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COMPARATIVE BIOAVAILABILITY STUDIES OF SOME COMMERCIAL CHLORAMPHENICOL SUSPENSIONS

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Plasma chloramphenicol levels were compared in six adult healthy human volunteers. Single oral doses of chloramphenicol palmitate suspensions equivalent to 500 mg of chloramphenicol base were administered. Taking product I as standard, the peak plasma concentrations were found to be lower in products II and III; relative bioavailability of product II and III being 62% and 27.8% respectively. The time for peak plasma concentration was the same i.e. 180 min. for three products. The reduced bioavailability of II and III can be attributed to poor formulation of chloramphenicol palmitate suspensions.

Key words: Bioavailability, Chloramphenicol, Suspension.

Introduction

The philosophy of bioavailability testing according to FDA is the measurement of pharmacological responses and clinical effectiveness [1].

Bioavailability studies always indicate variations in kinetics between animal species, manufacturers and dosage forms. Chloramphenicol, an important antibiotics, is marketed in Pakistan by both national and multinational companies. The present study was undertaken to evaluate the efficacy of 2 products manufactured by local companies and to compare them with the one that of an international company.

Bioavailability of chloramphenicol in animals has been frequently reported [2–5], however work on humans is comparatively much less.

Experimental

Six healthy adult human volunteers were asked to fast from midnight till the start of the experiment. A standard breakfast was given to all individuals before administring the dose which was conducted in cross over manner and each subject received 20 ml of suspensions equivalent to 500 mg of chloramphenicol base of each brand on different days with an interval of 15 days between the experiments.

Control blood samples equivalent to 3 ml were withdrawn from each individual. Suspension was then given to each of the individuals and 3 ml blood samples were withdrawn at intervals of 45, 90, 180, 360 and 720 min. Sodium citrate solution (2%) was used as an anticoagulant. Plasma was separated and assayed for chloramphenicol level based on the method described in the literature [6–9] and the results were correlated with those of microbiological assay [10] and gas liquid chromatography [11].

Standard curve was prepared by dissolving 100 mg chloramphenicol in 50 ml of distilled water; this was further diluted and the concentrations of 5, 10, 15, 20, 30 and 40 µg/ml were prepared by diluting with normal human plasma. To each of these plasma dilutions 4 ml of 0.1 M sodium phosphate buffer pH7 and 6 ml of isoamyl acetate were added.

The tubes were mixed well on a haematological mixer at 30 rpm. for 3 min., and then centrifuged for 10 min. Supernatant layer (3 ml) was pipetted out into clean tubes and to each tube 2 ml of 6.5 N sodium hydroxide and 3% isoniazid solution were added. These tubes were kept on a water bath at 30° for 30 min . and shaken for 15 sec., after every ten min. The tubes were centrifuged, clear isoamyl acetate was drawn off and the optical density of yellow under layer was measured using Bausch and Lomb Spectronic 20 at 430 nm. Control plasma readings were subtracted from their respective test readings. All the samples were run in duplicate. Absorbance versus concentration of standard solutions was plotted.

Results and Discussion

The bioavailability parameters of the drug are presented in Table 1. The peak plasma level (C_{max}) for product I was the highest while values of formulations II and III were significantly ($P<0.05$) lower than that of I. Time to reach C_{max}, T_{max}, was same for all the three preparations i.e. 180 min. However, the AUC value was highest for product I and lowest in case of formulation III and the values for II and III were significantly ($P<0.05$) lower than the standard product. Taking the formulation I as standard (100%), the relative bioavailabilities of product II and III were 62 and 27.8% respectively. The plasma concentration-time profiles of chloramphenicol, following the administration of various formulations of chloramphenicol suspension, revealed that plasma levels of the drug in case of products II and III were significantly lower than that of I at each of the time points (Fig 1).

Absorption half life ($t_{1/2}$abs) and absorption rate constant (K_{abs}) were nearly identical in the three products while C abs values for formulation II and III were significantly ($P<0.05$)

TABLE 1. BIOAVAILABILITY PARAMETERS (MEAN ± SEM, n=6) OF CHLORAMPHENICOL FOLLOWING AN ORAL ADMINISTRATION OF 20 ml (EQUIVALENT TO 500 mg OF CHLORAMPHENICOL BASE) OF DIFFERENT CHLORAMPHENICOL SUSPENSIONS TO HEALTHY VOLUNTEERS.

Parameter	Formulation I	Formulation II	Formulation III
C_{max} (ug/ml)	9.6 ± 0.6	6.5 ± 0.1*	5.4 ± 0.2*
T_{max} (min)	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0
$AUC_{e-\alpha}$ (ug.min/ml)	3788.9 ± 181.4	2363.5 ± 110.9*	1051.7 ± 42.3*
Relative bio-availability(%)	100	62*	27.8*

*P <0.05 compared with formulation I.

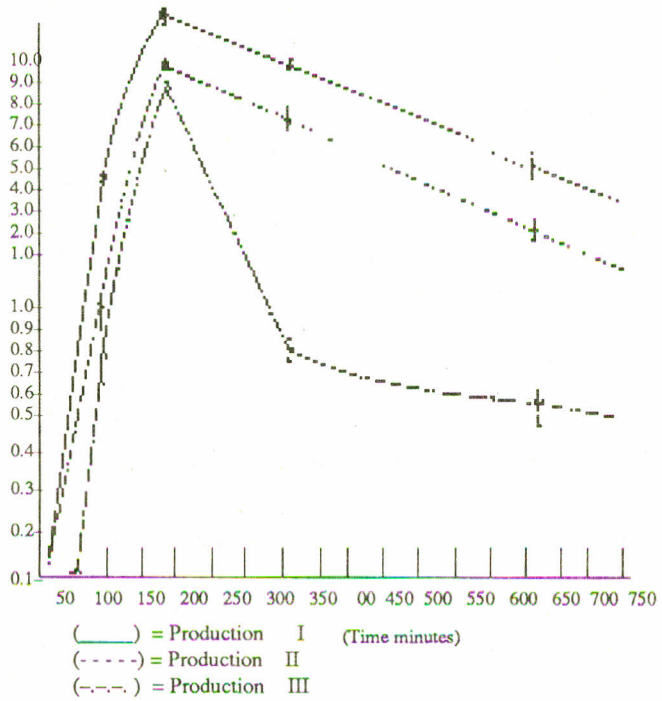

Fig 1. Comparison of mean plasma t SEM concentration of production I,II, and III

TABLE 2. ABSORPTION KINETIC PARAMETERS OF CHLORAMPHENICOL AFTER THE ADMINISTRATION OF VARIOUS CHLORAMPHENICOL SUSPENSIONS EACH EQUIVALENT TO 500 mg OF CHLORAMPHENICOL BASE

Parameter	Formulation I	Formulation II	Formulation III
C_o(abs)	34.0 ± 3.8	22.3 ± 1.9*	15.1 ± 1.5*
K_{abs} (min^{-1})	0.014 ± 0.001	0.012 ± 0.0004	0.013 ± 0.0004
$t_{1/2}$(abs) (min)	49.8 ± 2.3	57.1 ± 1.7*	51.8 ± 1.5*

Each value is the mean ± SEM; n=6.*P < 0.05 vs formulation I.

TABLE 3. MEAN ± SEM DISPOSITION KINETIC PARAMETERS OF CHLORAMPHENICOL AFTER ORAL ADMINISTRATION OF 20 ml OF THE VARIOUS CHLORAMPHENICOL SUSPENSIONS, EACH EQUIVALENT TO 500 mg OF CHLORAMPHENICOL BASE TO HUMAN SUBJECT

Parameter	Formulation I	Formulation II	Formulation III
$t_{1/2}$(elim) (min)	309.1 ± 25.8	277.6 ± 18.5*	156.3 ± 13.3*
B (min^{-1})	0.002 ± 0.0002	0.003 ± 0.0002	0.005 ± 0.004*
V_d (litre)	34.6 ± 2.8	46.9 ± 4.1	68.3 ± 6.5*
Cl (ml/min/kg)	1.2 ± 0.1	1.8 ± 1.0	4.5 0.4*

Values are means ± SEM; n=6, *P <0.05 compared with formulation I

lower than that of product I (Table 2).

The disposition kinetic parameters of chloramphenicol, are shown in Table 3, in which the elimination half-life ($t_{1/2}$ elim) of product II was not statistically different (P >0.05) while of product III was 49% less (P<0.05) when compared with standard product. Similarly, elimination rate constant (β) and plasma clearance (Cl) of the drug for product II were not statistically different (P >0.05) from product I while the values of these two parameters for product III were significantly (P<0.05) raised over those of standard product. Volume of distribution (V_d) of formulation II did not vary much from that of product I but V_d in case of formulation III was considerably increased over the value of standard product.

Table-4 shows the intra-and inter-subject variances. Here it may be seen that intra-subject variance did not achieve level of significance in any of the parameters, while in

TABLE 4. BIOAVAILABILITY AND PHARMACOKINETIC PARAMETERS SHOWING INTRA AND INTER SUBJECT VARIANCE (f-VALUE) OF CHLORAMPHENICOL FOLLOWING ORAL ADMINISTRATION OF 3 BRANDS OF THE DRUG TO SIX HEALTHY VOLUNTEERS IN A CROSS OVER MANNER.

Parameter	Intra subject Variance (f-value)	Inter Subject Variance (f-value)
T_{max}	**	**
C_{max}	0.68	26.95*
AUC	0.54	101.24*
C_o	0.76	12.28*
Kabs	0.35	2.47
$t_{1/2}$abs	0.35	3.17
$t_{1/2}$elim	0.55	14.04*
B	0.62	18.66*
V_d	3.19	20.88*
Clearance	3.05	105.01*

*P value less than 0.05. ** = Same value.

contrast the inter-subject variance was statistically significant ($P<0.05$) in all the parameter except K_{ab}s and $t_{1/2}$ abs.

All the bioavailability parameters ranked the three products in descending order of bioavailability as formulation I, II and III. The highest and lowest C_{max} values in this study were observed to be 9.6 and 5.4 µg/ml respectively. previously much higher C_{mx} values have been reported in paediatric patients (24.9–27.7 µg/ml) [14] and infants and children (11–15.1 µg/ml) [15]. The above authors proposed that higher Plasma levels of the drug in young-aged individuals might be due to poorly developed metabolic pathways and excretory processes. However, the age factor cannot influence any of the parameters in the present study as the comparative studies are carried out on the same subjects of the same age. The T_{max} in the present investigation was same for all the preparations i.e. 180 min. Although T_{max} cannot be considered too precise because of wide time intervals between time points at which blood samples were drawn, it is in line with those previously reported in man (210 min) by Ahmad *et. al* [16] and cats (90 and 180 min) by Watson [3]. Absorption half-life of all preparations was almost the same.

Bioavailability of chloramphenicol from chloramphenicol palmitate has been stated to be diminished by reduced secretion of digestive enzymes [3]. Furthermore Glazko *et. al.* [17] have suggested that bile and pancreatic secretion enhance the absorption of chloramphenicol from intestine.

However, in the present study the role of above mentioned factors seem not to have any significant effect on the bioavailabilities of different formulations because of the fact that the same healthy volunteers took part in this investigation.

Disposition kinetic parameters also ranked the three formulations in the same order as bioavailability parameters. Nonetheless, V_d in case of product III was unexpectedly increased in comparison to product I and II. This might be due to decreased plasma protein and/or tissue binding caused by any of the formulation ingredients employed for stabilizing suspension III. This phenomenon in turn might be responsible for increased plasma clearance and shorter half-life as compared to other 2 products. Hence from the present data it could be concluded that product I has highest and product III lowest bioavailability. Product II though had 37% less bioavailability as compared to product I yet had almost double than that of product III.

Comparative bioavailability studies (18-20) have indicated variations in drug bioavailabilities because of different reasons. After considering and evaluating various factors which affect bioavailability, in the present study it seems most likely that the differences in the bioavailabilities of the three products under investigation are due to the differences in formulation factors. The results of the present study further confirm the importance of drug fromulation and role of each and every ingredient used in the drug formulations. A poor formulation will undoubtedly give lower bioavailability and therapeutic response.

Data analysis. For the calculation of bioavailability and Pharmacokinetic parameters in Tables 1–3, the Chloramphenicol plasma level data were analysed using a computer program [12] for one-compartment model. This model was chosen as the preliminary examination of the data showed that such data could be best fitted to one-compartment model.

Statistical analysis. The analysis of variance based on randomized complete blocks design with one factor factorial was performed using M.STAT version 3 program [13] (IBM compatible). The statistical difference between the various parameters (bioavailability and Pharmacokinetic) was computed employing Duncan's Multiple Range test.

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