

Sperm head abnormality and mutagenic effects of aspirin, paracetamol and caffeine containing analgesics in rats

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Citation

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Abstract

The mutagenic effects of analgesics with aspirin, paracetamol and caffeine combinations were evaluated in a male rat model using a short-term in vivo mutagenicity test (sperm head abnormality assay). A 90-day exposure to recommended doses of the five analgesics, Aspirin (aspirin only), Cafenol (aspirin + caffeine), Panadol (paracetamol only), Pentax (paracetamol + caffeine) and Daga[†]

(aspirin + paracetamol + caffeine), significantly ($P < 0.001$) increased sperm head abnormality compared with controls; this exposure time also increased mutagenicity (or mutation indices) by at least 1.67-fold. The presence of caffeine in both Cafenol and Pentax analgesics reduced mutagenicity, while the presence of caffeine in Daga[†]

had a synergistic effect that significantly increased both the frequency of abnormal sperm heads to 13.5% and the mutation index to 8.00. Sperm head abnormality was also proportional to mutation index

INTRODUCTION

Analgesics with aspirin, paracetamol and caffeine combinations are ingested for many reasons including: general pains, headaches, fevers, cold, flu, rheumatoid arthritis, circulation problems (¹) and in cases of dependent-addiction (²).

In recent years, there has been an increasing awareness and realization of the genotoxic potentials of a wide variety of drugs, food additives, environmental pollutants (³, ⁴) and other socially acceptable compounds such as aspirin and caffeine (⁵). Habitual ingestion of aspirin and caffeine has been shown to be capable of inducing structural chromosomal aberrations in somatic cells in vivo (⁶) and reproductive toxicology (⁵). This awareness follows the recent development of appropriate, sensitive and practical methods for detecting and estimating the toxic effects of these substances and their impact on environmental health.

Aspirin is one of the most widely used analgesics available without prescription in several parts of the world (⁷). Mutagenic studies of aspirin in mammals are not common and are mostly limited to in vitro experiments. Kimmel et al. (⁸), reported the teratogenic effects of aspirin in Wister rats resulting in malformations of limbs, while Jones and Bodmer (³) suggested similar effects in humans at

recommended doses. It has been identified as a behavioural teratogen in humans at doses too low to produce differential birth-weight effects (⁶); and it has also been reported that maternal aspirin use during the first-half of the pregnancy has a high significant effect ($P < 0.0005$) on intelligence quotient (IQ) and attention in exposed children (⁹).

There are conflicting reports on potentials of paracetamol as an experimental carcinogenic agent, although evidences implicate metabolites of paracetamol and phenacetin as being active experimental carcinogenic agents (², ^{10,11}). Paracetamol has also been reported to inhibit DNA synthesis by 70-90% at 1hour following an oral dose in the spleen, testis, thymus, stomach, small intestine and bone marrow (¹²).

Caffeine is often used with aspirin and paracetamol to augment their antipyretic and analgesic effects (¹³). There is a strong relationship between the ingestion of various combinations of analgesics with caffeine resulting in approximately 2-fold increase in the risk of cancers of the pelvic, kidney, bladder and urinary tract (², ¹¹).

In view of such findings, this study set out to further explore the effect of caffeine on the mutagenicity of analgesics with aspirin, paracetamol and caffeine combinations in rats as model using short-term in vivo mutagenicity assay to assess

their effects on sperm head abnormality and mutation index.

MATERIALS AND METHODS

Forty-eight healthy isogenic strains of male albino rats (*Rattus norvegicus*) of about ten to eleven weeks old were obtained from the rat colony of the Department of Zoology and Environmental Biology, University of Calabar, Calabar-Nigeria for the study. The rats were housed in conventional cages and maintained under standard laboratory conditions in the animal house annex of the Department of Zoology and Environmental Biology, University of Calabar, Calabar with free access to water and commercial feed. The rats were divided into six groups of eight male rats for each using complete randomized design.

The following analgesics were obtained from reputable pharmacies: Aspirin (Aspirin 300mg), Cafenol (Aspirin 375mg and Caffeine 25mg), Panadol (Paracetamol 500 mg), Pentax (Paracetamol 500mg and Caffeine 16mg) and Daga⁺ (Aspirin 225mg, Paracetamol 250mg and Caffeine 30mg). The daily doses (D) were calculated using the formula: $D = \frac{MRT}{R}$ (where, M = Maximum recommended daily human dosage in tablets, R = Ratio of weight of rat to average adult human weight of 60 kg, T = Weight of each tablet), according to Ekaluo et al (5). Required daily doses were dissolved in about 60% average daily water intake determined during period of acclimatization, this was to ensure that the daily doses were consumed; before adding more water. The treatment lasted for 90 days.

The rats were sacrificed after 90 days of treatment, and the sperm suspension was obtained by mincing the epididymes with fine scissors into 1mg aliquots in physiological saline. The sperm suspensions were mixed with 1% eosin Y solution (10:1) for 30 minutes and air-dried smears were prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage abnormalities in every 200 spermatozoa observed on each slide and five air-dried smears were prepared on glass slides for each sample. Frequency of abnormal sperm heads was calculated according to Ekaluo (14). Mutation factor and mutation indices were also calculated as shown below. Differences between the means of the control and experimental groups were compared using the analysis of variance (ANOVA) test.

Figure 1

$$\text{Mutation Factor (MF)} = \frac{\text{Frequency of abnormal sperm heads (treated)}}{\text{Frequency of abnormal sperm heads (control)}}$$

$$\text{Mutation Index (MI)} = \frac{\text{Frequency of abnormal sperm heads (treated - control)}}{\text{Frequency of abnormal sperm heads (control)}}$$

RESULTS

General observations showed that all the rats in the study looked healthy and there was a general increase in body weights of all rats in both treatment and control groups during the treatment period. The increases in body weights of the rats indicated that the five analgesics had no adverse effect on growth and body weight of the rats.

A 90-day exposure to recommended doses of the five analgesics, Aspirin (aspirin only), Cafenol (aspirin + caffeine), Panadol (paracetamol only), Pentax (paracetamol + caffeine) and Daga⁺ (aspirin + paracetamol + caffeine), significantly ($P < 0.001$) increased sperm head abnormality at least by 2.5% when compared with control; this exposure also increased mutagenicity (or mutation indices) by at least 1.67-fold as shown on Table 1.

Figure 2

Table 1: Effect of caffeine on the frequency of abnormal sperm heads and mutagenicity of aspirin, paracetamol and caffeine combinations

Analgesic (Combination)	Frequency of abnormal sperm heads (%)	Increase in frequency of abnormality (%)	Mutation Factor	Mutation Index
Control	1.50	0.00	1.00	0.00
Aspirin (A only)	8.00*	6.50	5.33	4.33
Cafenol (A + C)	7.50*	6.00	5.00	4.00
Panadol (P only)	10.50*	9.00	7.00	6.00
Pentax (P + C)	4.00*	2.50	2.67	1.67
Daga ⁺ (A + P + C)	13.50*	12.00	9.00	8.00

A= Aspirin, C= Caffeine and P= Paracetamol

* Significantly increased above control, $P < 0.001$, ANOVA test.

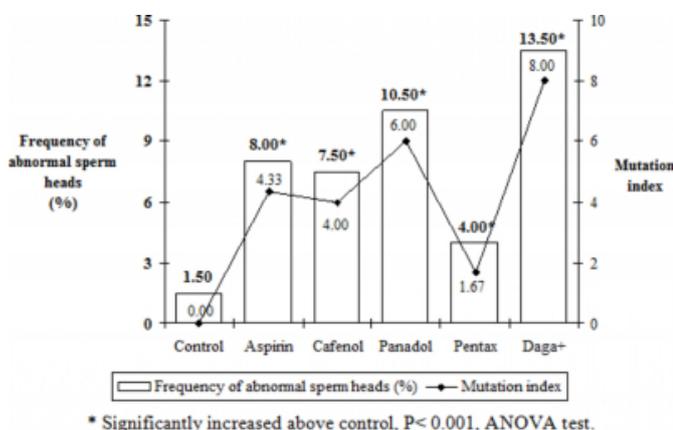
The presence of caffeine in Cafenol (aspirin + caffeine combination) reduced the sperm head abnormality effect of Aspirin (aspirin only) from 8.0% to 7.5%, and the mutation index from 4.33 to 4.00. The presence of caffeine in Pentax (paracetamol + caffeine combination) also reduced the sperm head abnormality effect of Panadol (paracetamol only) from 10.5% to 4.0%, and the mutation index from 6.00 to 1.67. However, the presence of caffeine in Daga⁺ (aspirin

+ paracetamol + caffeine combination) has a synergistic effect which significantly increased the frequency of abnormal sperm heads to 13.5% and the mutation index to 8.00; which are higher than what were recorded for other combinations (Aspirin, Cafenol, Panadol and Pentax).

Figure 1 shows the relationship between frequency of abnormal sperm heads and mutation index. The frequency of abnormal sperm heads recorded for rats treated with five analgesics with aspirin, paracetamol and caffeine combinations (Aspirin, Cafenol, Panadol, Pentax and Daga⁺) were proportional to the mutation indices.

Figure 3

Figure 1: Relationship between frequency of abnormal sperm heads and mutation index



DISCUSSION AND CONCLUSION

In the study, a significantly increased frequency of sperm head abnormalities was observed in the spermatozoa of treated rats. Increases in the incidence of abnormal sperm have been reported after treatment of male albino rats with formaldehyde (¹⁵) and analgesics (⁵). It has been reported that high temperatures, extreme nutritional deficiencies and some diseases can cause sperm abnormalities in a wide range of species including mice and man (¹⁶). Male Wister rats fed on a diet containing 36% of total calories as ethanol for 41 days caused similar sperm abnormalities as those reported in other mammalian species, and the mean frequency in control groups was 1.2%, with the range of 1.0-1.6% (¹⁷). The frequency of the control group here is also within this range. Various anti-inflammatory analgesics and certain steroids inhibit the production of prostaglandins (¹⁸), and inhibition of prostaglandins is known to interfere with spermatogenesis.

Although, the precise mechanism by which aspirin, paracetamol and caffeine containing analgesics cause sperm

head abnormalities has not been fully established, generally, damage to the sperm cell by substances may occur by one of three mechanisms: physiological, cytotoxic and genetic. The morphological abnormalities might have been caused by alterations (deletions, point mutation or a combination of both) in testicular DNA that in turn disrupts the process of differentiation of spermatozoa (¹², ¹⁹), exposure to chemicals that could produce pituitary-hypothalamic or sex hormonal effects which in turn could affect spermatogenesis (¹⁸, ²⁰, ²¹) and exposure of the seminal fluid to chemicals, resulting in functional or structural impairment of sperm cells (²⁰, ²¹).

The tested analgesics showed significant effects in an increasing order of Pentax, Cafenol, Aspirin, Panadol and Daga⁺. This is due to the active ingredient(s) found in the analgesics. The presence of Caffeine reduced the sperm head abnormality and mutagenicity of both aspirin and paracetamol in aspirin + caffeine combination (Cafenol) and paracetamol + caffeine combination (Pentax) respectively, while the presence of caffeine in aspirin + paracetamol + caffeine combination (Daga⁺) has a synergistic effect which significantly increased the frequency of abnormal sperm heads to 13.5% and the mutation index to 8.00. Sperm head abnormality was also proportional to mutation index and this conforms to the findings of Ross et al. (²), Jones and Bodmer (³) and Patierno et al. (¹¹).

The work has provided some data and information that may be useful for public health. There is the need for regular public health checks on the consumption of some socially acceptable compounds such as analgesics with aspirin, paracetamol and caffeine combinations, since the presence of caffeine in Daga⁺ had a synergistic effect that significantly increased both the frequency of abnormal sperm heads and the mutation index. The observed effects have been shown to be acute, reversible and did not persist after 60 days from the end of the treatment (⁵).

References

1. Faulkner, G., Prichard, P., Somerville, K. and Langman, M. J. S; 1998. Aspirin and bleeding peptic ulcers in elderly. *British Medical Journal* 297:1311 - 1313.
2. Ross, R. K., Pagainini-Hill, A., Landolph, R. J., Gerkins, V. and Henderson, B.E; 1989. Analgesics, cigarette smoking and other risk factors for cancer of the renal pelvis and ureter. *Cancer Research* 49: 1045 -1048.
3. Jones, A. and Bodmer, W. F; 1974. *Our future inheritance: Choice or Chance?* Oxford University Press, London.
4. Taylor, R. J;1980. *Food additives*. John Wiley & Sons, New York.
5. Ekaluo, U.B.; Udokpoh, A.E.; Udofia, U.U. and Ajang,

- R.O. (2005). Comparative toxicity of five commonly used analgesics on sperm count and sperm head abnormalities. *Global Journal of Pure and Applied Sciences* 11: 81-84.
6. Neel, J. V. and Bloom, A. D; 1973. The Detection of Environmental Mutagens. In: A. S. Baer (Ed.). *Heredity and Genetics: Reading in Social Genetics*. Macmillan, New York. pp. 44-58.
7. Giri, A. K; 1993. The genetic toxicology of paracetamol and aspirin (review). *Mutation Research* 296:199-210.
8. Kimmel, C. A., Wilson, J. G. and Schumacher, H. J; 1971. Studies on metabolism and identification of the causative agent in aspirin teratogenesis in rats. *Teratology* 4: 15-24.
9. Streissguth, A. P., Treder, R. P., Barr, H. M., Shepaid, T. H., Bleyer, W. A., Sampson, P. D. and Martin, D. C; 1987. Aspirin and acetaminophen use by pregnant women and subsequent child IQ and attention decrements. *Teratology* 35: 211-219.
10. Holme, J.A., Honslo, J. K., Bjørnstad, C., Harvison, J. P. and Nelson, S. D; 1988. Toxic effects of paracetamol and related structures in V79 Chinese hamster cell. *Mutagenesis* 3: 51-56.
11. Patierno, S. R., Lehman, N. L., Handerson, B. E. and Landolph, J. R; 1989. Study of the ability of phenacetin, acetaminophen and aspirin to reduce cytotoxicity, mutation and morphological transformation in C3H/10T $\frac{1}{2}$ Clone 8 mouse embryo cells. *Cancer Research* 49: 1033-1044.
12. Lister, C.F. and McLean, A.E; 1997. Inhibition of DNA synthesis by paracetamol in different tissues of the rat in vivo. *Toxicology* 116: 49-57.
13. Meyers, F. H., Jawetz, E., Goldfien, A; 1979. Review of medical pharmacology (6th edition). Lange medical publications. Taiwan.
14. Ekaluo, U.B. (2003). Mutagenic effects of aspirin and caffeine containing analgesics on Sperm abnormalities. *Journal of Nigerian Environmental Society* 1:156-162.
15. Odeigah, P.G.C; 1997. Sperm head abnormalities and dominant lethal effects of formaldehyde in albino rats. *Mutation Research* 389: 141-148.
16. Obe, G. and Ristow, H; 1979. Mutagenic, carcinogenic and teratogenic effects of alcohol. *Mutation Research* 65: 229-259.
17. Van Thiel, D. H., Gavaler, J. S., Lester, R. and Goodman, M. D; 1975. Alcohol-induced testicular atrophy. An experimental model for hypogonadism occurring in chronic alcoholic men. *Gastroenterology* 69: 326-332.
18. Hole, J.W; 1993. *Human anatomy and physiology* (6th edition). Wm. C. Brown publishers, Iowa.
19. Bruce. W. R. and Heddle, J. A; 1979. The mutagenic activity of 61 agents as determined by the micronucleus, Salmonella and sperm abnormality assays. *Canadian Journal of Genetics and Cytology* 21: 319-334.
20. Wyrobek, A. J. and Bruce, W. R; 1978. The Induction of sperm-shape abnormalities in mice and humans, In: A. Holander and F. J. de Series (Eds.). *Chemical Mutagens*. (Vol. 5). Plenum Press, New York, pp. 257-285.
21. Letz, G; 1990. Male reproductive toxicology, In: J. Ladou (Ed.). *Occupational Medicine*. Appleton and Lange. Connecticut, pp. 288-296.

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