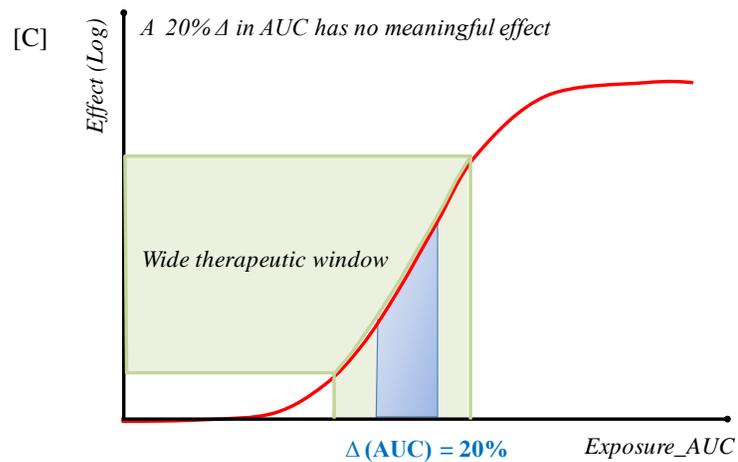
Figure 2: Comparison of moxidectin (MXD) availabilities in plasma and milk, expressed as partial areas under the plasma and milk concentration *vs.* time (AUC $_{(0-5\ days)}$ ± 1standard deviation) after pour-on administration (500 µg/kg) in licking-restricted (5 days licking restriction period) and free-licking dairy cows (n: 5).

*: p-value < 0.05 (source: Imperiale *et al.,* 2009).

According to the authors, licking restriction caused a significant decrease in moxidectin availability, both in plasma and milk matrixes. They concluded that licking had a considerable effect on the disposition of MXD after topical application in dairy cows.
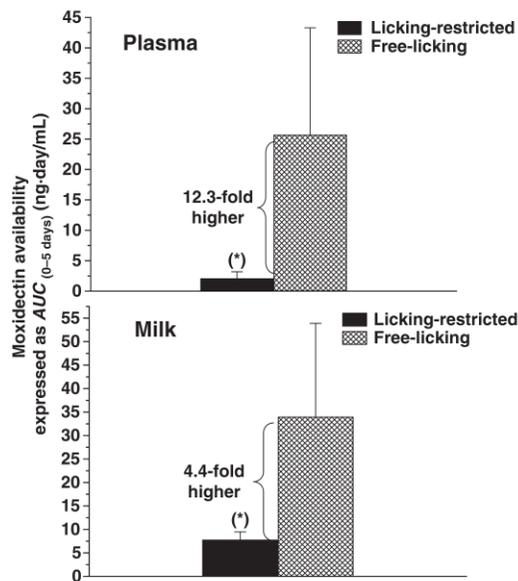
<u>Figure 3</u>: Endectocide (undisclosed compound) relative efficacy (RE) after pour-on application in free *vs.* tethered cattle (source: internal data). EPG: Egg count Per Gram.

RE on Ostertagia was null in tethered conditions, while almost complete in untethered conditions. For Cooperia, RE was two (at a 10 mg/kg dose) to three (at a 5 mg/kg dose) times greater in free cattle.

	Efficacy %	
	Cooperia	*Ostertagia*
10 mg/kg tethered	46.4	0
10 mg/kg free	99.8	100
5 mg/kg tethered	39.3	0
5 mg/kg free	99.8	99.7

$$RE = 100 * \frac{(EPG_{\text{before treatment}} - EPG_{\text{after treatment}})}{EPG_{\text{before treatment}}}$$

Figure 4: Two oral formulations could be bioequivalent, but have different withdrawal times (WTs). From: Toutain PL (2008). Bioequivalence: some challenge and issues. Informal CVMP/CMDv, Paris. Showing that A and B are bioequivalent does not guarantee that the upper one-sided 95% tolerance limit is below the maximum residual limit (MRL) with 95% confidence for both formulations.