Annals of Phytomedicine 6(2): 88-92, 2017
DOI: 10.21276/ap.2017.6.2.8; Print ISSN : 2278-9839 and Online ISSN : 2393-9885
Ann. Phytomed., 6(2): 88-92 (2017)

Original article

Safety profile of marbofloxacin following repeated intramuscular administration alone and piperine pretreated rats

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Received September 5, 2017: Revised October 27, 2017: Accepted November 7, 2017: Published online December 30, 2017

Abstract

A safety evaluation of marbofloxacin was carried out following repeated intramuscular (5 mg/kg of body weight for 5 days) administration alone and piperine pretreated (10 mg/kg, P.O. for 5 days) rats. The blood samples were collected into sterile K_3EDTA and non-heparinized test tube on 0 day (before treatment) and 6th day (after treatment) monitored by estimation of various haemato-biochemical parameters.The average values of hematological parameters, *viz*., HB, PCV, TLC, TEC, DLC, MCV, MCH and MCHC were estimated before and after drug administration. The average values of blood biochemical (ALT, AST, Total protein, Albumin, Globulin, LDH, Creatinine, BUN, Bilirubin and AKP) parameters were measured before and after drug administration. The values of HB, PCV, eosinophil and monocytes were found be significant (p<0.05) when compared to before treated values. No significant alterations in mean values of all serum biochemical parameters were observed after treatment compared to (0 day) before treatment. All the rats were found no clinical signs and others adverse reactions.It is concluded that marbofloxacin following repeated intramuscular alone and piperine pretreated rats did not show major significant alterations in the haemato-biochemical parameters. Marbofloxacin was found safe and well tolerated antibacterial agents to be used against bacterial infections.

Key words: Marbofloxacin, piperine, rats

1. Introduction

Marbofloxacin is a fluorinated quinolone for exclusive use in veterinary medicine (Brown, 1996). It is a bactericidal agent having broad spectrum of antimicrobial activity against gram-negative, some gram-positive bacteria and mycoplasma spp. *In vivo* and *in vitro* efficacy against *Staphylococcus intermedius*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas spp*, *Pasteurella multocida*, and *Mannheima haemolytica* have been reported (Spreng *et al.*, 1995; Shojaee and Lees, 1997). Pharmacokinetic and dynamic properties of marbofloxacin make favors its useful for the treatment of respiratory, urinary and skin infections in domestic animals (Brown,1996; Ihrke *et al.*, 1999). Its spectrum of activity and pharmacokinetic profile suggest good tissue penetration, making the drug, a suitable for dermatological and pulmonary infections. Therapeutic efficacy of marbofloxacin largely depends on the optimum dosage regimen and safety impact. Report on safety profile of marbofloxacin are scanty. Therefore, the present study was planned with the objective of hematological and biochemical parameters evaluation after intramuscular administration of marbofloxacin alone and piperine pretreated in rats.

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2. Materials and Methods

2.1 Experimental animal

The present study was conducted on 12 Wister albino rats obtained from Cadila Pharmaceutical Pvt. Ltd., Ahmedabad, Gujarat, India. This study (Protocol No. JAU/JVC/IAEC/SA/08/2016) was approved by Institutional Animal Ethics Committee, College of Veterinary Science and Animal Husbandry, Junagadh working under the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guideline. All the animals were kept in Laboratory Animal House, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, JAU, Junagadh, Gujarat, India.

2.2 Animal husbandry

The rats were housed in standard polypropylene cages with stainless steel top grill. During entire study period, the animals were housed in the cool environment (23^0 to 26^{0}C temperature) with relative humidity ranged between 40 to 55%. Twelve hour dark and light cycle was maintained in animal room. Rat pelleted feed (VRK Biological System, Vadodara, Gujarat, India) containing 18% protein and distilled water were provided *ad libitum* to animals throughout the study period. All albino rats were randomly selected for the study.

2.3 Experimental design and drug administration

Twelve albino rats were divided in two groups as group I and II of marbofloxacin alone and piperine pretreated rats, respectively.

Marbofloxacin was administered at the dose of 5 mg/kg weight intramuscularly in deep quadriceps muscle using tuberculin syringe (dose volume was 0.15 to 0.25 ml). The piperine was administered orally by graduating syringe with intubation cannula at the dose of 10 mg/kg body weight.

2.4 Collection of samples

Blood samples from rats were collected from retro-orbital sinus under light anesthesia into sterile K_3EDTA and non-heparinized test tube at 0 day (before drug administration) and 6th day (after treatment) for haematological and serum biochemical analysis. Blood smears for determination of differential leukocyte count (DLC) were prepared from fresh blood at the time of blood collection. Blood samples (1 ml) collected in K_3EDTA test tubes were utilized for haematological evaluation and those collected in non-heparinized test tubes (1 ml) were allowed to clot at room temperature. Serum was separated after centrifugation (1200 g for 10 min.). The serum samples were transferred to cryo-vials and then stored at −20ºC until assayed for enzyme estimation.

2.5 Hematological and biochemical analysis

Hematological parameters, *viz*., hemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), differential leukocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were analyzed by using automated hematology analyzer with ready to use suitable kits. Whereas, DLC was carried out as per method described by Schalm (1967). Biochemical parameters, *viz*., serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum lactate dehydrogenase (LDH), serum alkaline phosphatase (AKP), serum creatinine, serum bilirubin (total), blood urea nitrogen, total protein, albumin and globulin were estimated (Biosystems S.A., Barcelona, Spain) by automatic biochemistry analyzer using suitable kits.

2.6 Statistical analysis

All data obtained were presented as mean ± Standard error (SE). Data were analyzed statistically by student's t-test to observe difference among the treatment groups (Snedecor and Cochran, 1980).

3. Results and Discussion

The safety study of marbofloxacin in rats has not been reported but safety profile of other fluoroquinolones like ciprofloxacin, enrofloxacin and levofloxacin was investigated in different species of animal. Safety impact of marbofloxacin following repeated intramuscular administration given at the rate of 5 mg.kg^{-1} of body weight in rats for before and after treatment days was monitored by studying various hematological parameters. Various hematological indices were determined and presented in Table 1.The values of all parameters observed at 0thday were compared with values observed on the 6th day. The values of hematological parameters were no significant differences found in group I and presented in Figure 1. The values of HB, PCV, eosinophil and monocyte were found significant alterations in post treatment when compare to before treated values of the same parameters in piperine pretreated group II (p<0.05) and remaining hematological parameters unaffected were depicted graphically in Figure 2.

Results of the study are supported by report of non-significant change in hematological parameters following daily intramuscular administration of marbofloxacin (2 mg/kg) and trovafloxacin (3 mg/kg) for five days in sheep (Mahmood and Hussein, 2013). The same conclusion was also obtained for long acting moxifloxacin in sheep (Modi *et al*., 2013a). Sadariya *et al*. (2010) found no significant difference alteration in hematological parameters after repeated intra muscular administration of moxifloxacin (5.0 mg/kg) at 24 h interval for 14 days in male and female wistar rats. Patel *et al*. (2014) reported significant alteration in the values of neutrophils, lymphocytes, total erythrocytes and haemoglobin, but within normal clinical range following single intravenous administration of marbofloxacin at 2.0 mg/kg in sheep. Kamble *et al*. (2017) observed no significant alterations in hematological parameters in sodium flouride induced toxicity in wistar rats.

The mean values of serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum lactate dehydrogenase (LDH), serum alkaline phosphatase (AKP), serum creatinine, serum bilirubin (total), blood urea nitrogen, total protein, albumin and globulin were estimated before on 0 day and on 6th day. All groups were no significant alterations (p<0.05) in all serum biochemical parameters (Table 2).

It is evident that of biochemical parameters observed before treatment and after treatment did not offer significant (p<0.05). Results of the study are supported by report of non-significant change in biochemical parameters following daily intramuscular administration marbofloxacin (2 mg/kg) and trovafloxacin (3 mg/kg) for five days in sheep (Mahmood and Hussein, 2013). Sadariya *et al*. (2010) found no significant alteration in serum biochemical parameters after repeated intramuscular administration of moxifloxacin (5.0 mg/kg) at 24 h interval for 14 days in male and female wistar rats. Other fluoroquinolones like ciprofloxacin when given repeated in calves at intramuscular dose in calves did not cause any significant alteration in the values of biochemical parameters (Bhavsar *et al.*, 2004). The same conclusion was also obtained for enrofloxacin in yak (Khargharia *et al.*, 2007) and long acting moxifloxacin in sheep (Modi *et al*., 2013b). Fluoroquinolones as a class are generally well tolerated; most adverse effects are mild in severity, self-limiting and rarely result in treatment discontinuation (Ball, 1999; Mandell and Tillotson, 2002).

4. Conclusion

Repeated intramuscular administration of marbofloxacin at the dose rate of 5 mg/kg body weight in rats was found safe and well tolerated. Lack of clinical signs of adverse reactions, toxicity and absence of significant biochemical and hematological alteration following intramuscular administration may be open a new path for insight into the approach for treatment of various bacterial diseases in rats and animals. In future the drug may be potential candidates to be used in the treatment of infectious diseases in animals.

Table 1: Effect of daily administration of marbofloxacin following intramuscular (5 mg/kg of body weight for 5 days) alone and piperine pre-conditioning (10 mg/kg, P.O. for 5 days) on mean values of hematological parameters in rats

Parameters	Day of collection	Treatment groups	
		Marbofloxacin alone group-I	Piperine pre-treated + marbofloxacin group-II
HB (g/dL)	0 day	14.28 ± 0.63	16.05 ± 0.15
	6 day	14.87 ± 0.50	14.45 ± 0.13**
PCV (%)	0 day	43.12 ± 0.81	51.15 ± 0.61
	6 day	45.87 ± 1.58	44.95 ± 0.63**
TEC (10^6/cmm)	0 day	8.59 ± 0.18	10.06 ± 0.14
	6 day	8.95 ± 0.33	8.86 ± 0.17**
WBC(10^3/cmm)	0 day	12.14 ± 0. 70	12.94 ± 0.49
	6 day	11.39 ± 1.39	12.66 ± 0. 87
Neutrophils (%)	0 day	18.33 ± 2.22	21.00 ± 2.54
	6 day	17.83 ± 1.89	20.00 ± 1.86
Basophil (%)	0 day	0.00 ± 0.00	0.17 ± 0.17
	6 day	0.17 ± 0.17	0.17 ± 0.17
Eosinophil (%)	0 day	0.67 ± 0.21	0.83 ± 0.31
	6 day	0.67 ± 0.21	0.50 ± 0.22*
Lymphocyte (%)	0 day	77.17 ± 2.88	71.83 ± 3.29
	6 day	77.17 ± 2.12	75.50 ± 2.20
Monocytes (%)	0 day	3.83 ± 0.79	6.50 ± 0.89
	6 day	4.17 ± 0.65	3.83 ± 0.48*
MCV (fl)	0 day	50.17 ± 0.40	50.83 ± 0.65
	6 day	51.33 ± 0.42	50.83 ± 0.48
MCHC (%)	0 day	33.13 ± 1.20	31.37 ± 0.56
	6 day	32.43 ± 0.17	32.02 ± 0.25
MCH (pg)	0 day	16.63 ± 0.57	15.97 ± 0.27
	6 day	16.62 ± 0.19	16.23 ± 0.16

*Significant at $p<0.05$, **Highly significant at $p<0.01$ when compared with respective values of normal rats.

Table 2: Effect of daily administration of marbofloxacin following intramuscular (5 mg/kg of body weight for 5 days) alone and piperine pre-conditioning (10 mg/kg, P.O. for 5 days) on mean values of biochemical parameters in rats

Parameters	Day of collection	Treatment groups	
		Marbofloxacin alone group-I	Piperine pre-treated + marbofloxacin group-II
ALT (IU/L)	0 day	42.69 ± 3.17	50.69 ± 5.48
	6 day	44.83 ± 3.42	61.12 ± 4.85
AST (IU/L)	0 day	141.36 ± 25.88	116.96 ± 14.77
	6 day	140.66 ± 9.24	152.75 ± 10.33
Total protein(g/dL)	0 day	7.26 ± 0.04	7.45 ± 0.10
	6 day	7.46 ± 0.11	7.38 ± 0.06
Albumin (g/dL)	0 day	3.61 ± 0.05	3.68 ± 0.04
	6 day	3.70 ± 0.03	3.68 ± 0.04
Globulin (g/dL)	0 day	3.66 ± 0.04	3.77 ± 0.08
	6 day	3.75 ± 0.72	3.71 ± 0.04
LDH (IU/L)	0 day	907.70 ± 121.14	655.90 ± 136.8
	6 day	825.75 ± 147.80	530.98 ± 94.05
Creatinine (mg/dL)	0 day	0.39 ± 0.02	0.44 ± 0.02
	6 day	0.46 ± 0.05	0.46 ± 0.02
Total Bilirubin (mg/dL)	0 day	0.13 ± 0.06	0.22 ± 0.01
	6 day	0.08 ± 0.04	0.14 ± 0.04
BUN (mg/dL)	0 day	17.28 ± 2.01	18.10 ± 1.23
	6 day	22.47 ± 2.87	18.07 ± 0.69
AKP (IU/L)	0 day	17.37 ± 3.82	63.80 ± 49.33
	6 day	60.32 ± 42.22	21.85 ± 8.39

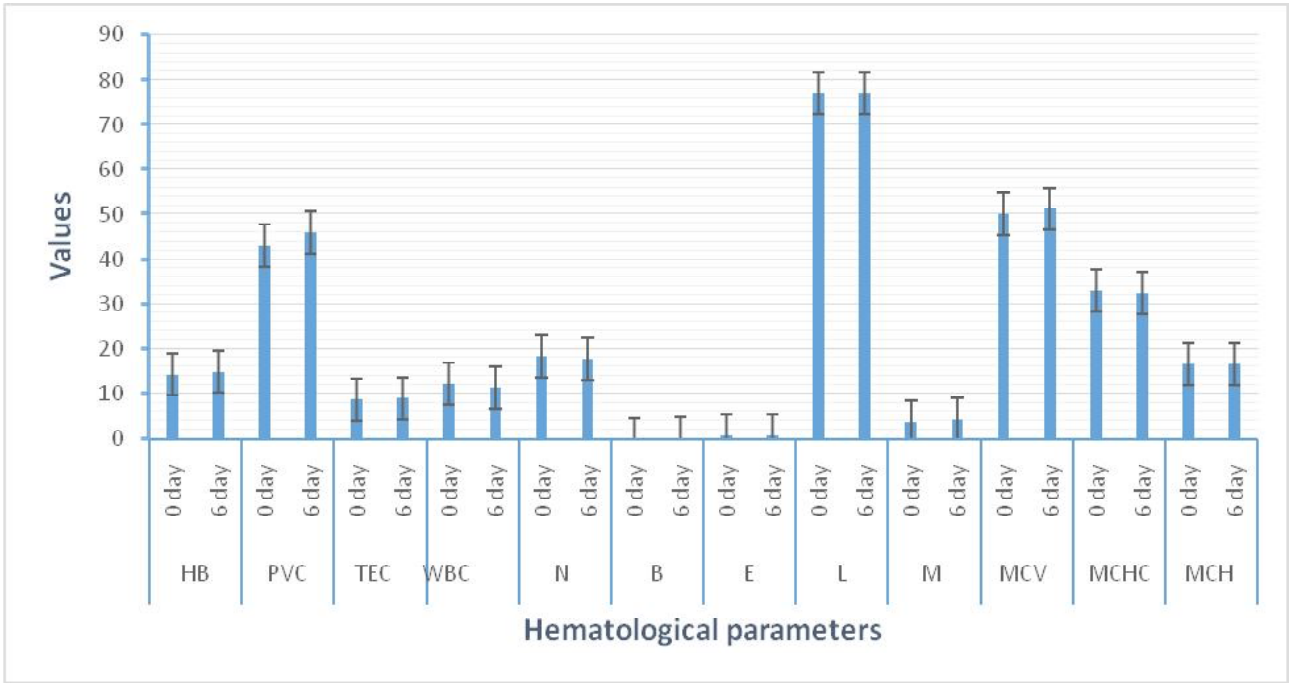

Figure 1: Effect of repeated administration of marbofloxacin following intramuscular on mean values of hematological parameters in rats.

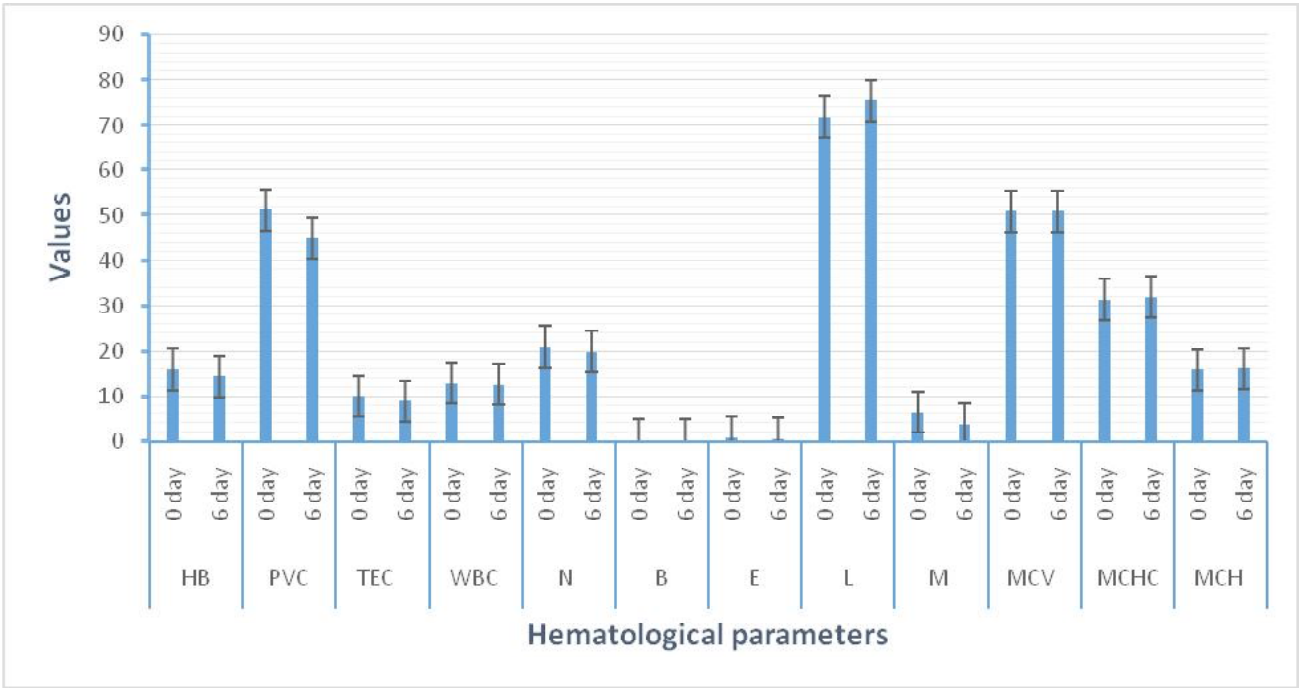

Figure 2: Effect of pre-conditioning piperine on marbofloxacin following intramuscular administration on mean values of hematological parameters in rats.

Conflict of interest

We declare that we have no conflict of interest.

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