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Bioavailability Studies of Marketed Haloperidol Formulations on Rabbits : A Clinical Utility

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The bioavailability of marketed formulations of haloperidol (HAL) was estimated in the rabbit model by single dose study design where intramuscular formulation was considered as a reference standard. Using a sensitive HPLC method, plasma concentrations of HAL were measured upto 72 hours. The C_{max}, t_{max}, AUC_{0-48} and relative bioavailability were compared using ANOVA and found not to be significantly different across the formulations. But plasma concentrations showed beyond toxic level (50 ng/ml) in all the formulations. Thus, monitoring of the HAL bioavailability for marketed formulations is needed for better therapeutic impact of chronic psychiatric treatment which may reduce the risk of HAL-induced extra-pyramidal side effects (EPS).

Clinically, individual variations in the pharmacokinetics of drugs, antipsychotics in particular are quite common. Monitoring the concentrations of a drug in the blood is essential to ascertain that the calculated dose actually delivers the plasma level required for therapeutic effect without producing any drug-related toxicity[1]. With some antipsychotic drugs, receptor sensitivity in individuals varies so that monitoring of plasma levels is needed to distinguish the patient who is supersensitive to the same. Plasma level monitoring may reduce drug-induced toxicity and thereby may increase the courses of a good therapeutic response for many anti psychotic Drugs[2]. Pharmacokinetic models allow more accurate interpretation of the relationship between plasma drug levels and therapeutic response. The bioavailability of any drug should not change one formulation to another formulation and must be within the optimum therapeutic level. Otherwise the drug in its dosage form may not be effective therapeutically or may show drug-induced toxicity.

Haloperidol is readily absorbed from the gastrointestinal tract and distributed to all tissues. It shows extensive first pass metabolism. Thus, an oral dose of haloperidol has average systemic availability of about 65%. It is metabolised in the liver and is excreted through the urine, bile and faeces[3].

Present study was initiated to compare the bioavailabilities of different marketed formulations containing haloperidol and to find out the probability of haloperidol induced toxicity in a rabbit model[4,5]. The bioavailability of five marketed tablet formulations (5 mg) and one oral solution (2 mg/ml) of haloperidol were estimated relative to that of an intramuscular injection formulation (5 mg/ml) employed as a reference product in rabbit model. The rationale of this work is to determine the bioequivalence of different haloperidol formulations available in Indian market.

EXPERIMENTAL

Five haloperidol tablet formulations (5 mg), one oral solution (2 mg/ml) and one intramuscular injection formulation (5 mg/ml) were purchased from a local pharmacy. Diphenyl amine (AR), ammonium acetate (AR), acetonitrile (HPLC grade), diethyl ether (LR) and other common chemicals were used as they received from different suppliers without further purification.

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Study design:

All the products included in the study were coded as F_1, F_2, F_3, F_4, F_5, F_6 (test formulations) and F_7 (reference standard). F_1 to F_5 are tablet formulations, F_6 is an oral liquid and F_7 is an injection. Healthy albino rabbits (1.5 to 2.5 kg) were divided into seven groups containing three (one male and two females) in each. In the single dose study, haloperidol formulations were administered through month and intramuscularly following an overnight fast of 12 h. Food was withheld for 5 h after the administration of the various haloperidol formulations. Water was given *ad libitum*. All the oral formulations were fed in 0.5% w/v corboxy methyl cellulose suspension with the help of an oral feeding needle. The study was carried out by taking 2.5 ml of blood samples from rabbit's marginal ear vein (using 0.2 ml of pre-chilled heparin as anticoagulant) at various time intervals for 72 h (i.e., 2, 4, 6, 8, 12, 24, 48 and 72 h.) The plasma was immediately separated by centrifugation at 3000 rpm for 15 minutes and thereafter stored at -20° until the time of analysis. Plasma drug concentrations were measured by a modified high performance liquid chromatography method[6].

Chromatographic conditions

A Water® HPLC system was used for the analysis. The column used was a micron Bondapak C 18 columm (10 µ, 30 cm* 3.9 mm i.d). A mixture of acetonitrile:1% ammonium acetate buffer (pH 6.85), (50:50 v/v) was used as mobile phase for haloperidol, at a flow rate of 1.5 ml/min with an operating pressure of 3000 psi. A Rheodyne® 7125 injector with 20 µl loop was used for the injection of samples. Detection was done at 245 nm with a sensitivity of 0.005 AUFS. The mobile phase was filtered through 0.45 µ membrane filter and degassed. The separation was carried out at the room temperature of about 20°. Standard stock solutions of haloperidol (Searle Ltd.) of 500 ng/ml concentrations were prepared in the mobile phase. A stock solution of 500 ng/ml of diethyl amine as internal standard was prepared separately in the mobile phase. From the standard solutions, mixed standard solutions of different concentrations were prepared and to each of these, 50 ng/ml of the internal standard diethyl amine was added.

Twenty microlitres of each standard solutions was injected and chromatograms were recorded. A computer-controlled data station with base line 810 software was used to plot the peak area versus concentration in ng/ml. Calibration curves were obtained by using peak area ratios of standards and internal standards (response factor) versus concentration. This was followed by injecting the sample solutions extracted from the plasma and the chromatograms were recorded. From the plasma, sample solutions were made and spiked with internal standard and response factor was used to calculate the concentration of each drug. From this concentration, amount of drug present in plasma was calculated.

The significance of the difference in respect of pharmacokinetic parameters amongst the different formulations considered for this work and amongst the rabbits at the same time of experiments is calculated by two way ANOVA method. Accordingly the probability of 'F' values obtained by two way analysis of variance from the table value at 5%.

Extraction of haloperidol from plasma

Half a millilitre of plasma was transferred into 10 ml glass stoppered centrifuge tubes and 50 ng/ml of the internal standard solution was spiked and thereafter acidified with 1.75 ml of 0.1 N HCl. To this, 2 ml of diethyl ether was added and the mixture was shaken for five minutes, then centrifuged at 5000 rpm for 15 minutes. The upper ether layer was aspirated off and 2 ml of aqueous layer was transferred into a test tube containing 0.5 ml of 1 N NaOH solution. To this, 3 ml of chloroform was added. The mixture was vortexed for 10 minutes and then centrifuged at 5000 rpm for 15 minutes. The aqueous layer was aspirated off, then the tubes were taken and centrifuged again to get a clear chloroform layer, 2 ml of which was transferred into 10 ml evaporating tubes and dried in a water bath at 80°. The dry residue was dissolved in 20 ml of mobile phase and then injected.

RESULTS AND DISCUSSION

One of the major problems in any single dose, bioavailability study of any drug is the measurement of low concentrations after time of the administration of the drug that are invariably encountered. Although the HPLC method employed in this study was sufficiently sensitive, it was not possible to monitor plasma haloperidol concentrations in all the rabbits over the entire 72 hours period of study.

The mean plasma concentrations versus time profiles of haloperidol obtained after administration of test and reference standard were different from the tablet formulations (test) to injectable formulations (reference). But amongst the tablet formulations as well between liquid

Table I - Mean Pharmacokinetic Parameters of Haloperidol in Albino Rabbits (n=3)

Code	C_{max} (ng/ml)	t_{max}(h)	k_{el} (h^{-1})	$t_{1/2}$(h)	AUC_{0-48} (ng-h/ml)	F_{rel} (%)
F_1	96.27	8.00	0.1031	6.84	2014.67	72.84
F_2	95.78	7.60	0.0947	7.34	1978.20	71.37
F_3	91.60	6.66	0.1014	6.84	1651.65	61.96
F_4	88.78	7.50	0.1022	6.77	1871.97	67.07
F_5	79.70	7.33	0.0971	7.12	1683.50	60.87
F_6	105.00	7.33	0.0978	7.23	2473.10	89.42
F_7	110.83	6.67	0.0947	7.46	2765.60	100.0

C_{max} - Maximum plasma concentration; t_{max} - Time to reach maximum concentration. K_{el} - Elimination rate constant; $t_{1/2}$ - Elimination half-life; AUC - Area Under Curve. F_{rel} - Relative bioavailability

Table II - Plasma Concentrations of Haloperidol in three Rabbits following Administration of single oral Doses of F_1 & F_6 and reference Products (F_7) VIA i.m. inj. (ng/ml)

Code		Time in Hours						
		2	4	6	8	12	24	48
F_1	Rabbit 1	19.43	42.74	85.23	96.27	79.03	37.96	3.57
	Rabbit 2	15.10	37.73	79.71	89.64	74.18	29.63	2.22
	Rabbit 3	23.76	47.75	90.72	102.90	83.88	46.29	4.92
F_6	Rabbit 1	48.74	66.59	103.97	105.00	92.26	50.49	4.62
	Rabbit 2	42.35	63.61	99.14	99.62	85.93	44.66	2.96
	Rabbit 3	55.13	69.57	108.80	110.38	98.59	56.32	6.28
F_7	Rabbit 1	67.01	88.52	110.83	107.66	95.90	59.73	5.83
	Rabbit 2	61.12	82.51	103.90	100.31	92.04	57.17	3.88
	Rabbit 3	72.90	94.53	117.76	115.01	99.76	62.29	7.78

Table III - Anova Analysis Data

Source of Variance	C_{max}	t_{max}	K_{el}	F_{rel}%
Formulations	5.77	0.459	0.0172	1.41
	(3.00)	(3.00)	(3.00)	(3.00)
Subjects	2.04	1.18	5.01	2.10
	(3.88)	(3.88)	(3.88)	(3.88)

Note: Values within the brackets are the table values.

formulations (test) and injectable formulations, they were almost superimposable on each other (fig 1). Haloperidol was readily absorbed from both tablet and liquid formulations but till second hour, the quantifiable plasma concentrations were too low to determine.

The C_{max} for all tablet formulations ranged from 79.7 to 96.3 ng/ml whereas for oral liquid it was 105 ng/ml against the reference standard of 110.8 ng/ml. The t_{max} value for all the test products was 8 h against the reference product of 6 h. The C_{max} of different test formulations analysed by two way ANOVA method was found to be significantly different (F=5.77) from that of reference standard but t_{max} value was insignificant (F=0.459). These results were compared by taking the mean plasma profiles (n=3). The variations in the individual plasma profiles is depicted in table 2 by the plots of the plasma

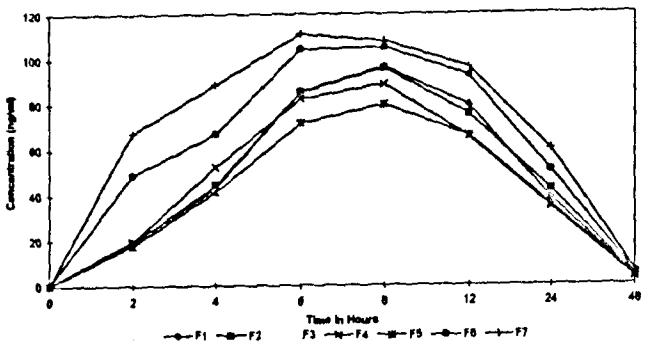

Fig. 1 : Mean Plasma Concentrations of Haloperidol

Mean plasma concentrations of haloperidol in albino rabbits (n=3) following administration of single oral doses (0.9 mg/kg) of test formulations (F1-F6) and via i.m. inj. of reference product (F7)

concentrations of haloperidol over time obtained for three representative subjects. The observed undulations in haloperidol plasma concentration may be due to enterohepatic recycling of the drug and/or some of its metabolites. Due to undulations, it was very difficult to determine the elimination rate constant (K_{el}) and plasma half life ($t_{1/2}$). But these parameters for the present study were estimated from the three data points for the straight lines available of each mean plasma concentrations versus time curve. The mean K_{el} values for test formulations were ranged from 0.0947 to 0.1031 h^{-1} and that of standard reference was 0.0947 h^{-1}. The plasma half-life ($t_{1/2}$) values for test formulations were varied from 6.77 to 7.34 h, where as standard reference it was 7.46 h. The differences in elimination rate constants (k_{el}) for all the formulations were insignificant when analysed by ANOVA method.

The relative bioavailability of test formulations as calculated from the test: reference ratios for the arithmetic means of the AUC $_{0\text{-}48}$h and C_{max} values is depicted in table-1. Thus, the mean relative bioavailability of test products was found to be in the range of 60.87 to 89.42% based on the reference standard as 100%. Results of the ANOVA of relative bioavailability parameters indicate that there was no significant effect of formulation of haloperidol on any of these AUC or C_{max} (F=1.41).

F-ratios concerning the formulations, C_{max} value is significantly different at 5% point because the calculated value F (5. 77) is more than the table value (3.00). But in all other cases, the F-values are less than table values as depicted in table-3. Concerning the animals, the K_{el} value is significantly different at 5% because the calculated value, F (5.01) is more than the table value (3.88). But other cases, they are insignificant.

Though the results were varied from the literature reports, it might be due to the animal model used in our experiments. But the results obtained in the present study clearly show some similarity in each cases of the different formulations on albino rabbits and thereby it can be concluded that the results are very much optimistic in respect of controlling the therapeutic response of haloperidol and can be used to monitore drug-induced side effects particularly the extrapyramidal syndrome.

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