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Re-Evaluate Interrelationship Dose-Response of Diminazene Aceturate Against to Infected Mice of *Trypanosoma Evansi* Bangkalan Isolates

(EVALUASI KEMBALI HUBUNGAN DOSIS - RESPONS DIMINAZEN ASETURAT TERHADAP MENCIT YANG TERTULAR TRYPANOSOMA EVANSI ISOLAT BANGKALAN)

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ABSTRACT

In vivo drug experiment involving *Trypanosoma evansi* (*T.evansi)* of Bangkalan isolated from outbreak of surra at 1988 in Madura Island, (Indonesia) infected mice were performed. Single dose of diminazene aceturate at the range of dose 5.0 to 9.7 mg/kg bw intra peritoneal were tested against 4th parasitaemic grade level of infected mice. The assesment of trypanocide dose was using probit test analysis at confidence interval 95% after observing trial groups data from thick blood smear examination of vein tail. Apparently the dose of diminazene at 6.858 mg/kg bwt had eliminated 50% of the bloodstream parasites and 9.138 mg/kg bw for 99% had eliminated parasites. The research result indicates that Bangkalan isolate of *T. evansi* had expressed reduced susceptible to diminazene more than 1.3 times than regimentation dose to research report on 2005 year at the same research subject namely mice (at about 5.22 mg/kg bw).

KeyWords **:** surra, chemotrypanocide, interchalating, kinetoplast, variant surface glycoproteins

ABSTRAK

Telah dilakukan penelitian obat antitripanosoma secara *in vivo* pada mencit tertular *Trypanosma evansi* dari isolat lapangan (isolat Bangkalan) yang menimbulkan wabah surra tahun 1988 di Pulau Madura. Dilakukan uji daya tripanosidal dosis tunggal *diminazen aceturate* dengan rentang dosis 5,0-9,7 mg/kg bobot badan (bb) secara intraperitoneal pada mencit kondisi parasitemia positif 4. Penilaian hubungan antara respons terhadap dosis, menggunakan analisis probit dengan tingkat kepercayaan 95%, hasil pemeriksaan darah vena ekor pada seluruh kelompok uji. Tampak bahwa dosis 6,858 mg/kg bb dapat mengeliminasi parasit pada aliran darah sebesar 50%, dan 9,138 mg/kg bb dapat mengeliminasi parasit sebanyak 99%. Hasil penelitian menunjukkan bahwa kepekaan *T.evansi* isolat Bangkalan terhadap *diminazen aceturate* makin menurun 1,3 kali dibandingkan dengan hasil penelitian tahun 2005 pada subjek penelitian yang sama yaitu mencit (sekitar 5,22 m/kg bb).

Kata-kata kunci **:** surra, kemotripanosidal, pengganjal, kinetoplas, *variant surface glyprotein*

INTRODUCTION

Surra diseases caused *T.evansi* for the first time make a outbreak in 1988 on Bangkalan - Madura (Bancaran village). Survey from Research Institute of Veterinary Science in the last of 1988 reported that early host infection of the outbreak was found in local horses. Three month after outbreak, the parasites were spreaded and found in other animals in Bangkalan (i.e. cattle breed and buffaloes) (Lazuardi, 1994). A Spesific treatment for *surra* disease in Indonesia has not been discovered yet, even though the endemic condition is spready in wide area of the East Region of Indonesia.

The Bangkalan isolates of *T.evansi* have a several specific charachterization as follows; (i) highest stability of surface proteins antigen variant than other isolates, (ii) antigenic susceptible for horses, cattles, buffaloes and dogs, (iii) still viables during the six to eight month at the deep temperature storage (– 20°C). Background characterization of Bangkalan isolates of *T.evansi* was caused that isolates easy to explore (Davison *et al.,* 1996)

The earlier studies having addressed to the powerfull trypanocide for *T.evansi* Bangkalan isolates was found on suramin (naphthylamine acid derivate) (Lazuardi, 1994). Unfortunately, since early 1998 the suramin as a drug of choice to *T.evansi* in Indonesia was not been produced any more. But in the early 1999, Directorate General of Livestock Services, Agriculture Department in Indonesia allowed other alternative of anti eucaryotic parasites agent, named isomethamidium chloride (phenantrydine derivates) and diminazene aceturate (diamidine derivate) (Directorate of Animal Health Care, 1999).

At that moment, all researchers in Indonesia knew that most of trypanocidal agents (i.e homidium bromide, homidium chloride, protidium bromide, quinapyramine derivates, pentamidine derivates and miselanous groups) were potentially resistant to *T.evansi* Bangkalan Isolates. Isometamidium chloride as agent trypanocide of *T.evansi* Bangkalan isolates was reported potencial trypanocidal drug in laboratory cases but already resistant to field cases (Prastyawati *et al*., 1992). Diminazene was reported as a potencial trypanocide in both of laboratory cases and field cases for Trypanosomiasis at single and double regiment (Silayo *et al.,* 1992; Gutierrez *et al.,* 2008; Gillingwater *et al.,* 2009). For anticipating resistant phenomenon via variant surface glycoproteins of parasites, a new regiment dose of diminazene aceturate for *T.evansi* Bangkalan isolates must be re-evaluated at least every five year.

In the year of 2004-2005, *in vivo* test of mice with single dose regiment of that in the dose of diminazene aceturate against *T.evansi* Bangkalan isolates was found 5.22 mg/kg bw via intra peritoneal (Lazuardi, 2005[a]; Lazuardi, 2007). After five years more, the single dose regiment of diminazen against *T.evansi* Bangkalan isolates was probably different. The research aims were to re-evaluated that single dose regiment of diminazen was killing parasites on 4th level grade of parasitaemic mice.

RESEARCH METHODS

Chemotrypanocide Drugs

Anti trypanosoma drug had been obtained from Hebei Kexing Pharmaceutical Co., Ltd address no. 114 of New Technology Industrial Development Zone, Luquan City, Hebei Province, The Peoples Republic of China. The drug profile was in the form of powder in small pocket in about 2.36 g each pocket which composed of 1.05 g diminazene and 1.31 g of antipyrine. The purity of diminazene were performed at 99.1% of 1,3-bis (p-pamidinophenyl) tryazene bis N-acetylglycine) as referred to certificate of analysis (COA) with approved by Tianhuiyan, analyzed by Weiguixiang and checked by Ningcunxia. The diminazene as a research object had been permitted by Director of Research Program in Airlangga University and under control of Indonesia Veterinary Pharmacy and Pharmacology Association (Indonesia Veterinary Pharmacy and Pharmacology Association, 2012). The diminazen were dissolved in aqua pro injection before use.

Trypanosomes

The registered stock (P 0104) *T. evansi* is a stock isolated from outbreak disease of surra in the year 1988 in Madura Island (Bancaran Area, Bangkalan Region), Indonesia, as described on protocol of material transfer agreement of Research Institute of Veterinary Science, Directorate General of Livestock Services and Animal Health, Agriculture Department of Republic Indonesia at RE Martadinata 30 rd, Bogor Indonesia. The origin Bangkalan isolates of *T. evansi* was used, because that isolates has a highest stability on the antigenic protein surface than other isolates from other trypanosomiasis endemic places of Indonesia (i.e: South of Sumatera and South of Sulawesi, East of Jawa, West and East of Nusa Tenggara) (Davison *et al.,* 1996).

Experimental Animals

Male of *Mus musculus albinos bulb/c* with about 20-25 g bw of each, and male Rat of *Rattus norvegicus wistar* strain with about 250-300 g of bw of each were obtained from Center of Veterinary Pharma, Directorate General of Livestock Services, Agriculture Department at Ahmad Yani 68-70 rd, Surabaya–Indonesia. They were kept in external parasites proof building and handled carefully. The study

protocol was approved and accepted from Special Task Force Bioethic Committee of Indonesia Veterinary Pharmacy and Pharmacology Association for handling and using animal (Indonesia Veterinary Pharmacy and Pharmacology Association, 2012).

Parasites Stocks

T. evansi Bangkalan isolates stocked from liquid nitrogen chamber (P 0104) were thawed and analyzed for re-assesment of the activity of parasites. The stocks of Trypanosomes were propagated onto three donor rats by intraperitoneally and kept them on isolated room in about three days. After three days, the parasites were accumulated from the donor rats by cardiac puncture and kept them in the temperature of 4°C in 3 mL heparin tube for assesing of minimum parasites stock at about 10^6 trypanosomes/mL blood). The parasites as a stock were added with 1 : 1 of phosphat buffer glucose and ready for inoculate agent (Kaminsky *et al.,* 1994).

Dose-response Test

The dose respons test, were used for twenty groups of seven mice of each, each groups as trial groups and inoculated 10^5 trypanosomes for each mice intraperitoneally and maintained parasites in bloodstream for about two days. For examining parasitemia condition were using analysis of blood smear from tail vein of the trials groups. After parasitemia condition at 4rd grade level as reffered to Lazuardi method (Lazuardi, 2005[b], Liao and Shen, 2010). The single dose test of diminazene were injected via intra peritoneal in the range of 5.0; 5.2; 5.5; 5.7; 6.0; 6.2; 6.5; 6.7; 7.0; 7.2; 7.5; 7.7; 8.0; 8.2; 8.5; 8.7; 9.0; 9.2; 9.5; 9.7 mg/kg bw. The dose respons were analyzed by thick blood smear method from tail vein of mice in trials groups. The positive and negative control groups were use 280 mice separated on two groups (140 mice for positive control and 140 mice for negative control). Positive control groups at number of 140 mice were inoculated with 10^5 trypanosomes by intraperitoneally each mice without the diminazene. Negative controls groups at number of 140 mice were injected at similar ranging dose test each mice without infected with parasites as reffered trial groups aboved. The 50% of dose-response test were examined by probit analysis of minitab 16th version after counted rasio of the mice with killed parasites versus infected mice on trial groups.

RESULT AND DISCUSSION

The effects of the *in vivo* activity of diminazene on *T.evansi* Bangkalan isolates are summarized in Table 1. The trypanocide dose of diminazen for elimination of 50% parasites in bloodstream was obtained at 6.858 mg/kg bw. The trypanocide dose of diminazene with empowered elimination activities on infected mice was obtained at about 9.138 mg/kg bw. Figure 1, was appeared that 30% of trypanocidal respons of diminazene obtained at 6.345 mg/kg bw, and 40% of trypanocidal respons of diminazene obtained at 6.611 mg/kg bw. The trypanocidal respon for 60% and 70% were obtained at 7.107 mg/kg bwt and 7.373 mg/kg bw.

Result report of diminazene at dose of 9.138 mg/kg bw against to *T. evansi* Bangkalan isolates was apparently bigger than the last research reported in 2005 (5.22 mg/kg bw). That fact showed that the parasites had performed a new derivates protein surface by expressing resistance to diminazen. It is firmly established that the surface protein of trypanosomes is dominated by coat of the variant surface

Table 1. The single dose-respons test of diminazene at ranging 5.0 to 9.7 mg/bw againts to *T.evansi* Bangkalan Isolates on infected mice

Trial groups	Dose (mg/kg bw)	Respons (elimination/infection)
1	5.0	0/7
2	5.2	0/7
3	5.5	0/7
4	5.7	1/6
5	6.0	2/5
6	6.2	2/5
7	6.5	¾
8	6.7	¾
9	7.0	¾
10	7.2	4/3
11	7.5	4/3
12	7.7	5/2
13	8.0	6/1
14	8.2	7/0
15	8.5	7/0
16	8.7	7/0
17	9.0	7/0
18	9.2	7/0
19	9.5	7/0
20	9.7	7/0

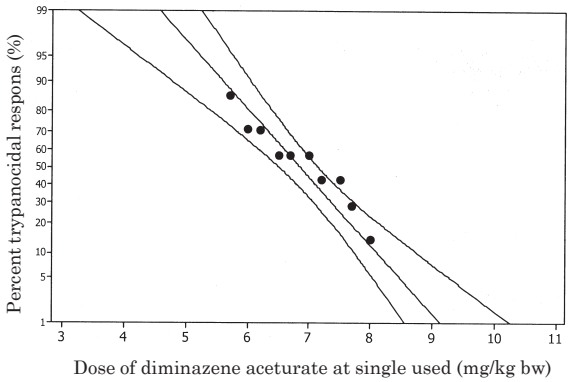

Figure 1. The survival plot of single dose-respons test at ranging 5.0 to 9.7 mg/bw for diminazene aceturate against *T.evansi* Bangkalan isolates on infected mice. The 50% of the dose-response was obtained at 6.858 mg/kg bw

glycoproteins. Some researcher predicted that variant surface glycoproteins made a new structures surface protein antigen as a part of parasites defence mechanism by productions a new copy of surface proteins antigen after exposure more than one chemotrypanocidal or after exposure other chemotrypanocidal agent than diminazene. For anticipate that problems at above, some researcher are make some opinion for producing some chemotrypanidal combination with prolong release vehicle (liposom) (Gillingwater *et al.,* 2010; Kroubi *et al.,* 2011). The major risk declined performance of variant surface glycoprotein was shown to make a new resistant parasites to diminazene at normal dose (Enyaru *et al*., 1998; Jia *et al.,* 2011; Jia *et al.,* 2012). The important compound of variant surface glycoproteins closely to diminazene exposure was space conformation of DNA protein base paired unit at a part of te parasites (kinetoplast regions), namely adenine-tyrocine and glycine-cytosine (A-T and G-C). Diminazene was active complexation with A-T and G-C of the kinetoplast as interchalating agent and caused a blocked biosynthesis activities. The chance of space conformation A-T and G-C of kinetoplast was known to make a chance of effective dose diminazene to block biosynthesis activity. If the dose of chemotrypanocidal agents are not adequate to kill parasites, the parasites at the second time would be producing a 10^7 new protein copies and also new space chemical structure conformations of DNA as a base pair unit.

CONCLUSION

These research can conclude that the parasites during the five years made a new copy of protein base pair that reduced susceptible of diminazene against the parasites as reffered to result research at much more dose than last trypanocide dose (at about 1.3 times). The new 50% trypanocidal dose of diminazene against to *T. evansi* Bangkalan isolates *in vivo* test was obtained at 6.858 mg/kg bw.

RECOMMENDED

From the reseach result, we can recommended that dose regiment of trypanocyde for diminazene to eliminate parasites in bloodstream better when used at recent dose regimented.

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