

PRELIMINARY COMMUNICATION

INVESTIGATIONS OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF PREDNISOLONE SOLID DISPERSION PREPARED WITH SKIMMED MILK

Nefise O. Sahin¹, Tadeusz Librowski^{2,#}

¹Mersin University, Faculty of Pharmacy, Department of Pharmaceutics, Yenisehir Kampusu, Mersin 33169, Turkey,

²Department of Pharmacodynamics, Jagiellonian University, Medical College, Faculty of Pharmacy,
Medyczna 9, PL 30-688 Kraków, Poland

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Prednisolone is an analgesic and anti-inflammatory drug. It possesses poor aqueous solubility and has irritant effects on stomach mucosa. In order to modulate its gastric side effect and increase aqueous solubility, physical mixture and solid dispersion of prednisolone were prepared with skimmed milk. In this study, anti-inflammatory and analgesic effects of these formulations were investigated in comparison to the plain drug itself employing hind paw edema test and Randall's analgesia test. Based on the data, it was determined that the drug maintained its pharmacological activity even after formulating in the form of physical mixture and solid dispersion.

Key words: *prednisolone, solid dispersion, anti-inflammatory effect, analgesic activity, hind paw edema test, skimmed milk*

[#] *correspondence*; e-mail: mflibrow@kinga.cyf-kr.edu.pl

INTRODUCTION

Glucocorticosteroids (GCCS) have been widely used in therapy of inflammatory diseases (both acute and chronic) for many years. Long-term therapy with these drugs is often necessary to control the symptoms of many rheumatic conditions. Long-lasting usage of GCCS in therapy causes many undesirable effects on cardiovascular system and bone metabolism. Bone loss is a most frequent side effect of a long-lasting application of GCCS. The mechanisms underlying anti-inflammatory activity of GCCS are very complicated. They interfere with the function several systems. In addition to the potential dangers associated with long-term use of GCCS at supraphysiological concentrations, there are problems associated with the withdrawal from steroid therapy. Because GCCS are the best anti-inflammatory agents available to date, therapeutic use would benefit greatly from a reduced undesirable side effects. The major limitation to the use of oral GCCS has always been concern about their safety [7, 10]. GCCS are effective when given orally, and obviously this is the preferred mode of administration for prolonged therapy. However, parenteral administration is required in certain circumstances. For example, intramuscular injection of water-soluble ester produces peak plasma steroid levels within 1 h. The GCCS are considered to be generally poorly water soluble and have demonstrated unpredictable dissolution rates.

Solid dispersion (SD) in water-soluble carriers have attracted considerable interest as a mean of improving the dissolution rate, and possibly enlargement of bioavailability, of a range of hydrophobic drugs [1, 2, 6, 7]. Prednisolone (PRED) is a corticosteroid anti-inflammatory, analgesic agent used in treatment of inflammatory diseases [8, 13, 18]. It is insoluble in aqueous solutions and causes gastric irritation upon oral administration [1, 3, 5]. It has been well-known that aqueous solubility can be enhanced with the aid of surface active agents or water-soluble salts [3, 7, 14, 15]. In order to improve dissolution and absorption rate of poorly soluble drugs, reduction of particle size has also been suggested in the literature [2]. Preparation of SD can provide reduction in particle size [1, 17]. In this study, physical mixtures (PM) and SD of PRED were prepared using skimmed milk (SM) in order to improve aqueous solubility while maintaining the original pharmacological activity of the

drug. We suppose that this method could result in the increased and more predictable dissolution rates, benefiting the therapeutic response. These SDs should be considered as potential dosage form modifications for the PRED and for other poorly soluble or insoluble drugs usually administered orally, where an increase in the bioavailability, enhancement of therapeutic effects and lowering of side effects are desirable. Thus, "hind paw edema" and Randall's analgesia test and hot-plate test were performed to investigate anti-inflammatory and analgesic activities of both PM and SD in comparison with the plain drug itself. Preliminary studies like ours are important for better predictability and increased understanding of the incorporation/release behavior of drugs from particulate delivery systems that can be made from and SD prepared using SM.

MATERIALS and METHODS

Animals

Male albino Wistar rats (150–200 g) and male albino mice (18–26 g) were used for anti-inflammatory and analgesia tests, respectively. The animals were housed and fed in a laboratory at constant temperature of 22°C under the standard conditions (12:12 h L:D cycle, standard pellet diet, tap water). Each experimental group consisted of 8 animals/dose and all the animals were used only once. Treatment of the used laboratory animals in the present study was in full accordance with the respective Polish and European regulations and was approved by the Local Ethics Committees of both universities.

Drugs

PRED was a gift from Mustafa Nevzat Pharm. Co, Turkey. Skimmed milk was used as purchased from Miss Dairy Products, Turkey. Carrageenan (Sigma Co, USA, Type IV), and all other reagents and chemical substances were of analytical grade.

In this study, the anti-inflammatory and analgesic activities of PRED, PM and SD were investigated employing hind paw edema and analgesia tests (Randall's test and hot-plate test method), respectively.

Preparation of skimmed milk powder

As described elsewhere [10], 25 ml of SM was freeze-dried until the humidity of the sample was

reduced to 3%. Based on preliminary studies, duration of the lyophilization process was determined as 72 h. The yield was 2.615 g for skimmed milk powder (SMP). The obtained product was sieved through 250 μm mesh.

Preparation of the physical mixtures

PM were prepared using the technique of Sahin et al. [10]; 500 mg of micronized drug was uniformly mixed with 2.615 g of SMP using an agate mortar and pestle. The prepared PM formulations were kept in a desiccator over calcium chloride at room temperature.

Preparation of the solid dispersion

SDs of PRED were prepared with the technique of Sahin et al. [10]; 500 mg of PRED was suspended in 25 ml of SM. Suspension was mixed with a magnetic stirrer in a water bath at 50°C until a homogeneous mixture was formed. Subsequently, it was frozen in the fluid nitrogen bath and lyophilized (Lyo-vac GT 2-Leybold, Heraeus). The resultant SD of PRED was sieved through 250 μm mesh.

Determination of anti-inflammatory activities of various formulations of PRED using carrageenan-induced hind paw edema test

Male albino Wistar rats (100–150 g) were used in “hind paw edema” test. Rats were divided into four groups, one of them being the control. In order to produce inflammation, 0.1 ml of 1% carrageenan solution in water was injected into hind paws subplantar tissue of rats, according to the modified method of Winter et al. [19]. The development of paw edema was measured pletysmographically. Prior to this administration, paw diameters were measured by dividers and recorded. After carrageenan injections, the following preparations were injected intraperitoneally (*ip*) PRED (10 mg/kg) to group I, PM (62.3 mg/kg equivalent to 10 mg/kg of PRED) to group II, and SD (62.3 mg/kg equivalent to 10 mg/kg of PRED) to group III, both in physiological saline (*ip*). Physiological saline was injected by the same way, to the control group. After these injections, paw diameters were measured at 1, 2, 3, 20 and 24 h, % of edema and edema inhibition was calculated according to the formulas given below.

Edema % = $(N' \times 100) / N$. Edema inhibition % = $(N - N' \times 100) / N$. N: paw diameters measured 1, 2, 3, 20 and 24 h after injection of carrageenan to

the control group – paw diameters at the beginning. N': paw diameters measured 1, 2, 3, 20 and 24 h after injection of carrageenan to the test groups – paw diameters at the beginning.

Analgesic activity

Analgesic activity was determined by assessing the inhibition of pain provoked by compression of carrageenan-inflamed rat paw and by the method of Randall and Selitto [9]. In Randall's test, pain threshold in the hind paw of rat affected by inflammation was measured using an analgesimeter 24 h after *ip* administration of the compounds. Mean pain thresholds were calculated for treated and control groups and the percent change in relation to the control was determined.

Hot-plate test

The hot-plate test was conducted according to the procedure described by Eddy and Leimbach [4]. In this test, reaction of mice to painful stimulus was measured. Mice were placed on the metal plate heated to $52 \pm 0.4^\circ\text{C}$ and covered with a glass cylinder (height: 25 cm, diameter 15 cm). The time (s) point of the first pain response (licking or jumping) was determined by a stop-watch and then, recorded. The experiments were conducted 30 min following the *ip* administration of the formulations and the plain drug.

Statistical analysis

The data are expressed as means \pm SEM. The obtained data were evaluated by the one-way analysis of variance (ANOVA), followed by Bonferoni's multiple comparison test; $p < 0.05$ was considered significant [12].

RESULTS and DISCUSSION

Our investigation aimed at finding out if a modification of the PRED by the use PM and SD prepared using SM in order to improve aqueous solubility, permits to obtain new drugs of similar or better anti-inflammatory activity, but lower side effects than the parent drug. The investigation of the anti-inflammatory activity of the formulations (PRED, PM, SD) are presented in Tables 1–3. The highest anti-inflammatory (data not shown) and analgesic activity was provided by formulation PM (after 3, 20, and 24 h observation, Table 3). There was no

Table 1. Anti-inflammatory activity of various PRED formulations in comparison with the plain drug

Compound (dose)	Increase in rat paw volume (mean \pm SEM)					
	0	1 h	2 h	3 h	20 h	24 h
Control	16.5 \pm 0.76	26.1 \pm 1.28	27.5 \pm 1.00	31.9 \pm 0.93	29.8 \pm 0.76	28.6 \pm 0.98
PRED 10 mg/kg <i>ip</i>	16.0 \pm 1.00	23.3 \pm 0.92	24.9 \pm 0.91	28.1 \pm 1.44	29.0 \pm 0.95	29.5 \pm 0.98
PM 62.3 mg/kg <i>ip</i>	16.5 \pm 0.61	22.2 \pm 0.93	22.9 \pm 1.24	24.7 \pm 1.53**	23.3 \pm 0.95**	22.5 \pm 0.92*
SD 62.3 mg/kg <i>ip</i>	16.3 \pm 0.41	23.1 \pm 0.68	22.4 \pm 0.78	24.5 \pm 0.58**	23.4 \pm 0.74**	23.7 \pm 0.95

The values represent means \pm SEM (n = 8 animals per group). ANOVA: F(23,168) = 21.452, p < 0.0001; * p < 0.01, ** p < 0.001 (Bonferroni's multiple comparison test)

Table 2. The results of Randall-Selitto test with regard to increase in paw edema

Compound (dose)	Increase in rat paw edema (mean \pm SEM)					Pain inhibition (%)
	1 h	2 h	3 h	20 h	24 h	24 h
Control	9.60 \pm 1.28	11.00 \pm 1.00	15.40 \pm 0.93	13.30 \pm 0.76	12.10 \pm 0.98	0
PRED 10 mg/kg <i>ip</i>	7.33 \pm 0.92	8.91 \pm 0.91	12.10 \pm 1.44	13.00 \pm 0.95	13.50 \pm 0.98	\uparrow 10.0
PM 62.3 mg/kg <i>ip</i>	5.66 \pm 0.93	6.40 \pm 1.24	8.20 \pm 1.53**	6.80 \pm 0.95*	5.99 \pm 0.92*	4.5
SD 62.3 mg/kg <i>ip</i>	6.75 \pm 0.68	6.10 \pm 0.78	8.20 \pm 0.58**	7.00 \pm 0.74*	7.42 \pm 0.95	7.2

The values represent means \pm SEM (n = 8 animals per group). ANOVA: F(19,140) = 9.134, p < 0.0001; * p < 0.01, ** p < 0.001 (Bonferroni's multiple comparison test)

significant difference between the time of reaction to pain stimulus values of PRED, PM and SD. The highest anti-inflammatory activity of formulation SD was observed after 3 h (after 20, and 24 h anti-inflammatory activity was diminished, data not shown). The compound PRED (10 mg/kg *ip*) potentiated the carrageenan edema and lowered the threshold pain in comparison with control (Tab. 2).

In order to obtain the favorable therapeutic effect of a drug, it is necessary to prepare the correct drug formula for desirable effects. New technological developments allow for improvement of the drug dosage form formulations. Methods of increasing the availability of poorly soluble drugs in order to improve their pharmacological properties still remain the main problems. The classic treatments of the inflammatory states and rheumatic diseases are not always effective in controlling the disease [1, 2]. This necessitated development of novel agents for treating rheumatic diseases [7]. Most of these drugs are biological in nature and are targeted at specific sites of the cascade of inflammatory reactions [3, 5]. Many of these agents have to be administered parenterally, and their produc-

Table 3. The influence of the tested formulations on pain reactivity in the hot-plate test in mice

Formulation/substance	Time of reaction to pain stimulus (s)	Analgesic activity (%)
Control	21.2 \pm 1.1	0
PRED	30.1 \pm 0.8*	42.0
PM	30.6 \pm 1.1*	44.3
SD	31.2 \pm 0.7*	47.2

The values represent means \pm SEM (n = 8 animals per group). ANOVA: F(3,28) = 24.787, p < 0.0001; * p < 0.001 (Bonferroni's multiple comparison test)

tion costs are very high. Some of the established rheumatic disease treatments utilizes bioreductive agents which are released in hypoxic tissues at the site of inflammation. GCCS like PRED are often used as standards with which other drugs with similar action are compared [5]. Not the least of the reasons for their status is that they are relatively inexpensive. However, they are not free of undesired side effects [11]. The obtained results demonstrated that preparation of PM and SD of PRED consider-

ably improved the anti-inflammatory and analgesic activity (Randall-Selitto test) in comparison to plain drug – PRED (Tab. 2). Based on the data obtained in this study, it can be concluded that anti-inflammatory activity of PM and SD formulations is significantly higher than that of the plain drug (Tab. 1). No loss of anti-inflammatory and analgesic activities was noticed for all formulations prepared with SM. On the contrary, a sustained activity was observed during the test period for SD formulations. All these findings indicate that SD and PM formulations of PRED with SM have a potential to be used as oral dosage forms since the drug formulated in these preparations does not lose its pharmacological activities. Further studies are needed to better characterize the effects of these modified compounds in other tissue such as the gastric mucosa.

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