Serial No	
Author 1	NWEKE, M. I.
Author 2	UCHEYA, R. E. .
Author 3Adeg	IGWEH, J. C.
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Keywords	
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Category	Physiology
Publisher	Nigeria Journal of Health and Biomedical Science
Publication Date	2006

Signature	

NWEKE MADUKA-G

Nigerian Journal of Health and Biomedical Sciences
January–June 2006, Vol. 5, No. 1, 51 – 56.

Abstracted on AJOL
http://www.inasp.info/ajol

Induced Gastrointestinal Tract (GIT) Derangement Following a Long-Term Administration of a Non-Selective Cyclooxygenase Inhibitor – Paracetamol in Pregnant Sprague–Dawley Rats.

R. E. UCHEYA, *J. C. IGWEH, *M. I. NWEKE
Department of Human Anatomy, College of Medicine, University of Nigeria, Enugu Campus. *Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus.

Correspondence to:
R. E. Ucheya
Department of Human Anatomy, College of Medicine, University of Nigeria, Enugu Campus.

SUMMARY
There are two recognized isoforms of cyclooxygenase enzymes, the cyclooxygenase – 1 and cyclooxygenase – 2, the former is involved in the biosynthesis of prostaglandins, in organs where these eicosanoids play certain protective roles in the gastrointesional tract (GIT) and the kidney, it also enhances mucus secretions and acts as a house keeping enzyme expressed constitutively in most tissues of the body, while cyclooxygenase – 2 is the inducible form expressed in response to proinflammatory cytokines and growth factors, indicating a role in inflammation and growth and also maintains haemodynamics. In pregnant state, several drugs are used out of necessity, despite their reported toxicity. The clinical conditions often necessitating the use of non–selective cyclooxygenase inhibitors during pregnancy include hypertension, thromboembolism, hyperthyroid-ism, epilepsy, diabetes mellitus, preterm labour, arthritis, pain and fever, among others. The aim of this study was to investigate the induced gastrointestinal derangement following a long-term administration of paracetamol in pregnant Sprague–dawley rats. Twenty female adult Sprague–dawley rats weighing between 160g – 180g (as the beginning of the experiment) were used for the study. The animals were randomly divided into two groups (A and B) of ten rats each. Group A animals received distilled water orally and served as control. While paracetamol treated animals (group B) received doses of 7.3mg/kg/day respectively by gavage. The animal weights were monitored at an interval of three days before gestation to 13th day after parturition. The animals were allowed feed and water liberally. Drug administration commenced from 10th day of gestation to the end of parturition. On the 13th day after parturition the maternal rats were then sacrifled for tissue processing. The results showed that the control animals had a normal architecture of the gastrointestinal tract. While the paracetamol treated animals showed a general derangement coupled with high degree inflammation of the stomach and intestinal lining, and a statistical significant weight loss ($P<0.005$) compared to the control animals. These findings reflect gastrointestinal tract impairment. We conclude that a long–term use of non-selective cyclooxygenase inhibitor – paracetamol in pregnant state has an erosive effect on the gastrointestinal tract and may possibly be the aftermath of gastrointestinal tract inflammation in women.

KEY WORDS: Cyclooxygenase inhibitor, paracetamol, gastrointestinal tract, inflammation, Pregnancy.

INTRODUCTION

Several drugs are in use in gynaecology, either in pregnant of non pregnant state, usually out of necessity, despite their reported toxicities and negative side effect (1). The case of thalidomide was a big disaster in the 1960s (2). However, in recent times, drug administration during pregnancy is done with utmost care. The clinical conditions necessitating the use of drugs during pregnancy include hypertension, thrombo-embolism, hyporthyroidism, epilepsy,

diabetes mellitus, preterm labour, arthritis, pain and fever, among others (3).

Minimum amounts of paracetamol for toxicity, to occur in majority of adults of average size (65 – 100kg/ 143 – 220lb) are in excess of 10 – 15g (150mg/kg or 68mg/lb), equivalent to 20 to 30 tablets. However, in lighter individuals toxic levels may be reached with fewer tablets, that is, 15 tablets (7.5G) in a person weighing 50kg (110lb). Alternatively, some individuals may be increase risk because of a higher sensitivity to the toxic effects of paracetamol when taken in over dose (4). The individual risk of toxicity following a paracetamol overdose can be difficult to assess (4). Increase sensitivity to the toxic effects of paracetamol may help to explain why, in rare circumstances, certain individual die after taking less than the estimated minimum threshold toxic dose (4). For these reasons both paracetamol and aspirin pack size were reduced in 1998 by the UK Department of Health in attempt to reduce the risks of accidental over dose associated with "Over–the–Counter" pain killers (5). From September 2002, packs were reduced to 16 tablets or capsules from general sales outlets and to 32 from pharmacies. For amounts of more than 100 tablets, a prescription is required. Pharmacists can, however, dispense up to 100 tablets in certain circumstances. Paracetamol packs also now carry more information on the risks of over dose on their labels (5).

The cyclooxygenase (COX) enzyme was first purified in 1976 and cloned in 1988 (6). In 1991, several laboratories identified a similar form of Cox – enzymes, and named it Cox–2. Thus, two isoforms of Cox occurs; Cox–1 and Cox–2. The former was reported to be the housekeeping enzyme express constitutively in virtually every tissue of the body (7) while Cox–2 is the inducible form. It was reported to be expressed in response to proinflammatory cytokines and growth factors. This implies there a role for cox–2 in both inflammation and control of cell growth (7). However, Cox–2 was also reported to be expressed constitutively in some organs. Vane and Booting (8) reported that constitutive Cox–2 mRNA expression was highest in the kidneys and urinary bladder. They further stated that Cox–2 expression was primarily in the renal outer medullary interstitial cell, and cortical expression in the macular densa (8). Khan *et al* (9) reported the role of macula densa Cox–2 in the regulation of renin in renovascular hypertension. Besides, Vane and Booting (8) were of the opinion that Cox–2 isoform is present constitutively in the brain and spinal cord, where it may be involved in neurotransmission particularly for pain and fever. Using immunohistochemical techniques, Hartner *et al* (10) showed that Cox–2 is the major isoform in the epithelium of the distal vas deferens, where it is also expressed constitutively. They concluded that Cox–2 from the distal vas deferens might play a role in erection, this is due to its ability to increase blood supply to the erectile smooth muscle of the penile organ by so doing causes an excitatory effect on the muscle thereby initiating erection.

Some investigators reported that Cox–1 is expressed at high levels in the collecting ducts and renal vasculature and to lesser extent in the papillary interstitial cells. Cox–2 was observed to be expressed in the macula densa, thick ascending limbs, papillary interstitial cells, podocytes and small blood vessels (11). The predominance of Cox–1 in the gastrointestinal mucosa and its role in mucous secretion in this region, as well as in normal blood flow in the kidneys, have already been reported (10). Prostaglandin production requires the Cox–1 or Cox–2. These enzymes mediate the conversion of arachidonate to prostaglandin (12). Thus, inhibition of the Cox enzyme forms the basis for the actions of the non–steroidal anti inflammatory drugs (NSAIDS), and of their reported side effects on the stomach, (GIT) and the kidneys Vane *et al* (12). Paracetamol is a non–selective cyclooxygenase enzyme inhibitor hence its ability to inhibit both cyclooxygenase–1 and cyclooxygenase–2 isoforms (12).

Dollery and Boobis (13) and Elseviers and De–Broe (14) found that the use of "over-the-counter" analgesics mixtures was a major causative factor of this problem. The causes of "over-the-counter" administration and abuse of drugs in Nigeria include the deteriorating economy, poor financial status of the people and illiteracy, which is the major predisposing factor to unawareness and ignorance of the effects of drug abuse. Consequently, many Nigerians including pregnant women are exposed to the use of the "over-the-counter" mixture of anagesics including non–selective cyclooxygenase inhibitors such as paracetamol.

Many workers had in the past reported the toxic effects of non–selective cyclooxygenase inhibitors–Paracetamol on the changes in haemostatic parameters following vitamin E supplementation during paracetamol administration in rats, Liver and kidney morphologies following vitamin E supplementation during caffeinated and non-caffeinated paracetamol administration in rats, in the non–pregnant state (15). They concluded that one may safely speculate that supplementation with vitamin E may be effective in remitting the deleterious toxic effects of paracetamol as evidenced by changes in the haemostatic parameters and supple-mentation with Vitamin E is also effective in reducing histological changes in the liver and kidney accom-panying paracetamol intoxication (15). Ucheya et al (16) reported the effects of long-term administration of non–selective cyclooxygenase inhibitors ibuprofen and paracetamol on glomerulogenesis in Sprague–dawley rats.

Most of the studies reported above are on the effects of non–selective cyclooxygenase enzyme

inhibitors in the non-pregnant state, while there are no reports of studies on the effects in pregnant state. The present study is designed to determine whether these drugs have demostratable adverse effects on the gastrointestinal tract in pregnant state. This is against the background that they were documented as tolerable (safe) during pregnancy, especially when taken below certain doses (13), secondly, their non-selectivity for cyclooxygenase enzyme inhibition (8) and thirdly, their indiscriminate use (5).

MATERIALS AND METHOD

Twenty Sprague–Dawley rats were procured from the Animal House of the University of Nigeria, Enugu Campus and allowed 2 weeks of acclimatization. They were housed in cages measuring 11 by 7cm and were allowed free access to food and water ad libitum. Individual identification of the animals were done by the number of strokes marked on their tails.

Mating of the Animals

The reproductive status and estrous period of the animals were determined by obtaining their viginal smears. After two complete regular cycles, timed mating of female animals were done on the night of the pro-estrous (N) phase of the cycle. In the morning following mating, vaginal smears were taken again. The presence of spermatozoa and squamus cells in the smear confirmed mating and fertilization of ovulated spermatozoa. The sperm-positive morning was thus designated day 0 of pregnancy.

Experimental Procedure

Twenty Sprague-dawley rats weighing between 160g – 180g were randomly divided into two groups (A and B) of ten rats each. Animals in group A received distilled water orally and served as control. The paracetamol treated animals (group B) received doses of 7.3mg/kg/day respectively by gavage. The animals were allowed feed and water liberally. Each rat was weighed at an interval of three days before the experiment to the 13th day after parturition. The treatment commenced from 10th day of gestation to the 13th day after parturition. The maternal rats in the experimental and control groups were weighed using Avery scale.

The maternal rats were then sacrificed by exposure to chloroform and opened up, in other to obtain their stomach, small and large intestine. They were immediately fixed in 10% Formalin for about 24hrs.

Photomicrographs of the stomach and the intestine in the prepared slides were then examined for histological changes. The mean weight of the animals before gestation was taken and designated as (a). The weight on the 10th day of gestation at the unset of drug administration was taken and the mean weight for each group at the 13th day after parturition was taken and designated as (b), after which the mean weight at 13th day after parturition was subtracted from the mean weight before gestation for each animal a group (a – b). The statistical method employed is the ANOVA. The weight of the paracetamol treated group and the control was then tested for a statistical significant difference by using the student T test to compare two variants (Paracetamol vs. Control).

RESULTS

Examination of the cross-sections of the photomicrographs of gastrointestinal tract of the control rats showed normal architecture of its microscopic appearance: A section of gland in the fundus of the stomach showed parietal cell which predominate in the upper region of the gland, whereas the zymogenic cells predominate in the lower region (Fig. 1a). The small intestine showed the villi (V), the intestinal glands (G), muscularis mucosae (MM), submucosa (SM), and the external and internal muscle layers (EM and IM) (Fig. 1b). A section of the large intestine showed its various layers and the absence of villi (Fig. 1c).

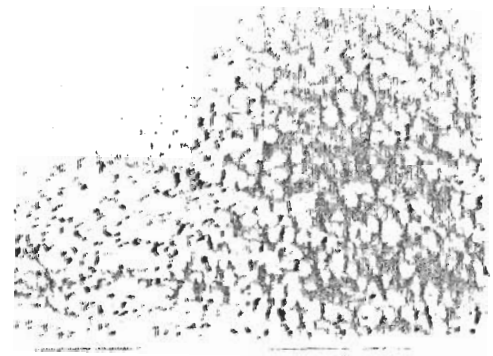

Fig. 1a: Cross-section of the gastrointestinal tract of control rats (group A). Stains: haematoxylin & eosin. Magnification: x 100.

Fig. 1b: Cross-section of the gastrointestinal tract of control rats (group A). Stains: haematoxylin & eosin. Magnification: x 40.

The photomicrographs of the paracetamol treated maternal rats showed derangement of the gastrointestinal tract architecture. Exudates in the lumen, mucosal ulceration and submucosal infiltration of GIT (Fig. 2a), ulceration, exudates in the lumen and a high degree inflammation resulting in haemorrhage of the GIT (Fig. 2b), mucosa and submucosal ulceration (haemorrhage) and submucosal infiltration of the GIT (Fig. 2c), mucosal ulceration (haemorrhage), submucosal infiltration plus smooth musclar atrophy of the GIT (Fig. 2d), smooth muscular atrophy of the GIT (Fig. 2e) and atrophy of the fundic cells (Fig. 2f).

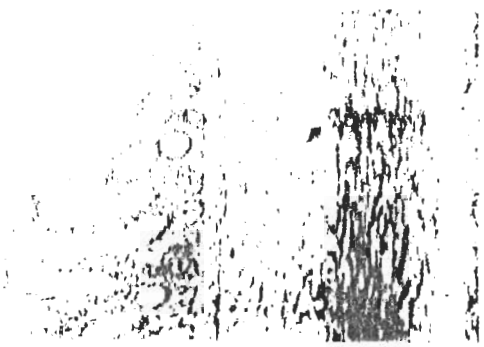

Fig. 1c: Cross-section of the gastrointestinal tract of control rats (group A). Stains: haematoxylin & eosin. Magnification: x 100.

Fig. 2a: Cross-section of the gastrointestinal tract of pregnant Sprague–Dawley rats treated with Paracetamol for 24days (10th day of gestation – 13th day after parturition) and sacrified on the 24th day. Stain: haematoxylin & eosin. Magnification: x 100.

Fig. 2b: Cross-section of the gastrointestinal tract of pregnant Sprague–Dawley rats treated with Paracetamol for 24days (10th day of gestation – 13th day after parturition) and sacrified on the 24th day. Stain: haematoxylin & eosin. Magnification: x 400.

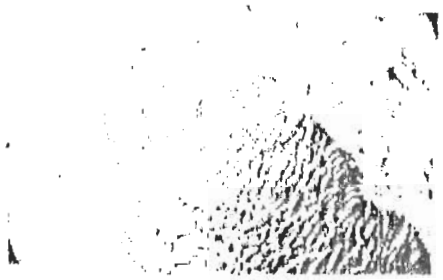

Fig. 2c: Cross-section of the gastrointestinal tract of pregnant Sprague–Dawley rats treated with Paracetamol for 24days (10th day of gestation – 13th day after parturition) and sacrified on the 24th day. Stain: haematoxylin & eosin. Magnification: x 40.

Fig. 2d: Cross-section of the gastrointestinal tract of pregnant Sprague–Dawley rats treated with Paracetamol for 24days (10th day of gestation – 13th day after parturition) and sacrified on the 24th day. Stain: haematoxylin & eosin. Magnification: x 100.

Fig. 2e: Cross-section of the gastrointestinal tract of pregnant Sprague–Dawley rats treated with Paracetamol for 24days (10th day of gestation – 13th day after parturition) and sacrified on the 24th day. Stain: haematoxylin & eosin. Magnification: x 100.

Fig. 2f: Cross-section of the gastrointestinal tract of pregnant Sprague–Dawley rats treated with Paracetamol for 24days (10th day of gestation – 13th day after parturition) and sacrified on the 24th day. Stain: haematoxylin & eosin. Magnification: x 40.

Table 1: Showing Effects of Long Term Administration of Paracetamol During Pregnancy on the Maternal Body Weight of Sprague–Dawley Rats.

Animal Group	Drugs administered	Mean weight B/F Pregnancy (a)	Mean Weight at 10th day of Pregnancy	Mean Weight 13th day after Parturition (b)	Mean Weight Difference (a–b)	Mean Weight Loss (%)
A	Control	172 ± 5.4	180 ± 3.1	173 ± 2.2	1 ± 3.2	0.58%
B	Paracetamol	169 ± 3.1	178 ± 5.1	140 ± 4.3	*29 ± 0.8	*17.15%

*Significantly different from value of control (*P<0.005; all weights in grams) Mean ± S. D.

The paracetamol treated maternal rats showed a mean weight loss of 29 ± 0.8g (17.15%) that was statistically significant (P<0.005) compared to the control rats (Table 1).

DISCUSSION

Our results showed that there was a general destruction coupled with a high degree inflammation (ulceration) of the gastrointestinal tract, which might be an indication of the haemorrhage, observed in the gastrointestinal lining of the treated animals. This however is a direct confirmation of the protective role of prostaglandins in the GIT which probably was inhibited because of the long term administration of cyclooxgyenase inhibitors, which on the other hand is mostly due to its indiscriminate use in pregnant state, as a result of over- the–counter sales of analgesics (14 & 15). This results further confirms the predominance of Cyclooxygenase–1 isoforms in the gastrointestinal mucosa and its protective role in mucous secretion in this region (6). Prostaglandin production requires the cyclooxygenase–1 or Cyclooxygenase–2. These enzymes mediate the conversion of arachidonate to prostaglandin (8). Thus, inhibition of the Cyclooxygenase enzyme forms the basis for the actions of the non–steroidal anti–inflammatory drugs (NSAIDS), and of their reported side effects on the GIT (6).

The drastic reduction in the mean weight of the maternal rats (P<0.005) probably must have been due to a compromised nutritional status of the maternal rats consequent on the gastrointestinal derangement observed.

CONCLUSION

A long-term administration of non selective cyclooxygenase inhibitor – paracetamol has an inflammatory/erosive effects on the gastrointestinal tract of Sprague dawley rats in pregnancy. Health talk on the indiscriminate use of non–selective cyclooxygenase inhibitor – paracetamol should be delivered during antenatal clinics.

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