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Evaluation of Piroxicam Injection

A. SINGHAI, S. JAIN AND N. K. JAIN*
Dept. of Pharmaceutical Sciences
Dr. H. S. Gour University, Sagar [M.P.] 470003

Piroxicam (PXM) is an analgesic, antipyretic and antiinflammatory agent, practically insoluble in water[1]. The increase in aqueous solubility of PXM using hydrotropes and cosolvents and formulation of its aqueous injections have been reported earlier[2]. These formulations were further evaluated *in vitro* (physical evaluation and haemolytic activity) and *in vivo* (pharmacokinetic studies). Some of the formulation have shown promising results.

IT is often necessary to administer a drug parenterally at a concentration which exceeds its aqueous solubility. The elimination of precipitation on dilution ensures a safer and more effective formulation[3,4,5]. In the present work the evaluation of formulated aqueous injections of PXM has been reported. These will be useful in rheumatic patients with peptic ulcer and gastrointestinal bleeding, in whom the oral administration of PXM is contraindicated. At the same time, there will be the possibility of reducing the drug dose and hence the adverse effects. By employing similar approach our team has reported formulation of aqueous injection of carbamazepine[5], norfloxacin[6] and ketoprofen[7].

EXPERIMENTAL

Materials

All the materials were used as reported earlier[2].

Physical evaluation of the selected formulations

The effect of dilution with intravenous fluids (normal saline/5% dextrose solution) was studied on the selected formulations of PXM namely, PSB_3, $PSMHB_1$ and $PPEG_4W_1$. Test dilutions of PXM solutions (formulated products) in different vehicles of different concentrations, were prepared with normal saline and 5% dextrose solution. Dilutions were made in duplicate at 25 ± 1°. The prepared dilutions (1:1 to 1:50) were examined visually using an Allen viewer for the presence of visible precipitate or microcrystals using a sample of intravenous fluid for comparison.

Haemolytic Studies

The drug as well as additives in parenteral preparations may have haemolytic effect on the RBC because of the difference in cellular activity against the erythrocytes. The rabbit blood used in this study was obtained from the marginal ear vein of a healthy male rabbit (mixed strain, 3.1 kg.). The blood was collected and immediately difibrinated by gentle rotation in a glass flask with glass beads, until the fibrin had separated. The defibrinated blood was poured into a small glass flask and aerated by gently swirling the flask for about 5 min. After defibrination, the RBCs were washed with normal saline and centrifuged until the supernatant was colourless. The RBCs were then diluted to original volume (1 ml) with normal saline solution.

*For correspondence

Table 1: Precepitattion of PXM in Formulation after dilution with normal saline and 5% dextrose solution

Formulation	Dilution	Time(h)											
		0.5	1	2	3	6	24	0.5	1	2	3	6	24
		Normal saline						5% Dextrose solution					
PSB_3*	1:1	-	-	-	+	+	+	-	-	-	+	+	+
	1:2	-	-	-	+	+	+	-	-	+	+	+	+
	1:5	-	-	-	+	+	+	-	-	+	+	+	+
	1:10	-	-	-	+	+	+	-	-	+	+	+	+
	1:20	-	-	-	+	+	+	-	-	+	+	+	+
	1:30	-	-	-	+	+	+	-	-	+	+	+	+
	1:40	-	-	-	+	+	+	-	-	+	+	+	+
	1:50	-	-	-	+	+	+	-	-	-	+	+	+
$PSMHB_1$**	1:1	-	-	-	+	+	+	-	-	+	+	+	+
	1:2	-	-	+	+	+	+	-	-	+	+	+	+
	1:5	-	-	+	+	+	+	-	-	+	+	+	+
	1:10	-	-	+	+	+	+	-	-	+	+	+	+
	1:20	-	-	-	-	+	+	-	-	+	+	+	+
	1:30	-	-	-	-	+	+	-	-	+	+	+	+
	1:40	-	-	-	-	+	+	-	-	-	+	+	+
	1:50	-	-	-	-	+	+	-	-	-	+	+	+
$PPEG_4W_1$***	1:1	-	-	+	+	+	+	-	+	+	+	+	+
	1:2	-	+	+	+	+	+	-	+	+	+	+	+
	1:5	-	+	+	+	+	+	-	+	+	+	+	+
	1:10	-	+	+	+	+	+	-	+	+	+	+	+
	1:20	-	-	+	+	+	+	-	+	+	+	+	+
	1:30	-	-	+	+	+	+	-	-	+	+	+	+
	1:40	-	-	-	+	+	+	-	-	-	+	+	+
	1:50	-	-	-	+	+	+	-	-	-	+	+	+

- = no precipitate, + = microcrystalline precipitate, * = PXM (4mg/ml) in 1.8M SB

** = PXM (3.34 mg/ml) in 1.6 M SMHB, *** = PXM (3.34 mg/ml) in 60=40 PEG-400:W,

Different concentrations of PXM were obtained separately by diluting the formulations with normal saline. A colorimetric method[8] was employed to determine the degree of haemolysis in each test solution. One tenth ml of RBC suspension was incubated with 10 ml of the different solutions (water, sodium chloride, hydrotropes and cosolvents) at 25±1° for 45 min. The unhaemolyzed cells were separated by centrifugation at 3000 rpm for 10 min. and the absorbance readings of the haemolysate

Table 2 : Haemolytic activity of PXM in different formulations*

PXM concentration (ug/ml)	Haemolysis (%) in formulation		
	PSB_3	$PSMHB_1$	$PPEG_4W_1$
5	2.53	4.81	8.41
10	4.88	6.37	10.32
20	8.71	11.54	15.87
30	15.03	20.03	27.11
40	30.13	32.13	38.84
60	45.27	54.22	63.17
80	86.81	91.37	97.28
100	98.43	99.89	100.60

*N= 3.

were noted at 550 nm. Each absorbance reading was compared with a total haemolysis reading obtained by taking RBC in water (1:10). The degree of haemolysis occurring in each test solution was calculated as a percent of total haemolysis.

Similarly, experiments were carried out to test the haemolytic activity of different concentrations of solubilizates (hydrotrope solutions as well as cosolvent blends) used in selected formulations at different sodium chloride concentrations (0.45- 1.8% w/v).

Pharmacokinetic Evaluations

Four groups, each of six healthy male rabbits (mixed strain, 2.5 — 2.9 kg.) were fasted overnight and 1 ml of blood sample was collected from the marginal ear vein, which served as a control. The formulation (PSB_3, $PSMHB_1$ and $PPEG_4W_1$) (20 mg/kg body weight) were injected intravenously in four different groups, each of six rabbits, into marginal ear vein and the animals were left free in cages. To another group of six male healthy rabbits (fasted over night) were administered powder of PXM proprietary tablet (Inflavan^R - 10 mg/kg body weight) with the help of a gastric canula and 20 ml of water. After different time intervals, blood samples (1.0 - 1.5 ml) were collected from the marginal ear vein using disposal plastic syringes prerinsed with 1% solution of heparin sodium in normal saline. Blood samples were centrifuged at 300 rpm for 5 min. to obtain plasma and stored in refrigerator until analyzed by the HPLC method[9].

From the data of plasma level studies of PXM the C_{max} and AUC_{0-14} were calculated. The significance levels of these results were calculated using student 't' test, and 't' values at $p = 0.05$.

RESULTS AND DISCUSSION

On the basis of the results of physical and chemical stability testing[4], the promising formulations, piroxicam-sodium benzoate (PSB_3) piroxicam-sodium m-hydroxy-benzoate 1 ($PSMHB_1$) and piroxicam/propylene-glycol 4-water1 (PPG_4W_1) were selected for *in vitro* and *in vivo* evaluation.

The administration of any parenteral formulation via intravenous route may result in the precipitation of drug on dilution with plasma or intravenous (i.v.) fluids. In the present study, the selected formulations of PXM were studied for the effect of dilution with normal saline/5% dextrose solution. The serial dilutions of each formulation were prepared in the ratio of 1:1 to 1:50 with normal saline or 5% dextrose solution and examined visually for the appearance

Table 3 : Haemolytic activity of cosolvents/hydrotropes at different NaCl Concentration

Hydrotrope/ Cosolvent conc. (%)	Haemolysis (% in vehicles																					
	Water					0.45% NaCl					0.9% NaCl					1.8% NaCl					
	PEG-400	PEG-600	SB	SMHB	PG	PEG-400	PEG-600	SB	SMHB	PG	PEG-400	PEG-600	SB	SMHB	PG	PEG-400	PEG-600	SB	SMHB	PG
2	4.98	4.32	2.24	1.69	7.11	4.28	4.11	2.01	1.61	7.02	3.59	2.78	1.89	1.38	6.23	2.41	1.24	0.81	0.78	4.53
5	8.71	8.13	3.57	3.71	13.97	8.03	7.79	3.48	3.58	13.58	7.14	5.48	2.43	2.49	12.14	5.11	3.12	1.38	1.24	8.81
7	12.57	11.73	16.38	15.02	19.89	12.12	11.02	15.32	13.31	19.52	11.21	9.23	13.47	11.68	17.23	8.33	6.71	8.37	8.13	15.12
10	18.69	17.27	37.49	36.11	30.18	18.15	16.43	39.49	39.37	29.23	16.69	14.23	32.51	33.07	27.49	12.59	11.57	18.11	27.19	23.68
15	34.73	33.13	99.87	88.43	45.43	33.24	30.73	80.14	85.52	45.01	31.47	27.27	33.81	81.14	43.61	21.12	19.38	46.01	69.04	36.59
20	65.88	64.39	99.84	99.48	70.08	64.17	63.18	98.01	98.48	69.11	60.87	58.27	96.24	94.29	67.48	49.57	47.01	90.47	83.73	58.71
25	79.32	76.13	99.23	99.89	88.45	78.29	74.28	98.89	99.37	82.21	76.22	72.21	98.11	96.69	85.31	65.17	65.43	96.58	92.13	78.66
30	94.74	90.47	100.24	100.12	99.48	92.38	89.87	99.69	99.78	98.84	90.59	87.23	98.97	98.81	96.51	85.42	80.12	98.13	97.20	93.24
35	100.60	98.76	100.37	100.68	100.12	99.10	98.68	99.10	100.13	99.73	98.33	97.21	98.33	99.69	99.12	96.73	94.71	96.73	100.11	97.81

of precipitate or microcrystals (Table 1). In a parallel study, the dilution effect was studied, simulating the flow of i.v. fluids in the body, using fabricated apparatus. The flow rate of aqueous fluids (normal saline / 5% dextrose solution) was maintained at 20 ml/min. while the rate of injection was varied from 0.5 to 2.0 ml/min. Dilution of PXM formulations with normal saline or 5% dextrose solution did not result in the immediate precipitation or transient cloudiness under all condition of dilution (Table 1). The observations reveal that all the test solutions based on hydrotrope solutions as vehicle (PSB_3, and $PSMHB_1$) remained clear for atleast 1 h while the formulations with cosolvent blends ($PPEG_4W_1$) remained clear for about 0.5 h, with normal saline as well as 5% dextrose solution. But slight to clearly visible microcrystalline precipitate was noted afterwards. Lack of immediate precipitation following dilution of formulation, solubilized using hydrotropes, can be attributed to the aqueous nature of these solvent systems. Such systems are likely to provide, upon dilution with aqueous fluids, a relatively stable supersaturated drug solution. All the formulations were observed to have better stability towards precipitate formation with normal saline than compared with 5% dextrose solution.

As the dilution ratio was increased, the appearance of precipitate was faster, but after much higher dilution (e.g. 1:30 to 1:50), the precipitate partly disappeared, This might be due to the redissolution of precipitate. It can be concluded that the formulations solubilized using hydrotropes are more stable towards precipitate formation upon dilution with normal saline or 5% dextrose solution than formulations solubilized using cosolvent system.

All the above selected formulations (PSB_3, $PSMHB_1$ and $PPEG_4W_1$) were subjected to *in vitro* haemolytic studies using RBCs of rabbit blood. The degree of haemolysis was estimated by a colorimetric method[8]. The haemolytic activity of PXM in different formulation at different drug concentration was studied (Table 2). The data clearly shows that all the selected formulation of PXM exhibit haemolytic

Table 4: Peak plasma level (C_{max}) and area under the curve (AUC_{0-14}) values in rabbit following administration of PXM formulations*

Product	Route	C_{max} (ug/ml)	AUC_{0-14} (ug.h/ml)	Comparative bioavailability (%)**
PSB_3	i.v.	26.91±6.48	51.43±6.19	133.2
$PSMHB_1$	i.v.	25.12±5.48	52.71±5.07	136.5
$PPEG_4W_1$	i.v.	23.44±6.71	49.29±5.11	127.7
PXM tablet	oral	9.18±1.88	38.61±4.71	-

* N = 6,

** = $\frac{\text{AUC i.v.}}{\text{AUC oral}} x\ 100$

effect. Its formulation at 500 μg/ml concentration resulted in nearly 100% haemolysis. The solubilizates were also individually evaluated in the concentration range 2-35 % to determine their haemolytic potential. The results show that all the solubilizates studied, exerted negligible haemolytic effect (< 20%), but highly significant haemolysis above 20 % of the solublilzate concentration. All the solubilizates produced nearly 100% haemolysis when studied in water in the concentration range of 30-35 %. In a parallel study, effect of varying concentrations of sodium chloride (0.45 - 1.8%) in the concentration range of 2-35% of the solubilizate was also studied. Sodium chloride at 1.8% concentration significantly reduced the haemolytic activity of different solubilizates, as evident (Table 3). Thus the haemolytic behaviour of these solubilizates can be ranked as SMHB > SB > PG > PEG-400 > PEG-600

It is important to note that the *in vitro* haemolysis in the present study would be unlikely to occur *in vivo,* as the intravenously injected solution will be diluted to about 25 times in the total blood volume.

After the *in vitro* evaluation, the PXM formulations (PSB_3, $PSMHB_1$ and $PPEG_4W_1$) 10 mg/kg body weight were studied in rabbit for plasma levels after i.v. administration. In the same study PXM tablets 10 mg/kg was administered orally. The peak plasma concentration (C_{max}) ranged from 23.44 - 26.91 μ/ml for PXM formulation (i.v.) while it was 9.18 μl/ml for PXM formulation (oral). To compare the bioavailability of different formulations, the area under the curve (AUC) were calculated (Table - 4). The results indicated that the AUC_{0-14} for PXM injection formulations are nearly same for all the three formulation studied. The AUC_{0-14} for PXM tablet is less than injection formulations.

To study the significance level for the variation among different formulation, student 't' test was applied. The 't' values are significant for PSB_3-PT and $PPEG_4W_1$ -PT and nonsignificant for PSB_3-$PSMHB_1$-$PPEG_4W_1$ for two-tail, 't' test. The 't' value at p = 0.05 is 2.228 for two-tail 't' test. It clearly indicates that the formulations given orally show significant variation when compared with any of the injection formulations. This suggests that the difference in bioavailability, is small, but significant.

Thus, it can be concluded that the developed parenteral formulations have better bioavailability with less variation in pharmacokinetic parameters than oral dosage form.

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