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SOME BIOCHEMICAL AND HISTOPATHOLOGICAL STUDIES OF PIRPROFEN IN RATS

BY

H. N. MOUSTAFA*; BUTHINA S. SAID; S.A. ASSAD AND E.A. ISHAK**

Faculty of Medicine, Cairo University and National Organization for Drug Control and Research.

* Forensic Medicine & Toxicology Department .

** Pathology Department.

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INTRODUCTION

Pirprofen (Rengasil)(R) is a new non-steroidal anti-inflammatory durg (NSAID) that not only shows excellent analgesic, anti-inflammatory, antipyretic and antinociceptive potency but also has a wide therapeutic margin (Chart & Maier, 1981; Chart et al., 1981). It is, also, useful in the management of rheumatoid arthritis, osteoarthritis and nonarticular rheumatism (Crouzet, 1982; Dionne & Weber, 1983) . Maier, (1984a) mentioned that pirprofen is a pronounced analgesic but without central, morphine-like attributes. The drug is available since 1982 in several countries and undergoing phase III investigation in the United States (Danan et al., 1985). Josef et al. (1981) noticed that its most side effects are gastrointestinal in nature, with nausea, indigestion, constipation, diarrhea and abdominal pain. Peptic ulceration and occult gastrointestinal blood loss have been reported in small percentage of patients receiving pirprofen (Dionne & Weber, 1983). Acute hepatitis and an asymptomatic increase in the level of serum aminotransferases have been described in patients treated by pirprofen (Castot et al., 1984). A total of five patients receiving pirprofen had

increases in alkaline phosphatase and/or aspartate transaminase > 10 percent above upper limits of normal (Roth, 1981). Jean-Pastor & Jouglard (1984) mentioned that five cases of liver damage were due to pirprofen. Danan et al. (1985) had reported the cases of two female patients aged 69 and 61 years suffering from fulminant hepatitis induced by pirprofen. The duration of drug administration before the onset of hepatitis was long, 7 and 9 months respectively, which ended with death of one patient from liver failure. Four cases of hepatic injury attributed to the use of pirprofen were reported to the Drug Monitoring Centres of Belgium, the Netherlands and the German Medical Association. The patients developed severe hepatic injury between 3 and 6½ months after starting therapy with 400-1200 mg pirprofen daily. Two patients died, whereas the other two patients made an incomplete recovery with a possible progression to post-necrotic cirrhosis (De Herder et al., 1986). Results have been published by a number of authors (Hurault de Ligny et al., 1985 and 1986 ; Bentata Pessayre et al., 1986; Eugene et al., 1987) indicating that acute renal failure and nephrotic syndrome developed in patients received pirprofen.

Inhibition of prostaglandin synthetase is well established as an important molecular mechanism underlying the biological effects of indomethacin, aspirin and most other non-steroidal anti-inflammatory drugs (Ku and Wasvary, 1975). Pirprofen is a drug whose pharmacological and therapeutic effects are similar to those of indomethacin (Ku & Wasvary,1975) and whose chemical structure (Fig. 1) resembles certain 2-arylpropionic acids that are among the most potent known inhibitors of prostaglandin synthesis (Ham et al., 1972).

The purpose of the present study was to evaluate the effect of pirprofen on the serum aminotransferases and prostaglandin $F_{2\alpha}$, in addition to the histopathological effects on the tissues of different organs in rats.

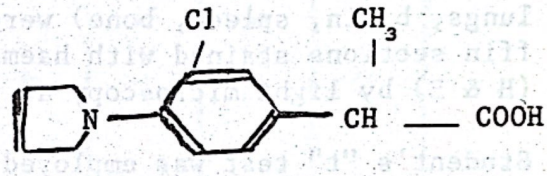
MATERIALS AND METHODS

Test Compound:

Pirprofen (Rengasil)$^{(R)}$ was supplied with Ciba-Geigy Egypt in the powder form (99.5% active ingredient). The drug is insoluble in water and was administered as uniform aqueous suspension in saline.

Fig. 1: Chemical structure of pirprofen (C21, 524-Su; Rengasil$^{(R)}$).

Structural Formula:



Chemical names: 2-[3-chloro-4-(3-pyrrolin-1-yl)-phenyl]-propionic acid.

Test animals:

120 adult albino rats of both sex each weighing 125-175 gm were used. Fourty animals were a control group and received saline only. Eightly animals served as a medicated group and treated with the drug in volumes not exceeding 0.5-1 ml/rat/day. Food and water were provided in sufficient amounts ad-libitum and daily dietary allowance was increased by 10% at a weekly intervals throughout the period of medication.

Dose amounted to 35 mg/kg body weight/day comparable to 1/10 LD_{50} of pirprofen (Chart & Maier, 1981) was administered orally every day for three weeks only because of the exceeding rate of deaths of the medicated animals which did not survive till the end of the designed period for study (4 weeks). Blood samples were withdrawn weekly from the retro-orbital

venous plexus and were subjected to the following biochemical investigations:

- Serum aminotransferases (S.G.O.T. & S.G.P.T.) were estimated according to the method of Reitman & Frankel (1957).

- Prostaglandin $F_{2\alpha}$ was evaluated by AIR technique of Jaffe and Behrman (1974).

Histopathological examinations of the different organs (liver, kidneys, heart, gastrointestinal tract, lungs, brain, spleen, bone) were performed on paraffin sections stained with haematoxylin and eosin (H & E) by light microscopy at the end of every week.

Student's "t" test was employed to evaluate significant changes of the data.

RESULTS

1. The results obtained for the effect of the test compound on serum aspartate aminotransferase (S. G.O.T.), serum alanin aminotransferase (S.G.P.T.) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) ar compiled in **Tables (1&2)**. These tables show the statistical analysis of the data and level of significance of the differences, in addition to the percentage change between the treated rats (after one, two and three weeks) and control non-treated rats. The data of the medicated groups of rats (after one, two and three weeks treatment) were, also, statistically analysed to determine the percentage change and level of significance of the differences between them **(Table 2)**.

2. **Histopathological Findings:**

Pirprofen produced the following histopathological changes in rats:

Table 1: Levels of statistical significance of aminotransferases activity and prostaglandin $F_{2\alpha}$ in adult albino rats treated with pirprofen.
N.B.: Values represent means of 10 rats of either sex. (N = 10).

Groups of rats	Mean values ($\bar{X}$) ± S.E. and levels of significance		
	Serum Aminotransferases		$PG_{F_{2\alpha}}$
	S.G.O.T. (Unit/ml)	S.G.P.T. (Unit/ml)	(Picogram/ml)
Control non-treated rats	40.4 ± 2.26	17.2 ± 0.55	341.8 ± 6.05
Rats treated for one week	60.0± 4.57**	36.8 ± 2.90***	234.8 ± 21.75***
Rats treated for two weeks	75.8 ± 3.57***	76.8 ± 4.83***	229.4 ± 17.67***
Rats treated for three weeks	87.8 ± 2.00***	80.6 ± 2.32***	150.6 ± 11.63***

** Significance level at $P < 0.01$.
*** Significance level at $P < 0.001$.

Table 2: Percentage increase and decrease and level of significance between different groups of rats.

% change between groups of rats		S.G.O.T. (Unit/ml)	S.G.P.T. (Unit/ml)	$PG_{F_{2\alpha}}$ (Picogram/ml)
Between control and treated	After one week treatment(1)	+32.67%**	+53.26%***	-31.30%***
	After two weeks treatment(2)	+46.70%***	+77.60%***	-32.88%***
	After three weeks treatment(3)	+53.99%***	+78.66%***	-55.94%***
Between treated groups 1,2,3	Between 1 & 2	+20.84%**	+52.08%***	-2.30 %
	Between 2 & 3	+13.67%**	+4.71%	-34.35%**
	Between 1 & 3	+31.66%***	+54.34%***	-35.86%**

** Significant difference at $P < 0.01$

*** Significant difference at $P < 0.001$

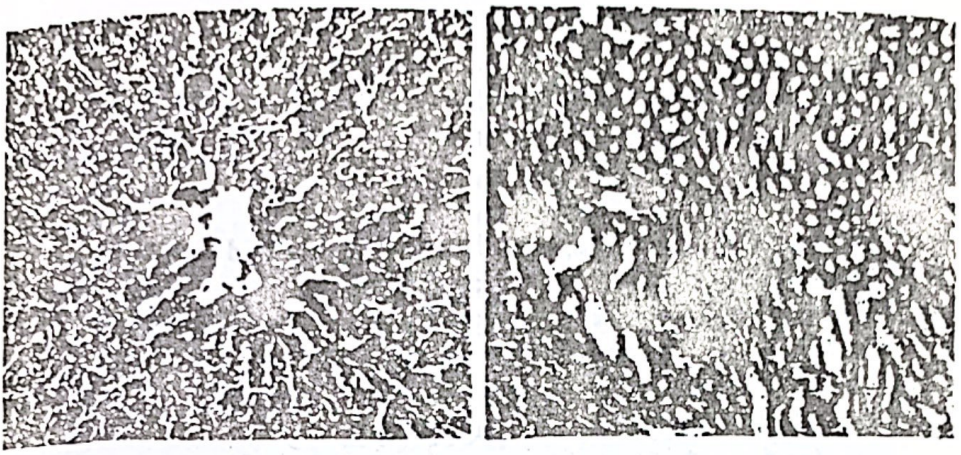


Fig. 2: Liver showing mild sinusoidal dilatation and focal mild fatty change (H & E X 150).

Fig. 3: Kidney showing moderate degenerative changes in tubules (H & E X 60).

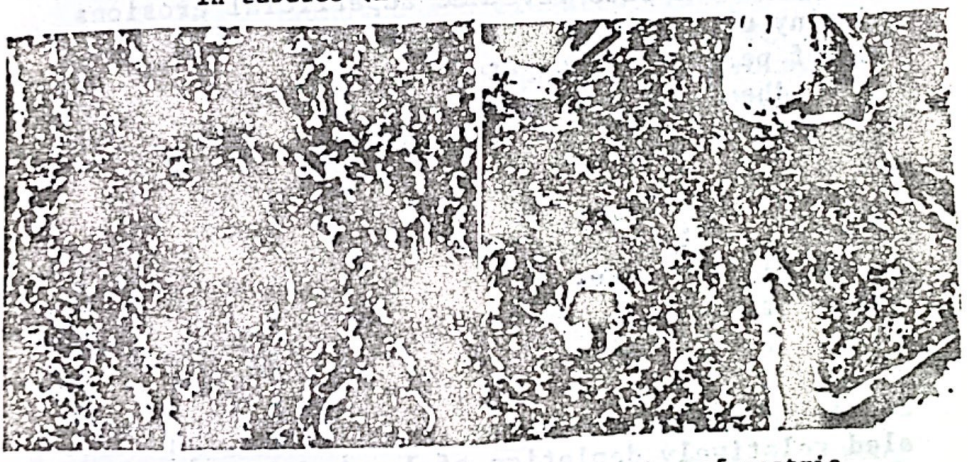


Fig. 4: Degenerative changes and congestion of gastric mucosa (H & E X 90).

Fig. 5: Bone and bone marrow showing mild hypocellularity (H & E X 90).

Liver: Congestion, mild sinusoidal dilatation and mild focal fatty degeneration were seen (**Fig. 2**). The focal fatty change has been more pronounced with prolonged use the drug.

Kidneys: No significant changes appeared after one and two weeks. Tubules showed degenerative changes (**Fig. 3**) and moderate congestion of their blood vessels with interstitial tissue oedema after three weeks treatment. Glomeruli did not reveal any significant change by light microscopy.

Gastrointestinal tract: Moderate congestion (**Fig. 4**) of the blood vessels of gastric mucosa was seen in most treated rats. At end of the study, the gastric mucosa of some rats revealed superficial erosions and tiny ulcerations; while one rat displayed evidence of perforation of stomach with granulation and early adhesion. The mesenteric lymph nodes draining the irritated G.I.T. ahowed moderate medullary sinus hyperplesia.

Bone marrow: Bone marrow showed progressive decrease in cellularity (**Fig. 5**) with increased duration of treatment, mostly affecting granulocytic series with minimal fatty replacement.

Spleen: Moderate congestion was found, Few rats revealed relatively depletion of lymph follicles.

The tissues of the other organs did not show any significant pathological change.

DISCUSSION AND CONCLUSION

The present study showed that pirprofen produced a significant increase in S.G.O.T. and S.G.P.T. after one, two and three weeks treatment (**Tables 1&2**).

These data could denote a liver function impairment and hepatocellular changes. The results of out histopathological study are compatible with those of the serum aminotransferases as evidenced by the presence of congestion, sinusoidal dilatation and fatty changes in the liver of medicated rats. These findings are partially in accordance with many published studies (Koch, 1983; Castot et al., 1984; Chalchat , 1985; Danan et al., 1985; Bentata Pessayre et al., 1986; De Herder et al., 1986; Fouin-Fortunet et al., 1986). During clinical trials of pirprofen, Koch (1983) observed elevation in transaminases and alkaline phosphatase values, as well as cases of hepatitis and icterus. Castot et al. (1984), also, noticed hepatitis with jaundice and elevated transaminases activity after therapy with pirprofen, 800 mg-1200mg daily for 3 - 9 months. In France, serum transaminases have raised in 36 patients treated with pirprofen (dosage 800 mg or 1200 mg daily) for 1 year in multicentres (Chalchat, 1985). Danan et al., (1985) reported the cases of two female patients suffering from fulminant hepatitis induced by pirprofen after 7 and 9 months administration, where the liver lesion consisted of massive predominantly centrilobular hepatic cell necrosis and microvesicular steatosis . Bentata pessayre et al. (1986) reported that, transaminases, alkaline phosphatase and bilirubin have increased in the blood of a female patient suffering from osteoarthritis and on pirprofen for about 3 months; in addition, liver biopsy has shown cholestatic and cytolytic hepatitis. Reports to the Drug Monitoring Centres of Belgium, the Netherlands and the German Medical Association of four patients with severe hepatic injury between 3 & 6.5 months after starting therapy with dosage 400 mg-1200mg pirprofen daily showed acute hepatocellular damage in the form of necrosis and benign fibrosis, and the reports mentioned that, all other possible diseases and preexistent liver diseases were excluded (De Herder,

1986). Also, liver biopsy of three patients with hepatitis due to pirprofen (dosage 800 mg daily) showed disseminated necrosis with inflammatory infiltrates (Fouin-Fortunet et al., 1986).

Danan et al. (1985) mentioned that, the mechanism for the liver lesion induced by pirprofen is unknown. The absence of hypersensitivity manifestations and the late response to readministration of pirprofen suggest a metabolic rather than an immunoallergic mechanism (Castot et al., 1984). This view is consistent with the observation that reactive metabolites are formed from benoxaprofen and other non-steroidal anti-inflammatory drugs in the presence of rat or human microsomes (Mitchell and Lietman, 1983). Also, this observation was in accordance with that of De Herder et al., (1986) who mentioned that, since clear immunoallergic signs were absent in cases of severe hepatic injury of pirprofen, metabolic idiosyncreasy may be the mechanism. On the other hand, Fouin - Fortunet, et al. (1986)said that the hepatic side effects of pirprofen may have been due to an immunoallergic mechanism.

Regarding the prostaglandin $F_{2\alpha}$, pirprofen produced inhibition in its synthesis in the tested rats,since it was very highly significantly ($P < 0.001$) decreased after one, two and three weeks by -31.3%,-32.88% and -55.94% respectively **(Tables 1&2)**. Our findings agree well with those obtained by many authors (Chart and Maier, 1981; Ku and Wasvary, 1975; Maier, 1984 b and 1985). Pirprofen is a relatively potent inhibitor of prostaglandin synthetase, the inhibitory concentration (IC_{50}) in vitro being 1.26 mcg/m (Chart and Maier, 1981). Studies with bovine cerebral cortex enzyme in the presence of added Cu^{2+} have shown that pirprofen is effective as an inhibitor of prostaglandin $F_{2\alpha}$ synthesis (Ku and Wasvary, 1975). Pirprofen is, also, a potent inhibitor of sheep seminal vesicle

prostaglandin E_2 synthase in vitro (Ku & Wasvary, 1975). This was expected, of course, in view of the fact that the compound acts competitively with respect to arachidonic acid which serves as the precursor of both prostaglandins through a common pathway (Ku & Wasvary, 1975). Pirprofen might, also, be expected to interfere with the formation of prostaglandins G_2 and H_2, peroxide intermediates in the synthesis of prostaglandins E_2 and $F_{2\alpha}$ from arachidonic acid (Ku & Wasvary, 1975). Maier, (1984 b) noticed that pirprofen inhibits arachidonic acid metabolism by interfering with cyclo-oxygenase, but not with lipoxygenase. Rengasil(R) interferes with the biotransformation of arachidonic acid into various prostanoids (e.g. prostaglandins, thromboxanes, prostacyclins), but does not inhibit metabolism to leukotrienes (Maier, 1985). It is believed that the analgesic, antipyretic and antidiuretic properties whcih pirprofen displays in aniaml experiments may be related to this prostaglandin synthetase inhibiting activity (Chart and Maier, 1981).

The pathological effects of pirprofen on the kidneys at the end of the study were degenerative changes in the tubules with moderate congestion and oedema in the interstitial tissue. Koch, (1983) said that,the toxic effects of pirprofen on the kidneys reported in animal studies were interstitial nephritis developed in rats, and there was infiltration of the renal cortex by lymphocytes and fibroblasts in dogs. Some studies have been published indicating that nephrotic syndrome and acute renal failure displayed in patients receiving pirprofen (Hurault de Ligny et al., 1985 &. 1986; Bentata Pessayre et al., 1986; Eugene et al., 1987). Renal biopsy showed tubular damage with epithelial atrophy and interstitial damage with inflammatory infiltration (Hurault de Ligny et al., 1985). In another study , renal biopsy showed interstitial nephritis (Hurault de Ligny et al.,

1986) . Acute overdoses of other non - steroidal anti-inflammatory drugs (NSAIDs) have caused nonoliguric renal failure (Appleby, 1981). This is believed to be the result of prostaglandin synthesis inhibition, which plays a role in maintaining renal blood flow (Anderson et al., 1976).

On the light of pathological lesions in the kidneys in our study and those of other studies reviewed here, we believe that the nephrotic syndrome reported with pirprofen is probably due to minimal change glomerulonephritis. However, the possible acute renal failure reported in the literature could only be explained on the basis of tubular necrosis, inspite of fact that we did not encounter such an aggressive change. The clear degenerative changes in the tubules in the present study, that could be aggravated with prolonged use, could lead to possible tubular necrosis.

Our study showed moderate congestion of the gastric mucosa in most treated rats. At the end of the study, the gastric mucosa of some rats revealed superficial erosions and tiny ulcerations; one rat displayed evidence of perforation of stomach with granulation and early adhesion. These findings were similar to those observed by Koch, (1983) who found that gastrointestinal erosion and ulceration developed in rats and dogs given 5 and 1 mg pirprofen, respectively , per kg per day. 20 mg/kg per day caused intermittent, occult gastrointestinal blood loss in monkeys (Koch, 1983). During clinical trials, isolated occurrences of peptic ulcer have been observed (Koch, 1983).Dionne and Weber, (1983), also, mentioned that peptic ulceration and occult gastrointestinal blood loss have been reported in a small percentage of patients receiving pirprofen. It is noticed that, our findings coincide with the previously mentioned ones.

The adverse effects reported here of digestive system, hepatic and renal degeneration justifies certain precautions in patients treated with pirprofen. The incidence of hepatotoxicity and nephrotoxicity can be limited by prescribing the drug for severe inflammation only and by supervising aminotransferases and renal function if pirprofen is given for longer than two weeks. We advise that, the drug administration must be discontinued as soon as an increase in serum aminotransferasis level or renal dysfunction is noted.

SUMMARY

Subchronic toxicity study of pirprofen (Rengasil(R)) was performed in rats. This included the effect of the drug on the serum aminotransferases (S.G.O.T. & S.C. P.T.) and prostaglandin $F_{2\alpha}$, in addition to, the histopathological effects on the tissues of the different organs.

The study revealed that, pirprofen has an adverse effect on the hepatocellular functions in the form of an increase in aminotransferases activity and hepatocellular degeneration. It, also, produced inhibition of prostaglandin $F_{2\alpha}$ synthesis. Degenerative changes occured in the tubules of the kidneys, as well as congestion of their blood vessels and oedema in the interstitial tissue. The gastric mucosa showed moderate congestion of the blood vessels. At the end of the study which continued for three weeks, the gastric mucosa of some rats revealed superficial erosions and tiny ulcerations; while one rat displayed evidence of perforation of the stomach.

Though we can recommend the short term medication period to avoid all the previous side effects appeared.

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