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General Pharmacology of Artesunate, a Commonly used Antimalarial Drug: Effects on Central Nervous, Cardiovascular, and Respiratory System

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Artesunate, a semi-synthetic derivative of artemisinin, is used primarily as a treatment for malaria. Its effects on the central nervous system, general behavior, and cardiovascular, respiratory, and other organ systems were studied using mice, rats, guinea pigs, and dogs. Artesunate was administered orally to mice at doses of 125, 250, and 500 mg/kg and to rats and guinea pigs at 100, 200, and 400 mg/kg. In dogs, test drugs were administered orally in gelatin capsules at doses of 50, 100, and 150 mg/kg. Artesunate induced insignificant changes in general pharmacological studies, including general behavior, motor coordination, body temperature, analgesia, convulsion modulation, blood pressure, heart rate (HR), and electrocardiogram (ECG) in dogs *in vivo*; respiration in guinea pigs; and gut motility or direct effects on isolated guinea pig ileum, contractile responses, and renal function. On the other hand, artesunate decreased the HR and coronary flow rate (CFR) in the rat *in vitro*; however, the extent of the changes was small and they were not confirmed in *in vivo* studies in the dog. Artesunate increased hexobarbital-induced sleeping time in a dose-related manner. Artesunate induced dose-related decreases in the volume of gastric secretions and the total acidity of gastric contents, and induced increases in pH at a dose of 400 mg/kg. However, all of these changes were observed at doses much greater than clinical therapeutic doses (2.4 mg/kg in humans, when used as an anti-malarial). Thus, it can be concluded that artesunate is safe at clinical therapeutic doses.

Key words: Artesunate, General pharmacology, Antimalarial drug, Central nervous system, Cardiovascular system, Respiratory system

INTRODUCTION

Malaria, caused mostly by *Plasmodium falciparum* and *P. vivax*, remains one of the most important infectious diseases in the world. The World Health Organization (WHO) recommends artemisinin-based combination therapies (ACTs) for the treatment of malaria. Artesunate, a semi-synthetic derivative of artemisinin, and other artemisinin derivatives are novel drugs in the treatment of malaria (Price, 2000). Artesunate is generally well-tolerated, safe, and has few adverse effects (Amold *et al*., 1990; Cao *et al*., 1997; Ha *et al*., 1997). In addition to the well-known anti-malarial activity of artesunate, a cytotoxic action of artesunate against cancer cell lines of different tumor types was recently identified (Chen *et al*., 2003; Dell'Eva *et al*., 2004; Efferth *et al*., 2001, 2003; Singh and Lai, 2001). Because the use of artesunate has increased greatly, further research on the safety of artesunate is necessary.

There have been several reviews on the toxicity of this family of drugs in animals (Brewer *et al*., 1998; Park *et al*., 1998; Ribeiro and Olliaro, 1998). However, adverse effects of artesunate have not been described, probably because they cannot easily be differentiated from malaria-related effects.

The present studies were conducted to examine the general pharmacological properties of high doses of artesunate. The study of its general pharmacological properties is intended to provide an indication of potential side effects resulting from the secondary pharmacological activity of high doses. The doses used in the present studies were selected to fully examine the potential pharmacological activity of artesunate, and therefore determine clinically effective doses of this drug.

MATERIALS AND METHODS

Animals. The experiments were performed using CrjBgi: CD-1 (ICR) mice, CrjBgi:CD (Spargue-Dawley) rats, Hart-

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ley guinea pigs (Bio Genomics Inc., Seoul, Korea) and Canis familiaris (Beagle dogs, Marshall Farm Co., USA). The animals were kept in a storage room under the conditions of constant temperature (23 ± 3°C), relative humidity (50 ± 10%), and illumination (12 h light/dark cycles) until the initiation of the experiment. All animals were fed with standard animal chow daily and had access to drinking water *ad libitum*. Animals were randomized into groups. All animal handling procedures were performed in accordance with guidelines of the Institutional Animal Care and Use Committee of our institute (IACUC).

Drugs and chemicals. Artesunate, a white or light yellowish crystalline powder (Fig. 1), was provided by Shin Poong pharm, Co. (Ansan, Korea), dissolved in 0.5% MC (methylcellulose) solution, filtered and administered orally to mice at doses of 125, 250 and 500 mg/kg and to rats and guinea pigs at 100, 200 and 400 mg/kg. For dogs, test drugs were administered orally in gelatin capsules at doses of 50, 100 and 150 mg/kg. The test drug at concentrations ranging from 1×10^{-7} to 1×10^{-5} M in buffer solution was used for the isolated ileum test and isolated rat heart test.

Diazepam was donated by Shinogie (Osaka, Japan) and hexobarbital·Na (Bayer Leverkusen, Gemany), chlorpromazine·HCl (Fluca, Buchs, Swiss), strychnine·HCl, theophylline, propranolol, pentylenetetrazole·HCl (Sigma, TX, USA) and morphine (Samsung pharm, Co., Seoul, Korea) were commercially purchased. D-mannitol and tartaric acid were gift from Jong Kun Dang Co. (Seoul, Korea). Furosemide was donated by Il-Yang Pharm. (Yong-In, Korea). CKD-732 was dissolved in 5% mannitol solution with mild shaking at room temperature and the solution was sterilized by filtration (0.22 μm). Hexobarbital, chlorpromazine, strychnine, pentylenetetrazole were dissolved in distilled water and diazepam was suspended in the aqueous solution of Tween 80 (0.05 %). The doses given were, vehicle control, 10, 30, 40 and 50 mg/kg and the drugs were administered intravenously. Propranolol was administrated intravenously. Morphine was administrated intraperitoneally.

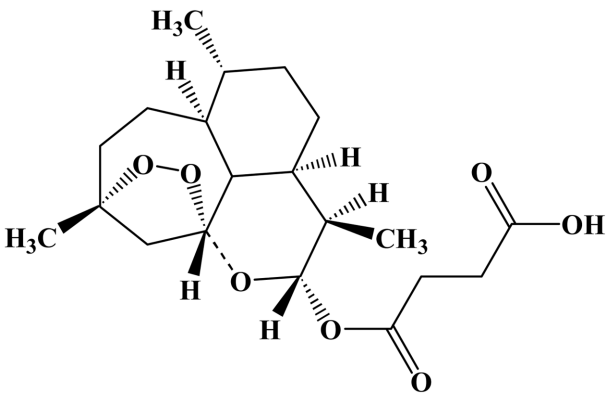

Fig. 1. Chemical structure of artesunate.

Apparatuses. A motility meter PAS (San Diego Instrument, CA, USA), rotarod (Ugo basile, Comerio-Varese, Italy), rodent shocker (HSE, March, Germany), hot-plate (Letica, Barcelona, Spain), thermometer; MGA-III (Shibaura Electronics, Tokyo, Japan), organ bath (Letica), Langendorff apparatus (HSE), flowmeter (Transonic Systems, NY, USA), pressure transducer (HSE), rodent ventilator (Harvard 683, MA, USA), plethysmometer (HSE), and Na/K/Cl analyzer (Bayer, MA, USA) were used in this study.

General behavior. Groups of 4 male and 4 female mice were used. Using the modified method of Irwin (Irwin, 1968), the general behavior of the animals was observed at 0, 30, 60, 120, 240, and 360 min after drug administration.

Spontaneous locomotor activity. Groups of 8 male mice were used for single-dose experiments with the test drug. Mice were placed in a plastic cage (260W × 250L × 400H) and the locomotor activity was measured using a motility meter for 5 min from 30 to 360 min after administration of the test drug (Svensson and Thieme, 1969).

Rotarod test. Groups of 8 male mice were used for single-dose experiments with the test drug. The animals able to stay on the rotarod (16 rpm) for more than 3 min were preselected and randomly divided into 5 groups. The animals in each group were placed on the rotarod at 0, 30, 60, 120, 240, and 360 min after administration of test drug. The numbers of mice falling within 1 min from a rotating rod were counted (Dunham and miya, 1957).

Body temperature. Groups of 4 male and 4 female mice were used for single-dose experiments with the test drug. The rectal temperature was continuously monitored with an electrothermometer (MGA III) after oral administration of test drug.

Hexobarbital-induced hypnosis. Groups of 8 male mice were used for single-dose experiments with the test drug. The animals were pretreated with drugs 30 min prior to the hexobarbital (70 mg/kg) injection. Following the hexobarbital injection, the duration of sleep was measured by examining the righting reflex. The period during which the mice lost their righting reflex was counted as the time of sleep.

Analgesia using acetic acid. Groups of 8 male mice were used for single dose experiments with the test drug. Animals were injected with 1% acetic acid (0.01 ml/g b.w., i.p.) 30 min after the administration of the test drugs. The number of writhings was counted for 5 min after the acetic acid injection and it was compared with that produced by

acetic acid alone (Koster *et al*., 1959).

Analgesia using the hot-plate method. Groups of 8 male mice were used for single-dose experiments with the test drugs. Thirty minutes after drug administration, each mouse was placed on a hot-plate (Letica) warmed with a water bath to 57°C. The time to the beginning of responses such as raising and licking the feet was measured.

Convulsions induced by pentylenetetrazole. Groups of 8 male mice were used for single-dose experiments with the test drug. Pentylenetetrazole (100 mg/kg, i.p.) was injected 30 min after the administration of the test drug, and the incidence of clonic convulsions was determined (Swinyard *et al*., 1952).

Convulsions induced by strychnine. Groups of 8 male mice were used for single-dose experiments with the test drug. Strychnine (1 mg/kg, i.p.) was injected 30 min after the administration of the drugs. The incidence of extensive tonic convulsions was determined.

Electroshock-induced convulsions. Groups of 8 male mice were used for single-dose experiments with the test drug. An electroshock (10 mA, 0.3 s, AC) was given to the ears of mice 30 min after the administration of the test drug. The incidence of extensive tonic convulsions was determined (Woodbury and Davenport, 1952).

Respiration. Groups of 4 male guinea pigs, weighing 350~410 g were used for single-dose experiments. The drugs were orally administered and the animals were positioned in a double-chamber plethysmometer (HSE) in such a way that the head was in the nasal chamber and the rest of the body in the cylindrical thoracal or body chamber. The responses of the animals were measured at 0, 30, 60, 120, and 240 min after drug administration. The respiratory rate and tidal volume were calculated based on the flow signal from the body chamber, which was sealed off from the nasal chamber with an airtight silicon sleeve around the necks of the guinea pigs.

Effects on isolated smooth muscle. Male Hartley guinea pigs, weighing 440~510 g, were stunned by a blow to the head and bled from the carotid arteries. The longitudinal muscle preparations (about 5 cm in length) were isolated from segments of the terminal ileum. The preparations were suspended in a 10 m*l* of a water-jacked organ bath containing Kreb's solution aerated continuously with 95% O_2 and 5% CO_2 at 37°C and their responses were recorded through an isotonic transducer (Harvard). The composition of the nutrition solution was Kreb's-Henseleit bicarbonate solution: NaCl, 6.9; KCl, 0.35; $CaCl_2$, 0.265; $MgSO_4$, 0.295; KH_2PO_4, 0.163; $NaHCO_3$, 2.1; glucose, 1.8; and EDTA, 0.009 g/*l*. The drugs were applied with the single-dose technique. The contractile responses to drug alone and responses to acetylcholine, histamine, and $BaCl_2$ were expressed as a percentage of the maximal response to acetylcholine (ACh) (5×10^{-7} M), histamine (2×10^{-6}) and $BaCl_2$ (2×10^{-3}). These doses are based on concentration-response curve of each agonist, EC_{50} (ACh, 1.1×10^{-4} M; histamine, 9×10^{-7} M; $BaCl_2$, 2.03×10^{-2} M), concentrations at maximum response (ACh, 5×10^{-3} M; histamine, 2×10^{-5} M; $BaCl_2$, 2×10^{-1} M).

Intestinal transport. After mice weighing 25~35 g were fasted for 3 h, they were used as groups of 8 animals. A charcoal meal, consisting of 5% activated carbon powder suspension in 10% gum arabic solution, was orally administered at a volume of 10 m*l*/kg 30 min after the administration of the test drugs at a volume of 10 m*l*/kg 30 min later, and the animals were killed by cervical dislocation and their intestines were immediately isolated and cooled with ice. The distance between the pylorus and the top of the charcoal and the total length of the intestine were measured. The transport rate was defined as the proportion of the former to the latter.

Gastric acid secretion. Groups of 5 rats weighing 230~280 g were fasted for 24 h. Under ether anesthesia and with rats in the supine position, their abdomens were opened along the midline. The pylorus was ligated and then drugs were given intraduodenally at a volume of 1 m*l*/kg. Five hours after the closing of the abdomen, the stomach was removed. The gastric juice was examined for the amount of gastric acid and total acidity with a titration method with 0.1 N NaOH using a titrator (Shay *et al*., 1945).

Renal function. Groups of 5 male rats weighing 190~230 g were used. Animals were acclimated to metabolic cages and allowed free access to solid food and water for 24 h. After deprivation of food and water and measurement of body weight, physiological saline 2.5%/b.w. and 3 h later 2.5% saline and test compound were administered orally. Then the animals were returned at once and their urine was collected for 5 h. Urinary electrolytes, Na, K, and Cl were measured with a Na/K/Cl analyzer, and the urine volume and pH were measured.

Isolated rat heart studies. For this study, isolated hearts were used according to the published methods after some modifications (Bolli, 1991; Watts and Maiorano, 1987). Male Sprague-Dawley rats weighing 300~450 g were anesthetized with sodium pentobarbital (100 mg/kg, i.p.). The tail vein was injected with heparin (20 IU/kg) and then the trachea was intubated while the rats were mechanically ventilated with a rodent ventilator. Their hearts were perfused *in situ* with oxygenated modified Krebs-Henseleit bicarbon-

ate buffer (described above) with retrograde aortic cannulation. The hearts were then excised and moved to the Langendorff apparatus, where they were perfused with an oxygenated modified Krebs-Henseleit bicarbonate buffer containing (in mmol/*l*) NaCl 116, $NaHCO_3$ 24.9, KCl 4.7, $MgSO_4$ 1.1, KH_2PO_4 1.17, $CaCl_2$ 2.52, glucose 8.32, and pyruvate 2.0 at a constant perfusion pressure (75 mmHg). A water-filled latex balloon attached to a metal cannula was placed in the left ventricle through the pulmonary vein and connected to a pressure transducer for measurement of left ventricular pressure (LVP). The hearts were allowed to equilibrate for 15 min, at which time left ventricular end diastolic pressure (LVEDP) was adjusted to 10 mmHg, and the balloon volume was maintained throughout the experiment. Then baseline contractile function, HR, and coronary flow (CF) were measured. Cardiac contractile function was calculated by subtracting LVEDP from left ventricular peak systolic pressure (LVSP), yielding left ventricular developing pressure (LVDP). The double product (DP), another important parameter for assessing cardiac performance, was calculated by multiplying HR by LVDP. Throughout the experiment, all these parameters were measured or calculated before and 10 min after pretreatment with the drug and 30 min after the onset of reperfusion with buffer. The drugs were administered using the single-dose technique.

Effects on blood pressure, heart rate, ECG and body temperature in beagle dogs. Groups of four male beagle dogs with weights between 8.6 to 9.0 kg were used. Radiotelemetry devices (Truett and West, 1995) were implanted as described below.

Animals were anaesthetised with pentobarbital at doses of 35 mg/kg, and the hair on the ventral body surface from the xiphoid to the inguinal area and the hair on the flank near the dorsal midline were removed. The pouch was formed under the skin cranial to the incision by separating the skin from the underlying tissues, then the device was inserted into the pouch. An incision was made to expose the femoral artery and vein, the femoral artery was dissected from the surrounding fat and connective tissues using blunt dissection. All excess tissues from the desired vessels were cleared to allow good haemostasis, followed by catheterisation. Two ligatures were placed under the vessel using a non-absorbable suture and tunnelled from the inguinal incision to the flank incision using a trocar. The catheter was passed through the trocar and pulled into the inguinal site. An incision was made on the left and right side of the thorax for placement of ECG leads. The leads were passed through the trocar, and each lead's wire coil was placed underneath the skin (positive lead anchored on the left lateral thorax and negative lead on the right side). The skin incision was filled with sterile saline solution and closed using a non-absorbable suture material. After operation, the area was thoroughly disinfected. The animals were placed in a warm environment and monitored overnight. After two days, data on systolic pressure, diastolic pressure, blood pressure, heart rate, body temperature, and ECG were collected from the individual telemetry receiver located in each dog cage. The telemetry receivers were connected to a desk-top computer used to store and analyse data. The software package used was DataQuest A.R.T. (DataSciences, Inc.). Data were collected for two minutes at intervals of 0, 30, 60, 120, 240 and 360 minutes after administration of vehicle control drug. On the following day, the responses of the animals were examined at intervals of 0, 30, 60, 90, 120, 240 and 360 minutes after administration of 50 mg/kg. The same method was employed for the experiments using doses of 100 and 150 mg/kg.

Statistical analysis. Data from the treated groups were statistically compared to those of the vehicle control group using the following methods: Data was summarized as mean ± S.D. and ± S.E.M. Variance of numerical data was checked by the Bartlett test. If the variance was homogeneous, the data was subjected to ANOVA. Otherwise, they were analyzed by the Kruskal-Wallis H test. If either of the tests showed a difference between groups, the data were analyzed by the multiple comparison procedure of the Dunnett's or the Scheffe's test. The data were considered to be significant when the P value was less than 0.05 or 0.01.

RESULTS

Artesunate was administered orally to mice at doses of 0,

Table 1. Effects of artesunate on spontaneous locomotor activity (group summary)

Drugs	Dose (mg/kg)	Counts/5 min					
		0 min	30 min	60 min	120 min	240 min	360 min
Vehicle	0	98.6 ± 25.1	70.1 ± 35.3	36.3 ± 15.6	55.4 ± 33.7	53.3 ± 24.1	64.8 ± 28.9
Artesunate	125	86.0 ± 20.5	81.5 ± 23.4	43.6 ± 24.7	49.5 ± 32.8	43.4 ± 25.7	58.9 ± 18.8
	250	110.0 ± 24.2	83.5 ± 35.2	36.6 ± 16.4	53.3 ± 32.5	46.5 ± 26.3	70.8 ± 22.4
	500	103.9 ± 36.5	67.3 ± 35.5	45.8 ± 21.2	59.9 ± 27.0	50.1 ± 28.9	55.1 ± 19.1
Diazepam	10	91.4 ± 14.9	0.4 ± 0.5**	11.4 ± 14.1*	7.8 ± 14.1**	12.6 ± 11.0*	24.9 ± 17.0**

Test articles were administered orally as a single dose. Each value represents the mea ± S.D. (n = 8). Significant difference from control group (*; $p < 0.05$, **; $p < 0.01$).

Table 2. Effects of artesunate on motor coordination (group summary)

Drugs	Dose (mg/kg)	Incidence of ataxia (no. of mice)					
		0 min	30 min	60 min	120 min	240 min	360 min
Vehicle	0	0	0	0	0	0	0
Artesunate	125	0	0	0	0	0	0
	250	0	0	0	0	0	0
	500	0	0	0	0	0	0
Diazepam	6	0	6	3	0	0	0

Test articles were administered orally as a single dose (n = 8).

125, 250, and 500 mg/kg, to rats and to guinea pigs at doses of 0, 100, 200, and 400 mg/kg, and to beagle dogs at doses of 0, 50, 100, and 150 mg/kg.

Artesunate at all doses and time points showed no effect on general behavior (data not shown), locomotor activity (Table 1), motor coordination (Table 2), analgesia (Table 3), or seizure activity (Table 4). Furthermore, no notable effects were observed in cardiovascular functions in the isolated heart (with the exception of heart rate (HR) and coronary flow rate (CFR); Fig. 2), contractile response of isolated ileums (Table 5), body temperature (Table 7), intestinal transit (Table 10), or respiratory function (Table 11).

Table 3. Effects of artesunate on analgesia using acetic acid and hot-plate in mice (group summary)

Drugs	Dose (mg/kg)	Acetic acid test	Hot-plate test
		Wriths (No.)	Licking time (s)
Vehicle	0	21.5 ± 3.3	6.2 ± 2.9
Artesunate	125	18.8 ± 3.8	6.5 ± 0.7
	250	19.8 ± 4.1	7.6 ± 2.5
	500	18.8 ± 5.7	8.4 ± 2.6
Ketoprofen	10	14.0 ± 4.5**	
Codeine phosphate	100		14.4 ± 5.5**

Test articles were administered orally as a single dose. Each value represents the mean ± S.D. (n = 8). Significant difference from control group (**; $p < 0.01$).

In a study of renal function, artesunate increased the excretion of Na^+ at doses of 200 mg/kg (124.5 ± 7.3 vs. 153.1 ± 9.3) and 400 mg/kg (124.5 ± 7.3 vs. 150.4 ± 6.9; Table 6), but this was not dose-dependent.

In the study of the isolated heart, artesunate significantly decreased CFR and HR at a dose of 1×10^{-5} mol/*l* (Fig. 2), but these effects were not observed in the cardiovascular system of the dog (Table 8).

We examined whether artesunate affected hexobarbital-induced sleeping time. Artesunate prolonged the sleeping time at doses of 250 mg/kg (44.1 ± 10.9 vs. 88.9 ± 32.1; p <

Table 4. Effects of artesunate on convulsions induced by pentylenetetrazole, strychnine, and electroshock in mice (group summary)

Drugs	Dose (mg/kg)	Pentylenetetrazole		Strychnine		Electroshock	
		Convulsion (No.)	Protection (%)	Convulsion (No.)	Protection (%)	Convulsion (No.)	Protection (%)
Vehicle	0	8	0	8	0	7	0
Artesunate	125	8	0	8	0	7	0
	250	8	0	8	0	6	0
	500	8	0	8	0	6	0
Diazepam	10	0	100				
	20			3	62.5		
	30					1	87.5

Test articles were administered orally as a single dose (n = 8).

Table 5. Effects of artesunate on isolated guinea pig ileum (group summary)

Drugs	Dose (Log[M])	Contractile responses (% of pre-drug response)			
		Alone	ACh.	His.	$BaCl_2$
Vehicle	0	0.0 ± 0.0	64.2 ± 4.4	186.4 ± 52.2	188.5 ± 41.7
Artesunate	−7	0.0 ± 0.0	64.3 ± 5.2	186.3 ± 44.7	190.6 ± 41.9
	−6	0.0 ± 0.0	63.8 ± 5.7	190.5 ± 52.4	191.6 ± 40.2
	−5	0.0 ± 0.0	64.8 ± 4.9	187.5 ± 44.0	187.3 ± 46.7

Each value represents the mean ± S.D. (n = 6). Ach, Acetylcholine; His, Histamine.

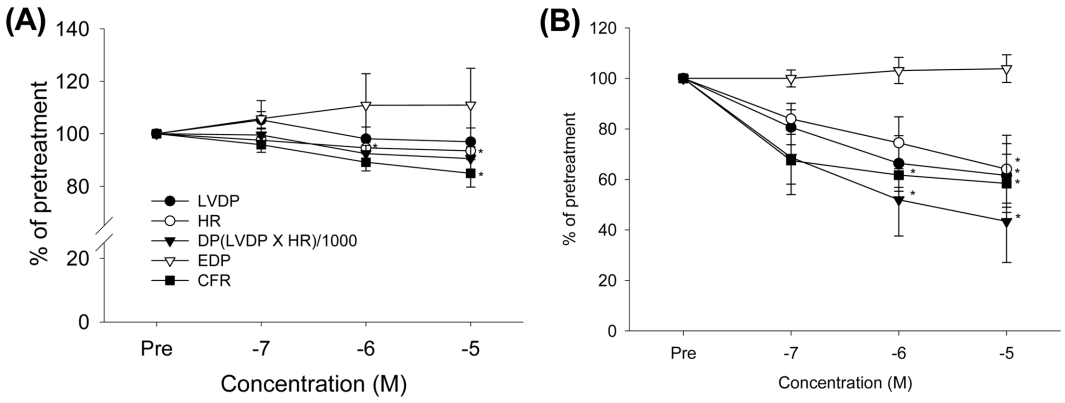

Fig. 2. (A) Effect of Artesunate on Left Ventricular Developing Pressure (LVDP), Heart Rate (HR), Double Product (DP), Left Ventricular End Diastolic Pressure (LVEDP), and Coronary Flow Rate (CFR). (B) Effect of the β-receptor inhibitor propranolol as positive control. Each column indicates the mean and S.E.M. ($n = 6$). Significant difference from control group, $^*P < 0.05$.

Table 6. Effects of artesunate on renal functions in rats (group summary)

Drugs	Dose (mg/kg)	pH	Urine volume (m*l*)	Na^+ (mmol/*l*)	K^+ (mmol/*l*)	Cl^- (mmol/*l*)
Vehicle	0	6.1 ± 0.7	6.52 ± 0.44	124.5 ± 7.3	42.1 ± 12.4	152.9 ± 15.6
Artesunate	100	6.3 ± 0.4	6.66 ± 1.24	144.3 ± 28.4	39.6 ± 10.8	150.5 ± 17.3
	200	6.6 ± 0.4	7.16 ± 2.11	153.1 ± 9.3*	39.1 ± 9.8	158.7 ± 15.6
	400	6.4 ± 0.4	7.00 ± 2.57	150.4 ± 6.9*	39.4 ± 13.0	152.9 ± 10.4
Furosemide	15	6.0 ± 0.4	10.96 ± 0.77**	130.7 ± 6.18	26.0 ± 2.4	159.9 ± 5.3

Test articles were administered orally as a single dose. Each value represents the Mean ± S.D. (n = 5). Significant difference from control group (*; $p < 0.05$, **; $p < 0.01$).

Table 7. Effects of artesunate on body temperature in mice (group summary)

Drug	Dose (mg/kg	Rectal temperature (°C)					
		0 min	30 min	60 min	120 min	240 min	360 min
Vehicle	0	34.7 ± 0.4	34.6 ± 0.3	34.7 ± 0.3	34.6 ± 0.2	34.6 ± 0.2	34.5 ± 0.2
Artesunate	125	34.7 ± 0.4	34.6 ± 0.3	34.5 ± 0.3	34.6 ± 0.3	34.6 ± 0.3	34.6 ± 0.4
	250	33.9 ± 0.5	33.9 ± 0.5	33.8 ± 0.6	33.9 ± 0.4	33.9 ± 0.4	33.9 ± 0.4
	500	34.0 ± 0.5	34.0 ± 0.5	34.0 ± 0.5	34.0 ± 0.4	34.0 ± 0.5	34.0 ± 0.5
Chlorpromazine	6	34.4 ± 0.4	32.2 ± 0.3**	32.2 ± 0.2**	32.2 ± 0.1**	33.2 ± 0.3**	33.6 ± 0.3

Test articles were administered orally as a single dose. Each value represents the mean ± S.D. (n = 85). Significant difference from control group (**; $p < 0.01$).

Table 8. Effects of artesunate on blood pressure, heart rate and ECG in male dogs using radiotelemetry (group summary)

	Blood pressure (mmHg) & Heart rate (BPM)				
	Drugs	Vehicle	Artesunate		
	Dose (mg/kg,p.o.)	0	50	100	150
Systolic pressure	0 min	126.1 ± 11.5	131.0 ± 16.2	123.9 ± 14.4	122.3 ± 9.6
	30 min	119.1 ± 9.5	120.7 ± 14.4	124.8 ± 14.5	117.6 ± 11.3
	60 min	115.2 ± 10.6	120.6 ± 13.1	123.4 ± 11.5	122.1 ± 11.7
	90 min	118.3 ± 11.7	117.5 ± 14.1	121.9 ± 9.7	121.4 ± 12.9
	120 min	115.6 ± 10.8	120.9 ± 12.9	120.6 ± 10.7	120.6 ± 13.3
	240 min	120.4 ± 13.1	122.5 ± 12.0	124.7 ± 11.2	122.2 ± 12.8
	360 min	119.2 ± 14.7	120.8 ± 12.7	124.9 ± 11.4	121.4 ± 13.3

Table 8. Continued

		Blood pressure (mmHg) & Heart rate (BPM)			
	Drugs	Vehicle	Artesunate		
	Dose (mg/kg,p.o.)	0	50	100	150
Diastolic pressure	0 min	88.2 ± 13.2	89.2 ± 8.6	83.4 ± 10.3	84.9 ± 13.2
	30 min	81.7 ± 12.8	80.8 ± 10.9	95.0 ± 8.3	80.3 ± 11.6
	60 min	77.9 ± 12.5	80.6 ± 10.4	82.9 ± 10.4	85.5 ± 11.7
	90 min	79.1 ± 8.1	78.5 ± 11.9	93.3 ± 10.8	84.2 ± 12.8
	120 min	77.8 ± 10.4	80.2 ± 10.0	91.2 ± 7.6	82.4 ± 11.4
	240 min	79.3 ± 12.5	82.0 ± 14.2	85.2 ± 9.7	82.5 ± 12.0
	360 min	79.3 ± 16.7	79.7 ± 12.8	84.5 ± 11.6	81.9 ± 13.1
Mean blood pressure	0 min	102.4 ± 11.0	105.5 ± 7.1	99.5 ± 7.7	100.1 ± 9.4
	30 min	97.2 ± 9.3	96.4 ± 8.6	101.1 ± 6.8	95.5 ± 8.6
	60 min	93.4 ± 9.1	96.4 ± 7.2	99.2 ± 6.1	100.5 ± 8.8
	90 min	94.9 ± 5.2	94.1 ± 9.5	99.0 ± 5.8	99.3 ± 10.0
	120 min	93.2 ± 7.8	96.1 ± 7.1	97.2 ± 4.4	98.0 ± 9.3
	240 min	95.7 ± 10.4	98.0 ± 10.8	101.2 ± 6.6	98.5 ± 8.9
	360 min	95.0 ± 14.0	96.0 ± 10.0	100.9 ± 8.4	97.9 ± 10.2
Heart rate	0 min	89.4 ± 11.2	88.5 ± 6.7	84.9 ± 7.7	90.7 ± 9.2
	30 min	91.6 ± 14.2	84.2 ± 9.6	91.8 ± 13.8	91.5 ± 7.9
	60 min	91.7 ± 8.3	85.9 ± 8.1	88.3 ± 12.6	98.8 ± 5.3
	90 min	85.2 ± 4.3	84.8 ± 8.9	90.3 ± 16.1	90.5 ± 8.1
	120 min	85.2 ± 7.0	77.6 ± 7.4	85.0 ± 17.8	87.6 ± 7.1
	240 min	74.1 ± 8.0	80.0 ± 7.5	82.9 ± 8.5	81.5 ± 10.8
	360 min	80.0 ± 10.6	80.2 ± 8.3	78.7 ± 7.0	81.3 ± 10.7
		ECG (sec)			
Q-T interval	0 min	0.191 ± 0.013	0.189 ± 0.017	0.197 ± 0.024	0.203 ± 0.021
	30 min	0.194 ± 0.016	0.195 ± 0.016	0.194 ± 0.030	0.204 ± 0.018
	60 min	0.196 ± 0.011	0.154 ± 0.086	0.197 ± 0.026	0.196 ± 0.016
	90 min	0.198 ± 0.010	0.196 ± 0.019	0.198 ± 0.023	0.204 ± 0.018
	120 min	0.200 ± 0.012	0.202 ± 0.013	0.205 ± 0.024	0.208 ± 0.018
	240 min	0.207 ± 0.014	0.208 ± 0.015	0.213 ± 0.021	0.220 ± 0.023
	360 min	0.201 ± 0.019	0.205 ± 0.015	0.218 ± 0.019	0.205 ± 0.015
P-R interval	0 min	0.116 ± 0.010	0.114 ± 0.010	0.114 ± 0.014	0.111 ± 0.012
	30 min	0.113 ± 0.010	0.112 ± 0.010	0.113 ± 0.001	0.111 ± 0.013
	60 min	0.112 ± 0.009	0.112 ± 0.010	0.113 ± 0.012	0.111 ± 0.012
	90 min	0.121 ± 0.029	0.113 ± 0.011	0.113 ± 0.013	0.113 ± 0.010
	120 min	0.112 ± 0.008	0.114 ± 0.010	0.115 ± 0.013	0.111 ± 0.010
	240 min	0.113 ± 0.010	0.114 ± 0.010	0.115 ± 0.011	0.112 ± 0.011
	360 min	0.114 ± 0.010	0.112 ± 0.010	0.112 ± 0.010	0.111 ± 0.010
QRS interval	0 min	0.027 ± 0.001	0.026 ± 0.001	0.027 ± 0.001	0.028 ± 0.001
	30 min	0.026 ± 0.001	0.027 ± 0.001	0.027 ± 0.001	0.027 ± 0.001
	60 min	0.027 ± 0.001	0.020 ± 0.001	0.027 ± 0.001	0.027 ± 0.001
	90 min	0.026 ± 0.001	0.026 ± 0.001	0.027 ± 0.001	0.027 ± 0.001
	120 min	0.027 ± 0.001	0.026 ± 0.001	0.027 ± 0.001	0.027 ± 0.001
	240 min	0.027 ± 0.001	0.026 ± 0.001	0.028 ± 0.001	0.027 ± 0.001
	360 min	0.027 ± 0.002	0.026 ± 0.001	0.026 ± 0.001	0.028 ± 0.001

Test articles were administered orally as a single dose. Each value represents the mean ± S.D. (n = 4).

0.01) and 500 mg/kg (44.1 ± 10.9 vs. 102.8 ± 39.4; $p < 0.01$; Table 9).

In an experiment on gastric function (Table 12), artesunate increased pH (1.40 ± 0.31 vs. 2.29 ± 0.53, $p < 0.01$) at a dose of 400 mg/kg. Also, artesunate decreased secretion volume (7.3 ± 1.08 vs. 4.3 ± 0.89, 3.5 ± 0.7, 3.2 ± 0.82, at doses of 100, 200, and 400 mg/kg, respectively; $p < 0.01$) and total acidity (0.5.56 ± 38.6 vs. 43.4 ± 23.2, 38.39 ± 15.5,

Table 9. Effects of artesunate on hexobarbital-induced hypnosis

Drugs	Dose (mg/kg)	Sleeping time (min)	Control ratio (%)
Vehicle	0	44.1 ± 10.9	100.0
Artesunate	125	61.0 ± 15.5	138.2
	250	88.9 ± 32.1**	200.0
	500	102.8 ± 39.4**	232.9
Ketoprofen	10	96.3 ± 14.1**	218.1

Test articles were administered orally as a single dose. Each value represents the mean ± S.D. (n = 8). Significant difference from control group (**; p < 0.01).

Table 10. Effects of artesunate on intestinal transport in mice

Drugs	Dose (mg/kg)	Transition ratio (% ratio)
Vehicle	0	50.8 ± 7.6
Artesunate	125	52.1 ± 6.1
	250	52.0 ± 6.2
	500	57.2 ± 10.0
Hyoscyamine	10	31.5 ± 11.4**

Test articles were administered orally as a single dose. Each value represents the mean ± S.D. (n = 8). Significant difference from control group (**; p < 0.01).

29.72 ± 10.5, at doses of 100, 200, and 400 mg/kg, respectively; $p < 0.01$) in a dose-dependent manner. The relative magnitude of the reduction in gastric acid secretion was one one-hundredth of that of atropine.

DISCUSSION

Artesunate, a water-soluble artemisinin derivate extracted from the Chinese herb Qinghaosu, is also commonly used as an effective and safe anti-malarial drug. A recent study focused on artesunate as a new and effective anti-cancer drug (Michaelis *et al*., 2010).

In the present study, general pharmacological effects of artesunate on the central nervous, cardiovascular, and respiratory systems were investigated using mice, rats, guinea pigs, and dogs.

In the study of renal function, artesunate significantly increased the excretion of Na^+ at doses of 200 and 400 mg/kg (Table 6). However, these significant differences were not dose-dependent.

Several antimalarial drugs, notably quinidine and halofantrine, produce clinically significant delays in ventricular repolarization, resulting in a prolongation of the electrocardiographic QT interval on the electrocardiogram (ECG) (White, 2007). High doses of artemether and artemotil have

Table 11. Effects of artesunate on respiration in guinea pigs

Respiration rate (%)						
Drugs	Dose (mg/kg)	Time (min)				
		0 min	30in	60 min	120 min	240 min
Vehicle	0	0.0 ± 0.0	2.3 ± 11.9	−1.7 ± 10.5	0.2 ± 5.3	1.3 ± 10.4
Artesunate	100	0.0 ± 0.0	−7.3 ± 11.7	−13.9 ± 8.1	−7.2 ± 10.1	−4.1 ± 6.3
	200	0.0 ± 0.0	−2.9 ± 14.0	−3.4 ± 9.4	1.2 ± 4.0	−0.2 ± 3.4
	400	0.0 ± 0.0	−10.9 ± 11.9	−6.6 ± 6.8	−1.8 ± 4.3	−2.6 ± 1.4
Tidal volume (%)						
Drugs	Dose (mg/kg)	Time (min)				
		0 min	30 min	60 min	120 min	240 min
Vehicle	0	0.0 ± 0.0	2.9 ± 39.9	−9.6 ± 18.6	7.8 ± 33.2	0.2 ± 26.3
Artesunate	100	0.0 ± 0.0	17.3 ± 23.3	20.2 ± 16.3	7.8 ± 33.2	8.6 ± 19.7
	200	0.0 ± 0.0	−4.3 ± 20.2	−4.4 ± 11.7	−13.0 ± 12.2	−10.3 ± 17.8
	400	0.0 ± 0.0	−1.0 ± 32.7	10.0 ± 25.1	12.3 ± 31.5	14.9 ± 22.5

Test articles were administered orally as a single dose. Each value represents the mean ± S.D. (n = 4).

Table 12. Effects of artesunate on gastric secretion in rats

Drugs	Dose (mg/kg)	Volume (m*l*)	pH	Total acidity (μEq)
Vehicle	0	7.3 ± 1.08	1.40 ± 0.31	105.56 ± 38.6
Artesunate	100	4.3 ± 0.89**	1.65 ± 0.26	43.40 ± 23.2**
	200	3.5 ± 0.70**	1.62 ± 0.29	38.39 ± 15.5**
	400	3.2 ± 0.82**	2.29 ± 0.53**	29.72 ± 10.5**
Atropine	1	4.3 ± 0.74**	1.30 ± 0.11	68.64 ± 16.0**

Test articles were administered orally as a single dose. Each value represents the mean ± S.D. (n = 5).

been associated with QT prolongation in dogs, raising the possibility of a class effect with the artemisinin derivatives (Classen *et al*., 1999). In the study of the isolated heart, artesunate significantly decreased CFR and HR at a dose of 1×10^{-5} mol/*l* (Fig. 2), but these effects were not observed in the cardiovascular system of the dog (Table 8). In a study on intravenous administration of artesunate, patients with severe *P. falciparum* malaria did not show significant cardiovascular effects (Richard *et al*., 2009).

Artesunate also prolonged the hexobarbital-induced sleeping time at doses of 250 and 500 mg/kg (Table 9). This suggests that artesunate possesses a sedative effect. This hexobarbital-induced sleeping time is considered to be a sensitive way to assess agents for central nervous system depressant actions (Carlini, 1973). Studies on the administration of high doses of artemether and arteether to dogs and rats demonstrated neurotoxicity (Classen *et al*., 1999; Brewer *et al*., 1994a, 1994b).

In experiments on gastric function (Table 12), artesunate increased pH at a dose of 400 mg/kg and decreased secretion volume and total acidity at doses of 100, 200, and 400 mg/kg in a dose-dependent manner.

However, all of these changes were observed at doses much greater than clinical therapeutic doses (2.4 mg/kg in humans when used as an anti-malarial). Thus, it can be concluded that artesunate is safe at clinical therapeutic doses.

In summary, artesunate at higher dose levels induced an increase in hexobarbital-induced sleeping time, decreases in HR and CFR of the isolated heart *in vitro* (but not *in vivo*), and a decrease in gastric secretion; however, it had no effect on other general pharmacological reactions, even at the highest doses tested.

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