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BIOCHEMICAL EFFECTS OF THE SPECTRA DOSES OF SPARFLOXACIN ON HEALTHY ALBINO RATS

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ABSTRACT

The fluoroquinolones have become an increasingly popular class of antibiotics for use in a variety of infections. This study investigated the effect of the spectra doses of sparfloxacin on the clinical chemistry parameters of healthy albino rats. The rats were evenly distributed into four groups such that each group had similar mean weights. Group 1 (control group) was administered 2.0 ml of 0.9% normal saline solution throughout the experimental duration. Group 2 (sub therapeutic dose) was administered a loading dose of 0.7 mg/kg body weight by oral route on day 1 and 0.35 mg/kg body weight p.o on days 2 to 7 while Group 3 (therapeutic dose) was administered a loading dose of 0.35mg/kg body weight p.o on day 1 and 0.175 mg/kg body weight p.o on days 2 to 7. Group 4 (toxic dose) was administered a loading dose of 3.5 mg/kg body weight p.o on day 1 and 1.75 mg/kg body weight p.o on days 2 to 7. The elevated levels of alanine aminotransferase (ALT) in the sub-therapeutic group are suggestive of liver-related malfunction or injuries to the liver parenchyma. It can also be seen that sub-therapeutic doses of sparfloxacin has some inductive effects on the ALTs but inhibitory effects by the toxic doses. Since no elevated levels of aspartate aminotransferase (AST) were seen in the treated groups, this suggests sparfloxacin is not hepatotoxic. There was no statistical difference in the creatinine levels of the control as well as all the treated groups. Thus it can be concluded that the renal function is not affected as serum creatinine level is a more reliable indicator of renal function

Keywords: Blood chemistry, liver enzymes, rats, sparfloxacin

INTRODUCTION

Fluoroquinolones are one of the most commonly prescribed classes of antibiotics [1]. The fluoroquinolones have become an increasingly popular class of antibiotics for use in a variety of infections. Newer members in this class have been developed with a broader spectrum of activity including better coverage of gram-positive organisms, enterobacteriaceae and some pathogenic streptococci. They are bactericidal agents that act by inhibiting two bacterial enzymes, DNA gyrase and topoisomerase IV, with roles in DNA replication thereby leading to cell death [2]. In most climes and health institutions, fluoroquinolones like ciprofloxacin and levofloxacin have enjoyed an appreciable degree of prescription and administration. However, other quinolones have had their licensed indications restricted in certain countries due to varying extent of toxicities. These include sparfloxacin in 1995, norfloxacin and moxifloxacin [3 - 4] whereas others have been outrightly withdrawn from the market, at least in some countries, following serious ADRs and safety concerns, including gatifloxacin [5], trovafloxacin and alatrofloxacin [6].

It has been opined that sparfloxacin maintained a therapeutic concentration of ≥ 0.125 µg/ ml from 0.042 h to 12 h while showing immunomodulatory effects on the humoral immune response and cell

mediated immunity as evidenced by an increased lymphocyte count as compared to both saline control as well as antigen control [7]. The pharmacokinetics of sparfloxacin is dose-dependent and has been studied in a cross-over study after single-dose administration of 200, 400, 600 and 800 mg orally to 12 healthy volunteers [8].

Uncommon but serious adverse drug reactions (ADRs) associated with fluoroquinolones include central nervous system (CNS) toxicity, phototoxicity, cardiotoxicity, arthropathy, and tendon toxicity with children and elderly being at greater risks [9 - 10]. Tendonopathy may manifest during, as well as sometimes long after fluoroquinolone therapy has been discontinued [11]. Events that may occur in acute overdose are rare and include renal failure and seizure [12]. The interactions of the fluoroquinolones with different receptor complexes such as blockade of the γ-amino butyric acid-a (GABAa) receptor complex within the central nervous system, leading to oxidative stress [13] and excitotoxic type effects has been proposed to be the mechanisms of toxicity of fluoroquinolones [9]. Even though current works show that blood abnormalities occur in less than one percent of patients, however, not much work has been done on the effects of different doses of sparfloxacin on the clinical chemistry parameters of healthy subjects. Hence this research work was set out to investigate the effect of the spectra doses of sparfloxacin (Figure 1) on the clinical chemistry parameters of healthy albino rats.

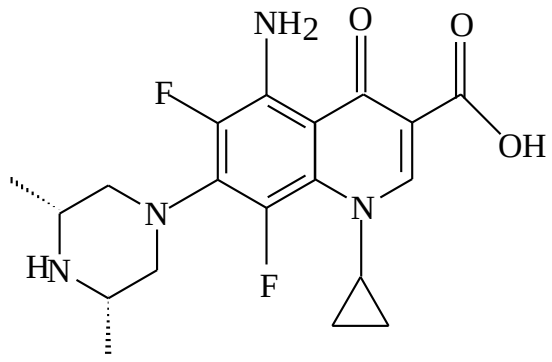

Figure 1: Sparfloxacin

MATERIALS AND METHODS

Experimental animals: Twenty four Wistar rats of either sex, weighing 80-150g, in-house bred at the animal house of the College of Medicine of the University of Lagos, were used in this study. The animals were cared for and used in accordance with the Institute of Laboratory Animal Research (ILAR) guidelines for care use of animals in experimental studies [14]. Animals were housed under standard laboratory conditions, air conditioned with 12-15 filtered fresh air changes per hour, environmental temperature between 17-23°C, relative humidity 30-70%, with 12 h fluorescent light (6.00 am to 6.00 pm) and 12 hours dark cycle. Rats were housed individually in standard wire mesh bottom wooden cages with hard wood chips as beddings (size: approximately L 410 x B 280 x H 140 mm), with stainless steel top grill having facilities for pellet food and Aquaguard filtered cum-purified water ad-libitum.

Experimental protocol: Following two weeks of acclimatization period, the animals were evenly distributed into four groups with each group having similar mean weights. The control group was administered 2.0 ml of 0.9% normal saline solution throughout the experimental duration. Group 2 (sub therapeutic dose) was administered a loading dose of 0.7 mg/kg body weight by oral route on day 1 and 0.35 mg/kg body weight p.o on days 2 to 7 while Group 3 (therapeutic dose) was administered a loading dose of 0.35mg/kg body weight p.o on day 1 and 0.175 mg/kg body weight p.o on days 2 to 7. Group 4 (toxic dose) was administered a loading dose of 3.5 mg/kg body weight p.o on day 1 and 1.75 mg/kg body weight p.o on days 2 to 7. On the eight day, blood was collected from the retro orbital plexus of each animal into lithium heparinised tubes. Plasma was separated in a refrigerated ultracentrifuge at 3500rpm for 15mins and analysed for: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase (CK), albumin (Alb.), urea, creatinine (Crea.), cholesterol (Chol.). The serum samples were assayed for alkaline phosphatase using the phenolphthalein method [15], alanine transaminase and aspartate transaminase were determined by the procedure of Rietman and Frankel [16]. Urea was assayed using urease – Bertthelot method [17], total cholesterol using enzymatic endpoint method [18 - 19] and creatinine assay was done by alkaline picrate method [20].

Statistical analysis: All values were expressed as Mean (±SD). Data were analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's test for multiple comparisons with the level of significance considered at $p < 0.05$ (Graph pad Prism 5).

RESULTS

The study was carried out to investigate the effect of sparfloxacin on the clinical chemistry parameters in

healthy rats. Table 1 shows the biochemical parameters investigated following the administration of loading doses of 0.35, 0.70 and 3.50mg/kg body weight sparfloxacin on day 1 and maintenance doses of 0.175, 0.35 and 1.75mg/kg on days 2 to 7 respectively to the sub-therapeutic, therapeutic and toxic dose groups. The study showed that there was no statistical significant difference in the ALT levels between the control group and the therapeutic dose group. However, a significant statistical difference in the ALT levels was seen between the control group and the sub-therapeutic and toxic dose groups. The AST levels in all the treated groups were significantly decreased when compared to the control group. All the treated groups showed significant statistical difference (increase) relative to control. The trend however is inversely proportional to the dose of sparfloxacin administration with a marked rise seen in the sub-therapeutic dose of sparfloxacin. A significant statistical difference (increase) in the creatinine levels in the treated groups with the highest value seen in the toxic dose group. The inverse relationship between glomerular filtration rate (GFR) and creatinine might as well suggest a lowering of the GFR of sparfloxacin or renal insufficiency. The cholesterol levels in all the treated groups were statistically significant when compared to that of the control group. As can be seen from the table, an inverse relationship is seen between sparfloxacin dose and cholesterol levels therefore indicative of the potential of sparfloxacin in lowering cholesterol levels.

DISCUSSION

Our study showed an elevation in the liver function enzymes which represented statistical significant difference except for ALT levels in the therapeutic dose group. Elevations in serum ALT are more specific for liver related injuries or diseases [21]. Hence the elevated levels in the sub therapeutic group are suggestive of liver related malfunction or injuries to the liver parenchyma. It can also be seen that sub therapeutic doses of sparfloxacin have some inductive effects on the ALTs but inhibitory effects by the toxic doses. Albeit earlier research has shown that elevated levels of AST are seen in myocardial infarction and liver injury. AST is less specific than ALT [22]. Therefore, therapeutic doses of sparfloxacin results in little or no damage to tissues containing these enzymes. Since no elevated levels of AST was seen in the treated groups, this is suggestive of sparfloxacin not being hepatotoxic. Slight or moderate elevations of both AST and ALT activities have been observed after administration of various medications such as NSAIDS, antibiotics, antiepileptic drugs, HMGCoA reductase inhibitors (such as statins), or opiates [23]. Medical research has shown that blood usually contains low levels of AST and when the body tissue or an organ such as the heart or liver is diseased or damaged, there is a release of additional AST into the bloodstream [24]. The AST – ALT ratio is sometimes a useful prognostic parameter especially in alcoholic liver disease [25]. The AST/ALT ratios of the groups were control (0.49), sub – therapeutic dose (0.37), therapeutic dose (0.27) and toxic dose (0.47). Since the ratio is less than 1 in all groups, this is indicative of no drug – related inflammation in the liver. The lowest ratio was seen in the therapeutic dose and this is suggestive of the safety of 0.70 mg sparfloxacin per kg bodyweight. Like chlorpromazine and sulphonamides, sparfloxacin has been observed to increase serum ALP concentrations. Most of the fluroquinolones are eliminated primarily through glomerular filtration and tubular secretion in the kidney [26]. The serum creatinine level is a more reliable indicator of renal function than the blood urea nitrogen (BUN) [28]. Serum creatinine concentration increases in the presence of impaired renal function. Since no significant difference was seen in the creatinine levels of the treatment groups, it can be suggestive of no impaired renal function in the groups. However a statistical significant decrease in the urea was seen in the toxic dose group. Cholesterol, an important parameter of the lipid profile was seen to be significantly decreased ($p< 0.05$) in all the groups. An inverse relationship between the dose of sparfloxacin administered and cholesterol levels was profiled in the study. Serum creatine kinase has been shown to increase in response to muscle myalgia or muscle injury. Sparfloxacin administered at all treatment doses can be said to have been implicated in the rise consequent to muscle injury with higher effects seen in the sub therapeutic dose group. An overview of the chronic and sub chronic administration of sparfloxacin is therefore questionable with respect to the clinical chemistry of subjects. Hence there is the need for appropriate therapeutic drug monitoring (TDM) of the biochemical parameters in subjects placed on sparfloxacin at various doses

CONCLUSION

The elevated levels of liver function tests are of clinical concern in subjects placed on long term administration of sparfloxacin. Sparfloxacin has been implicated in the elevation of liver enzymes on administration of maintenance and loading doses. It is therefore recommended that following sparfloxacin administration should be therapeutic drug monitoring

(TDM) on subjects with the appropriate intervention. Additional monitoring is also indicated to ensure that the appropriate clinical chemistry parameters are brought to baseline or whether a trend of elevations is noted on multiple occasions.

Table 1: Effect of Sparfloxacin on the Clinical Chemistry parameters of healthy rats

Biochemical Parameters	Control	Treated groups (dose in mg/kg body weight)		
		0.35	0.70	3.50
Creatinine (mg/dl)	0.69 ± 0.01	0.75 ± 0.01	0.74 ± 0.01	0.77 ± 0.01
Cholesterol (mg/dl)	86.5 ± 0.71	82.5 ± 3.54*	65.0 ± 1.41*	54.0 ± 1.41*
Albumin (g/dl)	2.58 ± 0.01	2.58 ± 0.01	2.26 ± 0.25	15.5 ± 0.71*
Creatine Kinase (U/l)	1236.0 ± 6.36	4302.5 ± 0.28***	3031.5 ± 3.54**	2689.5 ± 13.4*
Urea (mg/dl)	21.5 ± 0.01	23.5 ± 0.71	25.5 ± 0.71	15.5 ± 0.71*
Alkaline phosphatase, ALP (U/l)	233.5 ± 0 .07	185.5 ± 0.71*	301.5 ± 0.07*	187.5 ± 0.71*
Aspartate Transaminase, AST (U/l)	123.7 ± 0.07	108.5 ± 0.07*	68.1 ± 0.07*	88.5 ± 0.21*
Alanine transaminase, ALT (U/l)	253.2 ± 0.07	295.7 ± 0.07*	251.7 ± 0.07	187.3 ± 0.07*

Values are expressed as Mean ± SD, One Way ANOVA followed by Dunnet's Test,

* = P<0.05; ** = p<0.01; *** = p<0.001. Treated groups are compared with Control group.

REFERENCES

1. Etminan M, Forooghian F, Brophy JM, Bird ST, Maberley D. JAMA, 2012; 307(13):1414 – 1419
2. Drlica K, Zhao X. Microbiol Mol Biol Rev, 1997; 61(3):377 - 392.
3. The European Medicines Agency (EMEA) Restricts Use of Oral Norfloxacin Drugs in UTIs. United Kingdom: Doctor's Guide, 2008
4. The European Medicines Agency (EMEA) Danish Medicines Agency EMEA recommends restricting the use of oral moxifloxacin-containing medicines, 2008
5. Schmid, RE. Drug Company Taking Tequin Off Market. Associated Press, 2006
6. WHO Pharmaceuticals Newsletter (World Health Organisation) 07 (8). 1999
7. Nirala RK, Jaichandran C, Ranjan R, Bauri RK. Asian J Phar Biol Res, 2011; 1 (3): 343-349.
8. Montay G, Bruno R, Vergniol JC, Ebmeier M, Le Roux Y, Guimart C, Frydman A, Chassard D, Thebault JJ. J Clin Pharmacol, 1994; 34:1071–1076
9. De Sarro A, De Sarro G. Curr. Med. Chem, 2001; 8(4): 371 – 384.
10. Iannini PB. Curr Med Res Opin, 2007; 23 (6): 1403 – 1413
11. Saint F, Gueguen G, Biserte J, Fontaine C, Mazeman E. Rev Chir Orthop Reparatrice Appar Mot, 2000; 86 (5): 495 – 497.

12. Nelson LH, Flomenbaum N, Goldfrank LR, Hoffman RL, Howland MD, Neal AL. Goldfrank's toxicologic emergencies. New York: McGraw-Hill, Medical Pub. Division, 2006
13. Saint F, Salomon L, Cicco A, de la Taille A, Chopin D, Abbou CC. Prog Urol, 2001; 11 (6): 1331 – 1334
14. Guide for the Care and Use of Laboratory Animals. Institute for Laboratory Animal Research (ILAR) Commission on life Science, National Research Council, 1996.
15. Babson LA, Greeley SJ, Coleman CM, Phillips GD. Clin Chem, 1966; 12: 482- 490.
16. Rietman S, Frankel S. Am J Clin Pathol. 1957; 28: 56 – 58
17. Weatherbum MW. Chemica Analytica 1967; 39 (8): 971 - 974.
18. Trinder P. Ann Clin Biochem. 1969; 6: 24 – 27
19. Roeschlau P, Bernt E, Gruber WJ. Clin. Chem Clin Biochem, 1974; 12(5): 226.
20. Tietz NW, Pruden EL, Siggard-Anderson O. Electrolytes, Blood Gasses and Acid base Balance. In Tietz NW and Andresen BD (eds.).Textbook of Clinical Chemistry, Saunders Publishers, Philadelphia, 1986, pp 1188-1196.
21. Cornelius, CE. Serum Enzyme Activities and Other Markers for Detecting Hepatic Necrosis, Cholestasis or Cocarcinoma. In: Clinical Biochemical of Domestic Animals. 4th Ed, Academy Press, 1989, pp: 381-386.
22. Hayes, AW. Principles and methods of toxicology. 5th Ed New York, NY: Informa Healthcare USA, Inc, 2008
23 Burtis CA, Ashwood ER, Bruns DE, Tietz NW Tietz fundamentals of clinical chemistry Saunders Elsevier, 2008
24 Gregory TE, Simon JB Medical Review, 2011
25 Alatalo PI, Koivisto HM, Hietala JP, Puukka KS, BloiguR, Niemela OJ. Am J Clin Nutr, 2008; 88 (4):1097 – 1103.
26 Jha KC, Datta BK, Patra PH, Bhar MK, Singh A, Sar TK, Chaterjee US, Mandal TK, Chakraborty AK.. RJBClS, 2010; 1(4) 801 – 808
27 Kakadiya J, Shah N. JITPS, 2010; 1 (6), 270-273.