Egypt. J. Chem. Environ. Health, 1 (1):349-355 (2015)

Biochemical and cytogenetic Investigations into the effects of Enrofloxacin in rats

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Abstract

Oral administration of enrofloxacin at doses of 7.5 and 10mg for 30 successive days into mature rats significantly increased the frequencies and percentage of micro nucleated polychromatic erythrocytes (MPCEs) and ratio of polychromatic erythrocytes normochrotic erythrocytes (PCE / NCE) was recorded.

The effect of enrofloxacin on body weight was studied in 2 groups of 10 mature rats each was orally administered by the tested drug at a dose of 7.5 and 10 mg/ 100gm.b..wt. for 30successiundays. Enrofloxacin showed insignificant increase in bodyweight of the rats at the doses. The tested drug significantly increased the activities of serum Aspartate amino transaminase(AST), Alanine amino transaminase (ALT) and the level of urea, but decreased the alkaline phosphatase (AP) activity and level of total protein

Introduction

Fluoroquinolones are anti-microbial agents, with broad spectrum of bacterial activity against both gram-positive and gram negative bacteria. (Oliphant et al 2002 and Shenoy et al 2011) They are bacterial agents that exert their bacterial action by inhibiting the action of bacterial enzymes DNA gyrase (layrence and parker 2008)

Fluoroquinolones are well tolerated in patients but their uses have been associated with some adverse effects (Wolfson and Hooper 1985 and Grayson ,1999). Enrofloxacine is a second generation fluoroquinolone antibacterial agent (Martinez et al., 2006)

The effect if enrofloxacin on polychromaticand normochrromatic cells seems to be deficient and requires more investigation. Accordingly this work was conducted to clarify the effect if enrofloxacin on polychromatic, normochromatic, body weight and serum enzymatic activies and levels of total protein, creatinine and urea.

Material and Method

Enrofloxacin (1-cyclopropyl-7-(4-ethyl -1- piperazinyl)–b- Fluoro-1,04-dihydro-4-oxo-3-quinolone carboxylicacid) was produced by El Nasr pharmaceuticals chemicals company, Egypt.

Animals Thirty mature rats of an average body weight 150-180 gm were used. Rats were fed on ordinary ration and water adlibtium.

I. Cytogenetic:

In order to investigate the possible mutagenic effects of enrofloxacin (in low and large dose),the micronucleus test was performed to detect chromosomal damage associated with the treatment. Micronuclei were identified as dark blue staining bodies in the cytoplasm of the poly chromatic erythrocytes (PCEs).

Animals were divided into , 3 groups of 10 mature rats each were used first was kept as a control, the second and third groups were orally administered enrofloxacin at doses 7.5 and 10 mg / 100g.b.w.t for 30 successive days (long duration).

Following the protocol established by Salamon et al., (1980), bone marrow cells of rats were extruded with a pin into a clean dry glass slides and homogenized with two drops of fetal calf serum. Cells were smeared on the slide, air dried, fixed in absolute methanol and stained with Giemsa in phosphate buffer pH 6.8.The poly chromatic erythrocytes (PCEs,1000 / animal) were screened for micronuclei, and the changes in the mitoic activity (Hart and Engberg –Pederson, 1983; Al– Bekairi et al., 1991)were assessed on the basis of the ratio of polychromatic to normochromatic erythrocytes (PCE / NCE ratio).

II- Effect of enrofloxacin on body weight and biochemical analysis:

Rats of each group were weighted at the beginning of the experiment, then every week for 30 days. The changes in body weight was calculated and recorded.

Individual blood samples were obtained from rats, left to clot and sera were separated for biochemical analysis. The activities of AST, ALT and AP were determined by Reitman and Frankel (1957) and Roy (1970) while total protein, urea and creatinine were estimated as explained by Peters (1968), Kaplan (1965) and Husdan and Rapopart (1968).

The results were subsequently analyzed by following the statistical methods established by Snedcor (1969) in order to determine whether a dose group was positive or negative.

Result and Discussion

I. Cytogenetic :

The effect of enrofloxacin on the frequencies and percentage of micro nucleated polychromatic erythrocytes (MPCEs) as well as the ratio of PCE/NCE in control and treated animals are recorded in table (1).

Oral administration of enrofloxacin at 7.5 and 10mg/100 gm b.w.t. to mature rats for 30 days recorded significantly increased the percentages of MPCEs (1.71% and 2.07%respectively) when compared with corresponding control values. The PCE/ NCE ratio significantly increased of compared with control.

The significant increase in PCE/ NCE ratio according to Guyton (1991) and enhancement of mitotic activity of bone marrow cells could be considered as a sign of to toxicity and /or damage of some organs of the body. Limited information is available about genetic activity of enrofloxacin (Zowail et al., 2009, Majed et al.,2015, Fatai et al., 2013 and Priyadharshini, 2013).Enrofloxacin at doses 7.5 and 10mg/100 gm.b.w.t affected seriously the examined body organs enough to enhance the production of PCEs in bone marrow, there by attempting to supply the demand for RBCs in the body. Our study, conducted by lebeket al., (1982) found that plasmids mediated TR strains of staphylococcus auras was sensitive to enrofloxacin than other tetracycline and minocylrne drugs.

Enrofloxacin is a second generation of fluroquiolone antibacterial agent (Martinez et al., 2006) against bacteria especially mycoplasma (Grayson 1999). However, the cytogenetic and bioche mical studies have not been investigated.

The effect of oral administration of enrofloxacin at doses of 7.5 and 10 mg /100gm. b.w.t. daily for 30 successive days on activities of AST, ALT and urea level induced significant increased but significantly decreased AP activity and level of total protein(table 2). Similar results were previously obtained by Fatai et al2013; priyadharshini2013;olusegun & Emmanucl 2014 and starling et al.,). The increase of AST and ALT activities reflects the degree of tissue damage. A good deal of researchers (Duncan and Prasse, 1981) claimed that the activity of AST increased in inflammatory and degenerative changes in liver because of increase liberation of enzymes from hepatic cells. In addition, concomitant administration of tested drug increased serum urea level which is specific indicator of renal damage (Rule et al., 2004).

On other hand, the level of total protein was significantly decreased (Fatai et al., 2013).

The increase in body weight after oral administration of enrofloxcin is in significant(table) which coincides with the study of Gamguilhem et al., 2008 and Priyadharshini, 2013) who reported that administration of ciprofloxacin and did not affect growth of male rats.

Enrofloxacin had be proved to be mutagenic ,also it included degeneration in the visceral organs when used in repeated dose for a long period.

Table (1): Effect of enrofloxacinon the incidence of MPCEon the relation of PCE to NCE in rats mean± S.E (n=10)

G	Dose/mg 100g.b.wt.	PCE Screaned	MPCE		No	NCE Screaned	PCE/NCE Ratio
			No	Average			
C	-	10000	56	11.2 ± 0.06	1.12	4366	4.66 ± 0.1
Enrofloxain	7.5	10000	86	17.2 ±0.08***	171	2534	7.98 ± 0.096***
	10	10000	104	20.7 ± 0.14	2.07	2784	7.198 ± 0.11***

Table (2): Showing oral administration of enrofloxain on serum enzymatic activity and biochemical constituents in serum of rats. mean ±S.E (n=10)

G	Dose/ mg 100g. b.wt.	ALT U/L	AST U/L	AP U/L	Urea mg%	Creatinine mg%	Total protein gm%
C	-	3.58 ± 0.12	3.72 ± 0.13	12.96 ± 0.21	29.54 ± 0.16	0.53 ± 01.3	8.27 ± 0.21
Enrofloxain	7.5	4.90 *** ± 0.12	4.7 *** ± 0.17	11.27 *** ± 0.29	25.86*** ± 0.18	0.82 ± 0.38	7.65 ± 0.4
	10	5.72 *** ± 0.14	6.84 *** ± 0.11	9.09 *** ± 0.2	33.12*** ± 0.17	0.57 ± 0.17	6.74 ± 0.16***

*** significant at $p < 0.001$

Table (3): Effect of enrofloxain on body weight of rats mean± S.E (n=10)

G.	Dose/mg 100g.b.wt.	Body might in grams entry week			
		1w	2w	3w	4 w
C	-	84 ± 2.64	90 ± 1.8	99 ± 0.66	104 ± 4.6
Enrofloxain	7.5	88 ± 2.47	91 ± 3.67	106 ± 4.59	116.2 ± 4.12
	10	80±1.47	80*** ±1.8	91* ±3.36	97.b± 5.65

* Sig.at p<0.05

*** Sig. at P<0.001

References

Al-Bekairi, A.M.; Qureshi, S., chaudhry, M.A. and shah, A.H. (1991): " Uric acid as inhibitor of cyclophosphamide- induced micronuclei in mice". Mutat. Res., 262: 115-118.

Fatai, I.M., Image, N.O.A. and Gbenle, g.O. (2013): Biochemical investigations into the effects of co-administration of ciprofloxacin and nicosan. Afri- J. pharm. & pharmacol., 7 (39), 2674-79.

Duncan,J. and prasse, w(1981) :liver in veterinary laboratory medicine the Iwoa state university press, U.S.A(1): 45-64.

Gamguilhem, R.; Escoula, L. and Larrien, G. (2008): " Effect of the rout and sie of administration minocycline on the growth of rabbits. Annoles de Recherches veterinaires, 13 (2): 199-204.

Grayson,M.L. (1999): Ciprofloxacin. In kucers A, crowe. SM, Grayson ML, Hoy JF eds. The use of antibiotics: a clinical review of antibacterial, antifungal and antiviral drugs. Avon: the bath press, pp. 981-1060.

Guyton, A.C. (1991): " Textbook of medical physiology. " 8th Ed., " Red blood cells, anaemia and poly cythemia" (Editor M.J. wonsiewiez). An and polycythemia". (Editor M.J. wonsiewiez). An HBJ International Ed., W.B. sounders, P. 356.

Hart, J.H.W. and Engberg- Pederson, H. (1983): " Statistics of the mouse bone marrow micronucleus test: counting, distribution and evaluation of results. "Mutat. Res., 111: 195-207.

Husdan, H and Rapopart, (1968): " chemical determinfition of creatinine with deportenization." Clin. Chem., 14: 222.

Kaplan, A. (1965): "Ureanitrogen and urinary amoma Quated from meistes, s (1965): standard Methods of clinical chemistry". Academic press, Inc., New York, U.S.A.

Laurence,L.B. and K.L. Parker. (2008): Ciprofloxacin in chemotherapy of microbiol diseases. Academic press, New Yourk, 2008, pp. 709-839.

Lebek, G.; Semenitz, G.; Gstranuthaler, G. and pfaller, W. (1982): comparative investigation of tetracycline and minocycline on chromosomally- mediated and plasmid-mediated tetracycline –resistant and tetracycline–sensitive variants of a staphylococcus aureus strain by minimum inhibitory concentration, microcalormetric effects and ultrastcuctural atterations" Immun. Infec., 10 (5): 193-201.

Majed, M; Karem, H, A. and Sayer I.A. (2015): Flouroquinolones. Induced Antibacterial activity attenuation by pretreatment with vitamin B_{12}. Int.J. Pharmacol., 11 (1): 67-71.

Martinez, M; McDermott, P; walker, R. (2006): armacology of the fluoroquinolones. A prespectictive for the use in domestic animals. Vel.J.72, 10-28.

Oliphant,C.M. and. Green,G.M (2002). Quinolones: a comprehensive review. m. Fam. Physician, 66 (3): 455- 464.

Olusegun, K. A and Emmannel, B.O. (2014): Effects of ciprofloxacin and levofloxacin administration on some oxrdative stress markers in the rat. Int. J. Biol., Fd, Vet. & Agric. Eng., 8 (1), 72-76.

Peters, T. (1968): Determination of total protein clin. Chem., 14: 1147.

Priyadharshini, K.M. (2013): Ciproflaxacin induced body weight and haemotogical change in rats and anti-oxidant vitamin a, c and E as rescue agents. Int. J. Eng. Sci. Inven., 2 (2), 21-31.

Reitman, S. and Frankil, S. (1957): " Colorimetric method for the determination of serum transaminase activity. Am.J. Clin path., 28: 56-58.

Roy, A.V. (1970): " Arapid method for alkaline phosphatase estimation". Clin. Chem., 16: 431.

Rule, AD, Larson, TS, Bergstralh, EJ, Slezak, JM et al., (2004): Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. Annds. Int. Med, 141 (12): 929-937.

Salamon, M.; Heddle, J.; Stuart, E. and kat Z,M. (1980): " Towards an improved micronucleus test. Studies on 3 model agents, Mitous-ycin C, cycolophasphamide and dimethyl benzanthracene". Mnt. Res., 74: 347-356.

shenoy,S. Chakravaty,S. Nayak,A. candito,P.Z. Shanbhay,T. (2011): Anxiogenic affect of moxifolxacin in wistar rats. Inter. J. Appl. Brol. Pharm. Tech. 3 (4): 158-162.

Snedecor, G.W. (1969): " Statistical methods. " 4th Ed., the Iowa state. College press, Ames, Iowa.

Starling, k; Mano J. A .; G. Sinivasa and Jitendra, K.M. (2012): Effects of aflatoxin B on tissue residues of enrofloxacin and its metabolite ciprofloxacin in broiler chickens. Env. Toxical. & pharmacy., 33, 121-126.

Wolfson,J.S. and Hooper,D.C. (1985): The fluoroquindones: strnctures, mechanisms of action and resistance, and spectro of activity in vitro- Antimicrob. Agents chemother., 28: 581-586.

Zowail., M.E; khater, E.H.H. and El. Asrag, M.E.M. (2009): Protective effect of green tea extract against cytotoxicity induced by enrofloxacin in rat Egypt. A cad. J. biology. Sci., 1