Safety of Moxifloxacin following repeated intramuscular administration in Wistar rats

K.A. Sadariya, A.K. Gothi, S.D. Patel, S.K. Bhavsar* and A.M. Thaker

Department of Pharmacology and Toxicology,
College of Veterinary Science and Animal Husbandry,
Anand Agricultural University, Anand, Gujarat (INDIA).
* Corresponding author email : skbhavsar@aau.in, Mobile. No: +91-9825768508

Abstract

Moxifloxacin is a novel fourth generation fluoroquinolone with broad spectrum of antibacterial activity. The study was conducted to evaluate the safety of Moxifloxacin (5.0 mg/kg) after repeated intramuscular administration at 24 h interval for 14 days in male and female wistar rats. Hematological (Haemoglobin, RBC, WBC, MCV, MCH, MCHC, HCT and DLC), blood biochemical parameters (AST, ALT, ALP, Total Bilirubin, Total Serum Protein, Serum Albumin, Globulin, Serum Creatinine, Urea, Uric acid and Blood glucose) and histopathological examination of various tissues were carried out in the present study. Male and female animals of any group did not reveal any clinical symptoms and mortality attributable to the 14 days intramuscular administration of Moxifloxacin. The data were compared by unpaired two tail `t` test using Graph Pad Prism (Version 4.00). All above hematological and blood biochemical parameters were found to fluctuate within normal range during treatment period and the mean values were not significantly differ ($p < 0.05$) from corresponding control values. Moreover, no gross or microscopic changes were found in the liver, kidney, heart, spleen, stomach, intestine and joint cartilages of the treated wistar rats. Results indicate that daily administration of Moxifloxacin for 14 days seems to be safe and well tolerated in rats.

Key words: Moxifloxacin, Wistar rats, Safety study, Fluoroquinolone, Antibiotic, Antibacterial.

Introduction

Fluoroquinolones are synthetic bactericidal drug which act on bacterial DNA topoisomerases II and IV responsible for the nicking and negative supercoiling of double-stranded DNA. These compounds are considered to have a concentration-dependent effect. Moxifloxacin is a novel fourth generation fluoro-quinolone with a broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria, anaerobes and atypical organisms such as Mycoplasma and Chlamydia spp (Dalhoff et al., 1996). It has the highest potency against Staphylococcus aureus and Staphylococcus epidermidis. The drug thus seems to be extremely useful in a variety of infections including those of urinary tract, respiratory tract, soft tissues, bones and joints. Newer flouro-quinolone antimicrobials have been widely used in the clinical field because of their high clinical efficacy and to treat a broad range of infections. Report of the safety study of moxifloxacin in rat are scarce. Hence, the present study was planned to assess safety of moxifloxacin following multiple intramuscular doses given at the rate of 5 mg/kg repeated at twenty four hour intervals for 14 days in male and female wistar rats.

Material and Methods

Experimental animals

The present study was conducted on 24 adult healthy male and female wistar rats. The rats were procured from Animal Research Facility, Zydus Research Centre (ZRC), Ahmedabad, India and housed in individually ventilated polysulphone cages (IVC) under standard laboratory conditions at experimental animal room at Animal Research Facility, ZRC, Ahmedabad. The full system was kept in environmentally controlled room with 22 ± 3°C temperature and 30-70% humidity. Light/dark cycles of 12/12 hours were provided throughout the study period. Rats of 6-8 weeks age were selected after physical and behavioral examination. Nulliparous and non pregnant female rats were used in the present experiment. The live body weight range was within ± 20 % of the mean body weight for each sex at the time of randomization. Food and water were provided ad libitum. The experimental protocol for general procedures and use of animals was approved by the Institutional Animal Ethics Committee (IAEC). The rats were kept under constant observation for 7 days before commencement of experiment. All necessary

Table-1. Effect of daily intramuscular administration of Moxifloxacin (5mg/kg) for 14 days on various hematological parameters in male wistar rats.

Parameters	Control (Group-I)			Moxifloxacin (Group-II)		
	Mean ± SEM (n = 6)			Mean ± SEM (n = 6)		
	0 day	7th Day	14th day	0 day	7th Day	14th day
WBC(x103/µl)	10.44 ± 0.87	9.66 ± 0.82	12.08 ± 1.40	8.48 ± 0.12	7.75 ± 0.36	13.62 ± 1.02
RBC(x106/µl)	6.75 ± 0.10	6.91 ± 0.12	6.97 ± 0.69	6.50 ± 0.14	6.61 ± 0.14	7.18± 0.14
HGB(g/dl)	13.52 ± 0.14	13.70 ± 0.21	14.70 ± 0.21	13.15 ± 0.21	13.48 ± 0.20	14.33 ± 0.22
HCT(%)	43.12 ± 0.52	43.17 ± 0.69	42.02 ± 4.01	41.40 ± 0.77	42.10 ± 0.62	44.85 ± 0.76
MCV(fl)	63.87 ± 0.54	62.53 ± 0.76	60.95 ± 0.56	64.95 ± 0.58	63.75 ± 0.55	62.52± 0.49
MCH(pg)	20.05 ± 0.19	19.83± 0.25	22.73 ± 3.46	20.47 ± 0.16	20.42 ± 0.24	19.98 ± 0.16
MCHC(g/dl)	31.38 ± 0.19	31.68± 0.25	37.50 ± 3.46	31.77 ± 0.16	31.82 ± 0.24	31.95 ± 0.08
Platelets(x103/µl)	968.50 ±30.0	949.83 ± 62.27	888.17 ± 103.71	1009.17 ± 39.58	1121.33 ±53.47	1142.67 ± 62.20
Neutrophil (%)	10.50 ±1.12	11.52 ± 1.28	10.29± 1.11	10.77 ± 0.81	12.20 ± 1.32	7.78 ± 0.52
Lymphocyte (%)	86.43 ±1.61	84.47 ± 1.64	86.77 ± 1.43	86.57 ± 0.79	81.93 ± 1.62	87.98 ± 1.11
Monocyte(%)	1.29 ± 0.45	1.98 ± 0.60	1.46 ± 0.34	1.00 ± 0.22	3.36 ± 0.50	2.52 ± 0.42
Eosinophil(%)	0.89 ± 0.14	0.88 ± 0.11	0.58 ± 0.09	0.75 ± 0.07	0.58 ± 0.14	0.48 ± 0.06
Basophil(%)	0.88 ± 0.18	1.14 ± 0.13	0.88 ± 0.18	0.91 ± 0.07	1.52 ± 0.12	1.25 ± 0.25

managemental procedures were adopted to keep the rats free from stress.

Drugs and Chemicals

Moxifloxacin pure base powder was obtained from Ms. Zydus Research Centre, Ahmedabad. Paraffin wax, Hematoxylin and Xylene were procured from SD Fine Chemical Ltd., Mumbai. Dimethyl sulphoxide (DMSO), Twin-80, Eosin were procured from Qualigens fine chemicals, Mumbai. Reagents for Hematological analysis were purchased from Bayer (USA) and reagent for serum biochemical analysis was purchased from Randox laboratory Ltd., (UK).

Experimental Design and Drug administration

All animals of either sex were divided into two groups (each contain 6 male and 6 female animals). Group I served as control and received only vehicle (5% twin-80: 5% dimethylsulphoxide in milli-Q water) while group II received Moxifloxacin at doses of 5 mg/kg repeated at 24 hours interval for 14 days. Doses were calculated according to body weight of animals and administrated as per concentration strength of formulation. The drugs were administered by intramuscular injection using sterile 1 ml syringe and needle (26 G, 0.45mm x 13mm) in deep gluteal muscle. Safety of repeated intramuscular administration of moxifloxacin was assessed by studying haematology, serum biochemistry and histopathology of tissues. All animals were observed daily for mortality, physical examination, behavioral observations, body weight and food consumption throughout the period of study.

Blood samples were collected from retro-orbital plexus into clean sterilized plain and EDTA added centrifuge tube at 0 day (before drug administration) and on 7th and 14th day for serum biochemical and hematological analysis. The samples were analyzed by using automatic hematology analyzer (CELL-DYN®3700, Abbott Lab., USA) for assessment of hematological parameters. Serum biochemical parameters were analyzed using automatic biochemical analyzer (Daytona IR200, Randox Ltd., India). The statistical analysis was performed using Graph pad prism (version 4.0).

Results and Discussion

Male and female animals of any group did not reveal any symptoms attributable to the 14 days intramuscular administration of moxifloxacin. None of the animals found dead in both groups throughout the study period. There was non significant ($p < 0.05$) change in feed consumption as well as body weight of male and female rats of treated groups as compared to respective control groups.

Values of total WBC count, total RBC count, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration (MCHC), platelets and differential leukocyte count (neutrophil, lymphocyte, basophil, eosinophil and monocyte) for male and female wistar rat under safety study are presented in table 1 and 2. Results of various biochemical parameters like glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP), albumin, globulin, creatinine, urea and uric acid of male and female animals of all groups are presented in table 3 and 4. The mean values hematological and biochemical parameters of male and female treated animals for 14 days do not differ significantly ($P < 0.05$) from the corresponding values observed in control animals. There was non significant change in organ weight of treated and control animals. There were no gross pathological changes found at the time of post-mortem of rats of control and treatment group. Sections of liver, kidney, stomach, intestine, heart, spleen, joint cartilage and muscles of injection site were studied from all the 24 rats. There were no changes in cellular

Table-2. Effect of daily intramuscular administration of Moxifloxacin (5 mg/kg) for 14 days on various hematological parameters in female wistar rats.

Parameters	Control (Group-I)			Moxifloxacin (Group-II)		
	Mean ± SEM (n = 6)			Mean ± SEM (n = 6)		
	0 day	7th Day	14th day	0 day	7th Day	14th day
WBC(x103/µl)	5.86 ± 0.28	5.97 ± 0.61	7.41 ± 0.52	6.27 ± 0.47	5.61 ± 0.13	7.14 ± 0.47
RBC(x106/µl)	6.40 ± 0.10	6.13 ± 0.13	6.57 ± 0.15	6.69 ± 0.17	6.60 ± 0.12	6.30 ± 0.28
HGB(g/dl)	13.42 ± 0.24	12.97 ± 0.20	13.72 ± 0.17	13.47 ± 0.25	13.40 ± 0.20	12.78 ± 0.61
HCT(%)	42.55 ± 0.74	40.97 ± 0.67	43.08 ± 0.45	42.87 ± 1.04	42.42 ± 0.78	39.92 ± 1.86
MCV(fl)	66.48 ± 0.72	66.88 ± 0.86	65.67 ± 0.85	64.12 ± 1.10	64.3 7± 1.12	63.35 ± 1.02
MCH(pg)	20.95 ± 0.20	21.17 ± 0.19	20.87 ± 0.22	20.20 ± 0.32	20.38 ± 0.37	20.30 ± 0.39
MCHC(g/dl)	31.50± 0.15	31.65 ± 0.20	31.82 ± 0.11	31.47 ± 0.21	31.63 ± 0.17	32.03 ± 0.18
Platelets(x103/µl)	1007.67 ± 30.01	1028.83 ± 24.99	995.00 ± 84.25	960.67 ± 39.69	1015.00 ± 40.25	924.17 ± 120.64
Neutrophil (%)	13.33 ± 1.12	10.93 ± 1.22	11.41 ± 1.56	11.67 ± 1.37	15.77±1.62	13.21 ± 2.48
Lymphocyte (%)	81.95 ± 1.00	82.50 ± 1.89	79.63 ± 1.85	84.22 ± 2.36	78.77 ± 1.88	81.43 ± 2.28
Monocyte(%)	1.88 ± 0.39	3.59 ± 0.83	5.93 ± 0.97	1.82 ± 0.77	2.71 ± 0.68	3.55 ± 0.53
Eosinophil(%)	1.69 ± 0.39	1.37 ± 0.13	1.08 ± 0.15	1.29 ± 0.18	1.38 ± 0.13	0.79 ± 0.13
Basophil(%)	1.13 ± 0.17	1.60 ± 0.21	1.94 ± 0.31	1.04 ± 0.27	1.36 ± 0.16	1.24 ± 0.07

structures and no other abnormal microscopic lesions were found in treated rats from the control rats. It indicates that repeated intramuscular administration of moxifloxacin in male and female rats were well tolerated.

Results of our study were supported by report of non significant change in hematological and biochemical parameters following repeated administration of Ciprofloxacin in calves, Enrofloxacin in yak, Levofloxacin in layer birds and Moxifloxacin in human (Stass et al., 1998; Bhavsar et al., 2004; Khargharia et al., 2007; Patel et al., 2009). There were no adverse effects on body weight, food and water consumption, necropsy findings and histopathology following repeated oral administration of Fandofloxacin (125 mg/kg/day) in rats (Kim et al., 2003). Similarly no necropsy and histopathological alteration were reported following repeated oral administration of Levofloxacin (10 mg/kg at 12 h interval) in layer birds (Patel et al., 2009). In contrast, significant change in hematological and biochemical parameters following repeated administration Fandofloxacin in rats, Sinofloxacin in rats and dogs and Moxifloxacin in rats, mice and dogs were reported (Keutz and Schluter, 1999; Kim et al., 2003; Lu et al., 2008).

References

1. Bhavsar, S.K., Verma, M.P. and Thaker, A.M. (2004). Pharmacokinetics, tissue concentration and safety of multiple dose Intravenous administration of ciprofloxacin in cow calves. *J. Vet. Pharmacol.Toxicol*., 3 (1): 27-34.
2. Dalhoff, A., Petersen, U., and Endermann, R. (1996). Invitro activity of BAY 12-8039, a new 8-methoxyquinolone. *Chemotherapy.*, 42: 410-425.
3. Keutz, E.V. and Schluter, G. (1999). Preclinical safety evalution of Moxifloxacin, a novel fluoroquinolones. *Journal of antimicrobial Chemotherapy*., 43: 91-100.
4. Khargharia, S., Barua, C.C., Nath, N. and Bhattachrya, M. (2007). Blood Biochemical Studies of Enrofloxacin in Yak after Intravenous Administration. *Iranian J. Pharmacol. Therap.*, 6:137-138.
5. Kim, J.C., et.al. (2003). 26-week repeated oral dose toxicity study of the new quinolone antibacterial DW-

Table-3. Effect of daily intramuscular administration of Moxifloxacin (5 mg/kg) for 14 days on various biochemical parameters in male wistar rats.

Parameters	Control (Group-I)			Moxifloxacin (Group-II)		
	Mean ± SEM (n = 6)			Mean ± SEM (n = 6)		
	0 day	7th Day	14th day	0 day	7th Day	14th day
Glucose(mg/dl)	124.67 ± 2.41	141.55 ± 6.85	112.42 ± 3.90	132.17 ± 7.91	121.02 ± 5.37	124.08 ± 9.09
AST (U/L)	164.60 ± 6.91	194.93 ± 19.50	306.77 ± 38.97	146.83 ± 7.24	222.40 ± 20.91	192.25 ± 15.30
ALT (U/L)	31.38 ± 1.20	40.82 ± 0.57	54.88 ± 5.15	33.25 ± 1.44	48.40 ± 4.13	44.78 ± 1.82
ALP(U/L)	380.37 ± 34.51	311.18 ± 42.59	289.23 ± 32.56	424.93± 53.18	351.97± 32.77	331.62± 37.87
Total Bilirubin (mg/dl)	0.23 ± 0.03	0.11 ± 0.04	0.03 ± 0.02	0.18 ± 0.03	0.12 ± 0.01	0.03 ± 0.01
Total Protein (g/dl)	5.97 ± 0.08	6.27 ± 0.08	6.70 ± 0.10	5.82 ± 0.09	6.27 ± 0.11	6.55 ± 0.05
Albumin (g/dl)	3.72 ± 0.04	3.87 ± 0.05	3.95 ± 0.05	3.65 ± 0.04	3.78 ± 0.04	3.90 ± 0.05
Globulin(g/dl)	2.25 ± 0.06	2.40 ± 0.04	2.75 ± 0.07	2.17 ± 0.05	2.48 ± 0.08	2.65 ± 0.02
Creatinine (mg/dl)	0.45 ± 0.01	0.65 ± 0.10	0.64 ± 0.04	0.54 ± 0.08	0.58 ± 0.01	0.60 ± 0.06
Urea(mg/dl)	58.62 ± 3.82	54.72 ± 1.77	56.13 ± 2.97	54.68 ± 1.88	56.20 ± 1.34	56.47 ± 1.87
Uric acid(mg/dl)	0.95 ± 0.02	1.32 ± 0.15	1.46 ± 0.03	1.17 ± 0.15	1.46 ± 0.12	1.65 ± 0.10

Table-4. Effect of daily intramuscular administration of moxifloxacin (5 mg/kg) for 14 days on various biochemical parameters in female wistar rats.

Parameters	Control (Group-I)			Moxifloxacin (Group-II)		
	Mean ± SEM (n = 6)			Mean ± SEM (n = 6)		
	0 day	7th Day	14th day	0 day	7th Day	14th day
Glucose(mg/dl)	122.10 ± 3.86	127.80 ± 8.64	105.82 ± 5.06	117.95 ± 2.99	120.55 ± 4.63	121.17 ± 5.56
AST (U/L)	148.17 ± 15.96	168.82 ± 20.32	277.67± 15.01	163.80 ± 11.01	220.13 ± 16.83	301.52 ± 31.23
ALT (U/L)	26.07 ± 2.15	33.72 ± 1.34	42.75 ± 2.86	26.13 ± 1.78	37.15 ± 3.70	44.93 ± 4.06
ALP (U/L)	311.83 ± 36.07	231.62 ±38.25	231.72 ± 29.89	251.37 ± 30.41	201.42 ± 22.13	202.48 ± 20.56
Total Bilirubin (mg/dl)	0.06 ± 0.02	0.11 ± 0.03	0.00 ± 0.00	0.07 ± 0.02	0.13 ± 0.03	0.02 ± 0.02
Total Protein(g/dl)	6.17 ± 0.06	6.38 ± 0.07	6.83 ± 0.04	6.03 ± 0.04	6.37 ± 0.10	6.70 ± 0.10
Albumin (g/dl)	3.93 ± 0.06	3.97 ± 0.05	4.05 ± 0.04	3.88 ± 0.03	3.98 ± 0.06	4.08 ± 0.09
Globulin (g/dl)	2.23 ± 0.03	2.42 ± 0.06	2.78 ± 0.02	2.15 ± 0.03	2.38 ± 0.07	2.62 ± 0.03
Creatinine (mg/dl)	0.44 ± 0.01	0.56 ± 0.01	0.62 ± 0.01	0.45 ± 0.01	0.61 ± 0.01	0.63 ± 0.01
Urea(mg/dl)	56.78 ± 2.68	53.77 ± 1.05	48.78 ± 0.76	53.92 ± 2.85	60.47 ± 3.96	56.57 ± 3.08
Uric acid(mg/dl)	1.04 ± 0.16	1.16 ± 0.10	2.54 ± 0.28	0.95 ± 0.07	1.40 ± 0.21	2.16 ± 0.26

116 in prague–Dawley rats. *Food and Chem. Toxicol.*,41(5): 637-645.

6. Lu, G.C., She, J.H., Jiang, H., Li, Z.Y. and Yuan, B.J. (2008). Sixty-day repeated dose toxicity of sinafloxacin in rats and dogs. *Food and chemical toxicology*., 46(2): 575-580.
7. Patel, J.H., et.al.(2009). Safety level of levofloxacin following repeated oral administration in White Leg Horn layer birds. *Veterinary world*., 2 (4): 137-139.
8. Stass, H., Dalhoff, A., Kubitza, D., and Schuhly, U. (1998). Pharmacokinetics, Safety, and Tolerability of Ascending Single Doses of Moxifloxacin, a New 8-Methoxy Quinolone, Administered to Healthy Subjects. *Antimicrobial agents and Chemotherapy.*, 42(8): 2060-2065.

* * * * * * * *