RESEARCH NOTE

ACUTE TOXICITY TESTS OF ANTIPLASMODIAL *N*-ALKYL AND *N*-BENZYL-1,10-PHENANTHROLINE DERIVATIVES IN SWISS MICE

Mahardika Agus Wijayanti[1,2], Eti Nurwening Sholikhah[2,3], Iqmal Tahir[4], Ruslin Hadanu[4], Jumina[4], Wisnu Nurcahyo[5], Supargiyono[1,2] and Mustofa[2,3]

[1]Department of Parasitology Faculty of Medicine, [2]Center for Tropical Medicine, Faculty of Medicine, [3]Department of Pharmacology and Toxicology, Faculty of Medicine; [4]Department of Chemistry, Faculty of Mathemathics and Natural Sciences; [5]Department of Parasitology, Faculty of Veterinary, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract. Five new derivatives of *N*-alkyl and *N*-benzyl-1,10-phenanthroline had been shown to inhibit growth *in vitro* of *Plasmodium falciparum* FCR3 and *in vivo* of *P. berghei*. Acute toxicity tests demonstrated that some of those compounds had wide therapeutic indices. Safety tests of five *N*-alkyl and *N*-benzyl-1,10-phenanthroline derivatives were conducted in five groups of Swiss mice by a single intraperitoneal injection with various amounts of the test compounds, with chloroquine as comparison. Signs of toxic effects were observed during 24 hours and observations were continued for 14 days on the surviving mice. Mice were weighed before and after the test period. There were immediate behavioral changes among mice in the high dose group including restlessness, tremor, convulsion and eventually death, which was postulated to be due to the test compounds acting on the nervous system. There was no dose-dependent histopathological changes in the internal organs. Histopathological changes, such as congestion, degeneration and necrosis, were not found. There are no significant differences in mean weight gain among the groups of mice treated with the different compounds and controls. These results indicated that those new *N*-alkyl and *N*-benzyl-1,10-phenanthroline antiplasmodial compounds were toxic at high dose, but at non-toxic doses had no effect on weight gain and no histopathological effects on the appearance of internal organs.

Keywords: antiplasmodials, histopathology, *N*-alkyl and *N*-benzyl-1,10-phenanthroline derivatives, safety test

Correspondence: Mahardika Agus Wijayanti, Department of Parasitology, Faculty of Medicine, Universitas Gadjah Mada, Jl Farmako, Sekip Utara, Yogyakarta 55281, Indonesia.
Tel/Fax: +62274546215
E-mail: dikafkugm@yahoo.co.id

INTRODUCTION

Halofantrine is an effective drug against chloroquine resistant-*Plasmodium falciparum*, but this drug is expensive, has no parenteral formulation and has

some adverse side effects (Winstanley, 2000). In order to overcome those disadvantages, five new *N*-alkyl and *N*-benzyl-1,10-phenanthroline compounds have been synthesized: (1)-*N*-methyl-phenanthrolinium sulfate, (1)-*N*-ethyl-phenanthrolinium sulfate, (1)-*N*-benzyl-1,10-phenanthrolinium chloride, (1)-*N*-benzyl-1,10-phenanthrolinium bromide and (1)-*N*-benzyl-1,10-phenanthrolinium iodide (Wijayanti *et al*, 2006). Their IC_{50} (concentration to inhibit by 50%) values of *in vitro* growth of *P. falciparum* FCR3 strain ranged from 0.18 to 0.36 M. *In vivo* antiplasmodial evaluation against *P. berghei* in Swiss mice showed 50% effective doses (ED_{50}) of 2.08-50.93 mg/kg and therapeutic indices (TIs) with the acute toxicity tests ranged from 2.06 to 7.57, except for 1-*N*-benzyl-1,10-phenanthrolinium iodide, which was 58.38. Among the five 1,10-phenanthroline derivatives 1-*N*-benzyl-1,10-phenanthrolinium iodide has the most potent *in vitro* and *in vivo* antiplasmodial activity (Sholikhah *et al*, 2006; Wijayanti *et al*, 2006).

In this study, safety effects of those five new antiplasmodials were evaluated in a mouse model, based on clinical manifestations, histopathological examination of internal organs and on body weight.

MATERIALS AND METHODS

Animals

A total number of 260 Swiss mice was divided into five experimental groups with one positive and one negative control groups. Each group of 10 Swiss mice (5 males and 5 females, weighing about 30-35 g) was injected intraperitoneally as a single dose with one of the five antiplasmodial componds [(1)-*N*-methyl-phenanthrolinium sulfate, (1)-*N*-ethyl-phenanthrolinium sulfate, (1)-*N*-benzyl-1,10-phenanthrolinium chloride, (1)-*N*-benzyl-1,10-phenanthrolinium bromide and (1)-*N*-benzyl-1,10-phenanthrolinium iodide] in four to five different doses with controls given an equal volume (0.2 ml) of normal saline. Chloroquine was used as a comparison (Table 1).

Mice were weighed at the start and at the end of the test period. The animals were observed for 24 hours for any signs of toxicity, such as convulsion, tremor, respiratory change, motor coordination, appetite change and death (Ngatidjan, 2006). Observations of surviving mice were continued for 14 days. All mice that died during the study period were subjected to post-mortem examination and tissue samples of the internal organs (brain, lungs, heart, stomach, liver, spleen, kidney and testis) were collected. Mice that survived to the end of 14 day test period were sacrificed on day 15 and their internal organs collected. This study had been approved by Medical and Health Ethics Committee, Faculty of Medicine UGM, No: KE/FK/114/EC.

Histopathological examination

Tissue samples were fixed in 10% formalin, embedded in paraffin wax for microsection, followed by staining with hematoxylin and eosin and examination under a light microscope.

RESULTS

The drug dosages, administered as a single dose intraperitoneally, were based on the ED_{50} values from *in vivo P. berghei* 4 day-suppressive test, the range dose was approximately $^1/_4$-16 times of ED_{50} except for (1)-*N*-benzil-1,10-phenanthrolinium iodide which was approximately $^1/_4$-90 times of ED_{50} due to its high therapeutic index. Lethal dose (LD) values ranged from 25.94 to 132.89 mg/kg body weight

Table 1
Mortality of mice on acute toxicity test of *N*-alkyl and *N*-benzyl-1,10-phenanthroline derivatives.

Compound	Dose (mg/Kg)	No. of mice	No. of death	Toxicity signs
(1)-*N*-methyl-1,10-phenanthrolinium sulfate	2.77	10	0	-
	11.08	10	0	-
	44.31	10	0	-
	177.22	10	10	+[a]
(1)-*N*-ethyl-1,10-phenanthrolinium sulphate	4.77	10	0	-
	19.09	10	0	-
	76.38	10	0	-
	305.52	10	10	+[a]
(1)-*N*-benzyl-1,10-phenanthrolinium chloride	1.39	10	0	-
	5.56	10	0	-
	22.26	10	0	-
	89.02	10	10	+[a]
(1)-*N*-benzyl-1,10-phenanthrolinium bromide	4.01	10	0	-
	17.52	10	3	+[a]
	75.74	10	10	+[a]
	288.09	10	10	+[a]
(1)-*N*-benzil-1,10-phenanthrolinium iodide	1.45	10	0	-
	5.58	10	0	-
	24.49	10	0	-
	93.56	10	2	+[a]
	187.12	10	10	+[a]
Chloroquine	0.11	10	0	-
	1.11	10	0	-
	10.63	10	0	-
	110.79	10	7	+[a]
Normal saline	0	10	0	-

[a]Unsteady gait, tremor and convulsion.

(BW), while LD of chloroquine was 69.57 mg/kg BW (Wijayanti *et al*, 2006).

There were behavioral changes noted within four hours after drug administration in the high dose groups (Table 1). These signs of toxicity included hypo- or hyperactive, tremor, convulsion, respiratory change, loss of apetite and eventually death within one to four hours. Toxicity signs only occurred within the first four hours. Toxicity sign and death of chloroquine-treated mice were similar to experimental groups. Unsteady gait, tremor and convulsion were observed within one to four hours after drug administration. Those signs only observed among highest dose, 110.79 mg/kg. During the following 14 days, activity and appetite, as assessed by no significant changes in average weight gained, among drug treated groups (1.0-3.6 g) and untreated control (1.0-3.0 g) were normal among of the surviving mice.

Histopathological alterations in organ structures were examined of mice treated with the *N*-alkyl and *N*-benzyl-

Table 2
Histopathological changes observed in organs of mice injected with single dose (i.p.) of 1-*N*-alkyl and 1-*N*-benzyl-1, 10-phenanthroline derivatives.

Compound/ dose (mg/kg BW)	Organ							
	Brain	Heart	Lung	Liver	Kidney	Spleen	Stomach	Testis
1-*N*-methyl-1,10-phen.SO_4								
2.77	-	-	Pneumonia	-	-	-	Villi erossion	-
11.08	-	-	-	-	-	-	Villi erossion	-
44.31	-	-	Pneumonia	Hepatitis	-	-	Villi erossion	-
177.22	-	-	Pneumonia	-	-	-	Villi erossion	-
1-*N*-ethyl-1,10-phen.SO_4								
4.77	-	-	-	-	-	-	-	-
19.09	-	-	Pneumonia	-	-	-	Villi erossion	-
76.38	-	-	Pneumonia	-	-	-	-	-
305.52	-	-	-	-	-	-	-	-
(1)-*N*-benzyl-1,10-phen.Cl								
4.01	-	-	-	-	Lymphocytes on tubulus	-	-	-
17.52	-	-	Pneumonia	-	-	-	-	-
75.74	-	-	-	-	-	-	Villi erossion	-
288.09	-	-	Pneumonia	-	-	-	-	-
1-*N*-benzyl-1,10-phen.Br								
4.01	-	-	-	Lymphocytes granulation	Lymphocytes on tubulus	-	Villi erossion	-
17.52	-	-	Pneumonia	Lymphocytes granulation	-	-	Villi erossion	-
75.74	-	-	Pneumonia	Lymphocytes granulation	-	-	Villi erossion	-
288.09	-	-	-	Lymphocytes granulation	Lymphocytes on tubulus	-	Villi erossion	-
1-*N*-benzyl-1,10-phen.Iod								
1.45	-	-	Pneumonia	Lymphocytes granulation	-	-	Villi erossion	-
5.58	-	-	Pneumonia	Lymphocytes granulation	Lymphocytes on tubulus	-	Villi erossion	-
24.49	-	-	Pneumonia	Lymphocytes granulation	Lymphocytes on tubulus	-	Villi erossion	-
93.56	-	-	-	-	-	-	-	-
187.12	-	-	Pneumonia	Lymphocytes granulation	-	-	-	-
Chloroquine								
0.11	-	-	-	Lymphocytes granulation	Lymphocytes on tubulus	-	Villi erossion	-
1.11	-	-	-	Lymphocytes granulation	Lymphocytes on tubulus	-	-	-
10.63	-	-	Pneumonia	Lymphocytes granulation	Lymphocytes on tubulus	-	-	-
110.79	-	-	-	Lymphocytes granulation	Lymphocytes on tubulus	-	-	-

(-), no histophathological abnormalities.

1,10-phenanthroline derivatives (Table 2). There were no dose-dependent histopathological changes on the internal organs. The histopathological lesions in some organs were not specifically due to the test compounds administration. Some lession noted on the lung, liver, stomach and kidney were proposed to be due to underlying conditions of the mice. Pneumonia interstitialis, which was noted in some lungs, could have been caused by inhalation of ammonia from mice urine in the cages. Lymphocytes granulation found in perivascular areas of some livers and kidneys coresponded with undetected previous inflamation. There were some villi erosion in the stomach which also did not correlate with the toxic effects as the compounds were administered intraperitoneally. There were no difference of histophatological lesions among experimental and chloroquine-treated mice.

DISCUSSION

1,10-Phenanthrolines are known to function as metalloprotease inhibitors of various microorganisms, such as *Prevotella ruminicola*, *Fibrobacter succinogenes* and *Megasphaera elsdenii* (Wallace *et al*, 1996). Yapi *et al* (2000) have shown that the antiplasmodial activity of these compounds can be improved by alkylation at N-10 position, but the antiplasmodial activity of these 1,10-phenanthroline analogs are unrelated to metalloprotease inhibition. We have previously reported that antiplasmodial activities of *N*-benzyl-1,10-phenanthroline derivatives are better than *N*-alkyl 1,10-phenanthroline derivatives (Wijayanti *et al,* 2006). Toxic effects occurred at higher doses of *N*-alkyl-1,10-phenanthroline derivatives compared to *N*-benzyl-1,10–phenanthroline derivatives. This phenomenon may be due to carbon chain of *N*-benzyl-1,10-phenantroline derivatives being longer so that more nonpolar parts of the molecules increase their lipid partition coefficients, thereby allowing better solubility in lipid compartment and increasing their biological effects (Sharma, 2005).

Sulfate, chloride, bromide and iodide are the anion salts of *N*-alkyl and *N*-benzyl-1,10–phenanthroline derivatives. Iodide is the weakest base so enhancing the amounts of such compounds penetrating biological membranes, with the possibility of the drugs to interact with receptors and perturbing biological activities (Fessenden and Fessenden, 1990; Siswandono and Soekardjo, 2000). This supports the results of this study that toxicity sign of (1)-*N*-benzyl-1,10-phenantrolinium iodide occured at much more higher dose than its ED_{50} compared to the three *N*-benzyl compounds. Toxicity sign was observed on mice which received approximately 45 times of ED_{50} while the others were 6-19.

Intraperitoneal administration of Swiss mice at high dosages of the five antiplasmodial *N*-alkyl and *N*-benzyl-1,10-phenanthroline derivatives resulted in acute dose-dependent signs of toxicity and death. Animal deaths occurred within one to four hours after drug treatment, therefore the deaths were postulated to be the action of these compounds acting on the nervous system as evidenced by seizures before death. Histopathological changes in the internal organs associated with the emergence of death were not found. The underlying tissue degeneration and tissue necrosis observed in the study were histophatological changes in the internal organs usually caused by toxic effects of other compounds or drugs and these conditions were not pursued further.

Chloroquine is known to exert dose-

related retinal toxicity. A previous study on C57/6J mice showed complete loss of the outer plexiform layer as well as photoreceptors and photoreceptor nuclei after intraperitoneal injection of 10 mg/kg chloroquine hydrochloride for a total of 62 days (Gynes *et al*, 2008). The retinal pigmented epithelium demonstrated focal atrophy, loss of nuclei and pigment irregularity. Findings in the inner retina were notable for the loss of Müller cells and the presence of membranous cytoplasmic bodies (Gynes *et al*, 2008). Those pathological conditions were not identified in this study.

In summary, this study revealed that the five new antiplasmodial *N*-alkyl and *N*-benzyl-1,10-phenanthroline derivatives were toxic to mice only at high doses (comparable to chloroquine), but had no histopathological effects on the internal organs. Mice given sub-lethal amounts had normal activity, had weight gain comparable to controls, and manifested no histopathological changes in internl organs during the two week test period.

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