

An animal model of clinical kinetic analyzed to diminazene aceturate in subjects with *Trypanosoma* infection

Mochamad Lazuardi

Abstrak

Diminazene aseturat dilaporkan menghambat aktivitas balik enzim transkriptase melalui mekanisme aksi pengganjulan pada beberapa protozoa eukariot parasitik seperti kerabat *Trypanosoma*. Telah dicari farmakokinetik plasma darah diminazene pada lima kambing terinfeksi yang diberi pengobatan 7 mg.kg-1 berat badan dosis tunggal intra muskular. Konsentrasi obat ditetapkan melalui Kromatografi Cair Kinerja Tinggi fase terbalik. Hasil menunjukkan rerata (\pm galat baku) Absorpsi, Distribusi, Metabolisme, Ekskresi (ADME) berpola tri-eksponensial dengan K_a (menit^{-1}) $5.10^2 \pm 26.10^3$, α (menit^{-1}), K_{12} (menit^{-1}) dan K_{21} (menit^{-1}) $18.10^3 \pm 1.10^2$, $14.10^3 \pm 1.10^2$ dan $1.10^3 \pm 1.10^3$. Rerata harga β (menit^{-1}) dan K_{13} (menit^{-1}) didapat $1.4.10^4 \pm 4.10^5$ dan $3.10^3 \pm 2.10^4$. Rerata harga T_{maks} (menit) dan K_{maks} ($\mu\text{g.ml}^{-1}$) didapat 53.71 ± 30.61 and 13.40 ± 8.13 . Rerata harga V_{ds} (L), Cl (ml.menit^{-1}), $T_{1/2\beta}$ (jam^{-1}) and Area di bawah kurva $0-\infty$ ($\mu\text{g.L}^{-1}.\text{menit}$) didapat 4.91 ± 3.12 , 14.29 ± 4.08 , 94.91 ± 33.23 dan 12.680 ± 2.722 . (Med J Indones 2006; 15:69-73)

Abstract

Diminazene aceturate has been reported to inhibit the reverse transcriptase activity by intercalating action mechanism of a number of protozoan eucaryot parasitic like *Trypanosoma* species. The pharmacokinetics of diminazene in the blood plasma of five infected goats treated with single intramuscular doses of 7 mg diminazene base kg^{-1} body weight was investigated. The concentrations of the drug were determined by reverse phase high performance liquid chromatography. Results show that the mean (\pm SD) Absorption, Distribution, Metabolism and Excretion (ADME) of the drug plasma followed a tri-exponential process with K_a (minutes^{-1}) were obtained at $5.10^2 \pm 26.10^3$, α (minutes^{-1}), K_{12} (minutes^{-1}) and K_{21} (minutes^{-1}) were obtained at $18.10^3 \pm 1.10^2$, $14.10^3 \pm 1.10^2$ and $1.10^3 \pm 1.10^3$. The mean values of β (minutes^{-1}) and K_{13} (minutes^{-1}) were obtained at $1.4.10^4 \pm 4.10^5$ and $3.10^3 \pm 2.10^4$. The mean values of T_{max} (minutes) and C_{max} ($\mu\text{g.ml}^{-1}$) were obtained at 53.71 ± 30.61 and 13.40 ± 8.13 . The mean values of V_{ds} (L), Cl (ml.minutes^{-1}), $T_{1/2\beta}$ (hours^{-1}) and $AUC^{0-\infty}$ ($\mu\text{g.L}^{-1}.\text{minutes}$) were obtained at 4.91 ± 3.12 , 14.29 ± 4.08 , 94.91 ± 33.23 and 12.680 ± 2.722 . (Med J Indones 2006; 15:69-73)

Keywords: Pharmacokinetics, Chagaz, Berenil, Veriben, Tri-exponential, AIC

The use of prophylactic trypanocidal drugs, in particular diminazene aceturate (Berenil, Veriben), remain the most widely applied measure for the prevention and control of man trypanosomiasis (chagaz diseases) and animal trypanosomiasis (sura diseases) under field condition. Despite this, guidelines for their use are of limited assistance or the optimal drug dosage for a given situation, or in offering an alternative strategy to adopt in situations where a reduction in the efficacy of prophylaxis is encountered after treatment. This latter possibility, with regard to other chemotherapeutics, is now

apparently occurring with increasing frequency in field and may be the result of the development of drug resistance in the trypanosoma population.¹

Diminazene aceturate is an aromatic diamidine (Figure 1), as a yellow powdered have a physical characteristic as followed. Slightly soluble in alcohol, not soluble in ether and chloroform.

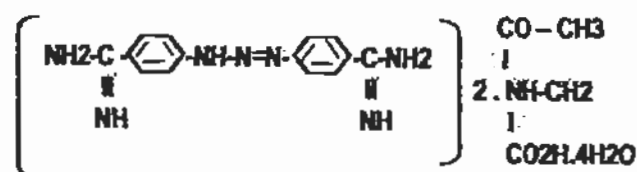


Figure 1. Molecular structure of diminazene aceturate

The diminazene aceturat have a molecular weight at 515.45 and contained of 51.25 % carbon, 5.67 % Hydrogen, 24.45 % of Nitrogen and 18.62 % of oxygen.² In general, the drug is effective when it is administrated as a single intramuscular dose of 3.5 to 7 mg.kg-1 body weight. However, treatment with that drug is not effective against trypanosomes that have invaded the CNS, and since the parasites which reemerge from the CNS after treatment do not appear to be drug resistant.³

Research study in animal model by infected bodies can be help us for explained Absorption, Distribution, Metabolism and Excretion (ADME) profile of diminazene during the therapeutics periods. That research models in Indonesia was never seen before. The current work was undertaken in an attempt to clarify the pharmacokinetics of diminazene aceturate in infected subject and the preliminary results are reported here.

METHODS

Research design of this work was used time series models with obtained kinetics parameter data research as a primary data.

Animals Preparation

Approval was obtained from the institutional Animal Ethics Committee of Veterinary Faculty Airlangga University, and animals were handled in accordance with the Indonesia animal welfare for scientific issued as described at International workshop of animal welfare in Surabaya.⁴

Adult male Etawa Breed Goats with a nominal weight of 50 kg and ages between 1 and 2 years were prepared as follows. Seven day before treatment all subject were acimated indoor at laboratory housing, and examined of BUN, Creatinin, SGPT and SGOT. Twenty fourth hours before treatment all subject was fasted.

Sample size

The sample size (N) of this research was used as described at equation 1 with assumption the value of

$$\left(z_1 - \frac{\alpha}{2} \right)$$

Equation 1

$$N = \frac{\left[\left(z_1 - \frac{\alpha}{2} \right) + z_{\beta} \right]^2}{\frac{(d)^2}{(S_a)^2 + (S_b)^2}}$$

In equation 1 was used at 1,96 (significance level of 0.05), Z_{β} was used at 1.645 (limit of error level at 5 %), the value of d was used at 3.62.⁵ The value of S_a was used at 1.7.⁶ The value of S_b was used 1.4.⁷

Parasites

The Trypanosoma evansi isolat Bangkalan were obtained from The Research Centre of Veterinarian, Directorate of Livestock Services, RE Martadinata 56, Bogor.

Diminazene aceturat

Diminazene aceturate at pharmaceutics grade (Berenil-RTU grade, each ml containing 70 mg of diminazene aceturate) was obtained from Hoechst Roussel Vet. Pvt. Ltd agency of Neeta Park, Airport Road, Yenwada, PUNE- 411,006 -India. All preparations were made up freshly for immediate use.

Chemicals

I-octanosulphonic acid sod. salt was supplied by Sigma-Aldrich chem corp., Methanol and Acetonitrile HPLC grade was obtained from Merck chem corp., Solid Phase Exctracton (SPE) ODS C18 1 ml was supplied by Sigma-Aldrich chem corp. Throughout the study deionized and aqua pro injection water (PT DIVIRSA) was used. The phosphate buffer was a mixture of 10mM Na₂HPO₄ and 10 mM NaH₂PO₄.2H₂O, NaCL and Glucose. These reagents and all other cemicals were of analytical grade and water used as such.

Apparatus

Chromatography was performed using a model quarternair a manual injector equipped with a 20 µl loop (Rheodyne 7725II HP), and a UV absorbance detector (Hewlett Packard VWD 1100) containing a dual path 14 µL flow cell (path legth 10 mm). Detection

of plasma samples and ultrafiltrate was performed at 370 nm. The detector signal was monitored by a HP Chem software program integrator.

Chromatography

The analytical column was a stainless steel (250 mm x 4.6 mm I.D) Ultrasphere C18 (5 µm) (Agilent)packed column. A guard column (10 mmx 2 mm ID) produced by Agilent (USA) was slurry handpacked and was installed in series with the analytical column. The mobile phase was composed of Acetonitrile : Water (70 % : 30 %) - containing ion pairing 0,005 M 1-octanosulphonic acid sod. salt and 0.1 % Triethyl aminine at pH 5.5. Chromatography was performed at a flow rate 0.5 ml/min. The eluent and the column were thermostated at 25 °C.

Research Protocol

- All subject were infected with 5 ml of *T. evansi* isolat Bangkalan (in phosphat bufer glucose) each goat every days during 5 days by jugular vein administrated. Each ml of Phosphat Bufer Glucose (PBG) containing 10^7 tryp.
- For analyzed developed of trypanosomes in blood stream, all subject were examined by whole blood film (WBF) test each 24 hours during 5 days.
- If the parasites were coming after examination by WBF, all subject were treatment with diminazene aceturate $7 \text{ mg.kg}^{-1} \text{ bw}$ by intra muscular administrated.⁸
- The whole blood plasma were sampling by jugular vein at 10, 30, 50, 70, 90, 120, 240, 720, 1440, 2880, 5760, 7200 and 10080 minutes post therapeutics. All whole blood plasma samples were prepared as mentioned at bellow⁹
 - One ml of methanol was added to each plasma samples (1 ml), mixed with vortex at 20"
 - Added with 2 ml of 0.05 M Na-EDTA solution and vortex 20 "
 - Centrifugation at 3500 rpm 10 min and removed any protein precipitate. Approximately supernate was adding na-EDTA solution to produced up to 4 ml of supernate containing 25 % of methanol
 - The free drug were seperated from blood plasma by SPE
 - The diminazene were obtained by eluted process of SPE with 90/10 Acetonitril/ distilled water contained 0.025 M 1-octanosulphonic acid sod. salt with 2 % of glacial acetic acid.
 - The eluent was evaporated by nitrogen gas and redissolved in 1 ml of mobile phase and filtrated with milipore (\varnothing 20 µm) and directly injected in triplicate onto HPLC system.

- The validation analyzed of HPLC condition were measured by VP (validation program) software for obtained the values of precision, accuracy, linearity and sensitivity.¹⁰
- All data were plotted at semi-log graphic as a time observed (minutes) vs. concentration ($\mu\text{g.ml}^{-1}$) and analyzed with linear exponential regression at two compartment models design for measured kinetics parameter.¹¹

RESULTS

Five of all subjects before infected trypanosomes were examined of BUN, Creatinin, SGPT and SGOT. The following table shows the characteristics of the subject and their values at Table 1.

Table 1

Subject	Chemical Clinics Characteristics			
	BUN (mg.dl ⁻¹)	Creatinin (mg.dl ⁻¹)	SGPT (U.L ⁻¹)	SGOT (U.L ⁻¹)
A	29.9	0.75	13	76
B	23.3	1.05	13	97
C	34.7	0.43	21	79
D	14.1	0.95	25	81
E	10.4	0.65	23	87

The Precision on plasma and accuracy of the test was evaluated by analyzed of coefficient variation (CV) and % recovery on 4th spike replicates ($0.260 \mu\text{g.ml}^{-1}$, $0.470 \mu\text{g.ml}^{-1}$, $1.088 \mu\text{g.ml}^{-1}$, $2.320 \mu\text{g.ml}^{-1}$) were obtained at range 3.075-4.878 % CV and 85.874-88.178 %. The linearity of calibration curves obtained with 5th spike plasma concentration at five replicates was good ($r^2 : 0.994$, $P < 0.05$, analysis of coefficient correlation from function : 1.971% , $Y = 42.78 \pm 1.551X + 0.8 \pm 0.97$). The limit of detection and limit of quantification of the test were obtained at $1.1 \eta\text{g.ml}^{-1}$ and $3.2 \eta\text{g.ml}^{-1}$.

Plasma concentration time-profiles of diminazene in 5 infected subject at the same periods are depicted in Figure 2. A three-compartment model adequately simulates these data, with an elimination half-life of about 3.95 days. All subject data were showed tri-exponential linear regression profiles.

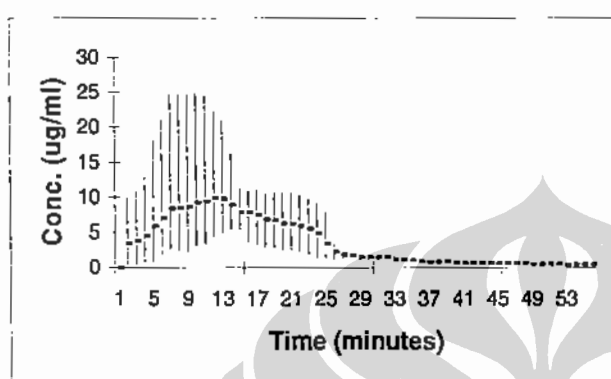


Figure 2. Concentration of diminazene ($\mu\text{g}\cdot\text{ml}^{-1}$) vs. time (min) in infected goat

The weighting (w_i) factor in all subject data were used 1, and resulting Akaike's Information Criterion (AIC) at range 64.902 to 124.5. Pharmacokinetics parameters obtained for individual animal, together with means and standard deviation, are shown in Tabel 2 with assumed absorption fraction of diminazene (F) at 1%.⁵

DISCUSION

The result of table 1 were showed normal values as described Blood et al., (12) and also Duncan and Prasse (13) at range BUN ($\text{mg}\cdot\text{dl}^{-1}$) 8-35, creatinin ($\text{mg}\cdot\text{dl}^{-1}$) up to 2.7, SGPT ($\text{u}\cdot\text{l}^{-1}$) up to 50, and SGOT ($\text{u}\cdot\text{l}^{-1}$) up to 307 ± 43 .

The extraction method, employing an ion-pairing reagent, produced minimal extraction of endogenous material. Almost complete recovery could be obtained in a two step extraction by addition of an organic solution (methanol) to the deproteinating and separated of analyte from matrix biology by SPE. The intra assay variation of spike plasma samples (% CV) was under regulation at $\leq 20\%$. The analytical recoveries from plasma were under regulation at range 80% to 120%.¹⁴ The minimum detectable quantity of diminazene extracted from plasma were gave a highest sensitivity then described Gummow et al.¹⁵ The advantages of this method over earlier techniques are enhanced selectivity and a simplified assay procedure. It is sufficiently sensitive to be applicable to the analysis of plasma samples derived from field studies of the clinical pharmacology of diminazene.

In semi-log models, visual inspection of observation concentration plotting against time (Fig. 2) showed the weighted model to be satisfactory at all stages of the concentration-time profiles. The analyzed of AIC in all subject were obtained minimum values as described by Lazuardi.¹¹

Table 2. Pharmacokinetic parameters obtained from infected goats treated with diminazene by deep im injection at a dose rate of $7 \text{ mg}\cdot\text{kg}^{-1}$ body weight

Parameter	Unit	Subject					Mean \pm SD
		A AIC 124.5	B AIC 72.37	C AIC 64.902	D AIC 99.13	E AIC 65.664	
Ka	Min^{-1}	51.10^{-3}	25.10^{-3}	22.10^{-4}	78.10^{-3}	73.10^{-3}	$5.10^2 \pm 26.10^{-3}$
α	Min^{-1}	28.10^{-3}	5.10^{-3}	143.10^{-4}	17.10^{-3}	26.10^{-3}	$18.10^{-3} \pm 1.10^{-2}$
K12	Min^{-1}	21.10^{-3}	32.10^{-4}	1.10^{-2}	$13.5.10^{-3}$	23.10^{-3}	$14.10^{-3} \pm 1.10^{-2}$
K21	Min^{-1}	4.10^{-4}	1.10^{-3}	1.10^{-3}	5.10^{-4}	2.10^{-3}	$1.10^{-3} \pm 1.10^{-3}$
Tmax	Min	43.327	99.7	68.914	29.733	26.875	53.71 ± 30.61
Cmax	$\mu\text{g}\cdot\text{ml}^{-1}$	12.183	10.608	6.908	27.544	9.776	13.40 ± 8.13
VDs	L	2.271	10.03	4.329	2.61	5.3	4.91 ± 3.12
β	Min^{-1}	834.10^{-4}	16.10^{-5}	$18.6.10^{-5}$	$9.51.10^{-4}$	$1.43.10^{-4}$	$13.36.10^{-5} \pm 4.10^{-5}$
K13	Min^{-1}	6.10^{-3}	1.10^{-3}	4.10^{-3}	3.10^{-3}	2.10^{-3}	$3.10^{-3} \pm 2.10^{-3}$
Cl	$\text{ml}\cdot\text{min}^{-1}$	16.816	13.304	19.852	9.515	11.96	14.29 ± 4.08
$T_{1/2\beta}$	Hours ⁻¹	138.5	72.19	61.93	121.451	80.5	94.91 ± 33.23
$\text{AUC}^{0-\infty}$	$\mu\text{g}\cdot\text{l}^{-1}\cdot\text{min}$	11.480	14.511	9.054	16.091	12.247	$12.680 \pm 2.722.10^3$

The kinetics parameter of all subject with infected condition were seen variation values. But generally, the observation of drug concentration in infected subject were lowest than healthy subject as described Lazuardi.⁹ The maximum concentration in subject of C were more lowest the other subject. But that subject was seen 2nd fastest then other subject for come in T maximum. The elimination half-life of subject C were more fastest then other subject but parameters of β in subject C were slowest than subject D (Table 2). The area under the curve of subject C was seen shallower than other subject, but the area under the curve of subject D was shown deeper than other subject. That phenomenon were shown the characterization of diminazene aceturate in infected bodies was different available. The polymorfism phenomenon of diminazene aceturate was predicted resulting from infection condition.⁹ The trypanosome infection resulted erythrolysis from all patient and make it a hypoproteinemia. That characterization will be resulted hypoplasmabumin and make it different available of diminazene in the bodies. Subject no c was seen have a strongest parasitaemic condition than other subject after 5 days post infection as described at Table 3.

Table 3. Parasitaemic condition each days after infection

Subject	Days post infection (parasitaemic condition)				
	1 st	2 nd	3 rd	4 th	5 th
A	+	++	+++	+++	+++
B	+	++	++	+++	+++
C	+	++	+++	++++	++++
D	+	++	++	+++	+++
E	+	+	++	++	+++

Acknowledgement

I am grateful to Professor Dr. A. Aziz Hubeis, Pharm, and Professor Dr. Rochiman Sasmita, DVM., MSc as a my promoter and co-promoter for supported.

CONCLUSION

The ADME characterization of diminazene aceturate in infected bodies were ununiformity values at mean range of elimination half-life at about 94.91 ± 33.23 minutes. The variabilities of observed concentration were depend on parasitaemic stage.

REFERENCES

- Whitellaw DD, Gault AE, Holmes PH, Sutherland A. Developmet of an enzym-linked immunosorbent assay for the detection and measurement of the trypanocidal drug. *Res Vet Sci* 1991; 50:185-9.
- Budavari S, O'Neil MJ, Smith A, Heckelman PE, editors. *The Merck Index : An encyclopedia of chemical drugs and biologicals*. 11th ed. Ran Way, New Jersey : Merck and Co., Inc. 1989. 3261.
- Mamman M, Peregrine AS. Pharmacokinetics of diminazene in lasma and cerebrospinal fluid of goats. *Res. Vet Sci* 1994; 57:253-5.
- CEO of The Indonesian Veterinarian Association, editors. *The workshop of International Concept on Animal Welfare with Reference to Indonesian cases, Legislation and Curriculum in Surabaya*; 2003 Oct 17-18; Surabaya, Indonesia. The Veterinary Faculty, Airlangga University.
- Aliu YO, Ødegaard S. Pharmacokinetics of Diminazene in Sheep. *J Pharmacokinetic Biopharm* 1985; 13(2):173-84.
- Mamman M, Aliu YO, Peregrine AS. Comparative Pharmacokinetics of Diminazene in Non infected Boran (*Bos indicus*) Cattle and Boran Cattle infected with *Trypanosoma congolense*. *Antimicrob Agents Chemother* 1993; 37(5):1050-5.
- Mamman M, Peregrine AS. Pharmacokinetics of Diminazene in Plasma and cerebrospinal fluid of goats. *Res Vet Sci* 1994; 57(2):253-5.
- Raina AK, Rakhesh Kumar, VS Rajora, Sridhar RP Sing. Oral Transmission of *Trypanosoma evansi* infection in dogs and mice. *Vet Parasitol* 1985; 18:67-9.
- Lazuardi M. The trypanocide assay of diminazene aceturate : The effective regimented dose design and toxicity investigated of consequence drug multiple administration on trypanosoma goats [dissertation]. Airlangga University. 2004.
- Wayudi TM, Nuraini F, Lazuardi M. The spiking method as an identifiyng of diminazene aceturate in plasina by High Performance Liquid Chromatography. Medical Faculty, Airlangga University. Office Medical Research Unit. 2005. Report no. 1/2004-2005.
- Lazuardi M. The equation analysis of linear pharmacokinetics models by Akaike's Information Criterion (AIC) of diminazene aceturate. *Folia Medica Indonesiana*. In press 2006.
- Blood DC, Radosúts OM, Arundel JH, Gay GC. *Veterinary medicine*. 7th ed. Oval road London NW1 7 DX ; Bailliere Tindall; 1986. 1463-4.
- Duncan JR, Prasse KW. *Veterinary laboratory medicine : clinical pathology*. 2nd ed. Ames, Iowa, USA; Iowa States University press; 1986. 232-3.
- Hartmann C, Massart DL, Mc Dowall. An analysis of the washington conference report on biological method validation. *J Pharm Biomed Anal* 1994; 12(11): 1337-43.
- Gummow B, Swan GE, Du Preez JL. Paired-ion extraction and high-performance liquid chromatography determination of diminazene in cattle plasma. : a modified method onderstepoort. *J Vet Res* 1995; 62(1): 1-4.