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Using Bioequivalence Approach to Assess Toxicity Effect of Herbal Products, Tyrel™ and Rumbion™

[1]Chunhao Tu and [2]Woon Yuen Koh
[1]Department of Pharmacy Practice, College of Pharmacy,
University of New England, 716 Stevens Avenue, Portland, ME 04103, USA
[2]Department of Mathematical Sciences, College of Arts and Sciences,
University of New England, 11 Hills Beach Road, Biddeford, ME 04005, USA

Abstract: Problem statement: Herbal products have been widely used in veterinary healthcare. Recently, an experiment was conducted in the Natural Animal Facility Pvt. Ltd., Bangalore, India on ten Albino Wistar female mice to assess the acute oral toxicity of two herbal products, Tyrel™ and Rumbion™. However, the researchers did not evaluate the toxicity effect of the two products in terms of the bioequivalence approach. Two drugs are considered bioequivalent when there is no significant difference between them in terms of their absorption rates. For this study, we used body weight instead of absorption rate due to the lack of absorption rate information in the data. Bioequivalence studies provide another approach to evaluate the safety of veterinary healthcare products. The objective of this study is to evaluate whether the two herbal products, Tyrel™ and Rumbion™ are bioequivalent in terms of their toxicity effect. **Approach:** In this study, Tyrel™ and Rumbion™ were orally administered to 5 mice at 5000 mg kg^{-1} body weight sequentially. The body weight of each mouse was repeatedly measured at three time points: day 0, 7 and 14. To assess their bioequivalence, we approximated the Area Under the Curve (AUC) of the body weight of each mouse versus time for each mouse for each herbal product. Bootstrapped confidence intervals were computed to test the hypotheses of bioequivalence of the two products. **Results:** The ratio of the average AUCs of Tyrel™ and Rumbion™ at 5000 mg kg^{-1} body weight was 1.008 (g) and the 90% bootstrapped confidence interval of the ratio of was (0.977, 1.053), which falls within the predefined bioequivalence limits, 0.8 to 1.25. **Conclusion:** Based on the bioequivalence study, we concluded that there was no significant difference between the toxicity effect of Tyrel™ and Rumbion™ at 5000 mg kg^{-1} body weight.

Key words: Bioequivalence, Area Under the Curve (AUC), herbal products, toxicity

INTRODUCTION

Recently, (Joshua *et al*., 2010) assessed the acute oral toxicity of the two herbal products, Tyrel™ and Rumbion™ on ten Albino Wistar female mice. However, the authors of (Joshua *et al*., 2010) did not evaluate the toxicity of these two herbal products using the Bioequivalence (BE) approach. Originally, BE is used to determine the difference between 2 drugs in terms of the absorption rate in: *in vivo* studies (Haidar *et al*., 2008a). Bioequivalence studies are an important process of developing new drugs (Haidar *et al*., 2008b). A test drug and a reference drug is considered bioequivalent if the 90% confidence intervals for the test/reference ratios of the area under the drug's plasma concentration versus time curve (AUC) fall within 0.8 and 1.25, which is the predefined BE limits (Haidar *et al*., 2008a; 2008b; Karalis *et al*., 2009). Adopting the concept of BE allows one to assess the safety of the medicines, such as assessing the toxicity effects in the medicines, as it is a requirement of veterinary medicine products (Carakostas and Colaianne, 1996).

Since the use of herbal medicines for livestock is popular, it is essential to evaluate the safety of herbal medicines (Joshua *et al*., 2010). Although safety assessment of herbal products such as Tyrel™ and Rumbion™ have been discussed in (Joshua *et al*.,

Corresponding Author: Chunhao Tu, Department of Pharmacy Practice, College of Pharmacy, University of New England, 716 Stevens Avenue, Portland, ME 04103, USA Tel: (207) 221-4175

2010), there is still a lack of a comprehensive statistical analysis regarding the BE aspects of these two herbal products. In this study, we apply BE approach to assess the safety of these two herbal products.

MATERIALS AND METHODS

Materials: The clinical study was conducted at the Central Animal Facility, Research and Development Centre, Natural Animal Facility Pvt. Ltd., Bangalore, India. In the study, ten 8-12 weeks old female Albino Wistar mice were given herbal products Tyrel^{TM} and Rumbion^{TM} orally at 5000 mg kg^{-1} body weight to evaluate their toxicity level. The mice were left to adapt for the environment for one week before dosing and food was withheld overnight before and 3 h after the administration of the products. However, water was not withheld. Over the period when the mice were given the products, their body weights (g) were measured and analyzed. The body weight of each mouse was repeatedly measured at three time points: day 0, 7 and 14. The detail protocol information can be found in Joshua *et al*. (2010).

Statistical methods: To evaluate the toxicity effect between the two herbal products, Tyrel^{TM} and Rumbion^{TM} at 5000 mg kg^{-1} body weight using the BE approach, we first define the hypotheses of a BE study as follows (Templeman, 2004):

H_0: $\mu_T/\mu_R \le \delta_1$ or $\mu_T/\mu_R \ge \delta_2$
H_a: $\delta_1 < \mu_T/\mu_R < \delta_2$,

Where:

μ_T and μ_R = The population means of AUCs of Tyrel^{TM} and Rumbion^{TM}, respectively
δ_1 and δ_2 = The upper and lower limits of the (1-α)% confidence interval, respectively

In the BE studies, the 90% confidence interval has been predefined. If the 90% confidence interval of the μ_T/μ_R falls within 0.8-1.25 of the BE limits, then one would reject the null hypothesis and claim that two pharmaceutically products are bioequivalent (Haidar *et al*., 2008a; 2008b). The AUC can be computed using the trapezoidal rule that is:

$$AUC = \int_{t_0}^{t_k} f(t)dt \simeq \frac{1}{2}\sum_{i=0}^{k-1}(f_i + f_{i+1})(t_{i+1} - t_i),\ i = 0,..,k$$

Where:

f(t) = Represents the body weight of each mouse given a herbal product and
f_i = An average body weight at day t_i (Wu and Houghton, 2010)

To test the hypotheses, we constructed the two-tailed 90% bootstrap Bias-Corrected and accelerated (BCa) confidence intervals (Bradley and Tibshirani, 1993) to estimate the ratio of the two AUCs by re-sampling 10,000 data sets for each herbal product, Tyrel^{TM} or Rumbion^{TM}. All analyses were computed in R version 2.11.1 (R Development Core Team, 2010) for windows. The R code, with documentation and data, is available from the author upon request.

RESULTS

Table 1 summarized the approximated AUC of each mouse under two various herbal products, Tyrel^{TM} or Rumbion^{TM} at 5000 mg kg^{-1} body weight. The average AUCs of Tyrel^{TM} and Rumbion^{TM} were 2541.7 (g) and 2520.7 (g), respectively and thus, the estimated ratio of the average AUCs of Tyrel^{TM} and Rumbion^{TM} was 2541.7/2520.7 = 1.008 (g). The 90% BCa confidence intervals for the AUCs were given in Table 2. The 90% BCa confidence interval for the AUC of Tyrel^{TM} was (2509, 2593) and that of Rumbion^{TM} was (2421, 2589). Moreover, the 90% BCa confidence interval of the ratio of the average AUCs of Tyrel^{TM} and Rumbion^{TM} was (0.977, 1.053), which falls within the 0.8-1.25 BE limits. Thus, one would reject the null hypothesis and conclude that Tyrel^{TM} and Rumbion^{TM} are bioequivalent in terms of their toxicity effect.

Table 1: The estimated AUC of each mouse over time by 2 herbal formulations at 5000 mg kg^{-1} body weight

Mouse	1	2	3	4	5	Mean (SD)
Tyrel^{TM}	2516.5	2642.5	2513	2555.0	2481.5	2541.7 (62.09)
Rumbion^{TM}	2555.0	2667.0	2569	2327.5	2485.0	2520.7 (126.01)

SD: Standard Deviation

Table 2: The 90% BCa confidence intervals based on 10000 bootstrap samples of AUC

Formula	Original	Bias	SE	90% BCa CI
Tyrel^{TM}	2541.700	0.2997	24.96980	(2509, 2593)
Rumbion^{TM}	2520.700	-0.5152	50.45180	(2421, 2589)
Ratio	1.008	0.0007	0.02258	(0.977, 1.053)

SE: Standard Error; CI: Confidence Interval; Ratio: Average AUC of Tyrel^{TM} / Average AUC of Rumbion^{TM}

DISCUSSION

This study, using the BE approach, showed that Tyrel^{TM} and Rumbion^{TM} at 5000 mg kg^{-1} body weight on the female mice are bioequivalent in terms of their toxicity effect. Thus, the overall toxicological effect of Tyrel^{TM} and Rumbion^{TM} at 5000 mg kg^{-1} body weight was considered similar.

In this study, we built a bridge to connect the BE studies and toxicity assessments for validating the safety of veterinary medicine. Although BE studies have been widely used to evaluate pharmaceutical products (Haidar *et al*., 2008a; Karalis *et al*., 2009), BE studies have not been given much attention for assessing toxicity effect in veterinary medicine.

CONCLUSION

Based on the bioequivalence study, we concluded that there was no significant difference between the toxicity effect of Tyrel^{TM} and Rumbion^{TM} at 5000 mg kg^{-1} body weight. Further study is needed to investigate the appropriateness of the BE limits for toxicity assessment using body weights in veterinary medicine.

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