Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Colorimetric Detection and Measurement of Paracetamol Exposure in Patients Prior Dispensing at a Pharmaceutical Care Centre

AWOFISAYO SO[1] AND UWANTA EJ[2]

[1]Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Uyo, NIGERIA.

[2]Department of Science Laboratory Technology, Akwa Ibom State College of Agriculture, Obio Akpa, NIGERIA.

ARTICLE DETAILS

Article history:
Received on 04 June 2011
Modified on 29 June 2011
Accepted on 20 September 2011

Keywords:
Colorimetric approach,
Paracetamol,
Detection,
Plasma levels,
Pharmaceutical care

ABSTRACT

The aim of the work was to detect and quantitatively assess the level of paracetamol in plasma samples of resident of the study area. Demographics of users and factors relating to the use are also examined. Plasma and urine samples were collected from 87 volunteers among buyers at a pharmaceutical care unit. The samples were screened for the presence of paracetamol and subsequently analyzed for the amount of paracetamol by colorimetric approach using Glynn and Kendal's method. Urine screening for paracetamol indicated a positive test for 80.4% of the samples. Prescriptions in the hands of volunteers had 49.3% features of paracetamol. The correlation between respondents screened positive for paracetamol and handling paracetamol containing prescription was 0.72. The range of paracetamol levels observed in plasma was 110-490 µg/ml. Routine screening of patients prior to dispensing of paracetamol in pharmaceutical care centres is imperative for control of possible liver damage due to continuous use and resulting accumulation of paracetamol and its metabolite in blood. .

INTRODUCTION

Paracetamol (N-acetyl-p-aminophenol) has been used as an analgesic and antipyretic for many years that it has become a household name. The drug was believed to be safe but toxicity was first noticed in the 1960's [1, 2]. In Nigeria, paracetamol is regarded as an over-the-counter drug (OTC). There are few, if any, reports of abuse involving paracetamol and the use of the drug is alarmingly on the increase. In some developed countries, the rate of consumption between 1994 and 1995 was estimated to be greater than 20g/person/year [3, 4]. In developing countries like the study area, where data on average consumption of paracetamol is not available, the resulting health problems obviously are unnoticed and unattended too.

Paracetamol is currently used either alone or in combination with other drugs such as opiates for analgesia or in other mixtures such as cold 'cures' for its mild antipyretic action [5]. The use of the drug and overdose has been reported a major cause of hospital admission.

Author for Correspondence:

Email: jdjide@yahoo.com

A study in Australia between 1985 and 1990 found 306 admissions for paracetamol overdose though all patients recovered with supportive therapy requiring no liver transplant [6]. Between 1993 and 1999 approximately 500 deaths were attributable to drugs containing paracetamol per year in England and Wales [7]. Some drugs have limitations on their availability for small adverse risks, paracetamol is not considered with the same critical concern [8]. In America where restrictions are placed on drugs including paracetamol, reviewing medical charts for 10 years between 1988 and 1997 of children (aged <18 years) with paracetamol overdose, 53% of cases were of unintentional ingestion while 3% was due to dosage error [9]. A study in the 1990's confirmed that users take paracetamol because it was easily available and inexpensive [10]. Advertising and awareness on the drug may also influence its excessive ingestion. Radio and television adverts of paracetamol in Nigeria gives a message that the drug is required or useful before getting to see a doctor [11]. In a study conducted on patients admitted with paracetamol overdose, the patients were asked about factors that might have deterred them from taking an overdose. 35% of the patients

would not have taken an overdose were it made a prescription only and 40% would have sought an alternative [12-15].

Paracetamol reduces the oxidized form of the COX enzyme, preventing it from forming pro-inflammatory chemicals. This leads to a reduced amount of Prostaglandin E2 in the CNS, thus lowering the hypothalamic set-point in the thermoregulatory centre.

Paracetamol also modulates the endogenous cannabinoid system. Paracetamol is metabolized to AM404, a compound with several actions; most important, it inhibits the uptake of the endogenous cannabinoid/vanilloid anandamide by neurons [16]. Anandamide uptake would result in the activation of the main pain receptor (nociceptor) of the body, the TRPV1 (older name: vanilloid receptor). Furthermore, AM404 inhibits sodium channels, as do the anesthetics lidocaine and procaine. Either of these actions by themselves has been shown to reduce pain, and a possible mechanism for paracetamol. However, it has been demonstrated that, after blocking cannabinoid receptors with synthetic antagonists, paracetamol's analgesic effects are prevented, suggesting its pain-relieving action involves activation of the endogenous cannabinoid system.

The exact mechanism how COX is inhibited in various circumstances is still subject of discussion. Because of differences in the activity of paracetamol, aspirin, and other NSAIDs, it has been postulated that further COX variants may exist. A recently discovered COX-1 splice variant termed COX-3 was considered to explain some of the knowledge gap; however newer findings do not support the hypothesis that it plays any significant role in the functioning of paracetamol. Paracetamol hepatotoxicity is, by far, the most common cause of acute liver failure in both the United States and the United Kingdom. Paracetamol overdose results in more calls to poison control centers in the US than overdose of any other pharmacological substance [17]. Signs and symptoms of paracetamol toxicity may initially be absent or vague. Untreated overdose can lead to liver failure and death within days. Treatment is aimed at removing the paracetamol from the body and replacing glutathione. Activated charcoal can be used to decrease absorption of paracetamol if the patient presents for treatment soon after the overdose. While the antidote, acetylcysteine, (also called N-acetylcysteine or NAC) acts as a precursor for glutathione, helping the body regenerate enough to prevent damage to the liver, a liver transplant is often required if damage to the liver becomes severe.

The work was aimed at establishing a format for screening and assessing the level of paracetamol in the urine and plasma samples of buyers as to ascertain the extent of use of the drug and possibly call for caution to the users prior to dispensing and restrictions through the regulatory authorities.

EXPERIMENTAL MATERIALS

Reagents

Sodium hydroxide, sodium nitrite, hydrochloric acid, sulfamic acid, trichloroacetic acid and ammonium hydroxide were analytical grade and purchased from Sigma Chemicals, Germany. Paracetamol powder was a gift from May and Baker Plc, USA.

Method

Detection of paracetamol in urine

Urine samples were collected from 87 (62 males and 25 females) with mean age (32.7±8.2 and 22.7±10.1 years), buyers at a pharmaceutical care unit of a private pharmacy while 70 volunteers (55 males and 15 females) with mean age (29.2±7.9 and 28.5±9.1years) consented to give 5ml of blood samples obtained by venipuncture.

Paracetamol-cresol-ammonia test (PCAT) was used to detect the presence of paracetamol in the urine of the volunteers. 0.5 ml of urine was taken from the collected samples and 0.5 ml of hydrochloric acid was added in a conical flask, allowed to stand for 10min and placed in a thermostated water bath at 100^{o}C. 2 drops of the mixture was taken into a beaker and 10 ml of water, 1ml of 1% o-cresol and 4 ml of 2M ammonium hydroxide were added. The blue colour produced (if paracetamol was present) was compared with the standard solutions of paracetamol prepared.

Calibration curve determination

The purity of the paracetamol powder was checked by melting pointbefore the assay was determined by the BP official method. 1g of paracetamol powder was dissolved in 10ml of dehydrated alcohol and diluted to 1L with water to give a stock solution of 0.1%. 20 ml aliquots of normal plasma were pipette into 5 beakers and 100, 200,400,600, and 800µL of the stock solution were added to give 50, 100,200,300 and

400µL of the drug in solution respectively. The absorbance of the various concentrations was determined at 254nm. Correlation of the concentration versus absorbance obtained for calibration curve determination and Glynn and Kendal's method gave a regression of 0.9987.

Colorimetric determination of paracetamol in plasma

The colorimetric assay performed was based on Glynn and Kendal's method of paracetamol determination in plasma.

0.5 ml of plasma samples collected was pipette into a centrifuge tube containing 10ml of 15% trichloroacetic acid and vortex mixed for 3 min and centrifuged. The clear supernatant obtained was decanted into a 10ml tube containing 0.5 ml 6N hydrochloric acid as 0.4 ml of sodium nitrite was added drop wise to the resulting solution. The content was allowed to stand for 2 min to allow the profuse liberation of nitrous acid. 15% of sulfamic acid was added to neutralize the remaining nitrous acid in the reaction mixture before the addition of 2.5ml of 15%sodium hydroxide. The absorbance of the various samples was taken at 430 nm against a control of the reagent blank of water.

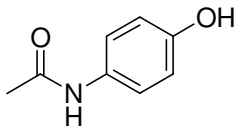

Figure 1: Structure of paracetamol

RESULTS

The absolute purity of the paracetamol powder was 99.81%. Beer's law was valid over the concentration range used in the calibration giving a linearity of concentration versus absorbance as expressed in Fig 1.

The screening of the urine samples gave the detection of paracetamol levels within the ranges as expressed in Table1 as made by visual observation against pre-constituted standard solutions of paracetamol. A total of 59 samples gave distinct blue colour indicative of appreciable levels of paracetamol in blood while 17 gave negative result. The calibration plot of the standard paracetamol solutions and the correlation coefficient for the curve are expressed in Fig. 2. The statistics of the mean plasma concentration (Cp) of paracetamol in males and females with the standard deviation and coefficient of variation are expressed in Table 1.

Table 1: The statistics of volunteers with positive paracetamol screening

Sex	Mean paracetamol level	Number of samples	Standard Deviation
Male	292.33	55	11.72
Female	274.00	15	9.70

Mean difference= 18.33 and 2 tail *P* value =0.5494

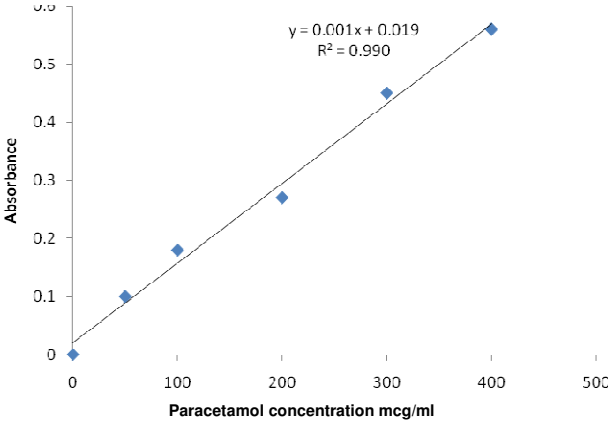

Figure 2: The calibration curve of absorbance and concentration of paracetamol

DISCUSSION

For the purpose of pharmaceutical care an attached quality control or drug monitor laboratory may be required within the pharmaceutical premises to aid a rapid pharmaceutical care intervention. Various assay methods have been documented for paracetamol with varying cost and sometimes involving technical skill and equipment. Titrimetric determination of paracetamol with cerium (IV) in acidic media using 1, 10- phenanthroline-iron (II) complex (ferroin) (BP official method) may be unsuitable for paracetamol in biological fluids because of presence of interfering substances. Glynn and Kendal's method gives more reliable measure of the free drug and is specific enough to rule out interfering drugs like caffeine, indomethacin, and other similar drugs. Colorimetric assay for paracetamol is accurate in aqueous and plasma samples over a clinically observable range of paracetamol concentrations.

The pattern of use of paracetamol in the study area revealed that the observation showed that paracetamol comes to mind when there is any sign relating to the consumer's perception of need for the drug. The plasma levels of paracetamol in females is not significantly higher than in males (P<0.001) (Table 2).

Table 2: Analysis of prescriptions presented for features of paracetamol

Parameters	Male N(62)	Female N(25)	Total N(87)
Total Prescriptions	62	25	87
Prescriptions containing paracetamol	21	10	31
Prescriptions containing paracetamol and handler having paracetamol level >300 µg/ml in plasma	8	5	13
Number of urine samples screened positive for paracetamol and plasma level less than 100 µg/ml	6	4	10

Levels of paracetamol above 200mcg/ml as observed in 80% of the study population as at the time of patronage and the fact that 37.5% of the prescriptions still contained the drug may be an indication that the blood level is on the rise in the study area. Chronic use of paracetamol can lead to liver damage [18]. The risk of liver damage due to paracetamol can be predicted from plasma paracetamol concentration. Screening of urine for presence of paracetamol levels can be a value–added service and by extension a form of pharmaceutical care in an environment where the regulation on sales of over the counter drugs is very relaxed. The distribution observed in this study gives an impression that paracetamol is considered a household commodity. Paracetamol poisoning is heard in Australia, the United States and U.K. with statistics on its attendant liver failure cases and other forms of intervention. Such data is not available in developing countries like Nigeria but consumption of the drugs does not appear different from what obtains in other part of the world. Very high and toxic levels of paracetamol in plasma can be noted through this method and the use of n-acetyl cysteine (NAC) to arrest the situation before it precipitates serious toxicity and the consequent signs [19].

CONCLUSION

The annexing or introduction of a mini-laboratory to government pharmacy units and community drug centres can be of great advantage in monitoring and early detection of overuse of drugs that can lead to possible accumulation and toxicity associated signs**.**

LIMITATION

The screening for paracetamol and determination of plasma paracetamol using Glynn and Kendal's method involves the liberation of nitrous acid which requires enough laboratory space and fume hood which may hinder routine performance of the determination. The method also requires a spectrophotometer that may be beyond the reach of a starter unit where this sort of determination as an extension of pharmaceutical care is required.

REFERENCES

[1] Thomas JS and Prescott LF. Liver damage and impaired glucose tolerance after paracetamol overdosage*. Br Med J.* 1966; (2): 506-7

[2] Dandson DGD and Eastham WN. Acute Liver necrosis following overdose of Paracetamol. *Br Med J*. 1966; 2: 497-9.

[3] Prescott LF. Paracetamol (Acetaminophen): A Critical Bibliography Review, 1st ed: London Taylor and Saviour, 1996.

[4] Spooner JB and Harney JG. The History and usage of paracetamol. *J. Int. Med. Res.* 1976. 4:1-6

[5] Gow PJ, Smallnovd RA, Angue PW. Paracetamol overdose in a liver transplantation center: An 8-years experience J. Restroenlent Hepatial 1999; 14:817-21.

[6] Mavin AJ, Wendon J, Williams R. A 7-years experience of severe acetaminophen induced hepatotoxicity (1987-1993). Gastroenterology 1995; 109; 1907-1917.

[7] Atete Z. Paracetamol related deaths in England and Wales, 1993-97. Health stat Q 2000; 7; 5-9.

[8] Pratt CM, Herolz RP, Ellis BE, Crowell SP, Lour W, Moye L. Risk of developing life threatening ventricular arrhythmias associated with tefebnadine in companion with over-the-counter antihistamines, ibuprofen and demastine. *Am J Cardiol* 1994; 73:346-52.

[9] Hawton K, Ware C, Mistry H, Hewitt J, Kingsbury S, Robert D, Weitizer H. why patients choose Paracetamol for self

paining and their knowkedge of its dangers. Br. Med. J. 1995: 310:164.

[10] Cormor S, Decks JJ, Hawton K, Simkin S, Keen A, Altman DG, Philo G, Bulstrode C. Effects of a drug overdose in a television drama on knowledge of specific dangers of self-poisoning: population based surveys: Br. Med J. 1999: 318:978-9.

[11] Hawton K, Ware C, Misty H, Hewitt J, Kingsbury S, Robert D, Weithizel H. Paracetamol self poisoning characteristics, prevention and harm reduction. Br. J. Psych 1996; 168:43-8

[12] Gunnel, D. Hawton K. Murray V, Garnier R, Bismulth Figg J, Simkin S. Use of paracetamol for suicide and non-fatal poisoning in the UK and France: are restrictions on availability justified? J. Epidermal Comm Health 1997: 51:175-9

[13] Prince M, Thomas SHL, James OFW, Kudson M Reduction in incidence of severe Paracetamol poisoning. Lancet 000: 355: 2047-8.

[14] Turnil JL, Burroughs AK, Moore KP. Change in occurrence of Paracetamol overdose in UK after introduction of blister pains. Lancet 2000, 355:2048 – 9.

[15] Sheen CL, Dillon JF, Bateman DN, Sampson K, Mac Donald TM. The effect on toxicity on reducing the size of available Paracetamol pack sizes Gut 2001 48 (Suppl. 1) A105.

[16] Hogestatt ED, Jonsson BA, Ermund A. Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase dependent arachidonic acid conjugation in the nervous system. J Biol. Chem.2005; 280 (36):31405-12.

[17] Khashab M, Tecor AJ, Kwo PY. Epiidemiology of acute liver failure. Curr Gastroenterol Rep. 2007; 9 (1): 66-73.

[18] Dally FF, Fountain JS, Murray L, Graudins A, Buckley NA. Guidelines for the management of paracetamol poisoning in Australia and New Zealand- explanation and elaboration.A consensus statement for clinical toxicologists consulting to the Australasian poisons information centres. Med J Aust. 2008; 185 (5):296-301.

[19] Graham GG,Scott KF. Mechanism of action of paracetamol. American Journal of therapeutics. 2005;12(1): 46-55.