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BIOEQUIVALENCE STUDY OF TWO ORAL OXYTETRACYCLINE FORMULATIONS (OXYTETRACYCLINE 50%® AND TETRAMED®) IN BROILER CHICKENS

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ABSTRACT

The present study was designed to assess the comparative bio-equivalence of **Oxytetracycline 50%®** and **Tetramed®** in healthy broiler chickens after oral administration of both products in a dose of 20 mg oxytetracycline/kg.b.wt. Twenty four broiler chickens were divided into two groups. The first group was designed to study the pharmacokinetics of **Oxytetracycline 50%®**, while the 2nd group was designed to study the pharmacokinetics of **Tetramed®**. Each broiler chicken in both groups were injected intravenously with 20 mg oxytetracycline standard/kg.b.wt. Blood samples were obtained from the wing vein and collected immediately before and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours after a single intravenous or oral administration. Oxytetracycline in both products obeyed a two compartments open model following I.V. injection in a dose of 20 mg/kg.b.wt. The disposition kinetics of **Oxytetracycline 50%® and Tetramed®** following oral administration of 20 mg oxytetracycline base/kg.b.wt. revealed that the maximum blood concentration [C_{max}] were 3.60 and 3.14 µg/ml and attained at [t_{max}] of 0.97 and 1.01 hours, respectively. The mean systemic bioavailability of oxytetracycline in **Oxytetracycline 50%® and Tetramed®** after oral administration in healthy chickens was 91.17 and 89.57%, respectively. In conclusion: **Tetramed®** is bioequivalent to **Oxytetracycline 50%®** since the ratios of C_{max}, AUC_{0-24} and $AUC_{0-\infty}$ (T/R) were 0.87, 0.88 and 0.87 respectively. These are within the bioequivalence acceptance range. **Tetramed®** and **Oxytetracycline 50%®** are therefore bioequivalent and interchangeable.

1. INTRODUCTION

Antibiotics are widely used in veterinary practice for the treatment and control of several bacterial diseases in both animals and poultry. Oxytetracycline is a broad spectrum antibiotic.[1] It produces its bacteriostatic effect by inhibiting protein synthesis. It has good absorption, wide distribution and is excreted by renal route. It is used in the treatment of systemic bacterial infections.[2]

Tetracyclines are one of the most extensively used antibiotics in the veterinary practice owing to its favourable pharmacokinetics and broad spectrum of antimicrobial efficacy. Tetracyclines are effective on gram-positive, gram-negative bacteria, Chlamydia, spirochetes and some protozoa.[3] Oxytetracycline is a tetracycline with broadspectrum antibacterial therapy, which normally requires some daily parenteral treatment.[4] Oxytetracycline is a valuable choice in both of systemic and localized or tissue infections due to its balanced distribution between the blood and tissues.[5,6]

The bioavailability and bioequivalence studies play an important role in determining therapeutic efficacy to register the generic drug products according to the Food and Drug Administration (FDA) regulations.[7] Bioavailability is defined as the rate and extent to which an active drug ingredient is absorbed and becomes available at the site of drug action. In case of bioequivalence it is defined as statistically equivalent bioavailability between two products at the same molar dose of the therapeutic moiety under similar experimental conditions.[7,8] The drug products are said to be bioequivalent if they are pharmaceutical equivalents or pharmaceutical alternatives and if their rate and extent of absorption do not show a significant differences statistically according to the FDA regulations.[7]

The aim of this study is to evaluate bioequivalence of two oral oxytetracycline powder Oxytetracycline 50%® and Tetramed® after oral administration of a single dose in broiler chickens.

2. MATERIALS AND METHODS

2.1. Drugs

Oxytetracycline 50%® was obtained from **VIRBAC Co, France** (it was used as reference product) and **Tetramed®** was obtained from **Medmac Co. Amman, Jordan (**it was used as test product). Both are formulated as water soluble powders in aluminium sachets of 100 gm package. Each one gram contains 500 mg oxytetracycline base (as hydrochloride) and the

excepient was lactose. Pure standard oxytetracycline 95% obtained from (Sigma-Aldrich Chemical Co., St. Louis, USA) was freshly prepared in sterilized normal saline to obtain a concentration of 200 mg oxytetracycline activity/ml, this was used for intravenous injection.

2.2. Broiler Chickens and Experimental Design

Twenty four Hubbard healthy one day old broiler chicks were obtained from Benha private poultry farm, Egypt. They were kept individually in cages, within a ventilated, heated room (20˚C), and 14 hours of day light. They received a standard commercial ration free from any antibiotics 30 days before starting the experiment to insure complete clearance of any anti-bacterial substances from their bodies. Water was offered *ad-libitum*. This study was done at laboratory of Pharmacology Department, Faculty of Veterinary Medicine, Benha University, Egypt.

2.3. Bioequivalence Study

Broiler chickens (30 days old and weighing 1.60 – 1.85 kg) were used to study the bio-equivalence of **Oxytetracycline 50%® and Tetramed®** after oral administration. Broiler chickens were divided into two groups. The 1st group (12 broiler chickens) was used to study the pharmacokinetics of **Oxytetracycline 50%®**. The 2nd group (12 broiler chickens) was used to study the pharmacokinetics of **Tetramed®**. Each broiler chicken in both groups was injected intravenously with 20 mg oxytetracycline standard activity/kg.b.wt. Broiler chickens were left for 15 days to ensure complete excretion of oxytetracycline from their bodies. Broiler chickens in the 1st group were administered orally (in drinking water) with **Oxytetracycline 50%®** in a dose of 20 mg oxytetracycline/kg.b.wt (1 gm/liter, while broiler chickens in the 2nd group were administered orally with **Tetramed®**.

2.4. Blood Samples

Blood samples were obtained from the wing vein (1 ml) and collected in test tubes immediately before and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours after a single intravenous or oral administration (groups 1 and 2). Samples were centrifuged at 3000 rpm for 10 minutes and the obtained sera were used for the estimation of oxytetracycline concentration. The serum samples were stored at −20˚C until drug assay.

2.5. Analytical Procedure

Rapid agar-diffusion assay for the quantitative determination of oxytetracycline in small volumes of blood by using *Bacillus subtilis* (*ATCC* 6633).[9]

The organism was washed from the agar slant (which has been incubated for 24 hour 37˚c). The resulting growth was washed with 50 ml of sterile normal physiological saline. The resulting suspension was centrifuged at 3000 r.p.m. for 10 minutes and the supernatant was resuspended in 50-70 ml normal saline and heated for 30 minutes at 70˚c. The final spore suspension was diluted with saline to obtain a density of 10^7 spores/ml by using Mc-forland and nephelometer barium sulphate standard. The diluted suspension was stored in refrigerator at 4˚c till used. About 1 ml of the suspension of Bacillus subtilis 10^7/ml was added to 100 ml agar at 55-60 ◌̊c. The mixture was shaken thoroughly till complete mixing of the test organism with agar. Petri dishes (20 cm x 20 cm) were used; about 25 ml of inoculated medium were poured to each dish by using sterile cylinder. After complete solidification, six wells were made on the surface of inoculated agar using stainless steel cylinder. The wells of each plate were filled with the serum sample. The plates were incubated at 37 ˚c for 16-18 hours. The diameter of each inhibition zone was measured.

The calibration curves of serum were prepared with different concentrations between 0.1 and 20 µg/mL using blank chickens serum.

Thereafter, the diameters of inhibition zones were measured with the aid of a transparent rule to the nearest millimeter. Each sample was replicated three times and analyzed similarly. The plot of oxytetracycline serum concentrations versus diameters of inhibition zone was linear with a correlation coefficient of 0.993. Serum concentrations of oxytetracycline were determined by comparing the zone of inhibition diameters with the standard curve. The absence of interfering endogenous compounds was demonstrated in antibacterial-free plasma obtained at time 0 (pretreatment) which showed no visible zone of inhibition around the impregnated disks. The limit of quantification (LOQ) defined visually as the smallest amount of drug that still produced a clearly distinguishable inhibition zone around the edges of oxytetracycline contained pores on nutrient agar media was 0.1µg/ml.

2.6. Pharmacokinetics and Statistical Analysis

Serum concentrations of oxytetracycline versus time data obtained during the study were utilized for calculating various pharmacokinetic variables using a compartmental and non-compartmental analysis using computerized program, WinNonline 4.1 (Pharsight, USA).

The peak concentrations, C_{max} and time to peak, T_{max} were obtained from the serum concentration-time data directly. The areas under the serum concentration of oxytetracycline

time curves from time 0 to the last sample collected (AUC_{0-24}) were calculated using linear trapezoidal method.[10] While $AUC_{0-\infty}$ was derived from AUC_{0-24} + $AUC_{24-\infty}$, where $AUC_{24-\infty}$ = C24/ß. For bioequivalence evaluation, the ratios of C_{max} (T/R), AUC_{0-24} (T/R) and $AUC_{0-\infty}$ (T/R) were calculated. Values within the bioequivalence acceptable range at 90% confidence interval, 0.80 – 1.25 were considered for accepting the null hypothesis of bioequivalence between the reference and the test brands.[11,12]

Statistical analysis on the plasma concentration-time and pharmacokinetic profiles were carried out with analysis of variance (ANOVA). Significant difference at $p < 0.05$ was determined using Dunett test. All data were reported as mean ± SEM.

3. ESULTS

The mean blood concentrations-time profile (µg/ml) of oxytetracycline after intravenous administration of 20 mg oxytetracycline/ kg.b.wt. in broiler chickens are shown in (Table 1 and Figure 1). The mean pharmacokinetic parameters of oxytetracycline after intravenous administration of 20 mg oxytetracycline/kg.b.wt. in broiler chickens are shown in Table 2. Oxytetracycline in both formulations after intravenous administration could be described in a two compartmentsopen model. Oxytetracycline intravenous administration in a dose of 20 mg/kg.b.wt. revealed a high volume of distribution (exceeded than one L/kg) calculated by steady state [*V*dss] method, which are factors made oxytetracycline is highly distributed in all body tissues. A factor revealed that oxytetracycline is the drug of choice for attacking the systemic infections caused by sensitive organisms. The mean serum concentrations of oxytetracycline in Oxytetracycline 50%® and Tetramed® following oral administration of 20 mg oxytetracycline/kg.b.wt. in broiler chickens are shown in (Table 3 and Figure 2).

The mean pharmacokinetic parameters of oxytetracycline in Oxytetracycline 50%® and Tetramed® after oral administration of 20 mg oxytetracycline/kg.b.wt. in broiler chickens are shown in (Table 4).

The disposition kinetics of oxytetracycline in Oxytetracycline 50%® and Tetramed® following oral administration of 20 mg oxytetracycline base/kg.b.wt. revealed that the maximum blood concentration [C_{max}] were 3.60 and 3.14 µg/ml and attained at [T_{max}] of 0.97 and 1.01 hours, respectively. The systemic bioavailability (F%) was 91.17 and 89.57% for Oxytetracycline 50%® and Tetramed® respectively. The mean ratio of C_{max} and AUC of the reference and tested formulations were within bioequivalence range and summarized in Table 5. All the experimental chickens remained healthy during and after the study.

Table 1. Mean (X ± S.E) serum concentrations (µg/ml) of oxytetracycline following intravenous administration of 20 mg oxytetracycline/kg.b.wt. in broiler chickens (n = 12).

Time post Administration (hour)	Mean serum concentration (µg/ml)	
	Group 1	Group 2
0.08	12.34±0.58	12.01±0.56
0.16	10.13±0.51	9.92±0.53
0.25	8.19±0.49	7.96±0.47
0.5	6.28±0.46	6.01±0.49
1	5.18±0.41	4.97±0.40
2	3.21±0.38	2.29±0.37
4	2.51±0.40	2.24±0.32
8	1.67±0.12	1.38±0.12
12	0.98±0.03	0.81±0.04
24	0.41±0.02	0.37±0.02

Table 2. Mean (X ± S.E) pharmacokinetic parameters of oxytetracycline following intravenous administration of 20 mg oxytetracycline/kg.b.wt. in broiler chickens (n = 12).

Parameter	Unit	Group 1	Group 2
C^o	µg ml^{-1}	15.23 ± 0.92	14.87 ± 0.97
α	h^{-1}	4.15 ± 0.19	4.14 ± 0.16
β	h^{-1}	0.09 ± 0.01	0.09 ± 0.01
$t_{1/2(\alpha)}$	h	0.16 ± 0.01	0.16 ± 0.01
$t_{1/2(\beta)}$	h	7.41 ± 0.24	7.42 ± 0.23
AUC	µg $ml^{-1}h^{-1}$	43.81 ± 4.23	39.02 ± 4.09
AUMC	µg $ml^{-1}h^{-2}$	404.24 ± 27.31	353.71 ± 25.62
MRT	h	9.22 ± 0.46	9.06 ± 0.48
CL	L kg^{-1} h^{-1}	0.45 ± 0.04	0.51 ± 0.05
Vd_{ss}	L kg^{-1}	4.21 ± 0.28	4.64 ± 0.30

C° concentration at zero time (immediately after single IV injection); **α, β**; hybrid rate constants representing the slopes of distribution and elimination phases after IV injection , respectively; $\mathbf{T_{0.5(\alpha)}}$ distribution half-life after IV injection; $\mathbf{T_{0.5(\beta)}}$ elimination half-life after IV administration; **AUC**; area under serum concentration-time curve; **AUMC** area under moment curve; **MRT** mean residence time; $\mathbf{Vd_{ss}}$ volume of distribution at steady state; **Cl** total body clearance.

Table 3: Mean (X ± S.E) serum concentrations (µg/ml) of oxytetracycline following oral administration of 20 mg oxytetracycline/kg.b.wt. in broiler chickens (n = 12).

Time post Administration (hour)	Mean serum concentration (µg/ml)	
	Oxytetracycline 50%® (Reference)	Tetramed® (Test)
0.08	0.91±0.08	0.89±0.07
0.16	1.61±0.11	1.34±0.10
0.25	2.46±0.14	2.01±0.12
0.5	3.39±0.16	2.87±0.17
1	3.99±0. 5	3.47±0.14
2	2.99±0.11	2.69±0.10
4	2.41±0.13	2.11±0.11
8	1.69±0.08	1.39±0.06
12	1.08±0.06	0.98±0.08
24	0.54±0.02	0.48±0.01

Table 4: Mean (X ± S.E) pharmacokinetic parameters of oxytetracycline in Oxytetracycline 50%® and Tetramed® following oral administration of 20 mg oxytetracycline/kg.b.wt. in broiler chickens (n = 12).

Parameter	Unit	Oxytetracycline 50%® (Reference)	Tetramed® (Test)
K_{ab}	h^{-1}	3.65 ± 0.17	3.43 ± 0.18
K_{el}	h^{-1}	0.115 ± 0.01	0.117 ± 0.01
$t_{1/2(ab)}$	h	0.18 ± 0.01	0.20 ± 0.02
$t_{1/2(el)}$	h	6.03 ± 0.21	5.92 ± 0.20
C_{max}	µg ml^{-1}	3.60 ± 0.18	3.14 ± 0.14
t_{max}	h	0.97 ± 0.03	1.01 ± 0.04
AUC	µg $ml^{-1}h^{-1}$	39.94 ± 3.74	34.95 ± 3.28
AUMC	µg $ml^{-1}h^{-2}$	430.69 ± 25.45	377.84 ±23.89
MRT	h	10.78 ± 0.44	10.81 ± 0.51
MAT	h	1.56 ± 0.07	1.75 ± 0.08
F	%	91.17 ± 5.07	89.57 ± 5.01

k_{ab}; K_{el} absorbtion and elimination rate constant after oral administration; **$T_{0.5(ab)}$** absorbtion half life after oral administration; **$T_{0.5(el)}$** elimination half life after oral administration; **C_{max}** maximum plasma concentration; **T_{max}** time to peak plasma concentration; **F** (bioavailability); fraction of drug absorbed systemically after oral administration.

Table 5. Bioequivalence between Oxytetracycline 50%® (reference) and Tetramed® (test) formulations.

Bioequivalence	C_{max}	AUC_{0-24}	$AUC_{0-\infty}$
Oxytetracycline50%®	3.60±0.18	39.94±3.51	39.94±3.74
(Reference) Tetramed® (Test)	3.14±0.14	34.84±3.34	34.95±3.28
Point estimate	0.87	0.88	0.87
Acceptable range	0.80-1.25	0.80-1.25	0.80-1.25
Conclusion	**BE**	**BE**	**BE**

BE-Bioequivalence

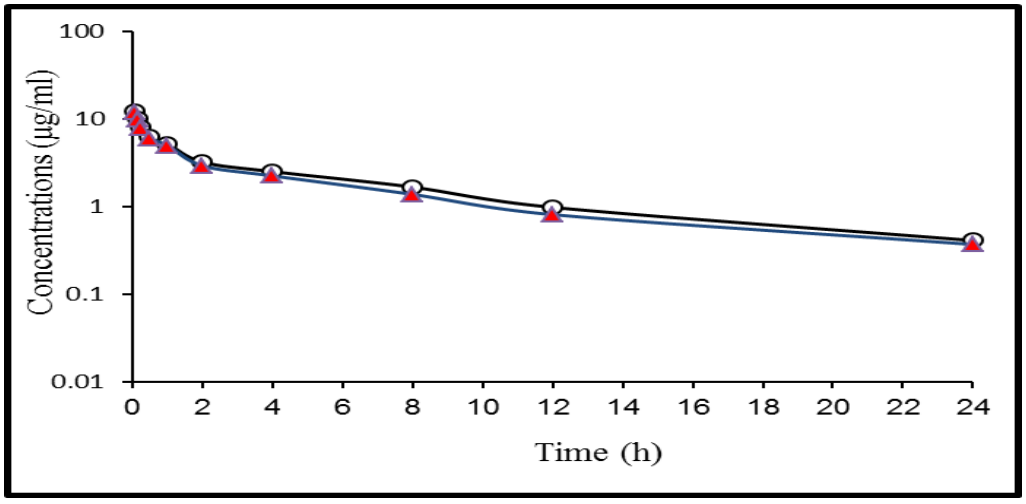

Figure 1: Semilogarthimic plot showing the serum concentrations-time profile of oxytetracycline in Group 1 (○-○) and Group 2 (▲-▲) following intravenous administration of 20 mg oxytetracycline/kg.b.wt. in broiler chickens (n = 12).

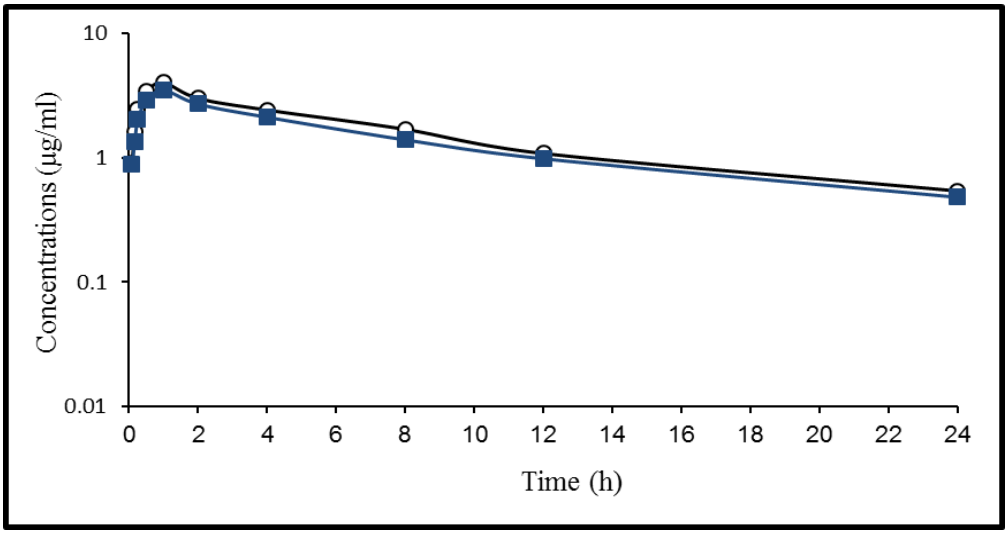

Figure 2: Semilogarthimic plot showing the serum concentrations-time profile of oxytetracycline in Oxytetracycline 50%® (○-○) and Tetramed® (■-■) following oral administration of 20 mg oxytetracycline/kg.b.wt. in broiler chickens (n = 12)..

4. DISCUSSION

Antibiotics are widely used as veterinary drugs or as feed additives to promote growth.[13,14,15] Oxytetracycline in both groups after i.v. administration could be described in a two compartments-open model. This indicated that, oxytetracycline distributed in the body of broiler chickens in two compartments; a central one which represent blood and highly perfused organs (kidney-liver-spleen-heart) and a 2nd peripheral compartment which represented by skin and connective tissues, these results were similar to that obtained for oxytetracycline in sheep[16] and lactating goats.[17]

The effectiveness of a drug is partly dependent on its formulation, route of administration and metabolic pattern.[18] These factors determine the plasma concentration-time profile of the drug. Following administration of a single oral dose (20 mg/kg b.w) of oxytetracycline formulations to healthy broiler chickens, therapeutic concentration were achieved 5 minutes post administration in all the chickens. The concentration was detected up to 24 hours in the serum of chickens given the (**Oxytetracycline 50%®** as a reference product and **Tetramed®** as a tested product).

Oxytetracycline in both groups was eliminated with half-lives [$t_{0.5(\beta)}$] equal to 7.41 and 7.42 hours, respectively. The long $t_{0.5}$ is a clear characteristic of oxytetracycline. A range of $t_{0.5(\beta)}$ to be 8-14 hours. This range is in fair agreement with that of the present study.[19]

High volume of distribution (4.21 and 4.64 L/kg) and a low total body clearance (0.45 and 0.51 L/kg/h) for **Oxytetracycline 50%® and Tetramed®**, respectively; indicates that oxytetracycline is rapidly absorbed, widely distributed and slowly eliminated in the body after oral administration in chickens. In this respect, high volume of distribution of oxytetracycline was recorded in sheep.[16]

The disposition kinetics of **Oxytetracycline 50%® and Tetramed®** following oral administration of 20 mg oxytetracycline base/kg.b.wt. revealed that the maximum blood concentration [C_{max}] were 3.60 and 3.14 µg/ml and attained at [t_{max}] of 0.97 and 1.01 hours, respectively. C_{max} was 6.09 µg/ml in sheep[20], 5.7 µg/ml in calves[21] and 4.4 µg/ml in dogs.[22]

The oral bioavailability of **Oxytetracycline 50%® and Tetramed®** was 91.17 and 89.57%, respectively; indicated a good absorption from GIT. This is indicated that both products are

advised to be given orally in case of acute bacterial attacks in blood and other organs. This values indicated the high extent of absorption following oral administration in chicken more than calves (46.35%).[23]

No significant differences were observed between the pharmacokinetics parameters of the two formulations; these results were showing the bioequivalence of the two formulations were according to the criteria established by FDA.[7]

It has been postulated that maximum efficacy in a clinical setting with oxytetracycline is achieved when serum concentrations of the drug are barely at or above the MIC level for the pathogen in question, for as long as possible within the dosing interval. These indicated a therapeutic blood concentration for most bacteria infecting chickens. These concentrations exceeded the MIC of most bacteria infecting chickens (MIC_{90} ranged between 0.025 ~ 0.56 µg/ml) for *Mycoplasma* spp., *E. coli*, *Salmonella* spp., *Haemophilus* spp., *Pasturella multiocida*, Shigella, *clostridia* spp.,) as mentioned by.[1,24]

Bioequivalence study is a test to assure the clinical efficacy of a generic versus brand drugs,.[7] Bioequivalence refers to a comparison between generic formulations of a drug, or a product in which a change has been made in one or more of the ingredients or in the manufacturing process, and a reference dosage form of the same drug.[18] This study shows that the bioequivalence ratio for mean AUC_{0-24}, $AUC_{0-\infty}$ and C_{max} (T/R) of **Tetramed®** versus the reference products (**Oxytetracycline 50%®**) were 0.88, 0.87 and 0.87 respectively. These values were within the recommended range at the level of 90% confidence interval, 0.80 – 1.25.[25] The two formulations of oxytetracycline oral tested in this experiment could therefore be considered bioequivalent.

CONCLUSIONS

Based on the above pharmacokinetic and statistical results that calculated in the current study, we concluded that **Tetramed®** was bioequivalent to **Oxytetracycline 50%®** and both products can be used as interchangeable drug in veterinary medicine practice especially in poultry.

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