AN EXPERIMENTAL STUDY OF ANALGESIC NEPHROPATHY IN RABBIT A LIGHT MICROSCOPIC STUDY

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Abstract

This experimental study of analgesic nephropathy was conducted on rabbit divided into four equal groups (A,B,C,D). Group A served as control. The drugs acetylsalicylic acid (Aspirin) 600 mg/Kg/day and Paracetamol (Acetaminophen) 300 mg/Kg/day alone as well as combination of Acetylsalicylic acid (300 mg/Kg/day) and Paracetamol (150 mg/Kg/day) were administered orally to groups B,C and D respectively for 4 weeks. After four weeks, the animals were sacrificed. Histopathological evaluation of renal tissue of each animal revealed that control group "A" animals did not show any abnormality. The acetylsalicylic acid treated group "B" showed acute pyelonephritis (71%), mild pyelitis (14%) and congestion of renal pelvis (14%). The group "C" receiving paracetamol revealed acute pyelonephritis (14%), acute pyelitis (43%), pelvic congestion (14%) and no change in 28% animals. The group "0" receiving combination of acetylsalicylic acid and Paracetamol showed interstitial nephritis (14%), acute pyelitis (14%), cloudy swelling (14%) and pelvic congestion in 43% animals. This study indicates that acetylsalicylic acid is more injurious to kidneys than paracetamol and these drugs do not have potentiating effect (JPMA 38:113 , 1988).

INTRODUCTION

Since the fifth decade of the present century, increasing efforts have been made to analyse the association between nephropathy and abuse of analgesics or a mixture of analgesics. Despite numerous clinical observations and experimental studies in animals, the crucial details of the problems remained uncertain until 1961. A critical analysis of analgesic nephropathy was reported by Schreiner[1] and Gilman[2]. Although phenacetin has been implicated by some as the nephrotoxic component of analgesic mixtures, it was not confinned that only this, or any other particular ingredient was also the causative factor. A number of previous workers were of the view that chronic use of any of the analgesic antipyretic or analgesic mixtures may in the susceptible animal or man, cause renal or hepatic injury.[3] The commonly used analgesic thugs like acetylsalicylic acid and paracetamol are available in the form of tablets, elixirs, syrups and mixtures. If we consider the easy availability of these drugs, the clinical problems of analgesic nephropathy are not surprising. This analgesic abuse has created problems for urologists, pharmacologists, physicians and surgeons. A large number of clinical and experimental studies have been undertaken by several workers[3-9] to find out the genesis of the toxic effects of these thugs. The data on the toxicity of these drugs on kidneys, as reported by other workers, were available but to our knowledge no report was available of any experiment with the large doses for prolonged periods of time. The present study has been designed to observe the effects of these drugs in higher doses (the doses which approximate those used by the patients with chronic painful ailments like chronic headache, lumbar pain and arthritis, etc.) on the kidney of rabbits. It was also desired to observe the potentiation effects of these drugs.

MATERIAL AND METHODS

This experimental model for the study of analgesic nephropathy was designed at Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. Twenty eight healthy adult male rabbits (average weight 1-2 Kg) were selected for this study. These animals were divided into four equal groups (A,B,C and D) of seven each. All these animals groups (both control and experimental) were kept on 14:10 L/D schedule and at temperature of 24 ± 2°C at animal house of Jinnah Post-graduate Medical Centre. These animals were given crushed carrots and leucerene (the natural diet of rabbit). The acetylsalicylic acid B.P. (aspirin, U.S.P.) and Paracetamol, B.P. (Acetaminophen U.S.P.) were obtained from Glaxo Laboratories Ltd, Karachi, Pakistan, in pure powder form. These drugs were administered orally (mixed with crushed carrots) to the experimental animals groups ("B", C and D) for four weeks. The experimental schedule, grouping and drug treatment of animals is summarised in Table 1.

Table 1. Grouping of Animals and Drug Treatment.

Group & No of animals	Drug Administration	Remarks
A 1 3	None (Normal animal house diet only).	Pure control
A 2 4	Excipient (500 mg/kg b.wt/day	Treated con-trol
B 7	Acetylsalicylic and 600 mg/Kg. b.wt/day	Experimental
C 7	Paracetamol (300 mg/kg. b. wt/day)	Experimental
D 7	Acetylsalicylic and 300 mg/Kg. b.wt/day Paracetamol 150 mg/Kg. b. wt/day	Experimental

Four out of seven control animals were administered excipient which is used commercially in the preparation of tablets of these drugs so as to eliminate a possible variable factor in thug toxicity. Each animal was also closely watched, throughout the duration of the experiment, for changes in body weight, colour of conjunctiva and response to food and light. At the end of four weeks, each animal was sacrificed by the method of air embolism and both kidneys and liver were removed at autopsy. (There was no animal death during the experiment). Both the kidneys and liver tissues (for a separate

study) were preserved in 10% buffered formalin (10 ml of 40% formaldehyde dissolved in 90 % of water) and alcoholic formalin (10 ml of 40% formaldehyde added to 90 ml of 80% ethyl alcohol). The renal tissues were processed, sectioned and stained. In addition to the routine haematoxylin and eosin staining, special stains like periodic acid schiff (to demonstrate glycogen and Mucopolysaccharide) and Mallory's trichrome (to demonstrate connective tissue) were also performed on each kidney tissue. One additional block was also taken from each kidney for direct frozen section and Oil red "0" staining was performed on each of them. All the sections were studied by light microscopy and results recorded.

RESULTS

All animals in both, the control and experimental groups, revealed changes in their average body weights during the experiment as summarized in Table II.

Table II. Changes in Animal Weights during Experiment.

Group	Average animal weight at the start of experiment (gm)	Average animal weight at autopsy (gm)	Remarks regarding increase or decrease in weight
A (n = 7)	1537	1542	0.33% (Increase)
B (n = 7)	1532	1494	3.5 % (Decrease)
C (n = 7)	1533	1484	3.4 % (Decrease)
D (n = 7)	1532	1480	3.6 % (Decrease)

The average weights of kidneys of experimental groups B,C & D were reduced as compared to control group (A) as shown in Table III.

Table III. Changes in Kidney Weights in Each Group.

Group	Average animal weight (gm)	Average weight of kidneys (gm)	Ratio of kidney weight to body weight	P. Value at 0.05
A (A1&A2) (Control) (n = 7)	1542	9.3	Average	– normal limit
B Experimental (n = 7)	1478	8.6	Decreased	∠0.05
C Experimental (n = 7)	1485	8.7	Decreased	∠0.05
D Experimental (n = 7)	1479	8.5	Decreased	∠0.5

The summary of morphological changes in kidneys of both the control and experimental groups is presented in Table IV.

Table IV. Comparison of Microscopic findings in Kidneys of each Group.

Groups	Diagnosis	Number	Percentage
A (7)	NAD	7	100%
B (7)	Acute pyelonephritis	5	71%
	Mild pyelitis	1	14%
	Congestion renal pelvis	1	14%
C (7)	Acute pyelonephritis	1	14%
	Acute pyelitis	3	43%
	Pelvic congestion	1	14%
	NAD	2	28%
D (7)	Interstitial nephritis	1	14%
	Acute pyelitis	1	14%
	Cloudy swelling	1	14%
	Pelvic congestion	3	43%
	NAD	1	14%

We concluded that the misuse of acetyl¬salicylic acid and paracetamol in higher doses and for prolonged period of time is injurious for the kidneys. Acetylsalicylic acid induced more injurious (toxic) effects on kidneys as compared to paracetamol. It was also observed that these drugs do not have potentiating effect if adminis¬tered in combination.

DISCUSSION

The association of renal disease and abuse of analgesics has been known since 1950. Gilruan suggested that a relationship existed between prolonged analgesic abuse and a form of chronic renal disease whose features were those of interstitial nephritis with papillary necrosis2. A large number of clinical reports also appeared in literature on many occasions from various parts of the world about the injurious effects of phenacetin abuse which was used as part of various analgesic mixtures[1-9]. The use

of excipient or placebo (Cornstarch or Lactose) administered in treated control animals excluded the possibility of toxic effects of this ingredient which is normally used in the commercial preparation of tablets. The acetyisalicylic acid induced renal lesions in this study were in consistent with the findings of Angervall[5]. He studied the effects of acetylsalicylic acid, phenacetin and N-acetyl-p-aminophenol on albino rats and observed the changes resembling interstitial nephritis. Clausen[7] also confirmed the similar findings in rabbit by administering acetylsalicylic acid and phenacetin in higher doses over a period ranging from 3—12 months. These morphological changes as observed in this study are also supported on the basis of clinical and autopsy findings in an adult female who died of uraemia as reported by Sankerkin[11]. This lady consumed about 8 Kg of para-aminophenol drugs (Phenacetin and its metabolites) over a period of 40 years for the treatment of migraine. Both the kidneys revealed the changes of interstitial nephritis alongwith renal papillary necrosis. The renal pelvic congestion, as detected in both acetylsalicylic acid and paracetamol receiving groups, can be compared with the experimental clinical study of Prescott[9] who selected healthy volunteer adult females free from previous history of renal disease. These ladies were divided into five groups. Each group received separate drugs like acetylsalicylic acid, paracetamol, phenacetin and caffeine (experimental groups) for 2-4 weeks. The control group only received corn-starch or Lactose only. The urinary findings revealed increased epithellal cells and RBC casts because of irritative effects of salicylic and acetic acids (reactive metabolites of acetylsalicylic acid) excreted by kidney which are responsible for renal pelvic congestion. The place tablets did not reveal any urinary abnormality which can be compared with our treated control animals who did not show any morphological changes in kidneys. Scott[12] also reported similar renal changes in both clinical and animal studies. Young[13] also reported similar changes in model studies of analgesic nephropathy in animals. We could not find the study of potentiating effect of these drugs in the available literature.

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