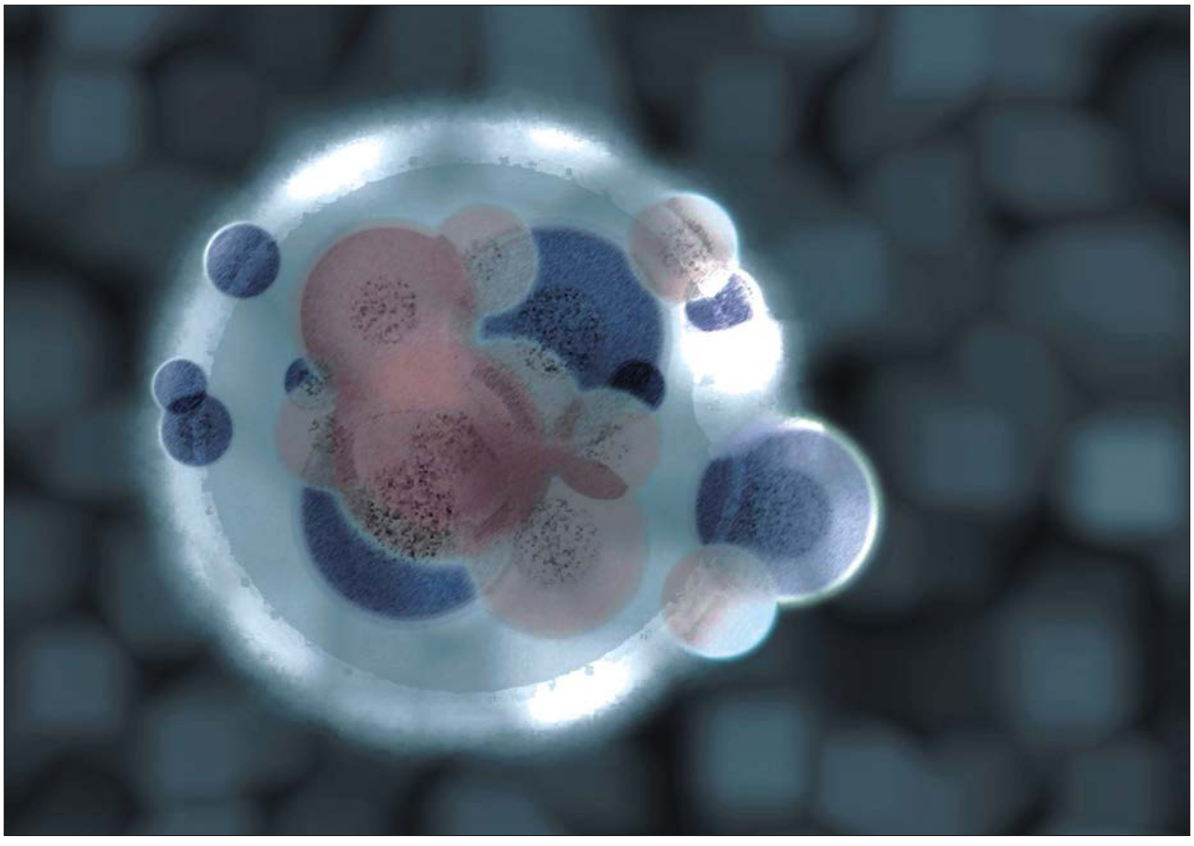

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Effects of Oral Administration of Aspirin and Paracetamol on Plasma and Brain Protein, Tryptophan Levels and Monoamine Oxidase Activity in Rats

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Abstract: The effects of oral administration of aspirin and paracetamol on plasma and brain protein, tryptophan concentrations and monoamine oxidase activity were investigated. Fifty-four virgin albino Sprague-Dawley rats were used for the study and divided into three groups. The first group of rats was fed rat chow with water *ad libitum* and oral administration of 0.05% (w/v) aspirin and the second group of rats was fed rat chow with water, *ad libitum* and oral administration of 0.05% (w/v) paracetamol for 35 days. The third group of rats serves as the control and fed with rat chow and water *ad libitum* for 35 days. The rats were sacrificed by decapitation after starving them overnight and their blood and brain were collected quickly. Plasma and brain protein and tryptophan concentrations and brain monoamine oxidase activity were assayed. Results showed that oral administration of aspirin and paracetamol do not significantly ($p<0.01$) affect body weight, feed and water intake of rats. But these drugs significantly elevated plasma protein, tryptophan and decreased brain protein and tryptophan concentrations. Oral administration of aspirin significantly inhibited brain monoamine oxidase activity, but was not affected by paracetamol. Data of the present study indicate that aspirin, but not paracetamol could alter the metabolism and neurotransmission mediated by certain biogenic amines catalyzed by monoamine oxidase in the brain.

Key words: Aspirin, paracetamol, brain, protein, tryptophan, monoamine oxidase activity, rats

INTRODUCTION

Aspirin, paracetamol, ibuprofen and naproxen are examples of non-narcotic analgesics (Chan *et al*., 2005; McQuaid and Laine, 2006; Goodman-Gillman *et al*., 1990; Breda *et al*., 2002). Aspirin (acetylsalicylic acid) has analgesic, anti-inflammatory and antipyretic actions. It is used in headache, transient musculoskeletal pain and dysmenorrheal and it is preferably given after meals because of gastric irritation effects (Temple, 1981a; Meredith *et al*., 1978). Aspirin inhibits an enzyme called cyclooxygenase, this is involved with the production of prostaglandins and thromoxanes, which are hormones with a wide range of functions in the body, including pain transmission to the brain. Therefore with cyclooxygenase blocked, there are less prostaglandins and thromoxanes and so less pain (Goodman-Gillman *et al*., 1990; Breda *et al*., 2002).

Paracetamol (N-acetyl-p-aminophenol) is a para-aminophenol derivative and the major metabolite of phenacetin. It has a weak inhibitory effect on peripheral biosynthesis, but it is a potent inhibitor of prostanglandin production within the Central Nervous System (CNS). This presumably accounts for its analgesic and antipyretic properties (Breda *et al*., 2002; Goodman-Gillman *et al*., 1990). Paracetamol reduces activity of cyclooxygenase, reducing production of prostaglandins, similar to aspirin. But what makes paracetamol different, is that the inhibition is ineffective when in the presence of peroxides. So the effects of paracetamol are restricted to the central nervous system and so it does not have the blood thinning or anti-inflammatory properties of aspirin (Goodman-Gillman *et al*., 1990; Dargan *et al*., 2002).

Previous studies have demonstrated that some non-steroidal anti-inflammatory drugs (NSAIDS), specifically aspirin, have acute negative effects on sleep in human and animals (Murphy *et al*., 1994; Chan *et al*., 2005; Vale and Kulig, 2004) and platelet aggregation (Seymour *et al*., 1984). In equivalent dosage, paracetamol is as effective as aspirin for most painful conditions, but is generally believed to be less effective when pain is associated with acute inflammation (Kwon *et al*., 1997; Macdonald, 2002).

Serotonin (5-hydroxyl tryptamine, 5HT) is a neurotransmitter and has been implicated in the control of sleep (Goodman-Gillman *et al*., 1990). Tryptophan is an essential amino acid and one of the precursors of proteins and serotonin (Champe and Harvey, 1994; Ebuehi and

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Akinwande, 1994). Monoamine neurotransmitters, such as serotonin and catecholamines, which can be inactivated by monoamine oxidase (MAO) to produce their corresponding aldehydes (Costa and Sandler, 1972).

There is paucity of information from the wealth of literature on the effects of analgesics on the plasma and brain proteins and neurotransmitters metabolism (Costa and Sandler, 1972; Seymour *et al*., 1984; Murphy *et al*., 1994; Chan *et al*., 2005; McQuid and Laine, 2006). It is not known whether brain protein, tryptophan and MAO activity are involved in the relief of mild to moderate pain of disease aetiology. Since aspirin and paracetamol are widely used as general purpose mild analgesics, it is pertinent to decipher whether these drugs affect plasma and brain proteins, tryptophan levels and MAO activity. The present study will provide this information.

Human brain tissue is hardly available for direct biochemical analysis, therefore an indirect approach using animal model, such as rats, was used for the study and data obtained could be extrapolated to humans. The specific objectives of the study are to ascertain the effects of oral administration of aspirin and paracetamol on plasma and brain protein, tryptophan levels and brain monoamine oxidase activity in rats.

MATERIALS AND METHODS

Drugs and source: Emzor Aspirin tablets (a brand of aspirin) and Paracetamol manufactured by EMZOR Pharmaceutical Industries, Isolo, Lagos, Nigeria, were purchased from University of Lagos Community Pharmacy. EMZOR Pharmaceutical Industry is one of the reputable and government approved pharmacy in Nigeria. Each tablet contains 300 mg acetylsalicylic acid BP and recently manufactured as indicated on the date and batch number. 0.05% (w/v) paracetamol solution was prepared by dissolving 0.05 g of paracetamol tablet in 100 mL of deionized water. 0.05% (w/v) aspirin solution was prepared by dissolving 0.05 g of aspirin tablet in 100 mL of deionized water.

Drug administration and animal feeding: Fifty-four virgin Sprague-Dawley male rats weighing 138.0±5.42 g were collected from the Animal Centre of the College of Medicine of the University of Lagos, Lagos. They were put in groups of three rats per cage, kept in a room with a temperature of 28±2°C and illuminated for 12 h per day (07:00-19:00 h). The rats were fed commercial chow containing 21% protein with water *ad libitum* and acclimatized 2 weeks before use. Animals were cared for in accordance with the principles of the Guide to the care and use of Experimental Animals. After the acclimatization, the rats were divided into three groups containing 18 rats each. The first group of rats was fed rat chow with water and oral administration of 1.0 mL of 0.05% (w/v) aspirin/100 g body weight/day for 35 days. The second group of rats was fed rat chow with water and oral administration of 1 mL of 0.05% (w/v) paracetamol /100 g bodyweight/day for 35 days, while the third group was fed rat chow with water *ad libitum* for 35 days, which represents the control. The oral administration of aspirin or paracetamol solution was carried out between 08:30 and 09:30 h daily, using sterile syringes with minimal and gentle handling of the rats. The body weight, feed and water intake of the rats were computed daily during duration of drug administration.

Determination of plasma, brain tryptophan and protein concentrations: After the 35 days of drug administration, all the rats were starved overnight and sacrificed the following day by decapitation. The brains were quickly excised and weighed. Plasma and brain tryptophan in rats fed chow with water and orally administered 0.05% (w/v). aspirin or paracetamol or only rat chow with water *ad libitum* for 35 days, were determined by method of Udenfriend *et al.* (1962) as modified by Ebuehi and Akinwande (1994). The protein was determined according to the method of Lowry *et al.* (1951).

Preparation of enzyme system: The method of Catravas *et al.* (1977) was adopted to prepare the enzyme systems of monoamine oxidase (MAO) in the rat brain. Two gram brain tissue was thawed and homogenized in 10 volumes of 0.25 M sucrose containing 1 mM $MgCl_2$ using Teflon glass homogenizer placed in ice bath. The brain homogenate was centrifuged at 10,000 g for 30 min. The supernatant fluid was carefully decanted and the mitochondrial pellet was suspended in an equal volume of homogenization medium and was used for the enzyme assay. No MAO activity was observed in the 10,000 g supernatant.

Determination of monoamine oxidase activity: Monoamine oxidase activity was assayed by a modification of the method of Weissbach *et al.* (1960). The assay mixture contained 0.5 mL of 0.05 M Tris-HCI buffer, pH 7.4, 1 mg of serotonin (5-hydroxy tryptamine creatinine sulphate) and 0.2 mL with deionized water. The incubation was carried out at 37°C for 30 min in a water bath. The assay of monoamine oxidase activity was by observing the rate of serotonin disappearance. It utilizes a direct spectrophotometric method read against a blank cuvette prepared by replacing serotonin with water.

Increase in absorbance was measured at 330 nm using DU-7400 Beckman spectrophotometer and expressed as the change in the absorbance at 330 nm per unit time.

Statistical analysis: Data were subjected to analysis of variance and the significant differences were further tested by the Duncans test (Snedecor and Cochran, 1969).

RESULTS

The mean body weight gain of rats orally administered aspirin or paracetamol (0.05% w/v) were 1.09 or 1.14 g day^{-1}, respectively, while the control rats was 1.26 g day^{-1} for 35 days. The mean feed intake of rats orally administered aspirin or paracetamol were 17.2 or 18.5 g day^{-1}, respectively, while the control rats was 20.4 g day^{-1}. The mean water intake of rats orally administered aspirin or paracetamol were 10.95 or 11.63 mL day^{-1}, while the control rats was 12.47 mL day^{-1}.

There was a progressive increase in the body weight, feed and water intake of rats administered aspirin or paracetamol or chow and water *ad libitum* for 35 days. However, there were no significant ($p<0.01$) differences in body weight, feed and water intake of rats administered drugs or water for 35 days.

The concentrations of plasma tryptophan and protein of rats fed rat chow and water, with oral administration of aspirin or paracetamol were significantly ($p<0.01$) higher than in rats fed chow and water (Table 1). There was no significant difference in the plasma tryptophan and protein concentrations in rats orally administered aspirin or paracetamol for 35 days. The concentrations of brain tryptophan and protein of rats fed rat chow and water, with oral administration of aspirin or paracetamol were significantly ($p<0.01$) higher than in the control rats (Table 2). There was no significant difference in the brain tryptophan and protein concentrations in rats orally administered aspirin or paracetamol for 35 days.

The absorbance values of brain assay mixture of rats fed chow with water and oral administration of aspirin or paracetamol were significantly ($p<0.01$) lower in the absorbance at 330 nm than in control rats (Table 3). The specific activities of brain MAO in rats fed rat chow and water, or orally administered paracetamol were not significantly ($p<0.01$) different, but both groups were lower than in rats administered aspirin (Table 4).

Table 1: Concentrations of plasma tryptophan and protein of rats fed rat chow and water, with orally administered aspirin or paracetamol or fed chow with water for 35 days[1, 2]

Food with drug administered	Plasma tryptophan ($\mu g\ mL^{-1}$)	Plasma protein (mg/100 mL)
Rat chow with water and oral administration of 0.05% (w/v) aspirin	0.704±0.004[a]	0.610±0.09
Rat chow with water and oral administration of 0.05% (w/v) paracetamol	0.679±0.005[a]	0.592±0.078[a]
Rat chow with water	0.594±0.007[b]	0.503±0.064[b]

[1]Values are expressed as mean±SE, [2]Values carrying different superscripts vertically are significantly different ($p<0.01$)

Table 2: Concentrations of brain tryptophan and protein of rats fed rat chow with water and orally administered aspirin or paracetamol or fed chow with water for 35 days[1, 2]

Food with drug administered	Brain tryptophan ($\mu g\ g^{-1}$)	Brain protein ($\mu g\ g^{-1}$)
Rat chow with water and oral administration of 0.05% (w/v) aspirin	0.412±0.0055[a]	0.326±0.075[c]
Rat chow with water and oral administration of 0.05% (w/v) paracetamol	0.454±0.0073[a]	0.341±0.062[c]
Rat chow with water	0.719±0.0086[b]	0.635±0.081[d]

[1]Values are expressed as mean±SE. [2]Values carrying different superscripts vertically are significantly different ($p<0.01$)

Table 3: Absorbance measurement at 330 nm of the brain assay mixture of rats administered 0.05% (w/v) aspirin or 0.05% (w/v) paracetamol, with rat chow and water for 35 days[1]

Time of incubation (min)	Rat chow and water with 0.05% (w/v) aspirin	Rat chow and water with 0.05% (w/v) paracetamol	Rat chow with water
0	0.0470±0.0006	0.0510±0.0003	0.0760±0.0075
10	0.1560±0.0005	0.1685±0.0004	0.1890±0.0051
20	0.2610±0.0007	0.2704±0.0005	0.2970±0.0040
30	0.3082±0.0008	0.3156±0.0009	0.3396±0.0030

[1]Values expressed as mean±SE

Table 4: Brain monoamine oxidase specific activities of rats administered 0.05% (w/v) aspirin or 0.05% (w/v) paracetamol, with rat chow and water for 35 days[1, 2]

Time of incubation (min)	Rat chow and water with 0.05% (w/v) aspirin	Rat chow and water with 0.05% (w/v) paracetamol	Rat chow with water
10	0.4296±0.0005[a]	0.4631±0.0008[b]	0.4450±0.0006[b]
20	0.3638±0.0009[b]	0.4020±0.0006[d]	0.4256±0.0008[d]
30	0.0266±0.0007[c]	0.1782±0.0005[f]	0.1679±0.0007[f]

[1]Values are expressed as mean±SE and expressed as micromoles/60 min mg protein, [2]Values carrying different superscripts horizontally are significantly different ($p<0.01$)

DISCUSSION

Data of the present study indicate that body weight, feed intake and water intake of rats were not significantly affected by oral administration of aspirin or paracetamol for 35 days. One could suggest that oral administration of aspirin or paracetamol do not affect appetite for food or water and body growth in rats. There is paucity of information on the effect of analgesics on body growth and protein and tryptophan metabolism. Ebuehi *et al.* (1999) had previously reported a reduction in body weight, feed and water intake of rats administered nicotine or tobacco for 30 days. The present data, therefore, indicate that oral administration of aspirin or paracetamol do not impair body growth of rats.

There was a gradual behavioral change in rats administered aspirin or paracetamol, as evident in the shift from calmness at commencement of the drug administration to being quite vicious and difficult to handle. It could be suggested that this behavioral change may be due to drug tolerance, culminating from oral administration of aspirin or paracetamol, which are both analgesics. Rats orally administered aspirin were sluggish and often slept after 5 min of drug administration, while those rats administered paracetamol slept after 30 min. This suggested that aspirin is a better sedative than paracetamol in rats.

The plasma tryptophan and protein concentrations were elevated by oral administration of aspirin and paracetamol in rats. This present finding is not in agreement with the previous reports of Kwon *et al.* (1997). The brain tryptophan and protein concentrations of aspirin and paracetamol orally administered rats were impaired. It could be opined that oral administration of these analgesics may inhibit protein synthesis or may prevent the uptake of amino acids into the tissue through the blood brain barrier for synthesis of proteins in the brain (Pardridge, 1988; Kwon *et al*., 1997).

Data of the present study showed that oral administration of aspirin showed a marked significant inhibition of brain monoamine oxidase activity as compared to in the paracetamol administered rats. It could be suggested that oral administration of aspirin may enhance catabolism or inactivation of biogenic amines, such as serotonin. However, very scanty information exist on the effect of analgesics on the brain monoamine oxidase activity, plasma and tissue tryptophan levels. The rapidity of uptake of aspirin following administration of a soluble formulation suggests significant absorption from the stomach (Macdonald, 2002; Prescott, 1983). There was no significant difference in the pharmacokinetic parameters of paracetamol derived from a soluble or plain formulation (Prescott, 1983; Breda *et al*., 2002). A comparison of the uptake of aspirin from the soluble aspirin formulation with paracetamol from either plain or soluble tablets showed that aspirin entered the plasma and achieved peak levels significantly more quickly, since, the half-life of paracetamol was significantly longer than that of aspirin (Kwon *et al*., 1997; Macdonald, 2002).

These findings suggest that onset of analgesia should be more rapid, following dosing with soluble aspirin, a conclusion supported by comparative efficacy, from studies conducted with differing formulations of aspirin (Vale and Kulig, 2004; Temple, 1981b). There is scanty information on the binding of paracetamol to plasma protein (Miligan *et al*., 1994). Aspirin is usually administered orally in adults and children and rapidly absorbed from the gastro intestinal tract. Aspirin is 99% metabolized to salicylate and other metabolites. The elimination half-life of aspirin in plasma is about 15-20 min (Macdonald, 2002; Dargan *et al*., 2002).

Data of the present study indicate that oral administration of aspirin or paracetamol by rats do not significantly affect body growth. Plasma and brain tryptophan and protein levels in rats were altered by oral administration of these analgesics. In addition, oral administration of aspirin inhibited brain monoamine oxidase activity. Therefore, it could be inferred that neural functions mediated by monoaminergic neurotransmission could be curtailed by oral administration of aspirin and not by paracetamol.

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