Chapter 11

Pharmacokinetic study in rabbits

11.1 PHARMACOKINETIC STUDIES IN RABBITS

For the in vivo study of the sublingual delivery, the method has been successfully applied to pharmacokinetic studies of buprenorphine[1], diltiazem[2] in rabbits.

The plots of drug plasma concentration vs time were plotted for salbutamol sulphate, ondansetron hydrochloride and lamotrigine after administration of their film and tablets formulation into the sublingual cavity of rabbits. Wagner nelson method was used for the calculation of the pharmacokinetic parameters[3-6].

11.2 PHARMACOKINETIC STEPS FOR CALCULATING PHARMACOKINETIC PARAMETERS[3-6]

Pharmacokinetic parameters were calculated as follow:

10.2.1) Maximum plasma concentration (C_{max}): It was determined directly from the plasma concentration time profiles.

10.2.2) Time to maximum plasma concentration (T_{max}): It was determined directly from the plasma concentration time profiles.

10.2.3) Area under the plasma concentration-time curve from time zero to t (AUC_{0-t}): It was calculated by using trapezoidal rule. According to trapezoidal rule, the area under the curve from time t_2 to time t_1 is calculated by following equation:

$$AUC_{t_1}^{t_2} = \frac{C_1 + C_2}{2} \times (t_2 - t_1)$$

Where, C_1 and C_2 is concentration at time t_1 and t_2.

10.2.4) Concentration at zero time (C_0):

Plot time versus ln(conc.) graph on excel graph or plot time versus concentration in semi logarithmic graph paper. Extrapolate the terminal linear phase to zero. It gives

intercept. The antilog of the intercept obtained by linear regression gives the concentration at zero time (C_0).

C_0 = Antilog (intercept).

10.2.5) Elimination rate constant ($-K_{el}$):

The plot of plasma concentration vs time was plotted on semi-logarithmic paper. The terminal portion (last three detectable concentrations) was essentially linear with the slope of $-K_{el}$ Calculate the slope of the terminal linear phase. K_{el} = -slope × 2.303

10.2.6) Elimination half life ($t_{1/2}$) :

It was determined by following equation:

$t_{1/2} = 0.693/K_{el}$

10.2.7) Area under the plasma concentration-time from time zero to infinity ($AUC_{0-\alpha}$): The trapezoidal rule written in its full form to calculate the AUC from t = 0 to t = α is as follows:

$$AUC_{t0}^{t\alpha} = \sum AUC_{tn-1}^{tn} + \frac{C_{pn}}{K_e}$$

Where,

C_{pn} = last observed plasma concentration at t_n and k = slope obtained from the terminal portion of the curve.

10.2.8) Absorption rate constant (K_{ab}):

The terminal linear portion of the curve with slope-K_{el} was extrapolated to t=0. The actual plasma levels were subtracted from the corresponding concentrations on the extrapolated linear portions. This gave a series of residual concentration (Cr). The

plot of natural log of residual concentration (ln Cr) vs time gave a straight line with solpe –Kab.

10.2.9) Absorption half life ($t_{1/2ab}$):

It was calculated as follows:

$(t_{1/2ab}) = 0.693/k_{ab}$

10.2.10) Volume of distribution (V_d):

It is the volume in which drug would have to be distributed to produce the measured plasma concentration.

$$V_d = \frac{F \times G_0}{K_{el} \times AUC_{0-\alpha}}$$

(Fraction of administered dose G_0 absorbed following oral administration)

10.2.11) Clearance (Cl):

It is the total volume of plasma from which the drug have been removed per unit time.

Clearance (Cl) = $(V_d \times 0.693)/t_{1/2el}$

10.2.12) Cumulative drug eliminated at t time:

It is calculated as follow:

Drug eliminated = $0.434\ K_{el} * t$

10.2.13) Fraction of drug absorbed at time t $= \frac{C + K_{el} \times AUC_0^t}{K_{el} \times AUC_0^\alpha}$

10.2.14) Total drug in plasma at t time = original conc. in plasma calculated by HPLC method at t time + cumulative drug eliminated at t time.

10.2.15) Residual concentration = Ln conc – conc at t time in extraplotted linear eliminated line of graph.

10.2.16) Area under momentum curve (AUMC):

AUMC is the area under the curve of graph of C_p×t versus t.

10.2.17) Mean residence time (MRT):

MRT = CAUMC/CAUC

Where, CAUMC = Cumulative AUMC

CAUC = Cumulative AUC.

11.3 CALCULATION OF DOSES OF THE DRUGS IN RABBITS

The dose of the drug in the rabbits was calculated, depending on the weight of the rabbits in mg/kg using the following formula:

HED (Human Equivalent Dose) = Animal dose (Animal weight/Human weight)$^{0.33}$

The maximum dose of salbutamol sulphate that can be given to human in single day is 16 mg. According to the above formula, the dose for the rabbits is calculated to be 0.79 mg/kg. In this study, the dose given to the rabbits is 2 mg/kg which is below the LD_{50} dose (450 mg/kg, subcutaneous route in mice)[7].

The maximum dose of ondansetron hydrochloride that can be given to human in single day is 16 mg. According to the above formula, the dose for the rabbits is calculated to be 0.79 mg/kg. In this study, the dose given to the rabbits is 2 mg/kg which is below the LD_{50} dose (4.6 mg/kg intraperitoneally in mice)[8].

The maximum dose of lamotrigine that can be given to human in single day is 400 mg. According to the above formula, the dose for the rabbits is calculated to be 18.49 mg/kg. In this study, the dose given to the rabbits is 1 mg/kg which is below the LD_{50} dose (4000 mg/kg, oral in human)[9].

10.4 PHARMACOKINETIC STUDY OF SALBUTAMOL SUPHATE

The control (conventional tablets), fast dissolving films and fast dissolving tablets of salbutamol sulphate (dose: 2 mg/kg) were administered into the buccal cavity of each rabbit. There were three groups of rabbits, one each for conventional tablets, fast dissolving films, fast dissolving tablets. In each group there were four rabbits. Blood samples were collected from the marginal ear vein at 0.25, 0.5, 0.75, 1, 2, 4, 6, 12 and 24 hrs after salbutamol sulphate administration. The heparinised blood samples were immediately centrifuged at 4000 rpm for 15 minutes and separated plasma was stored at -4 °C.

Plasma samples collected from the rabbits were analyzed using developed reverse phase HPLC method and the drug plasma concentration values were determined from the calibration curve. The average drug plasma concentration of all three formulations are shown in table 11.1. The average plasma drug concentrations versus time profiles for all three formulations are given in figure 11.1.

Table 11.1. Average plasma salbutamol sulphate concentration after administration of conventional tablet, fast dissolving tablet and fast dissolving film in rabbits (n=3).

Sr. No	Time (hr)	Plasma concentration in ng/ml ± S.D (n=3)		
		Conventional tablets	Fast dissolving tablet	Fast dissolving film
1	0	00.00 ± 00	00.00 ± 00	00.00 ± 00
3	0.25	00.00 ± 00	124.66 ± 6.23	146.27 ± 6.91
4	0.5	54.28 ± 3.42	231.64 ± 9.06	268.78 ± 13.86
5	0.75	89.36 ± 5.83	247.81 ± 10.67	233.84 ± 9.52
6	1	124.48 ± 6.85	236.52 ± 8.37	219.37 ± 10.51
7	2	231.57 ± 10.07	186.78 ± 7.25	163.84 ± 6.43
8	4	163.82 ± 7.44	104.38 ± 5.14	88.32 ± 5.11
9	6	94.11 ± 5.91	60.38 ± 4.29	55.73 ± 4.97
10	12	46.79 ± 4.38	22.74 ± 1.58	16.22 ± 1.24
11	24	24.72 ± 1.79	13.83 ± 0.86	10.11 ± 1.77

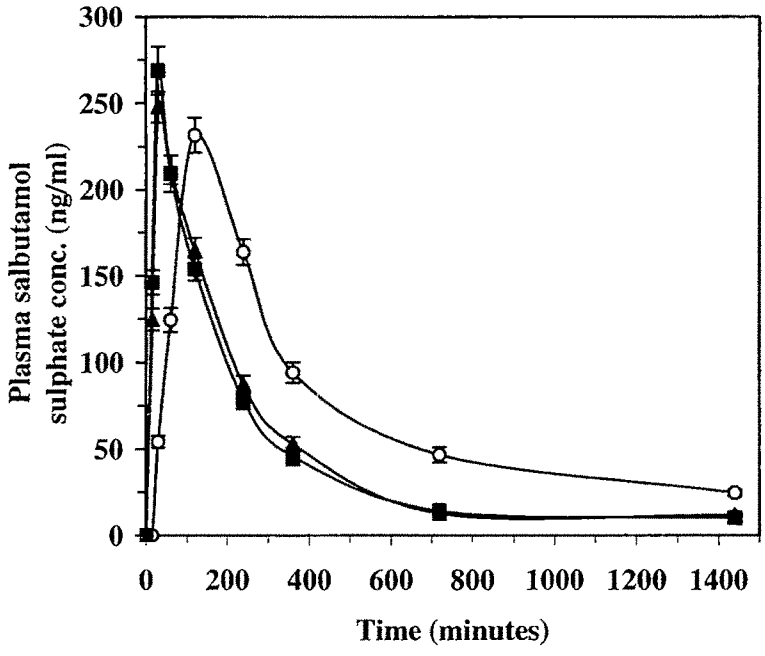

Figure 11.1. Comparison of pharmacokinetic profiles of different formulations of sabutamol sulphate. (○) Conventional tablet, (■) fast dissolving tablet, (▲) fast dissolving film. Each value is mean ± S.D. of three experiments.

The different pharmacokinetic parameters of the salbutamol sulphate formulations were calculated by using by Wagner-Nelson method. The pharmacokinetic parameters of different formulations of salbutamol sulphate are shown in table 11.2.

Table 11.2. Different pharmacokinetic parameters of different formulations of salbutamol sulphate calculated by Wagner-Nelson method.

Sr. No.	Parameters	Conventional tablet	Fast dissolving tablet	Fast dissolving film
1	K_{ab} (h^{-1})	2.49	11.16	11.44
2	K_{el} (h^{-1})	0.098	0.136	0.253
3	T_{max} (h)	2.0	0.50	0.50
4	C_{max} (ng/ml)	231.57	247.81	268.78
5	$T_{1/2}$ (h)	7.07	5.08	2.74
5	$AUC_{(0-24)}$ (ng h ml^{-1})	1734.58	1101.60	1053.34
6	$AUC_{(0-\infty)}$ (ng h ml^{-1})	1986.42	1188.39	1093.3
7	V_d (L/kg)	5.14	6.17	3.61
8	CI ($Lhr^{-1}kg^{-1}$)	0.503	0.791	0.914
9	$AUMC_{(0-24)}$ (ng h^2 ml^{-1})	12980.44	5753.63	5393.54
10	Mean residence time (h)	6.53	4.84	4.93

10.5 PHARMACOKINETIC STUDY OF ONDANSETRON HYDROCHLORIDE

The control (conventional tablets), fast dissolving films and fast dissolving tablets of ondansetron hydrochloride (dose: 2 mg/kg) were administered into the buccal cavity of each rabbit. There were three groups of rabbits, one each for conventional tablet, fast dissolving film, fast dissolving tablet. In each group there were three rabbits. Blood samples were collected from the marginal ear vein at 0.25, 0.5, 0.75, 1, 2, 4, 6, 12 and 24 hrs after ondansetron hydrochloride administration. The heparinised blood samples were

immediately centrifuged at 4000 rpm for 15 minutes and separated plasma was stored at -20 °C.

Plasma samples collected from the rabbits were analyzed using developed reverse phase HPLC method and the drug plasma concentration values were determined from the calibration curve. The average drug plasma concentration of all three formulations are shown in table 11.3. The average plasma drug concentration versus time profiles for all three formulation are given in figure 11.2.

Table 11.3. Average plasma ondansetron hydrochloride concentration after administration of conventional tablet, fast dissolving tablet and fast dissolving film in rabbits (n=3).

Sr. No	Time (hr)	Plasma concentration in ng/ml ± SD (n=3)		
		Conventional tablets	Fast dissolving tablet	Fast dissolving film
1	0	00.00 ± 00	00.00 ± 00	00.00 ± 00
3	0.25	00.00 ± 00	136.18 ± 6.14	152.08 ± 6.35
4	0.5	24.64 ± 1.63	314.33 ± 12.58	325.61 ± 14.82
5	0.75	51.96 ± 3.19	309.33 ± 9.77	298.78 ± 9.71
6	1	85.72 ± 4.82	289.67 ± 8.92	267.42 ± 8.48
7	2	321.48 ± 13.07	205.18 ± 7.92	198.34 ± 6.94
8	4	178.37 ± 6.93	108.49 ± 6.61	104.62 ± 6.85
9	6	111.93 ± 5.47	68.48 ± 4.74	64.07 ± 5.14
10	12	38.93 ± 2.99	26.05 ± 2.86	22.94 ± 3.81
11	24	16.38 ± 1.31	12.86 ± 1.03	13.05 ± 1.36

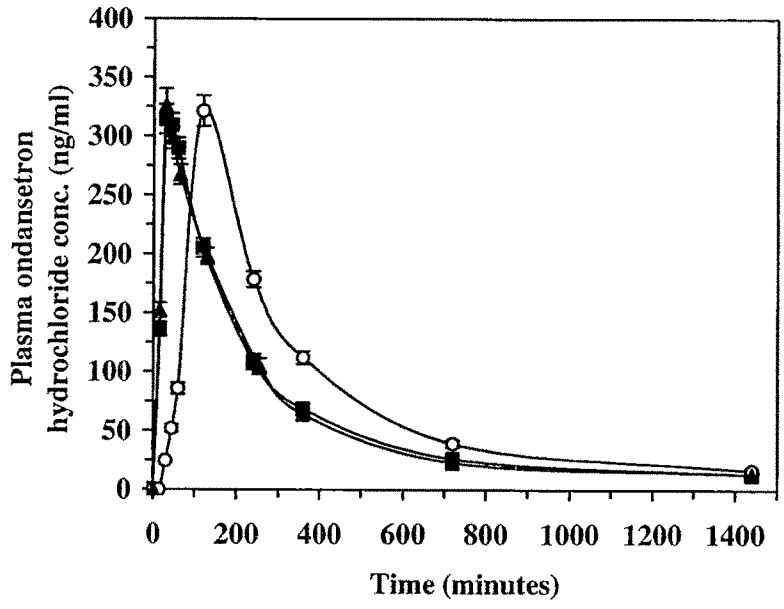

Figure 11.2. Comparison of pharmacokinetic profiles of different formulations of ondansetron hydrochloride. (○) Conventional tablet, (■) fast dissolving tablet, (▲) fast dissolving film. Each value is mean ± S.D. of three experiments.

The different pharmacokinetic parameters of the ondansetron hydrochloride formulations were calculated by using by Wagner-Nelson method. The pharmacokinetic parameters of different formulations of ondansetron hydrochloride are shown in table 11.4.

Table 11.4. Different pharmacokinetic parameters of different formulations of ondansetron hydrochloride calculated by Wagner-Nelson method.

Sr. No.	Parameters	Conventional tablet	Fast dissolving tablet	Fast dissolving film
1	K_{ab} (h^{-1})	4.6787	11.62	11.68
2	K_{el} (h^{-1})	0.1296	0.1184	0.116
3	T_{max} (h)	2.00	0.5	0.5
4	C_{max} (ng/ml)	321.48	314.33	325.61
5	$T_{1/2}$ (h)	5.34	5.85	5.94
5	$AUC_{(0-24)}$ (ng h ml^{-1})	1811.14	1481.28	1409.05
6	$AUC_{(0-\infty)}$ (ng h ml^{-1})	1937.51	1590.49	1521.06
7	V_d (L/kg)	4.07	5.31	5.64
8	Cl ($Lhr^{-1}kg^{-1}$)	0.528	0.629	0.657
9	$AUMC_{(0-24)}$ (ng h^2 ml^{-1})	11708.82	8079.06	7599.74
10	Mean residence time (h)	6.043	5.079	4.99

10.6 PHARMACOKINETIC STUDY OF LAMOTRIGINE

The control (conventional tablets), fast dissolving films and fast dissolving tablets of lamotrigine (dose: 1 mg/kg) were administered into the buccal cavity of each rabbit. There were three groups of rabbits, one each for conventional tablet, fast dissolving film, fast dissolving tablet. In each group there were three rabbits. Blood samples were collected from the marginal ear vein at 0.25, 0.5, 0.75, 1, 2, 4, 6, 12 and 24 hrs after lamotrigine administration. The heparinised blood samples were immediately centrifuged at 4000 rpm for 15 minutes and separated plasma was stored at -20 °C.

Plasma samples collected from the rabbits were analyzed using developed reverse phase HPLC method and the drug plasma concentration values were determined from the calibration curve. The average drug plasma concentration of all three formulations are shown in table 11.5. The average plasma drug concentrations versus time profiles for all three formulations are given in figure 11.3.

Table 11.5. Average plasma lamotrigine concentration after administration of conventional tablet, fast dissolving tablet and fast dissolving film in rabbits (n=3).

Sr. No	Time (hr)	Plasma concentration in ng/ml ± S.D. (n=3)		
		Conventional tablets	Fast dissolving tablet	Fast dissolving film
1	0	00.00 ± 00	00.00 ± 00	00.00 ± 00
3	0.25	00.00 ± 00	683.95 ± 19.05	257.44 ± 4.17
4	0.5	47.85 ± 5.37	677.38 ± 16.66	694.78 ± 16.96
5	0.75	110.58 ± 7.48	652.69 ± 12.06	665.03 ± 12.34
6	1	185.89 ± 6.83	649.43 ± 9.83	652.75 ± 8.91
7	2	656.14 ± 18.66	549.48 ± 11.52	583.66 ± 9.48
8	4	578.28 ± 14.03	480.89 ± 9.81	528.37 ± 7.13
9	6	502.62 ± 11.78	432.72 ± 7.94	461.5 ± 11.06
10	12	431.36 ± 9.55	375.11 ± 5.77	419.32 ± 14.85
11	24	329.63 ± 13.74	338.68 ± 10.26	335.11 ± 7.94

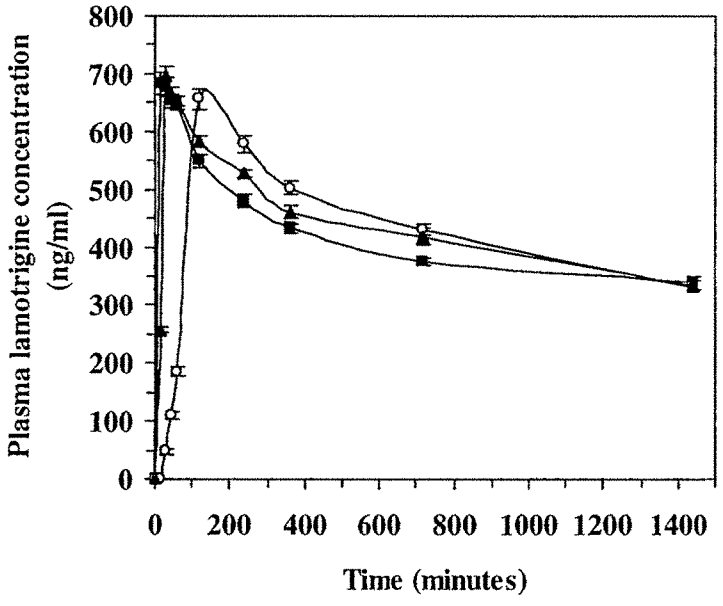

Figure 11.3. Comparison of pharmacokinetic profiles of different formulations of lamotrigine. (○) Conventional tablet, (■) fast dissolving tablet, (▲) fast dissolving film. Each value is mean ± S.D. of three experiments.

The different pharmacokinetic parameters of the lamotrigine formulations were calculated by using by Wagner-Nelson method. The pharmacokinetic parameters of different formulations of lamotrigine are shown in table 11.6.

Table 11.6. Different pharmacokinetic parameters of different formulations of lamotrigine calculated by Wagner-Nelson method.

Sr. No.	Parameters	Conventional tablet	Fast dissolving tablet	Fast dissolving film
1	K_{ab} (h^{-1})	5.381	26.29	22.23
2	K_{el} (h^{-1})	0.0294	0.0307	0.0297
3	T_{max} (h)	2.00	0.25	0.50
4	C_{max} (ng/ml)	656.14	683.95	694.78
5	$T_{1/2}$ (h)	23.57	22.57	23.33
5	$AUC_{(0-24)}$ (ng h ml^{-1})	10167.05	9834.34	10375.05
6	$AUC_{(0-\infty)}$ (ng h ml^{-1})	21378.95	20866.26	21658.21
7	V_d (L/kg)	3.11	3.059	3.048
8	Cl ($Lhr^{-1}kg^{-1}$)	0.0916	0.0939	0.0905
9	$AUMC_{(0-24)}$ (ng h^2 ml^{-1})	112854.0	105818.3	111231.9
10	Mean residence time (h)	11.09	10.76	10.72

From the data shown in the table 4, it was observed that the time to achieve the peak plasma concentration (T_{max}) was lower (30 min) in case of fast dissolving film and fast dissolving tablet in comparison to the control (2 hr) for all three drugs. Therefore, we can say that there was quicker onset of action in the case of fast dissolving film and fast dissolving tablet. Also, the data show that the peak plasma concentration was almost same for all three formulations of all drugs. The half lives of the fast dissolving film and fast dissolving tablets were less as compared to the control of all three drugs. The area under the Concentratoin v/s Time curve (AUC) for the fast dissolving film and fast dissolving tablets were less as compared to that of control for all drugs. The fast

dissolving films and fast dissolving tablets provide a quick onset of action which may was confirmed from the dissolution studies and in vivo studies and hence may be beneficial in asthmatic attack (for salbutamol sulphate), emesis (for ondansetron hydrochloride) and epileptic attack (for lamotrigine).

10.7 REFERENCES

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