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Full Length Research Paper

Pharmacokinetics of mequindox after intravenous and intramuscular administration to goat

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Pharmacokinetics and bioavailability of mequindox were determined after single intravenous (i.v.) or intramuscular (i.m.) administrations of 7 mg/kg body weight (b.w.) to 10 healthy adult goats. Plasma mequindox concentrations were measured by high performance liquid chromatography. Pharmacokinetics were best described by a two-compartment open model and an one-compartment open model for i.v. and i.m. groups, respectively. The elimination half-life and volume of distribution after i.v. and i.m. administrations were statistically different ($t_{1/2\beta}$, 1.8 to 1.5 h, P < 0.05 and V_d, 0.35 to 0.45 L·kg^{-1}, P < 0.05, respectively). Mequindox was rapidly ($t_{1/2a}$, 0.28 h) and almost completely absorbed (F, 99.8%) after i.m. administration. In conclusion, 2~3 times daily i.v. and i.m. administration of mequindox (7 mg/kg b.w.) in goats may be useful in treatment of infectious diseases caused by sensitive pathogens. The plasma disposition kinetics of mequindox in goats is reported for the first time.

Key words: Mequindox, pharmacokinetics, high performance liquid chromatography (HPLC), goats.

INTRODUCTION

Mequindox is a broad-spectrum antibiotic derived from the family of quinoxaline that also includes olaquindox and quinoceton. All three compounds act by inhibiting bacterial DNA synthesis. Olaquindox and quinoceton have been used to suppress intestinal pathogenic micro-organisms (such as *Escherichia coli*) and to promote growth of livestock and poultry (Ai et al*.,* 2003; Wan et al*.,* 2006). The mechanism of growth promotion of these two antimicrobials is generally believed to modify and promote the intestinal microflora functions (Corpet, 1999; Visek, 1978). However, olaquindox has been banned for food premix use in poultry in China due to its greater toxicity compared with quinoceton, and therefore its use has been limited (Wan et al., 2006). Mequindox, with chemical name 3-methyl-2-quinoxalinacetyl-1,4-dioxide, is a novel veterinary drug developed in China and has been mainly used to prevent and treat many intestinal infectious diseases in domestic animals, such as enteritis, alo laxata and swine dysentery (Yuan, 1998). It exhibits excellent broad spectrum antimicrobial activities against many kinds of Gram-negative bacteria such as *E. coli*, *Papanicolaou bacillus*, *Salmonella choleraesuis*, *Salmonella typhimurium* and *Bacillus proteus,* and against several Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus* (Huang et al., 2008; Zhao et al., 1982). Furthermore, mequindox has advantages including high efficiencies, low toxicities, fast absorption, high bioavailability and broad distribution in the body (Huang et al., 2008). Especially, it has an excellent activity against *Treponema hyodysenteriae* (Chen, 2002; Jia, 1998; Qu et al., 1995). Therefore, mequindox has been widely used as both feed additive and parenteral solution to prevent and/or treat infectious diseases in livestock in China. Little information is available about the toxic effects of mequindox despite the fact that high doses of mequindox are associated with oxidative stress and pathological toxicity in the kidney (Huang et al, 2010), thus, a long-term large-scale use of mequindox can cause great toxicity to domestic animals, and there are already many reports about mequindox poisoning in livestock such as swine, cattle, horse and chicken.

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Abbreviations: i.v., Intravenous; **i.m.,** intramuscular; **HPLC,** high performance liquid chromatography; **AIC,** Akaike's Information Criterion.

Consequently, further research of mequindox is in need. Disposition kinetics of both olaquindox and quinoceton have been reported in the literature for many different animal species involved in swine, piglet, chicken, carp etc. (Ai et al., 2003; Zhu et al., 1993; Li et al., 2002, 2005) while only limited information on the pharmacokinetic behavior of mequindox is acquired. Since the pharmacokinetic behavior of antibiotic drugs can be influenced by many factors such as formulation, the route of administration and animal species (Baggot and Mckellar, 1994), the characterization of specific disposition kinetic varies in different formulations and animal species, and is crucial for the appropriate therapeutic use of these agents. This study attempted to estimate the comparative pharmacokinetics of mequindox in goats after i.v. and i.m. administration in order to provide the basis of rational mequindox use in clinical veterinary medicine.

MATERIALS AND METHODS

Reagents

All reagents used during the extraction and analysis were of analytical reagent grade at least. Methanol for preparation of the mobile phase was of high-performance liquid chromatographic (HPLC) grade (Kermel Chemical Reagent Company, Limited, Tianjin, China). Mequindox standards (99.90% purity) and crude mequindox (98.90% purity) were provided by the Chinese Veterinarian Research Institute Medicine Factory of Chinese Academy of Agriculture Sciences (Lanzhou, China).

Animals

Twelve (12) healthy adult goats (6 males) with a mean (± S.D.) weight of 30 ± 6 kg were used. The animals were fasted overnight (approximately 12 h) before administration, and received only water *ad libitum*. All animals were maintained in accordance with the guideline of Regulations for the administration of affairs concerning experimental animals (Chinese State Council, 1988).

Experimental design

During the experiment, goats were randomly allocated into two groups of six animals each. Jugular venous catheters used for sampling and infusion were percutaneously placed and secured in protective bandages wrapped around the animal's neck. Catheter patency was maintained by daily flushing using a normal saline-heparin (10 U/ml) solution. Mequindox of a single dosage of 7 mg/kg b.w. was administered to 2 goat groups by i.v. and i.m., respectively. Blood samples (5.0 mL) were collected before administration (as control samples) and at 5, 10, 15, 30 and 45 min as well as at 1, 2, 3, 4, 5, 6 and 7 h after administration from each tested animal. All plasma samples were separated by centrifugation and stored frozen at -20°C until further analysis.

Analytical method

Frozen plasma samples were thawed at room temperature and then 1.0 ml sample was mixed with 4.0 µl methanol, followed by centrifugation (15 min at 12 000 r/min, 4°C). Supernatants were filtered by 0.45 ml filter, and then injected into a Beckman HPLC system (Los Angeles, USA) for analysis (20 µl for each sample). The HPLC was equipped with two 125 Solvent Module pumps, an injector with a 20-µl loop, a 166 UV-VIS detector was used for the quantification of mequindox. Detection was accomplished by the UV absorption at 261 nm. The reverse phase C18 (4.6 × 250 mm, 5µm particle size) column was used. The mobile phase used was 35% methanol in double-distilled water, and the flow rate was 1.0 ml/min. Chromatographic analysis was performed at ambient temperature.

Calibration curve

One gram (1 g) mequindox standard was weighed and dissolved in 1000 ml methanol to give a stock solution of 1 mg/ml. This solution was used to prepare standards of 0.5, 1, 2, 4, 8 and 16 µg/ml in 0.5 ml drug-free goat plasma. Standard solutions were extracted and analysed in the same way as unknown samples. Calibration curves were obtained by plotting the area of mequindox against the corresponding concentration of mequindox spiked in goat plasma. The HPLC method for mequindox in goat plasma was validated by assessing extraction efficiency and inter- and intra- day reproducibility at concentrations of 0.5, 1.0 and 5.0 µg/ml.

Statistical analysis

The plasma concentration-time profiles in each individual animal were fitted by using residual method (Liu, 2003). The Akaike's Information Criterion (AIC) (Yamaoka et al., 1978) and coefficient of variation were used to select the best model to define the plasma concentration-time data for each animal. For the estimation of pharmacokinetic parameters, the time to reach peak concentration (T_{max}) and peak concentration (C_{max}) were read from the plotted concentration-time curve in each individual animal, the area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity, while volume of distribution (V_d), total body clearance (Cl_B), distribution and elimination half-lives ($T_{1/2ka}$, $T_{1/2ke}$) and bioavailability (F) were calculated according to standard equations (Gibaldi and Perrier, 1982) .

Pharmacokinetic parameters were presented as mean ± S.D. and were statistically compared by the Student's t-test. Mean values were considered significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Mequindox was found to be accurately resolved as a single sharp peak within 4 minutes and the retention time of mequindox were approximately 3.2 min (Figure 1). No interference was found from normal endogenous serum constituents, which indicated that the chromatographic condition was feasible.

The calibration curve prepared from goat plasma spiked with known amounts of drugs was linear between 0.1 and 16.0 µg/ml mequindox. Correlation coefficients of calibration curves were greater than 0.9995. In goat plasma, at three concentrations of 0.5, 1.0 and 5.0 µg/ml, analytical recoveries of mequindox were 100.96 ± 0.02, 95.26 ± 0.12 and 97.98 ± 0.02% (n = 5); intra-day coefficients of variation (n = 5) were 3.74, 2.97 and 3.84%; inter-day coefficients of variation (n = 5) were

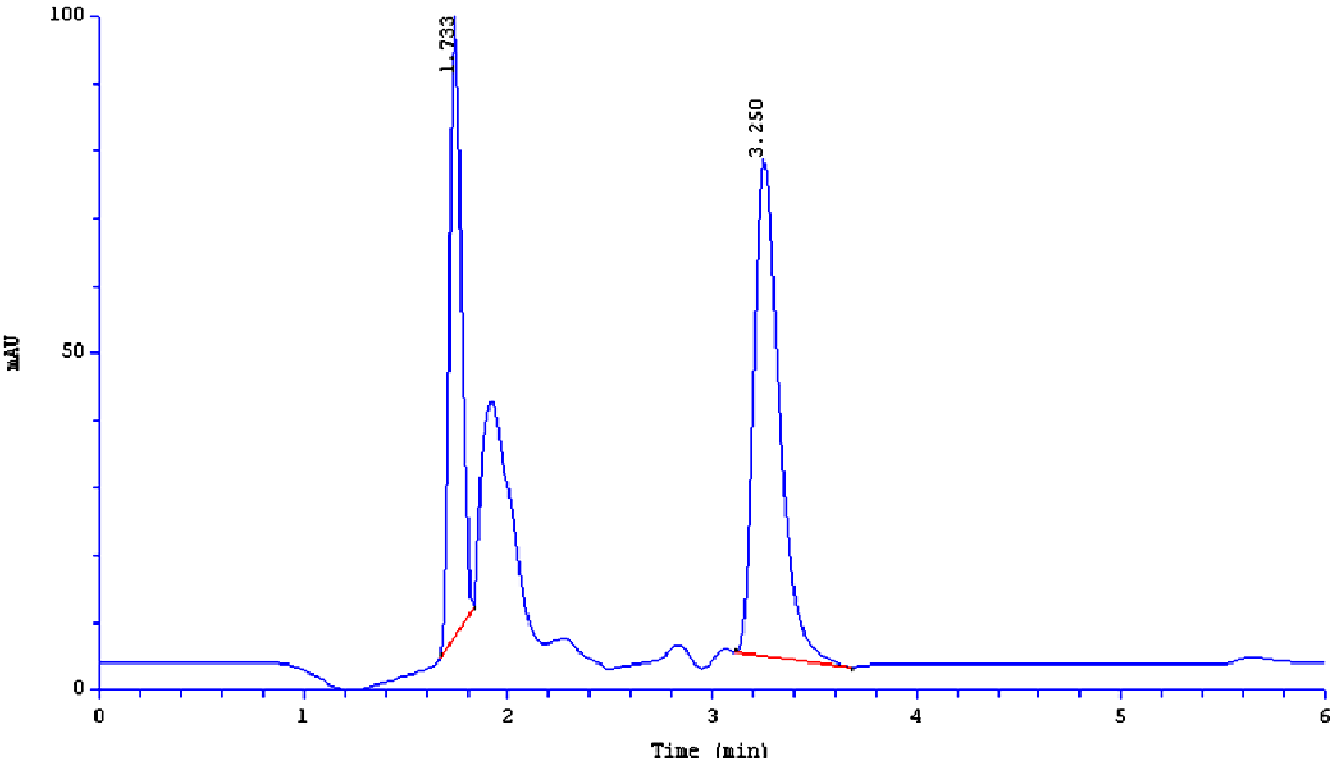

Figure 1. Representative chromatogram of mequindox in a goat plasma sample.

7.98, 4.32 and 7.75%, respectively.

Plots of the predicted mean concentration vs. time is shown in Figure 2. Based on the comparisons of AIC values and the range of R^2 values in one-, two- and three-compartment open model, mequindox concentration in plasma shows a bi-exponential curve after i.v. administration, presenting a phase during which concentrations decrease quickly, followed by a slower decrease phase, meanwhile, indicating the use of a two-compartment open model for analysing the data from i.v. group. In addition, the disposition of the drug after i.m doses can be described adequately by a one-compartment open model.

The plasma concentrations for i.v. and i.m. routes were determined by HPLC (Table 1). The highest initial mequindox plasma concentration after i.v. administration was detected in the first plasma samples taken 5 min post-injection. Table 2 summarizes the mean pharmacokinetic parameters for mequindox obtained after i.v. and i.m. administration to goats according to the compartmental method. After i.v. administration, the initial plasma concentration (C_0) was 14.65 ± 1.28 μg·ml^{-1}, apparent volume of distribution 0.349 ± 0.154 l·kg^{-1} and area under the plasma concentration–time curve from zero to infinity (AUC) 34.459 ± 6.244 μg·ml^{-1}·h. After i.m. administration, maximum plasma concentration (C_{max}) was 11.01 ± 2.94 μg·mL^{-1} and was reached at 0.667 ± 0.129h (t_{max}), terminal half-life ($t_{1/2\beta}$) was significantly shorter in i.m. group (1.494 ± 0.226 h) than for the i.v. group (1.784 ± 0.394 h, $P < 0.05$), the total body clearance (CL_B/F) after i.m. administration (0.21 ± 0.04 L·kg^{-1}·h^{-1}) was similar with the group treated with i.v. injection (0.209 ± 0.039 L·kg^{-1}·h^{-1}), the apparent volume of distribution V_d/F was obtained significantly higher in i.m.group (0.452 ± 0.103 L·kg^{-1}) than that in i.v. group (0.349 ± 0.154 L·kg^{-1}, $P < 0.05$). The bioavailability (F) of mequindox after i.m. administration was 99.8%.

To the best of our knowledge, this is the first investigation of the disposition of mequindox in goat after i.v. and i.m. administration. Mequindox is best fitted to a two-compartment open model following i.v. treatment and is best described by one-compartment open model following i.m. treatment. The drug was found quickly eliminated from goat with a terminal $t_{1/2\beta}$ of 1~2 h after i.v. and i.m. administration. Consequently, it should be considered to minimize the interval time when mequindox is administered to goats. The difference in the elimination half-lives between the two routes (1.78 h with i.v. versus 1.49 h with i.m.) was statistically different ($P < 0.05$), which may have contributed to a larger V_d value following i.m.. In the present study, the absolute bioavailability of i.m. mequindox was 99.8%, which indicates that i.m. achieves plasma drug concentrations equivalent to or even somehow better than i.v.. This is rare, but still could be a reasonable phenomenon in pharmacokinetics study. This could possibly explained that, in the i.v. administration process, the body fluid in the related tissues played a role similar to drug absorption enhancer.

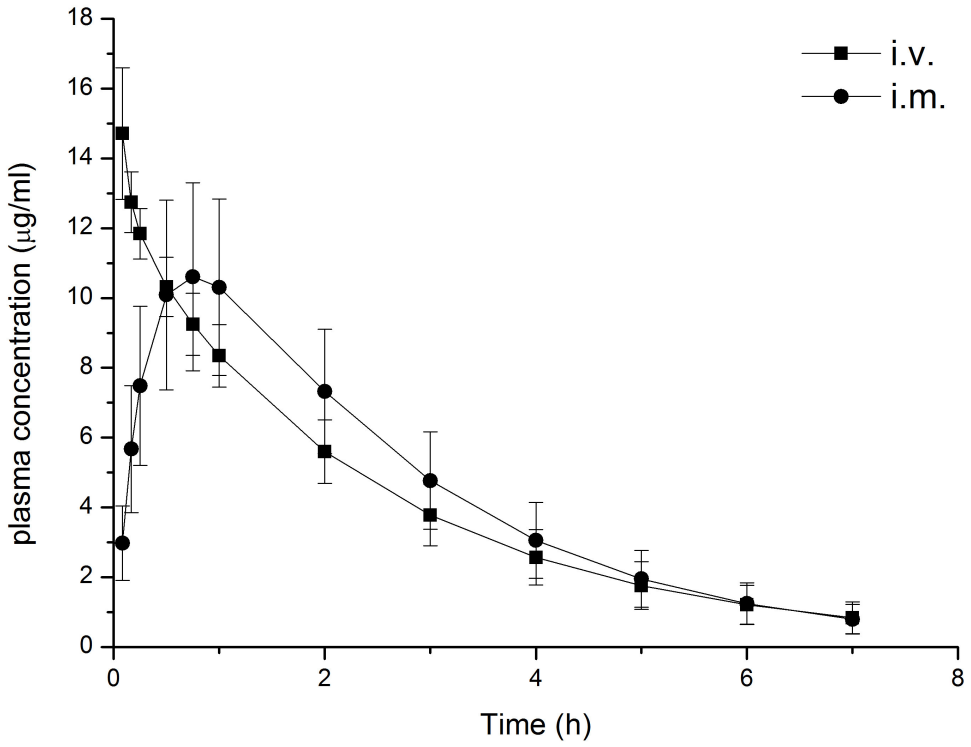

Figure 2. Mean (±S.D.) plasma mequindox concentration in goat following i.v. and i.m. administration of 7 mg/kg.

Table 1. Mequindox mean concentrations (μg/ml) in goat plasma after single i.v. and i.m. administration of 7 mg/kg b.w. (mean ± S.D., n = 6).

Time (h)	i.v.	i.m.
0.083	14.89 ± 2.05	3.12 ± 0.88
0.167	12.54 ± 0.86	5.49 ± 1.95
0.25	11.57 ± 0.64	7.56 ± 2.46
0.5	10.49 ± 0.38	9.83 ± 2.92
0.75	9.07 ± 0.94	11.09 ± 2.76
1	8.22 ± 0.53	10.05 ± 2.18
2	5.46 ± 1.10	7.70 ± 1.60
3	3.98 ± 0.24	4.80 ± 1.61
4	2.52 ± 1.05	2.99 ± 1.14
5	1.76 ± 0.78	1.93 ± 1.21
6	1.25 ± 0.67	1.12 ± 0.49
7	0.46 ± 0.37	0.76 ± 0.42

i.v. Intravenous; i.m., intramuscular.

Table 2. Pharmacokinetic parameters of Mequindox in goats after i.v. or i.m. administration of 7 mg/kg b.w. (mean ± S.D., n = 6).

Parameter	Treatment	
	i.v.	i.m.
C_0 (μg·mL^{-1})	14.65 ± 1.28	NA
C_{max} (μg·mL^{-1})	NA	11.01 ± 2.94
T_{max} (h)	NA	0.667 ± 0.129
$t_{1/2\alpha}$ (h)	0.597 ± 0.825	NA
$t_{1/2\beta}$ (h)	1.784 ± 0.394*	1.494 ± 0.226*
$t_{1/2a}$ (h)	NA	0.284 ± 0.196
V_d/F (L·kg^{-1})	0.349 ± 0.154*	0.452 ± 0.103*
CL_B/F (L·kg^{-1}·h^{-1})	0.209 ± 0.039	0.21 ± 0.04
AUC (μg·mL^{-1}·h)	34.459 ± 6.244	34.402 ± 6.862
F (%)	100 as presuppose	99.8%

C_0, Initial serum concentration; C_{max}, peak serum concentration; T_{max}, time to peak serum concentration; $t_{1/2\alpha}$, half-life of distribution; $t_{1/2\beta}$, half-life of elimination; $t_{1/2a}$, half-life of absorption; V_d, the apparent volume of distribution; Cl_B, total body clearance; CL_B and V_d represent their true values divided by the systemic availability (F); AUC, area under the concentration-time curve; F, bioavailability; NA - not applicable. For intravenous administration, F =1 * P < 0.05 (values with this symbol are significantly different).

The pharmacokinetics of mequindox have been investigated in swine (Chen, 2002) and sheep (Huang et al., 2008) in previous studies. It was found that mequindox distributed in swine tissues in 10 min and elimination half-life was 2 h following i.m. dosing, showing a more rapid absorption than that of goats, however, not faster compared with the terminal $t_{1/2\beta}$ in goats (1.5 h). Mequindox could not be determined in swine serum after

8 h post dosing, which is similar to current goat results. Compared with values estimated in sheep after i.v., mequindox terminal $t_{1/2\beta}$ in sheep (2.06 h) is longer than the value obtained in goats (1.78 h). The clearance of mequindox found in the current study in goats (0.209 $L \cdot kg^{-1} \cdot h^{-1}$) is similar to the values found in sheep (0.218 $L \cdot kg^{-1} \cdot h^{-1}$). The volume of distribution for sheep (0. 643 L/kg) is much higher than goats (0.349 L/kg), indicating that this drug distributed more extensively in sheep.

The pharmacokinetic behavior and plasma concentrations of mequindox after single i.v. and i.m. administration dose of 7 mg/kg b.w. in goats indicate that 2 ~ 3 times daily administration could be used for treatment of infections caused by many susceptible organisms. Moreover, the pharmacokinetic parameters of mequindox in goats following i.m. administration are characterized by short elimination half-life, high bioavailability, and high volume of distribution. These results suggest that mequindox may be a suitable agent for treatment of infectious diseases in goats. However, further studies are needed to establish repeated dosage regimens and clinical efficacy.

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REFERENCES

Ai XH, Chen ZW, Zhang CG (2003). Studies on pharmacokinetic and tissue concentration of olaquindox in comnlon carps. Acta Hydrobiologica Sinica. 27: 273-277.

Baggot JD, Mckellar QA (1994). The absorption, distribution and elimination of anthelminitic drugs: the role of pharmacokinetics. J. Vet. Pharmacol. Ther. 17: 409-419.

Chen ZL (2002). Veterinary pharmacology, 2nd ed. China Agricultural Press, Beijing, China.

Corpet DE (1999). Mechanism of antimicrobial growth promoters used in animal feed. C. R. Acad. Agric. Fr. 85: 197-205.

Gibaldi M, Perrier D (1982). Pharmacokinetics, 2nd ed. Marcel Dekker, Inc., New York, USA.

Huang XJ, Wang X, Ihsana A, Liu Q, Xue XJ, Su SJ, Yang CH, Zhou W, Yuan ZH (2010). Interactions of NADPH oxidase, rennin-angiotensin-aldosterone system and reactive oxygen species in mequindox-mediated aldosterone secretion in Wistar rats. Toxicol. Lett. 198: 112-118.

Huang YQ, Li YQ, Yang YJ, Miao XL (2008). Studies on the pharmacokinetics of Mequindox in sheep. J. Northwest A&F University. 36: 77-81.

Jia XH (1998). Clinical application of Mequindox. Shandong J. Anim. Sci. Vet. Med. 11: p. 48.

Li JY, Li JS, Xu ZZ, Du XL, Miao XL, Zhao RC, Liu JX, Xue FQ (2002). Studies on the pharmacokinetics of quinocetone in pigs and chickens. Acta Veterinaria et Zootechnica Sinica. 34: 94-97.

Li JY, Zhou XZ, Li JS, Zhao RC, Miao XL, Zhang JY (2005). The pharmacokinetics of quinocetone in pigs. Chin. J. Vet. Drug, 39: 1-3.

Liu CX (2003). Practice pharmacokinetics, 1st ed. China Science and Technology Press of Medicine and Drug, Beijing, China.

Qu QS, Gao YH, Liu ST (1995). Study on effect of Mequindox to swine diarrhea. Tianjing J. Anim. Sci. Vet. Med. 12: 30-31.

Visek WJ (1978). The mode of growth promotion by antibiotics. J. Anim. Sci. 46: 1447-1469.

Wan X, Yao PJ, Jin H, Liu H, Chen C (2006). Advances in the study of Quinocetone. Chin. J. Vet. Drug, 40: 41-44.

Yamaoka K, Nakagawa T, Uno T (1978). Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. J. Biochem. 100: 609-618.

Yuan ZH (1998). Veterinary medicine guide, 1st ed. China Agricultural Press, Beijing, China.

Zhao R, Xia W, Wang Y, Yan X, Zhou Z, Li J, Cai J (1982). Initial observation of the bacteriostasis activity and toxicity of MAQO and MCEQO. J. Tradit. Chin. Vet. Med. 1: (51-54).

Zhu ZZ, Chen ZL, Feng QH (1993). Studies on pharmacokinetics and tissue concentration of olaquindox in chickens. Acta Veterinaria et Zootechnica Sinica. 24: 258-264.