A HISTOPATHOLOGICAL STUDY OF ANALGESIC HEPATOTOXICITY IN RABBIT

Pages with reference to book, From 41 To 43

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Abstract

This study of analgesic hepatotoxicity was conducted on rabbit divided into four equal groups (A—D). The group "A" served as control. The group "B" animals were administered acetylsalicylic acid (600 mg/Kg/day) orally,while group "C" animals received paracetamol (300 mg/Kg/day). The group "D" was given both the acetylsalicylic acid (300 mg/Kg/day) and paracetamol (150 mg/Kg/day). An autopsy was performed on all the animal groups at the end of 4 weeks. On gross and microscopic examination of liver sections, the control animals did not reveal any hepatic abnormality while the group "B" animals showed acute hepatitis (57%), acute cholangitis (14%) and focal necrosis (14%). The group "C" revealed acute hepatitis in 86% cases. The group "D" receiving combination of drugs showed acute hepatitis (71%) and acute cholangitis (14%). This study indicates that paracetamol is more toxic to liver as compared to acetylsalicylic acid in higher doses (JPMA 38: 41 ,1988).

INTRODUCUON

The salicylates and para-aminophenol derivatives i.e. acetaminophen, paracetamol and phenacetin are commonly used as simple house hold analgesics. When one considers their abuse and easy availability the high incidence of nephrohepatotoxicity does not seem to be surprising. The problem of analgesic abuse not only relates to those who indulge in self medication but also to the involvement of much larger population. Paracetamol is increasingly being used as suicidal agent these days. In 1970, twenty deaths and 890 hospital admissions for suicidal paracetamol overdosage have been recorded in England and Wales. This increasing problem of analgesic hepatotoxicity usually results in death due .to massive hepatonecrosis but the milder degree of liver damages are also frequent[1-7] Toxicity of this thug is thought to be due to the biotransformation of the drug and subsequent protein binding and cell injury due to toxic metabolite. This hepatotoxicity is said to be mediated by a toxic reactive metabolite formed from the parent compound by cytochrome-P450 mixed — function oxidase system of the hepatocytes. This metabolite is detoxified by binding to glutathione. When excessive amounts of metabolites are formed in the liver, glutathione level in the liver falls and metabolites are covalently bound to hepatocytes. This process is believed to lead to hepatic necrosis usually of centriobular type[3]. The patients on salicylate therapy for acute rheumatic fever, Juvenile and adult rheumatoid arthritis and systemic lupus erythematosus may develop acute hepatitis and even chronic active hepatitis. [8] This study was undertaken to assess the intensity and extent of hepatotoxic effects of these commonly used drugs in higher doses for prolonged periods of time.

MATERIALS AND METHODS

Twenty eight healthy adult male rabbits (average body weight 1—2 Kg) were selected for this study. They were equally divided into 4 groups (A—D) and kept under the 14 : 10 LID schedule at a temperature of 24±2°C. Each animal was kept in separate cage and put on normal animal house diet of carrots and lucerene. The drugs, acetylsalicylic acid and paracetamol were obtained from Glaxo Laboratories (Pakistan) in pure powder form and administered orally to experimental groups (B,C and

D) by mixing in crushed carrots. The dose was calculated 1Kg body weight. The animal grouping and drug treatment is given in Table I.

TABLE – I

Grouping of Animals and Drug Treatment.

Groups	Number of Animals	Drugs used	Duration of Dosage
A 1 (Pure control)	3(1–3)	None (Normal Animal) (House diet only)	–
A 2 (Treated control)	4(4–7)	Excipient (500 mg/Kg/day)	4 Weeks
B (Experimental)	7(8-14)	Acetylsalicylic acid (600mg/ Kg/day)	4 Weeks
C (Experimental)	7(15-21)	Paracetamol (300 mg/Kg/day)	4 Weeks
D (Experimental)	7(22-28)	Acetylsalicylic acid (300 mg/ Kg/day) and Paracetamol (150 mg/Kg/day)	4 Weeks

All the animals were closely watched for their general health during 4 weeks of experiment. At the end of experiment, all the animals were killed by the method of air embolism. There was no animal death

during the experiment. The livers were removed at the time of autopsy. Suitable blocks of liver tissues were taken and fixed in 10% buffered formalin and 10% alcoholic formalin (for preservation and demonstration of glycogen and mucopolysaccharides by PAS stain). Another piece of liver tissue from each animal was taken for frozen section and demonstration of fat by oil red "0" staining. In addition to routine hamatoxylin and eosin staining, the special stains like van-Gieson (to demonstrate reticulin), periodic acid schiff (to demonstrate glycogen and mucopolysaccharides) and Mallory's trichrome (to demonstrate connective tissue) were also performed.

RESULTS

All the animals were observed for changes in their body weights and general health during the experiment. The group "A" animals on the average revealed 0.33% increase, while group B, C and D animals showed 3.5%, 3.4% and 3.6% decrease in their body weights. The average group weight of livers/Kg body weight revealed significant decrease (P Value <0.05) in both the group C and D. On microscopic examination, the control group did not reveal any abnormality except in one animal of treated control group (A 2) there was one small focal area of lymphocytic infiltration around an area of bile duct which was not sufficient to call it cholangitis. The experimental groups (B, C and D) revealed some histological changes in the Liver (Table II).

TABLE – II

Frequency of Hepatic abnormalities in various Groups.

Groups	Number of Animals	Diagnosis	No.	Percentage
A(A and A) 1 2 (Controls)	7	NAD	7	100%
B (Experimental)	7	Acute hepatitis	4	57%
		Acute cholangitis	1	14%
		Focal necrosis	1	14%
		NAD	1	14%
C (Experimental)	7	Acute hepatitis	6	86%
		NAD	1	14%
D (Experimental)	7	Acute hepatitis	5	71%
		Acute cholangitis	1	14%
		NAD	1	14%

NAD – No abnormality detected.

DISCUSSION

The association of paracetamol abuse and hepatotoxicity is gaining importance because of its usage as a suicidal agent either alone or in combination with other analgesic mixtures. A dose of 15—50 gm/day is dangerous and may result in death of a person due to hepatonecrosis[1]. Similarly the hepatotoxicity of salicylates has been mentioned by Sherlock[8]- She described that patients on salicylates therapy for acute rheumatic fever, juvenile and adult rheumatoid arthritis and systemic lupus erythematosus may

develop acute hepatitis and even chronic active hepatitis. In this study we have also observed varied intensity and extent of hepatotoxicity due to acetylsalicylic acid (Group "B") and paracetamol (Group "C"). A small focal area of lymphocytic infiltration around one bile duct in one of the treated control animal may possibly be due to the individual susceptibility to excipient. Rest of the Group "A" animal did not reveal any hepatic abnormality. The acetylsalicylic acid induced hepatic lesions i.e. acute hepatitis, acute cholangitis and focal necrosis are consistent with the morphological hepatic findings described by Sherlock. [8] The hepatotoxicity In paracetamol receiving group "C" showed acute hepatitis in 86% cases. This well marked hepatonecrosis can be compared wth short term acute effects of paracetamol toxicity as studied by Mitchell[3] and Walker[6]. Mitchell and his co-workers[3] carried out their studies on rats by administration of paracetamol (acetaminophen) in larger oral doses (300—750 mg/Kg/day) by using intragastric tubes and studied the acute hepatotoxic effects ranging from 34 to 72 hours. Moreover, they also studied the protective effect of glutathione on paracetamol induced hepatotoxicity. Similarly Walker et al[6] also studied the short term effects of paracetamol induced hepatotoxicity (24—48 hours) in mice by using oral dose of 500 mg/Kg/ day. The diversity in the intensity and extent of hepatonecrosis and cholangitis reported in this study have also been observed in similar experimental short term studies by Davidson[2] ,Gazzard[4], Dixon[5] , Chiu[7], and Proudfoot[9].

REFERENCES

1. Prescott, L.F., Wright, N., Roscoe, P. and Brown, 5.5. Plasmaparacetamol half-life and hepatic necrosis in patients with paracetamol overdosage. Lancet, 1971;1: 519.
2. Davidson, D.G.D. and Easthan, W.N. Acute liver necrosis following overdose of paracetamol. Br. Med. J., 1966;2: 497.
3. Mitchell, J.R., Jollow, DJ., Potter, W.Z., Gillette, J.R. and Brodie, B.B. Acetaminophen — induced hepatic necrosis. IV. Protective role of glutathione. J. Pharmacol. Exp. Ther., 1973; 187: 211.
4. Gazzard, B.G., Hughes, R.D., Postmann, B. and Williams, R. Protection of rats against the hepato-toxic effects of paracetamol. Br. 3. Exp. Pathol., 1974;55:601.
5. Dixon, M.F., Dixon, B., Aparicio, S.R. and Loney, P.D. Experimental paracetamol — in duced hepatic necrosis; a light and electron microscope and histochemical study. 3. Pathol., 1975; 116:17.
6. Walker, R.M., Racz, WJ. and McElligott, T.F. Acetaminophen-induced hepatotoxicity in mice. Lab. Invest., 1980;42:181.
7. Chiu, S. and Bhakthan, N. M.G. Experimental acetaminophen — induced hepatic necrosis; biochemical and electron microscopic study of cysteamine protection. Lab. Invest., 1978; 39:193.
8. Sherlock, S. Diseases of the liver and biliary system. 6th ed. Oxford, Blackwell, 1981,p.302.
9. Proud foot, A.T. and Wright, N. Acute paracetamol poisoning. Br. Med. 3., 1970; 3 : 557.