BIOEQUIVALENCE OF TWO BRANDS OF GENTAMICIN INJECTION IN GOATS

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ABSTRACT

Bioequivalence of two brands of gentamicin injection were studied in twelve clinically healthy goats. Single dose, equivalent to 5 mg gentamicin/kg.b.wt. was administered intramuscularly to the subjects in a parallel two way crossover design. The studied non-innovator brand (Opigent 10%) were biologically equivalence with Garamcin 4% as innovator (no statistically significant difference were observed using ANOVA at p≤0.05) in any in vivo indicator of the bioequivalence parameters, where, the area under plasma concentration-time curve (AUC0-24) was 118.14±3.36 and 118.12±4.75 µg/ml.h and maximum plasma concentration (Cmax) was 18.68±0.09 and 18.77±1.00 µg/ml for the studied innovator and non-innovator consequently.

INTRODUCTION

Gentamicin are widely used in veterinary medicinal as a chemo-therapeutic agent due to it have a broad-spectrum activity against varieties of G+ve and G-ve microorganisms. Gentamicin, generally bactericidal, a responsive host defense system is essentially for successful use. The bactericidal activity of gentamicin is occurred by reversible binding to bacterial ribosome’s and to inhibit protein synthesis. Gentamicin enter micro-organism in part by diffusion and in part by an energy-dependent carrier-mediated system that is responsible for the high level of these antibiotics achieved in susceptible bacteria (Brander; et al, 1991, Richard & Clasronce, 1986 and James; et al, 1996).

In last years, numerous formulations of gentamicin injection introduced for medicinal applications by several pharmaceutical companies and have been chemically equivalents rather than innovator product. Consequently, the biologi-
cal equivalency of the available formulations has become important and comparative bioequivalence studies have become necessary for evaluation the quality and acceptability of such non-innovator products.

There are a several factors affect biological equivalence of the same chemically equivalence drugs. These factors are specifications of active component, specifications of finished products, excipients, formulations and stability of finished products (BP pharmacopoeia, 2010 and USP pharmacopoeia, 2010).

Several regulations were issued for bioequivalence studies for human drugs as a indicator of drug quality (Food and Drug Administration; 1992, European Medicine Agency; 2009 and Central Administration of Pharmaceutical Affairs; 2010) but limited for veterinary drugs (European Medicine Agency; 2001 and Joao, et al; 2008), not implemented and not an item of drug registration.

So, the present study was undertaken to provide a scoping on biological equivalence of the available chemically equivalent non-innovators gentamicin injection using innovator’s as a standard in clinically healthy goats.

**Drugs:**
For this study, two brands of gentamicin injectable formulations were used. The innovator was Garamycin 4% from Memphis (Egypt) under license of Shering-Plough, USA and the non-innovator was Opigent 10%, Opipharma, Egypt.

**Animals:**
Twelve clinically healthy goats (2 males & 10 females, 20-30 kg body weight and 6 - 8 months age) were used for the study. The animals were fed barseem and kept under observation before starting and during the experiment.

**Experimental design:**
The animals were divided into two groups (each of six goats; 1 males & 5 females). For this study, two brands of gentamicin injection (Garamycin 4% as innovator and Opigent 10% as non-innovator) manufactured in Egypt were used. Single intramuscular dose of each brand equivalent to 5 mg of gentamicin/kg.b.wt. (Brander; et al, 1991) were administered to the animals in a parallel two way crossover design with a time interval of 15 days elapsed between each treatment, as a wash-out period, allowing time for essentially complete drug elimination from the body of the treated animals (Code of Federal Regulations and Food & Drug Administration; 1989).

**MATERIAL & METHODS**
Blood sample were collected from jugular vein of treated animals in a heparinized sterile test tubes just prior each treatment and at 15&30 minutes 1, 2, 4, 8, 12 and 24 hours post drug administration. Plasma were separated by centrifugation at 3000 rpm for 15 minutes and kept at -20ºC till analysis.

Bioassay:
Gentamicin concentration in plasma samples was assayed by microbiological technique with an agar diffusion method (Grove & Rondall, 1955 and Benett et al., 1966) using *E.Coli* (ATCC 10536) as a standard test organism (BD, USA). The correlation coefficient ($r^2$) of linearity of standard curve was 0.99.

Data analysis:
Drug concentrations in plasma were analyzed and calculated according to (Baggot, 1977 and Riviere, 1999). Differences in calculated parameters of two drugs were tested for statistical significant using ANOVA test (Snedicor and Cochran, 1987).

RESULTS
Mean plasma concentrations of gentamicin (µg/ml) were plotted against time (h) after single intramuscular administration of 5 mg gentamicin /kg.b.wt. to twelve clinically healthy goats in a parallel two crossover design (Figures 1&2).

Comparative bioequivalence parameters of the studied gentamicin brands were represented in Tables (1). The two drugs were reached maximum plasma concentration within 0.50 hours and maximum plasma concentration (Cmax) was 18.86±0.09 µg/ml for innovator (Garamycin 4%) and 18.77±1.00 µg/ml for non-innovator (Opigent 10%).

The obtained area under plasma concentration-time curve (AUC0-24) was 118.14 ± 3.36 and 118.12±4.75 µg/ml.h for innovator (Garamycin 4%) and non-innovator (Opigent 10%) respectively.
Table (1): Bioequivalence parameters (Mean±SE) of innovator (Garamycin 4%) and non-innovator (Opigent 10%) gentamicin injection following intramuscular administration (5mg/kg.b.wt.) to twelve clinically healthy goats in a parallel two way crossover design (n=12).

<table>
<thead>
<tr>
<th>Bioequivalence parameters</th>
<th>unite</th>
<th>Innovator (Garamycin 4%)</th>
<th>non-innovator (Opigent 10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0-24</td>
<td>µg/ml.h</td>
<td>118.14 ± 3.36</td>
<td>118.12 ± 4.75</td>
</tr>
<tr>
<td>Cmax</td>
<td>µg/ml</td>
<td>18.86 ± 0.09</td>
<td>18.77 ± 1.00</td>
</tr>
<tr>
<td>tmax</td>
<td>h</td>
<td>0.50 ± 0.02</td>
<td>0.50 ± 0.04</td>
</tr>
</tbody>
</table>

N.B.: Not statistically significant at \( p \leq 0.05 \)

Figure (1): Mean plasma concentrations of Garamycin 4% (5.0 mg/kg.b.wt.) after single intramuscular administration in goats (n=12).
Figure (2): Mean plasma concentrations of Opigent 10% (5.0 mg/kg.b.wt.) after single intramuscular administration in goats (n=12).

**DISCUSSION**

Bioequivalence means comparative bioavailability which indicate more than one similar dosage forms reach to the general circulation at the same relative rate and relative extent. It is an important indicator for evaluating the quality and acceptability of non-innovator drugs in comparative with innovator one.

There are several factors affect biological bioequivalence of the same chemically bioequivalence drugs. These factors are specifications of active component (optical rotation, heavy metals, relegated substances, potency, etc.), specifications of finished products (pH, assay of active ingredients, etc.), excipients, formulations and stability of the finished products. The pharmaceutical literature is replete with formulation factors that may influence absorption and drug preparation, assuming in the first place that has an active component of known purity and potency. The issue then becomes, what are the potential interactions that can occur between the active ingredients and the excipients that make up the formulation? Additionally, what the effects of the practitioner’s compounding techniques (materials used, mixing efficacy, etc.) on the amount of the active ingredients ultimately appearing in the formulation? These strategies are often encountered in pharmacological...
kinetics as they are affect the parameters estimated after drug administration. These factors are the primary determinants of differences in efficacy between so-called pioneer and generic drug products (Riverie, 1999).

The items used to evaluate bioequivalence of the two studied brands of gentamicin in clinically healthy goats are area under plasma concentration-time curve (AUC0-24), maximum plasma concentration (Cmax) (World Health Organization, 1974 and Code of Federal Regulations and Food & Drug Administration, 1989).

Area under plasma concentration-time curve (AUC0-24) is an indicative for the relative amount of drug absorbed while maximum plasma concentration (Cmax) is an indicator for extent of absorption (Baggot, 1977), where, the obtained area under plasma concentration-time curve (AUC0-24) was 118.14 ± 3.36 µg/ml.h for Garamycin and 118.12±4.75 µg/ml.h for Opigent and maximum plasma concentration (Cmax) was 18.86±0.09 and 18.77 ± 1.00 µg/ml for innovator and non-innovator respectively.

The obtained results denote to non-significant differences between the studied non-innovator brand (Opigent 10%) in comparative with the innovator one (Garamycin 4%). But, this paper invite who working in pharmaceutical industries, who exported the drugs and inspectors to put on our considerations a special attention toward bioequivalence studies to obtain a good evaluation about the efficacy or quality of the drugs in addition to the field trials (which not only enough) and finally to put the drugs on its right line to obtain a good picture about what we use.

Finally, we concluded that, the studied non-innovator brand (Opigent 10%) was biologically equivalent to the innovator one (Garamycin 4%).

REFERENCES:


Central Administration of Pharmaceutical Affairs (2010): Guidelines for bioequivalence studies for
marketing authorization of generic products.


**Food and Drug Administration; Center for Drugs and Biologics** (1992): Guidelines for the format and content of the human pharmacokinetics and bioavailability.


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**Acknowledgments**

Thanks to -Allah’s mercy- Prof. Dr. Abdalla Metwaly Al-Bauomy (Professor of Pharmacology, Chairman of Pharmacology and Forensic Medicine & Toxicology Department, Beni-Suef University) for his learning of pharmacokinetic calculations.
الملخص العربي
التكافؤ الحيوي لمستحضرين جنتاميسن حقن في الماعز

د. محمد محمد أحمد
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تم دراسة التكافؤ الحيوي لمستحضرين جنتاميسين حقن في ماعز سليم ظاهريا بعد إعطاء جرعة واحدة لكل من ما سمه الماء طريقة الحقن العضلي (5 مجم / كجم وزن) باستخدام نظام الحقن المتوازي الببالي على مدة 15 يوم.

اشترت النتائج التي أن المساحيق تحت المنحنى لتركيز الدواء في البلازما على مدى 24 ساعة من إعطاء الدواء كانت 118,14 ± 0.2 و 118.12 ± 0.5 ميكروجرام / مل. ساعه وان اعلي تركيز للدواء كان 18.18 ± 0.09 و 18.77 ± 0.20 ميكروراجام/مل لكل من المستحضر المرجعي (جاراتيسين 4%) والمستحضر غير مرجعي (أوبي جينت 10%) بالتابع.

أثبتت الدراسة أن مستحضر أوبي جينت 10% (مستحضر غير مرجعي) متكافئ بيولوجيا مع مستحضر جاراتيسين 4% (مستحضر مرجعي) وذلك بعد إجراء المقارنة الإحصائية للنتائج باستخدام اختبار انوفا على مؤشرات التكافؤ الحيوي (المساحيق تحت منحنى تركيز الدواء في البلازما وعلي تركيز الدواء في البلازما).