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BIOAVAILABILITY AND DISPOSITION KINETICS OF AMOXICILLIN;

IN NORMAL AND WATER DEPRIVED (DEHYDRATED) RABBITS

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ABSTRACT… OBJECTIVE: The study was planned to observe the bioavailability and disposition kinetics of amoxicillin in adult rabbits (irrespective of sex) under healthy and dehydrated conditions. **Design:** Comparative. **Place and duration of study:** The study was conducted at the department of pharmacology, University of Veterinary and Animal Sciences, Lahore from April 2013 to October 2013. **Methodology:** Initially all rabbits were weighed and their packed cell volume (PCV) and other biochemical parameters were observed under normal conditions. Bioavailability and disposition kinetics of amoxicillin (10mg/kg body weight) were studied in normal rabbits following oral and intravenous route of drug administration. After 10 days washout period, these rabbits were made dehydrated by keeping the animals off water but not food. The animals with 10% decrease in body weight were declared dehydrated. Their parameters were again measured. Treated rabbits were administered amoxicillin orally and intravenously (10mg/kg body weight). Samples were drawn at prescribed time. Amoxicillin was assessed in plasma by using microbiological assay method. Plasma concentration was analyzed using non compartmental method. **Results:** The water deprived or dehydrated rabbits showed a significant increase in the packed cell volume, blood glucose and plasma globulins as compared to the normal rabbits. However, there was a significant ($p<0.05$ & $p<0.01$) decrease in body weight, total proteins, albumins and albumin globulin ratio of the dehydrated rabbits. The peak plasma concentration, volume of distribution and rate constant of elimination was lower in the dehydrated rabbits as compared to the normal rabbits. The plasma concentration of amoxicillin after intravenous administration in dehydrated rabbits had a significant ($p<0.05$ & $p<0.01$) larger area under curve, area under 1st moment curve, a longer half life and a larger mean residence time. **Conclusions:** The study in the dehydrated rabbits indicated the need of modification of dosage regimen.

Key words: Amoxicillin, Dehydration, Water depletion.

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INTRODUCTION

Loss of fluid from all fluid compartments of the body is called dehydration. Dehydration frequently occurs in body due to circumstantial water deprivation, excessive sweating in warm climate, during exercise and disease states such as polyurea, diarrhea, sever bleeding, vomiting and exudation from raw area. A multitude of physiological and biochemical changes has been attributed to temporary dehydration, which can significantly modify the bioavailability and disposition of the drug. These changes in drug disposition kinetics can result in altered sensitivity and toxicity of drugs requiring a new basis of drug selection and dosage modification. In drinking animals, dehydration by water deprivation occurs and receptor cells are stimulated by following stimuli and compensatory mechanisms;

1. Reduction of cellular water volume [1].
2. A reduction of intravascular volume [2, 3].
3. A release of rennin, angiotensin in response to hypovolaemia [4, 5, 6].

Amoxicillin is probably is the most interesting and valuable of the semi synthetic penicillins, because whilst it retains the typical gram positive activity of penicillins it also possesses greatly enhanced activity against gram negative bacteria. It is a drug of choice within the class for the treatment of typhoid, meningitis, endocarditis, septicemia, peritonitis and gonorrhea because it has better oral absorption, when compared with other β-lactam antibiotics. Amoxicillin is stable in gastric juice, produces less gastric disturbance and penetrate well into the purulent and mucoid sputum in distinction to ampicillin. (Brogden et al 1975) Amoxicillin 250-500 mg three times daily is equivalent to the same amount of ampicillin given four times daily. It acts by inhibiting the synthesis of bacterial cell wall. It prevents cross-linkages between the linear peptidoglycan polymer chains, that make up a major component of the cell walls of both Gram positive and Gram negative bacteria. However, it is susceptible to degradation by β- lactamase producing bacteria, and so may be given with clavulanic acid to decrease its susceptibility. Amoxicillin continues to be a useful antimicrobial drug and its low index of toxicity, freedom from sensitization and reliable absorption continues to make it an attractive agent in the treatment of variety of infections [7, 8].

So amoxicillin being an important member of antimicrobial drugs it should be determined whether dehydration itself consistently changes the way healthy individuals handle the drug.

METHODOLOGY

A total no of 12 healthy rabbits of either sex were obtained from department of live stock management, University of Veterinary and Animal Sciences, Lahore. A ten days period was allowed to condition the animal for handling and adaptation. The animals were routinely examined by veterinarian. The mean values for the body weight, packed cell volume and biochemical parameters are given in table I. The average body weight of the animals was 1.51± 0.34 kg ranging between 1.2 to 1.89 kg. A wash out period of 10 days was given between experiments for the two routes of drug administration. After study in normal rabbits these animals were used for the experimentally induced condition.

Initially all the rabbits were weighed then their blood pH, blood glucose, total plasma protein, total plasma lipids and albumin globulin ratio was determined. (Table I) A condition of dehydration was produced by keeping animal off water but not food. Body weight of animals was recorded daily. Animals with a 10% decrease in the body weight were declared dehydrated. All the above parameters were measured again after producing dehydration. Bioavailability and disposition kinetics were studied in all normal rabbits following intravenous injection (10 mg/kg body weight) and oral route of drug administration. (Table IV, V) Blood samples (2ml) were drawn from the jugular vein at 5, 10, 15, 30, 45, 60, 120, 240, 360, and 480 minutes.

A wash out period of 10 days was given after completion of bioavailability and disposition kinetics trial in normal animals. The experimental diseased condition was induced in all rabbits. The blood samples were collected from the rabbits suffering from dehydration, to determine their packed cell volume, pH, blood glucose, total lipids, total proteins albumin and globulin after the administration of amoxicillin through oral and intravenous routes separately. For determining the bioavailability and disposition kinetics of amoxicillin in normal and treated rabbits drug was administered as a single dose 10mg/kg body weight. The blood samples were collected in heparinized glass centrifuge tube and plasma was separated and used for analysis. Amoxicillin concentration in plasma was measured by microbiological assay by using sarcina lutea (ATCL9341) as test organism[9,10]. Plasma levels of amoxicillin in normal and diabetic rabbits were used to analyze their individual kinetic parameters using non- compartmental method of analysis and results were compared by student T-test[11]. (Table II, III).

RESULTS

Biochemical parameters of blood as well as body weights were measured in normal and water deprived rabbits. Average body weight loss in dehydrated rabbits was 218.00 grams which represented a mean decrease of 13% as compared to normal rabbits. However, packed cell volume increased to 8.82 % in dehydrated rabbits which was statistically highly significant ($p<0.01$)

than normal rabbits. The value of blood pH did not change in both groups, while the blood glucose concentration increased significantly ($p<0.05$) in dehydrated rabbits. The amount of total proteins increased significantly ($p<0.01$) in dehydrated group when compared with normal values. Albumin and albumin globulin ratio showed a significant ($p<0.05$) & ($p<0.01$) decrease in the dehydrated group when compared with the normal rabbits. Whereas a significant ($p<0.05$) increase of globulins is seen in dehydrated group of rabbits.

Plasma concentrations of amoxicillin in normal and dehydrated rabbits, after oral and intravenous routes of administration, are shown in table II and III respectively. The statistical comparison is also given along with. The maximum plasma concentrations of 6.66±1.18μg/ml and 5.62±1.25μg/ml were attained after 30 minutes in normal and dehydrated rabbits, respectively after oral administration. It was observed from the mean plasma concentrations data that there was significant ($p<0.05$) decrease in the plasma concentration of amoxicillin at 10, 30, 45, 240, 360 and 480 minutes after the drug administration in dehydrated group whereas no difference was observed between normal and dehydrated groups at 5, 15, 60 and 120 minutes after drug administration. Following intravenous administration of amoxicillin (10mg/kg body weight), plasma concentrations was in range from 7.18±0.64 to 2.85±0.69 μg/ml in normal rabbits while the plasma concentration of the dehydrated ranges from 7.51±0.50 to 3.41±0.56μg/ml. A significant ($p<0.05$) and ($p<0.01$) increase was observed in plasma concentration of amoxicillin in treated and dehydrated group at 240, 360 and 480minutes after drug administration, it was almost identical in the early part i.e. from 5 minutes to 120 minutes after drug administration in normal and dehydrated animals.

Disposition kinetic parameters of orally administrated amoxicillin in normal and dehydrated rabbits showed no significant difference from each other, except the volume of distribution (Vd). Volume of distribution was significantly lower in dehydrated rabbits than the normal rabbits. After intravenous administration the total area under the plasma concentration time curve (AUC) was significantly ($p<0.05$) higher in dehydrated rabbits. Dehydrated rabbits also showed a highly significant ($p<0.01$) rise in the total area under the 1st moment curve (AUMC), half life ($T_{1/2}$) and in the mean residence time (MRT) of the drug whereas, elimination rate constant (K- Terminal) and volume of distribution (Vd) were significantly ($p<0.01$) lower in dehydrated rabbits. Total body clearance (CL) was almost identical between the two groups.

Parameters	Unit	Normal Groups	Diabetic Groups
Body weight	Kg	1.68±0.23	1.46±0.28*
Packed cell volume	%	32.82±3.80	41.6±4.40**
Blood pH		7.20±0.10	7.2±0.06
Blood glucose	mg/dl	95.0±18.00	109.0±14.07*
Total lipids	mg/dl	746.0±188.0	764.0±105.0
Total proteins	g/dl	5.96±0.90	7.25±0.90**
Albumins	g/dl	4.46±0.0.90	3.70±0.30*
Globulins	g/dl	1.48±0.57	3.55±0.55*
A/G Ratio		3.02	1.04**t

Table-I. Mean ±S.D. (n=10) values for the body weight, packed cell volume and biochemical parameters of blood in normal and diabetic rabbits.

**= Significant difference (p<0.05); **= Highly significant difference (p<0.01)*

Time (Minutes)	Normal Groups	Diabetic Groups
5	4.99±1.25	4.15±1.11
10	5.82±1.24	4.66±1.21*
15	6.07±1.42	5.19±1.39
30	6.66±0.65	5.62±1.25*
45	6.66±1.18	5.58±1.05*
60	6.04±1.47	5.19±0.92
120	5.36±1.45	4.60±0.86
240	5.05±1.18	4.03±0.72*
360	4.26±1.17	3.49±0.68*
480	3.73±0.99	2.81±0.53*

Table-II. Mean ±S.D. (n=10) plasma concentration (μg/ml) of amoxicillin following oral administration of 10mg/dl dose in normal and diabetic rabbits.

**= Significant difference (p<0.05)*

Time (Minutes)	Normal Groups	Diabetic Groups
5	7.18±0.64	7.51±0.50
11	6.91±0.60	7.15±0.44
15	6.59±0.58	6.70±0.52
30	6.25±0.62	6.10±0.57
45	5.78±0.68	5.75±0.55
60	5.27±0.64	5.31±0.62
120	4.68±0.61	4.87±.57
240	3.87±0.76	4.41±0.54*
360	3.31±0.69	3.97±0.54**
480	2.85±0.69	3.41±0.56*

Table-III. Mean ± S.D. (n=10) plasma concentration (μg/ml) of amoxicillin following intravenous administration of 10mg/dl dose in normal and diabetic rabbits.

**= Significant difference (p<0.05)*

Parameters	Unit	Normal Groups	Diabetic Groups
AUC	μg h/ml/ kg	36.16±13.03	32.51±6.35
AUMC	μg.h²/ml/kg	125.90±56.98	116.07±23.08
K- Terminal	/hr	0.38±0.28	0.28±0.01
$T_{1/2}$	Hrs	2.23±0.63	2.47±0.06
MRT	Hrs	3.22±0.90	3.57±0.08
Cl	Ml/hr/kg	93.02±15.36	113.5±21.84
Vd	l/kg	0.37±0.31	0.32±0.06*
C(max)	Minutes	6.66±1.79	5.62±1.25
T(max)	μg/ml	45.00	30.00

Table-IV. Mean ± S.D. (n=10) Values for bioavailability and disposition kinetics of amoxicillin following oral administration of 10mg/dl dose in normal and diabetic rabbits.

**= Significant difference (p<0.05)*

Parameters	Unit	Normal Groups	Diabetic Groups
AUC	μg h/ml/ kg	29.16±6.27	36.08±4.23*
AUMC	μg.h²/ml/kg	88.10±35.50	130.40±17.0**
K- Terminal	/hr	0.35±0.06	0.28±0.01**
$T_{1/2}$	Hrs	2.03±0.34	2.5±0.05**
MRT	Hrs	2.92±0.49	3.61±0.07**
Cl	Ml/hr/kg	101.80±10.10	101.1±10.03
Vd	l/kg	0.36±0.07	0.28±0.03**

Table-V. Mean ± S.D. (n=10) Values for bioavailability and disposition kinetics of amoxicillin following intravenous administration of 10mg/dl dose in normal and diabetic rabbits.

*= Significant difference (p<0.05), **= Highly significant difference (p<0.01)

DISCUSSION

A multitude of physiological and biochemical changes have been attributed to the temporary dehydration, which can significantly modify the disposition of the drugs in the body. Among the various physiological effects of water deprivation, the most prominent manifestations are the loss of body weight[2,12,13,14] and decrease of both blood and plasma volume[2,13,15]. The short term water deprivation has pronounced effect on the secretion of hypothalmo- neurohypophysial system, causing release of extra amount of oxytocin and vasopressin in the systemic circulation. A gradual decrease of these hormones in the neurohypophysial glands was observed during five consecutive days of water deprivation in rats[16,17].

In the present study the loss in body weight in the dehydrated rabbits was found to be significant ($p<0.05$). This loss in body weight is quite in agreement with the previous studies made in rabbits[18] and humans[19]. In rats after 10 to 15 days of water deprivation the reduction in body weight was 50%[20]. The other studies on animals also proved a statistically significant body weight loss due to dehydration in animals[12,13,14,20,22]. In the present study, water deprivation lead to a highly significant ($p<0.01$) decrease of 13% in body weight of water deprived rabbits. Water deprivation leads to an increase in packed cell volume due to decrease in blood and plasma volumes[2,13,15]. The reduction in pulmonary blood volume in 24 hours water deprived rats suggested that the pulmonary system acts as a fluid reservoir mobilizing blood to the general circulation in case of depletion of circulating blood[13]. On the other hand osmotic pressure, plasma concentration and haematocrit value increases progressively as water deprivation continues, but the pH and acid base status of the blood remains essentially constant[12,14,17,23]. No statistical change was observed on the blood pH of water deprived rabbits in the present study. Blood sugar level was significantly ($p<0.05$) higher in water deprived rabbits. This increase in the level of blood glucose was also observed on an erythromycin study in dehydrated rabbits[18]. This increase might be attributed to the increased secretions of epinephrine from chromaffin cells triggered by various factors including hypotension that resulted due to dehydration. The elevated level of epinephrine increased blood sugar and lactic acid concentration via glycogenolysis from liver and glycolysis from muscles[24]. Total proteins concentration was significantly ($p<0.01$) higher in the water deprived rabbits. This higher plasma protein value was quite in agreement with the previous investigations on the water deprived animals[17, 23, 25, 26, 27]. A higher plasma protein value was described as change in the equilibrium between transcapillary albumin shift and lymphatic return. In addition, this increase can be attributed to the change in plasma volume. The study revealed statistically significant ($p<0.05$) decrease in the plasma albumin fraction of the water deprived rabbits. It might be the result of an increased catabolism or decreased synthesis of albumin by the liver due to the stress of dehydration. Plasma globulins showed a significant ($p<0.05$) increase in the dehydrated rabbits. As plasma globulins are increased frequently in stress and dehydration due to fever[28]. Plasma albumins and globulins are subjected to different influences and their concentration may therefore vary independently of one another. There was a significant ($p<0.05$) increase in the albumin globulin ratio which was associated with the changes in the albumins and globulins.

In water deprived rabbits, plasma concentration of amoxicillin after oral administration of amoxicillin suspension revealed a significantly ($p<0.05$) lower drug concentration at all blood sampling intervals, which might be attributed to higher osmolarity of blood, which can interfere with the rapid absorption of the drug. Instantaneous higher plasma concentration than those in normal rabbits ultimately reduces the concentration gradient responsible for passive absorption.

Plasma concentration of amoxicillin after intravenous administration in normal and water deprived rabbits showed a statistically significantly ($p<0.05$ & 0.01) higher level at 240, 360 and 480 minutes after the drug administration in water deprived rabbits as compared to the plasma concentrations of normal rabbits. A higher plasma concentration values in the later stage of sampling were also observed in a previous study while studying the bioavailability and disposition kinetics of erythromycin in normal and water deprived rabbits, but the plasma concentrations of the water deprived rabbits were lower than the normal rabbits in the early stage i.e. 0 to 120 minutes after drug administration[18]. Another study made on the dehydrated humans showed same pattern of results[29]. No relative change was observed in the peak time but the plasma concentrations were higher in the dehydrated rabbits resulting in 14.5 % increase in the AUC and 12.2 % increase in the elimination half life. These variation are statistically non significant.

As regards the bioavailability and disposition kinetics there was a non significant change in the total area under the plasma concentration versus time curve and total area under the 1st moment curve. Tmax was 45 minutes in normal rabbits and was 30 minutes in dehydrated rabbits. Value of the volume of distribution of water deprived rabbits was significantly ($p<0.05$) lower than the normal rabbits. This variation is attributed to the highly significant difference in the plasma concentration at the various sampling times in water deprived rabbits.

Area under the plasma concentration versus time curve showed a significant ($p<0.05$) increase in the water deprived rabbits after the intravenous administration of the drug. Total area under the 1st moment curve was also significantly ($p<0.01$) higher in the water deprived rabbits showing a wide coverage by the drug in the body. The elimination rate constant was statistically lower in the water deprived rabbits resulting in a longer half life as compared to the normal rabbits. The increase in amoxicillin half life in water deprived rabbits was 24%. An increase 94% in aspirin half life was reported in dehydrated rats[30]. An increase of 32% in erythromycin half life in dehydrated rabbits was reported by Ahmad (1990). A significant increase in the half life in humans was reported by[31]. Mean residence time (MRT) of drug increased in the water deprived rabbits significantly ($p<0.05$). This increase in MRT is attributable to the slower elimination and a longer half life of amoxicillin in water deprived rabbits. Area under the plasma concentration verses time curve and total area under the 1st moment curve were also higher due to mean residence time and the volume of distribution which was significantly ($p<0.01$) lower in the water deprived rabbits[30]. There was a slow disposition in water deprived rabbits which can be due to the slow metabolic activity and slow renal function[3].

CONCLUSIONS

The dehydrated rabbits after oral drug administration of amoxicillin showed a significant increase in packed cell volume, blood glucose and plasma globulins. There was a significant decrease in the total body weight, total proteins, albumin, and albumin globulin ratio of dehydrated rabbits. Plasma concentration of amoxicillin was significantly lower in the dehydrated rabbits as compared to the normal rabbits. The dehydrated rabbits showed a significantly larger area under curve, area under 1st moment curve a smaller rate constant of elimination resulting in a longer half life a larger mean residence time and a smaller volume of distribution.

These studies in rabbits indicate the need for modification of dosage regimen under experimentally induced dehydrated conditions and warrant clinical evaluation/ application.

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