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Renal safety evaluation of marbofloxacin: Ornidazole combination in sheep

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Abstract
A renal safety evaluation of combination of antimicrobial drugs *viz*. marbofloxacin and ornidazole was studied to assess clinical impact of their intravenous administration on various renal parameters in sheep. Six healthy male sheep were treated with combination of marbofloxacin (2.0 mg/kg body weight) and ornidazole (20 mg/kg body weight). The urine samples before and after intravenous drug administration of combination at different time intervals (6 h, 12 h, 1, 2, 3, 4, 5, 6 and 7th day after treatment) were collected and subjected to physical and chemical analysis and microscopic examination. Physical parameters including colour, odour, transparency and pH were studied. The chemical analysis included the assessment of glucose, ketones, proteins, bilirubin *etc*. The values of all the parameters before and after drug administration were estimated and compared for any alteration using statistical techniques of t-test of significance. The statistical comparison of physical and chemical parameters before and after treatment did not reveal any significant alteration and no animals showed any signs and symptoms of urinary discomfort. No microscopic abnormality was detected in urine sediments. The study indicates the intravenous administration of marbofloxacin and ornidazole combination is well tolerated by sheep, in terms of renal safety, at prescribed dosage.

Keywords: Marbofloxacin, ornidazole, renal safety profile, sheep

Introduction
Marbofloxacin, a third-generation fluoroquinolone, is exclusively developed antimicrobial drug for veterinary use (Meunier *et al.*, 2004) [1]. Marbofloxacin, having favourable pharmacokinetics parameters as well as low MIC values for target organisms, is approved for use in veterinary species *viz.*, cattle, pigs, dogs, horses and cats for respiratory, urinary tract, soft tissue and dermatological infections (Cotard *et al.*, 1995, Thomas *et al.*, 2001) [2, 3]. Ornidazole is a 5-nitroimidazole derivative anti-protozoal drug active against protozoa and anaerobic bacteria. It is converted to reduction products that interact with DNA to cause destruction of helical DNA structure and strand leading to protein synthesis inhibition and cell death in susceptible organisms. It is extensively used in hepatic and intestinal amoebiasis, giardiasis, anaerobic infections, prophylaxis of postoperative anaerobic bacterial infections, trichomoniasis of uro-genital tract and bacterial vaginitis (Mukherjee and Boshoff, 2011) [4].
The clinical occurrence of mixed infections in gastrointestinal tract, urinary tract, reproductive tracts and other organs caused by bacteria and protozoa is common, and use of single antimicrobial, many times proves ineffective therapeutically. Such mixed infections need use of combination of antimicrobials and anti-protozoal drugs to cover the extended spectrum of pathogens. The combination of fluoroquinolone with other antimicrobial drugs is of great clinical utility particularly in case of mixed infections involving Gram negative, Gram positive and anaerobic organism. One such promising combination is marbofloxacin and ornidazole. Such type of combinations are extensively used in human medicine and got the wider popularity among clinical community. The safety of such combination is of prime importance to be evaluated before clinical uses to exploit its therapeutic benefits in clinical veterinary medicine. The reports of renal safety of combination of marbofloxacin and ornidazole combination are not available in veterinary species, limiting the clinical use of this combination for treatment of infections. Looking to paucity of such scientific information, the present study was conducted to explore the renal safety of combination of marbofloxacin and ornidazole following single intravenous administration in sheep.

Materials and Methods

Experimental animals

Experiment was conducted in six healthy male Patanwadi sheep aged between 1 to 1.5 years and having body weight between 30 to 38 kgs, after getting approval of research protocol by Institutional Animal Ethics Committee (IAEC). All the animals were kept in individual pan of experimental sheep shed at Livestock Research Station, Sardarkrushinagar (Gujarat, India). The animals were subjected to acclimatization period of 15 days during which all the animals were subjected to daily clinical examination in order to exclude possibility of any diseases condition, which otherwise may interfere with results of experiment. The standard farm routine with feeding schedule was followed and access to ad libitum water was provided to all experimental animals to keep them from any stress and discomforts.

Drug administration

Exactly 1 % solution of marbofloxacin was prepared using distilled water and 0.01 M acetic acid solution. Similarly, 0.5 % solutions of ornidazole for intravenous administration were prepared using water for injection in 0.61 % sodium chloride. The miscibility of marbofloxacin solution and ornidazole was checked before administration. The ornidazole and marbofloxacin prepared were mixed in required quantity and administered intravenously as a combination at the dose rate of 2.0 and 20.00 mg/kg body weight, respectively.

Urine sample collection

Normal voided urine samples were collected from all animals before drug administration. The perineum area was clipped and shaved and sterilized with 70% alcohol swab. The urinary catheter (sterile AI sheath) was inserted in to urethral opening after proper lubrication and antiseptic application. The urine samples were collected in glass tubes at the designated time intervals *i.e.* 6 h, 12 h, 1, 2, 3, 4, 5, 6 and 7th day after treatment.

Physico-chemical analysis

Following parameters were selected for physical (colour, odour, transparency and turbidity, specific gravity, litmus reaction) and chemical (glucose, bilirubin, blood/RBCs, urobilinogen and protein) examination of urine. Reagent strips (*Uriscan urine strips*) were used to perform several semi-quantitative evaluations (urine pH, protein, glucose, ketones, bilirubin/ urobilinogen, and occult blood) simultaneously. The strips were kept in air tight bottle to avoid effect of moisture (Oyaert and Delanghe, 2019) [5]. The scientific basis of physico-chemical analysis and reagents required are described in the Table 1.

Table 1: Scientific basis and reagent required for physico-chemical analysis of urine

Sr. No.	Physico-chemical Parameter	Basis of test	Reagent Required
1.	Specific gravity	This test is based on apparent pKa change of certain pre-treated polymeric ion exchange resin in relation to ionic concentration. In the presence of an indicator, colours ranges from deep blue – green in urine of low ionic concentration through green and yellow green in urine of increasing concentration.	Bromothymol blue 5.6 mg
2	pH	The test is based on the double indicator principle that gives a broad range of colours covering entire urinary pH range. Colour changes from orange through yellow and green blue.	Methyl red 0.03 mg Bromothymol blue 0.50 mg
3	Glucose	The test is based on a double sequential enzyme reaction. One enzyme, glucose oxidase, catalyzes the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. A second enzyme, peroxidase, catalyzes the reaction of hydrogen peroxide with a potassium iodide chromogen to oxidise the chromogen to colors ranging from green to brown.	Glucose oxidase 460 unit Peroxidase 2100 unit Potassium iodide 13.9 mg
4	Bilirubin	This test is based on the coupling of bilirubin with diazotized dichloroaniline in a strongly acid medium. The colour range from various shades of pink to violet.	Sodium nitrite 0.733 mg
5	Ketone	This test is based on the development of colour ranging from blue pink, for a negative reading, to purple/marron when acetoacetic acid reacts with nitroprusside.	Sodium nitroprusside 10.0 mg
6	Blood	This test is based on the peroxidase –like activity of haemoglobin, which catalyzes the reaction of organic hydroperoxide and chromogen. The resulting color ranges from yellow through green dark blue. The appearance of green spots on reagent area indicates presence of intact RBC in urine.	3,3'5,5'tetramethyl benzidine 2.8 mg Cumene hydroperoxide 23.6 mg
7	Protein	The test is based on the color changes of indicator tetrabromophenol blue in the presence of protein. A positive reaction is indicated by a color change to yellow/green.	Tetrabromophenol blue 0.2 mg
8	Urobilinogen	This test is based on a modified Ehrlich reaction, in which p-diethylaminobenzaldehyde reacts with urobilinogen in strongly acid medium to produce color from pink to dark pink.	p-diethyl aminobenzaldehyde 14.583 mg

Microscopic examination

Urine samples were also examined microscopically for presence of crystals after centrifugation at 3000 RPM for 15 minutes. The sediments were observed under microscope.

Statistical analysis

Safety data generated after urine analysis were subjected to test for statistical significance using paired t-test. SPPS ver. 16.0 was employed for statistical analysis.

Result and Discussion

The results of present study provide insight into the safety profile of combination and provides therapeutic base for using such novel combination in sheep suffering from infectious diseases. There was no alteration observed in the colour, odour, transparency, turbidity, specific gravity and pH of urine in sheep before and after single dose intravenous administration of combination of marbofloxacin (2.0 mg/Kg) and ornidazole (20 mg/Kg) in sheep. The values of specific gravity and Urine pH are presented in Table 2. There was no

presence of any abnormal chemical constituents in urine of treated sheep. None of animals showed any signs related to urinary discomfort and urine frequency was found to be normal in all treated animal before and after drug administration. No microscopic abnormality was detected in urine sediments.

Table 2: Effect of single dose intravenous administration of combination of marbofloxacin and ornidazole on urine specific gravity and pH in sheep

Collection Time point	Specific gravity	Urine pH
0 day	1.02±0.003	7.83±0.167
6 h	1.02±0.004	8.33±0.211
12 h	1.02±0.004	7.83±0.167
1 day	1.02±0.003	8.00±0.258
2 day	1.02±0.002	7.83±0.167
3day	1.02±0.003	8.00±0.00
4 day	1.02±0.003	7.83±0.167
5 day	1.01±0.002	7.83±0.307
6 day	1.02±0.004	8.17±0.167
7 day	1.01±0.002	8.17±0.167

[#]No statistical difference was noted

Fluoroquinolone drugs like marbofloxacin being hydrophilic in nature and having low protein-binding, show rapid and extensive distribution. Low protein bindings greatly increase the drug excretion, as only unbound fraction is excreted *via* kidneys through the processes like glomerular filtration and active tabular secretion. The high value of marbofloxacin: creatinine clearance ratio (7.85) indicates that the both mechanisms glomerular filtrations as well as active tubular secretion are involved in the marbofloxacin excretion from the body (Munawar *et al.*, 2017) [6]. The elimination of ornidazole also depends on renal route of excretion. Thus, the kidneys being the major organ involved in excretion of marbofloxacin and ornidazole is subjected to risk of toxicity and adverse effects. Urinalysis is an important laboratory test that can be readily performed in veterinary practice and is considered part of a minimum database to revel functional status of kidneys. The specific gravity of the urine reflects the concentrating or diluting ability of the kidney (Benjamin, 1985) [7]. In present study, the colour (pale yellow to deep yellow) and odour (slight ammonic) of the urine samples were of normal in nature. In healthy sheep, specific gravity is variable ranging from 1.015-1.045 (average 1.030), depending on fluid and electrolyte balance of the body (Reece, 2009) [8]. In the present study, the value of specific gravity before and after drug administration showed non-significant difference and remains unaffected by intravenous administration of combination of marbofloxacin and ornidazole in sheep (Table 2).

The urinary pH range for the cow, sheep, goat, and horse is ~7.9±0.5. The diet of the animal is the major determinant of urinary pH levels. The pH level of urine is very variable and can range from as low as ~4.5 in cases of extreme acidosis to 8.5 in cases of extreme alkalosis. At the minimum pH level of ~4.5, urine contains 32 μM H^+, and at pH 6.0, urine contains just 1 μM H^+. In present study the values of pH of urine in all the sheep ranges from 7.83 (0 day) to 8.33 (at 6h) suggesting no any alteration by drug administration (Ryan *et al.*, 2002) [9]. Glucosuria is not present normally in sheep because the renal threshold for glucose is >180 mg/dL in most animals species. Urine samples collected from all sheep treated with combination were also found negative for presence of glucose before and after drug administration. The present study reveals that all animals treated did not show any abnormal presence of chemical constituents in urine including ketones, bilirubin, proteins, blood and haemoglobin. The transparency, turbidity, pH and specific gravity of urine before and after administration of ornidazole remained normal. The safety of marbofloxacin alone has also been documented in sheep based on haematology and plasma biochemistry evaluations by Patel *et al.*, 2014 [10]. Marbofloxacin has shown good tolerance in cattle species (Thomas *et al.*, 2001) [11]. Marbofloxacin has been reported as a very safe drug in other species like dogs suffering with Chronic Kidney Disease (Paradis *et al.*, 2001) [12] and cats (Ishak *et al.*, 2008) [13].

To conclude, the physical, chemical and microscopic examination of urine samples collected before and after single dose intravenous co-administration of marbofloxacin and ornidazole showed non-significant alterations and clinically all the values before and after co-administration of marbofloxacin and ornidazole remained within normal range.

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