Stability And Surrogate Markers Of Antimicrobial Activity Of Ampicillin Used In Drinking Water For Treatment Of Chickens

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ABSTRACT

Physical and chemical stability of ampicillin after reconstitution in drinking water was investigated under the effect of heat at three conditions (30, 40 and 50 °C) for 8 hours. Appearance of drug solution not changed from zero time and up to 8 hours after reconstitution, while pH of the drug solution was increased from 7.21 at zero time to 7.80, 8.45 and 8.25 for three conditions respectively. Assay of ampicillin at zero time was 99.45 % and still stable for 2 hours after reconstruction (99.22, 99.11 and 99.13 %), while at 4, 6, and 8 hours, the degradation of ampicillin was recorded with final concentrations of 86.27, 86.36 and 86.19% for the three storage conditions (30, 40 and 50°C) at 8 hours respectively. After single oral administration of ampicillin before (25 mg/ kg.bwt.) and after heat stress at 50°C and storage for 8 hours (equivalent to 21.67 mg/ kg.bwt.) in clinically healthy chickens, Pharmacokinetic / pharmacodynamic integration index was 5.06 and 4.65 hours respectively.

INTRODUCTION

A medicinal product is designed to possess certain desirable properties of which the following are of major importance. When the product is administered by the specified route, the active constituent should achieve the required rate and extent of bioavailability. The product itself should be efficacious, safe, and acceptable; it should be convenient in use and stable. The stability of a product relates to various reactions that may change the original properties of the preparation after reconstitution and storage. Other criteria of stability are the effects of such changes on the fitness of the product for use as a medicine *(1)*.

In this trial, ampicillin was used as a module to study the stability of antibiotics after reconstitution in drinking water. Ampicillin is a broad spectrum penicillin derivative used for treating respiratory, gastrointestinal, urinary and skin bacterial infections. It is 4-8 times more active against gram-negative bacteria and 50 times more resistant to gastric pH than penicillin-G, but is sensitive to beta-lactamase *(2,3)*. On the other hand, several factors affect the fate of drugs in animals *(4)* and these include species differences *(5)*.

The objective of the present study was to investigate the stability of ampicillin after reconstitution in drinking water with exposure to heat stress for a definite time and then to determine the comparative surrogate markers (Pharmacokinetic /pharmacodynamic integration index) of antimicrobial activity of ampicillin following oral administration in chickens before and after treatment.

MATERIAL AND METHODS

Ampicillin 20% (VETWIC-Egypt) water soluble powder was used for both; *in vitro* study (stability) and *in vivo* study (pharmacokinetic).

In vitro study

Samples preparation

Ampicillin was reconstituted in tape water (pH 6.8 and conductivity 368 µs) at recommended dose (1-1.5gm/liter) on fifteen bottles (one liter capacity) and stored in three conditions at 30, 40 and 50 °C (five bottles/ incubator) for 8 hours. After reconstitution of the drug 50 ml was taken from each bottle at 0, 2, 4, 6 and 8 hours for physical evaluation and drug assay.

Physical analysis

Physical analysis was carried depending on appearance and pH of drug solution *(6)*.

Drug assay

Concentration of ampicillin was determined in drug solutions by microbiological assay

method *(7)* using *Micrococcus Luteus* ATCC 9341 (BD, USA) as a standard test organism. The sensitivity of detection of ampicillin was 0.05 μg/ml and correlation coefficient (r^2) of linearity of standard curve was 0.99.

2.*In vivo* study

Twenty chickens; 1.75-2.0 kg b.wt. and 35 days old were divided into two equal groups (each of 10). The first group was administered ampicillin intracrop using specific stomach tube at a rate of 25 mg/kg.b.wt. as a control group and the second group was administered ampicillin after reconstitution in drinking water and storage for 8 hours at 50°C (equivalent to 21.67 mg/ kg.bwt.).

Blood sampling

After drug administration, blood samples were withdrawn from wing vein in sterile tubes prior and at 0.25, 0.5, 1, 2, 3, 4, 5 & 7 hours after drug dosing. Sera were separated and kept at -20°C till drug analysis.

Drug assay

Concentration of ampicillin was determined in serum by microbiological assay method *(7)* using *Micrococcus Luteus* ATCC 9341 (BD, USA) as a standard test organism. The sensitivity of detection of ampicillin was 0.05 μg/ml and correlation coefficient (r^2) of linearity of standard curve was 0.99.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of ampicillin against *E.Coli* O157:H7 (BD, USA) was performed using agar plate diffusion technique *(8)*.

Data analysis

The results were displayed as mean±SE and Student (*t*) test at $p \leq 0.05$ was used *(9)*. Pharmacokinetic data was performed using serum concentration-time profile *(10,11)*.

RESULTS AND DISCUSSION

Stability studies of drugs before and after reconstitution have an important role on the assessment of efficacy and acceptability of drugs, whereas, the drugs must be kept under a special conditions to protect its active ingredients from rapid degradation. There are a five types of stability of drugs must put in our consideration to judge the fitness of any drugs; chemical, physical, microbiological, therapeutic and toxicological stability *(12)*. However, chemical degradation of active ingredients resulted in reduction or loss of potency or production of undesirable metabolites (which may be toxic) as chemical degradation of tetracyclines which converted to epianhydrotetracycline, while, degradation of an excipient cause problems of physical or microbiological stability *(13)*.

According to International Conference on Harmonization (ICH) and Food & Drugs Administration (FDA) countries allover the world were classified into four zones, in each zone the drugs must be kept within a definite limits of temperature and relative humidity%. Egypt was located in Mediterranean zone; the typical storage conditions are 25±2 °C for temperature and 65±5% for relative humidity *(14)* and recently, the typical storage conditions become 30±2 °C for temperature and 70±5% for relative humidity% *(15)*.

In this study, physical and chemical data of ampicillin after reconstitution in drinking water and storage for 8 hours under heat stress (30, 40 and 50 °C) was represented in Table 1 and Figure 1.

Physical (appearance and pH) stability of ampicillin after reconstitution in drinking water and storage for 8 hours under heat stress (30, 40 and 50 °C) indicated that, the appearance of drug solution is not changed from zero time and up to 8 hours of storage and the pH of this solution was increased from 7.21 at zero time to 7.80, 8.45 and 8.25 at three conditions for 8 hours without significant changes, but give an evidence of drug degradation.

Table 1. Physical and chemical specifications of ampicillin after reconstitution in drinking water and storage for 8 hours under heat stress (30, 40 and 50 °C).

Time (hour)	Storage conditions								
	30 °C			40 °C			50 °C		
	Color of solution	pH	Assay%	Color of solution	pH	Assay%	Color of solution	pH	Assay%
0	Clear	7.21 ±0.23	99.45 ±0.92	Clear	7.21 ±0.41	99.45 ±0.29	Clear	7.21 ±0.07	99.45 ±0.91
2	Conform	7.32 ±0.90	99.22 ±0.24	Conform	7.33 ±0.23	99.11 ±0.12	Conform	7.39 ±0.09	99.13 ±0.36
4	Conform	7.44 ±0.07	96.54 ±0.61	Conform	7.45 ±0.11	95.49 ±0.23	Conform	7.60 ±0.10	95.30 ±0.58
6	Conform	7.61 ±0.11	90.68 ±0.32	Conform	8.00 ±0.22	90.93 ±0.71	Conform	8.05 ±0.07	90.69 ±0.24
8	Conform	7.80 ±0.23	86.27 ±0.58	Conform	8.45 ±0.06	86.36 ±0.80	Conform	8.25 ±0.11	86.19 ±0.07
Rate constant of degradation (+/-) from 2 to 8 hours	-	+ 0.01	- 0.024	-	+ 0.02	- 0.023	-	+ 0.02	- 0.023

Acceptance limit of assay % : 90% - 110

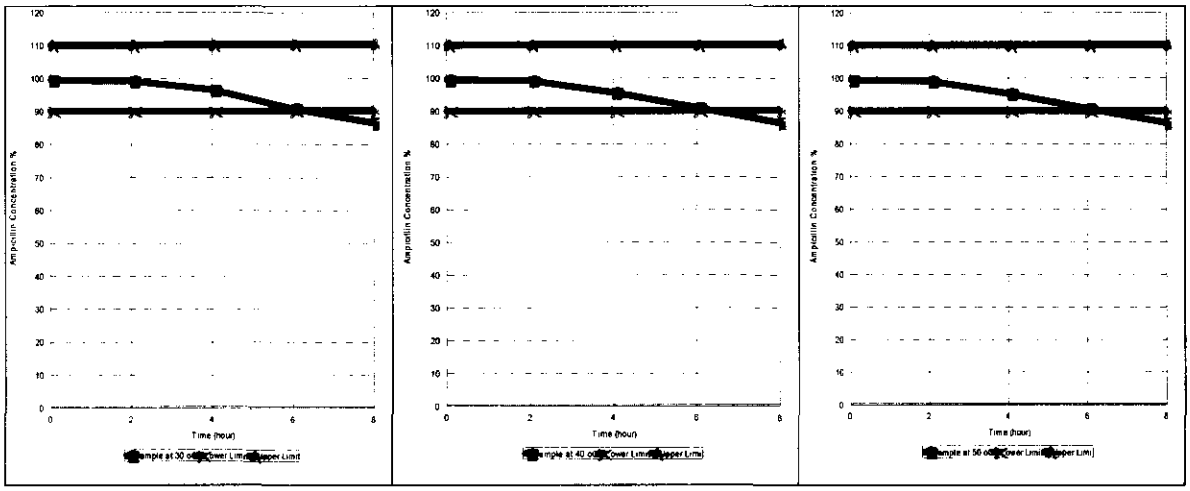

Figure 1. Assay % of ampicillin at 0, 2, 4, 6 and 8 hours after reconstitution in tape water and storage under heat stress at 30, 40 and 50 °C.

Chemical stability (assay) of ampicillin after reconstitution in drinking water and storage for 8 hours under heat stress (30, 40 and 50°C) indicated that, assay of drug at zero time was 99.45% and still stable for 2 hours after reconstruction (99.22, 99.11 and 99.13%) for the three storage conditions. At 4,6, and 8 hours of reconstitution the degradation of ampicillin was recorded with constant rate of -0.024, -0.023 and -0.023 and final concentrations of 86.27, 86.36 and 86.19 % at 8 hours for the three storage conditions (30, 40 and 50°C) which fall out acceptance limit of specification (90-110%) *(16)* without significant changes between the three storage conditions and indicated that, degradation of

ampicillin after reconstitution in drinking water is time dependent not heat dependent.

Mean serum concentration-time profile following single oral administration of ampicillin before (25 mg/ kg.bwt.) and after heat stress (50°C) and storage for 8 hours (equivalent to 21.67 mg/ kg.bwt.) in clinically healthy chickens was depicted in Figure 2, while, pharmacodynamic / pharmacokinetic parameters were shown in Table 2.

Table 2. Pharmacokinetic/pharmacodynamic parameters of ampicillin following single oral administration of (25 mg/ kg.bwt.) and after heat stress at 50°C for 8 hours (equivalent to 21.67 mg/ kg.bwt.) in clinically healthy chickens (n=10).

Parameters	Unite	Ampicillin	
		at 0 time	at 8 hours
Pharmacokinetic			
Cmax	$\mu g.ml^{-1}$	0.90±0.12	0.77±0.20
tmax	min.	0.75±0.10	0.75±0.09
AUCo-∞	µg/ml.h	2.41±0.12	2.06±0.13
Pharmacodynamic			
MIC (*E.Coli*)	µg/ml	0.125	
Pharmacokinetic/pharmacodynamic integration			
T ≥ MIC	-	5.06±0.35	4.65±0.32

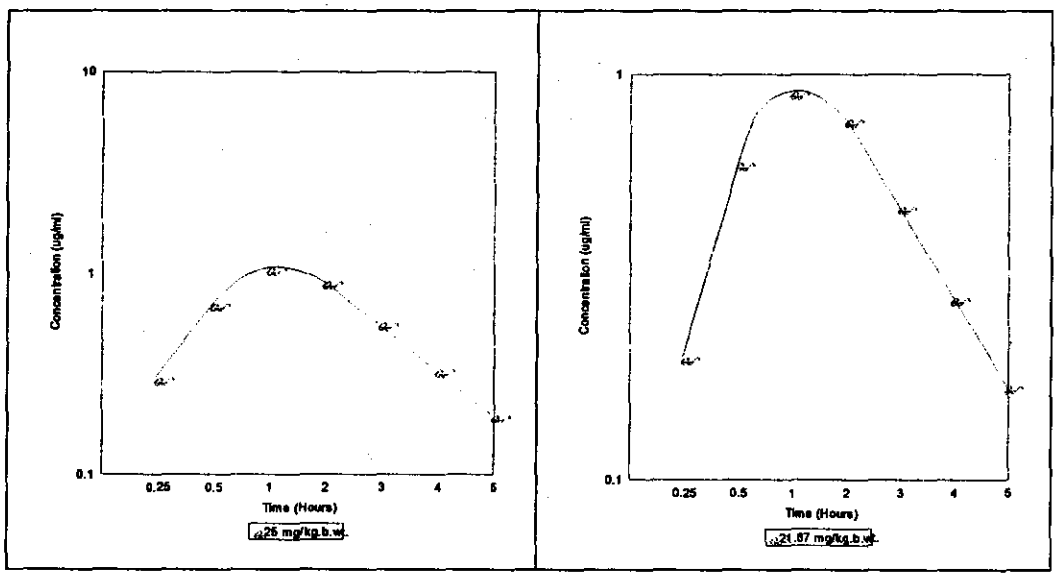

Figure 2. Mean serum concentration-time profile following single oral administration of ampicillin (25 mg/ kg.bwt.) and after heat stress at 50 °C for 8 hours (equivalent to 21.67 mg/ kg.bwt.) in clinically healthy chickens (n=10).

The aim of beta-lactam therapy is to keep the antibiotic plasma concentration above the minimum inhibitory concentration (MIC) at which bacteria are inhibited from growth *(17)*.

After single oral administration of ampicillin before (25 mg/ kg.bwt.) and after heat stress at 50°C and storage for 8 hours (equivalent to 21.67 mg/ kg.bwt.) in clinically healthy chickens, peak serum concentrations (Cmax) was 0.90 and 0.77µg /ml at maximum time at which the drug reach maximum concentration (tmax) of 1 hours and area under serum drug concentration curve (AUCo-∞) of 2.41 and 2.06 $\mu g.ml^{-1}.h$ respectively.

Time of drug concentration in serum>minimum inhibitory concentration (T> MIC) is the best pharmacokinetic/ pharmacodynamic integration index as a surrogate markers of antimicrobial activity for a concentration-independent drug as ampicillin and this index is required for clinical cure depending on the host defense or pathogen *(11)*.

Our results indicated that, time of drug concentration (T) in serum before (25 mg/ kg.bwt.) and after heat stress at 50°C and storage for 8 hours (equivalent to 21.67mg/ kg.bwt.) in serum of clinically healthy chickens overcome the minimum inhibitory concentration (MIC) of *E.Coli* (≥ 0.125 µg/ml) for 5.06 and 4.65 hours, respectively as a surrogate markers of antimicrobial activity

(T>MIC) with no statistically difference. Finally, we concluded that, ampicillin was stable under the effect of temperature at 30, 40 and 50 °C and acceptable for medicinal use for 6 hours after drug reconstruction in drinking water.

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الثبات والكفائه الميكروبية البديلة للامبسيللين المستخدم في الدواجن بعد إضافته لماء الشرب

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تم دراسة الثبات الطبيعي (اللون والأس الهيدروجيني) والكيماني (تركيز المادة الفعالة) للأمبسيللين بعد إذابته في ماء الشرب وحفظه عند ثلاث درجات حرارة (30، 40 و 50 درجه مئوية) لمدة 8 ساعات.أشارت النتائج إلى أن محلول الدواء لم يطرأ عليه تغيير في حين حدثت زيادة في الأس الهيدروجيني من 7.21 عند بداية الذوبان إلى 7.80، 8.45 و 8.25 عند 8 ساعات من المعاملة الحرارية بالتتابع في حين كان تركيز المادة الفعالة عند بادية الذوبان 99.45% وظل ثابتا لمدة 2 ساعة بعدها بدأ في التحلل وكان التركيز النهائي 68.27، 86.36 و 86.19% عند 8 ساعات من المعاملة الحرارية بالتتابع.

كما دلت مؤشرات التوافق الحركي مع الفاعلية الدوائية بعد إعطاء الدجاج جرعه واحده عن طريق الفم قبل (25 مجم/كجم وزن حي) وبعد التعرض لـ 50 درجه مئوية لمدة 8 ساعات (جرعه مكافئه 21.67 مجم/كجم وزن حي) إلى أن اعلي تركيز للدواء في البلازما ≤ اقل تركيز لمنع نمو ميكروب القولون العصوي كانت 5.06 و 4.65 بالتتابع.