THE EFFECTIVENESS OF 2,4-DIKLOROBENZOILTIOUREA AS ANTICONVULSANT WITH ELECTROSHOCK METHOD IN MICE

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ABSTRACT

2,4-diklorobenzoiltiourea is a derivative of benzoiltiourea which has acyclic tioureide group and similar structure with barbiturate derivatives. Barbiturate and its derivatives have anticonvulsant effects. The objective of this study is to determine the anticonvulsant effect of 2,4-diklorobenzoiltiourea which is measured with electroshock. This is an experimental study using four groups of mice which consist of two experimental groups, one control group and one standard group. The first two groups were given 25 mg/kg and 50 mg/kg suspension of 2,4-diklorobenzoiltiourea for each group. The control group were given suspension of Sodium CMC and the standard group were given 26 mg/kg Sodium Phenobarbital. Thirty minutes after treatment, all the animals through their eyes were introduced with 0,5 mA electric shock for 0,2 second as seizure inducer. Effect of anticonvulsant were identified from the reducing duration of seizures which are total of either tonic, clonic and tonic-clonic seizures.

The result the analysed statistically and showed that 2,4-diklorobenzoiltiourea has anticonvulsant effect and has similar strength with sodium phenobarbital.

INTRODUCTION

Seizure is an episode of brain dysfunction happened in limited duration caused by abnormal discharge of cerebral neurons. Seizure is one of the clinical manifestations of epilepsy (Katzung, 2001). Epilepsy is a chronic disorder of central nervous system which occurs spontaneously in short duration with declining to losing consciousness as the main symptoms and usually with recurrent seizures (DiPiro, 2008). Optimizing the treatment in patient with epilepsy is very important because it affects patient's life. Epilepsy can result in significant abnormalities such as dangerous psychological conditions, anxiety and depression, and also can cause death (Linn DW et.al. 2009).

Antiepileptic agents are effective for controlling seizure in 50%-80% of all patients. Agents working as central nervous system (CNS) depressant can be used for reducing seizures in patient with epilepsy. CNS depressant agents can be categorized in sedative hypnotics, anti-anxiety, muscle relaxants, anticonvulsants, and antipsychotics. Sedative-hypnotics agents are barbiturates and benzodiazepines (Rang, HP et.al 2004; Goodman, 1995).

In medicinal chemistry, development of new compounds begins with the study of structure-activity relationship of drug. Biological activities of an active compound are influenced by its physicochemical properties, such as lypophilicity (π), electronic (δ), and steric (Es) feature. Lipophilicity can be determined by calculating the value of log P. Several methods can be used in determining log P, such as $\Sigma\pi$ Hansh Fujita, Σf Rekker Mannhold, or computerized methods using ChemOffice (Siswandono, 2000).

Siswandono (1998) synthesized Benzoyl urea (compound) through acylation of a primary amine group of urea with benzoyl group of benzoyl chloride. Benzoyl urea is an ureide acyclic with a structure similar to bromisovalum or barbiturate derivates, which are known for its CNS depressant effect, thus it is expected that benzoyl urea has the same effect as CNS depressant. As its structure doesn't contain any bromide, benzoyl urea doesn't cause bromism.

Kesuma (2004) synthesized benzoyl thiourea and its derivates through acylation of an amine group of thiourea with benzoyl group of benzoyl chloride and derivates. 2,4-diklorobenzoiltiourea is a derivate of benzoyl thiourea with acyclic thioureida group and a structure similar to barbiturate derivative compounds. The substitution of O (oxygen) by S (sulphur) on urea compounds resulting to thiourea, in which the electro negativity of O is greater than S; therefore the polarity of S is lower than O. With two –Cl on aromatic ring of benzoyl thiourea at the position of C2 (ortho) and C4 (para), it is expected that the lipophilicity can be increased, so the substance can penetrate the blood brain barrier and can be used as a candidate of CNS depressant agents (Kesuma, 2004). Ideal CNS suppressant effect is achieved when the compound has octanol-water partition coefficient value of 100 or log P = 2 (Siswandono, 2000).

As Kesuma (2004) had tested CNS depressant activity of 2,4-dichlorobenzoyl thiourea with potentiation test against thiopental, this study examined another CNS depression activity, which is the anticonvulsive activity. The study of 2,4-dichlorobenzoyl thiourea as an anticonvulsant used male mice *(Mus muscullus)* as the experimental subjects (Smith dan Mangkoedjojo, 1998). The methodology that used in this research was electroshock treatment, because it was related to the response of test subjects to electric shock. The electric shock would cause seizure on the subject because it affected the balance of electric current in the neuron. An electroshock apparatus, (which was used in) MES (*Maximum Electroshock Seizure*), can increase the spread of abnormal seizure discharges in neurons and increase the release of the neurotransmitters such as norepinephrine and acetylcholine. The ability of drug in against the effect of Maximum Electroshock Seizure can be understood and indicated as the ability of the drug to prevent the spread of the seizure discharges in neurons, in treating major motor (grand mall) seizure (Thompson, 1990).

The (observed) test parameter was the comparison of the duration of seizure between the treatment and control group. If the duration of seizure in treatment group decreased (compared with control group), 2,4-dichlorobenzoyl thiourea had anticonvulsant effect. However, if the duration of seizure in

treatment group did not decrease or constant (compared with control group), 2,4-dichlorobenzoyl thiourea did not have anticonvulsant effect.

This study aimed to find a new drug candidate which can be used to treat seizures in epileptic patient with maximum therapeutic effects and minimum side effects.

METHODS

Materials

The materials used in this study was 2,4-dichlorobenzoyl thiourea synthesized by Kesuma (2004) through acylation of 2,4-dichlorobenzoyl chloride with thiourea in THF solvent under reflux condition at a temperature of 90° C for 2 hours. The other materials are Phenobarbital-Na, CMC-Na and aquadest.

Animals

The animals used in this study were male, naive, healthy and non disabled mice, with 2-3 months old, and weighing 20-30 g.

Instrument

The main instrument used in the study was *Maximum Electroshock Seizure* (MES). It induces seizures by delivering 50 miliAmpere electric current for 0,2 seconds. Seizures could be tonic, clonic, or even tonic - clonic. Other instruments used were beaker glass, analytic scales, animal scales and stopwatch.

Procedure

1. The Determination of Peak Effect Time

Before anticonvulsant effects test was performed, a peak effect time, the time that is needed by a compound to achieve maximum concentration in the blood so that the compound can give the maximum activity, had to be determined.

The peak effect time was determined by observing seizure duration of the mice at 15, 30, 45, 60, 90, and 120 minutes after the injection of 25 mg/kg of 2,4-dichlorobenzoyl thiourea suspension with the volume of 0.5 ml/25 g mice. The seizures were induced by *Maximum Electroshock Seizures*. The duration used in the experiment was determined by the shortest seizure duration in which the compound achieved the maximum concentration (Siswandono, 2003; Turner RA, 1965))

2. Evaluation of Anticonvulsant Effect in Mice

The mice were divided into four groups, that consist of two treatment group, one control group, and one standard group. The control group was given oral CMC Na suspension. Treatment group I received 25 mg/kg of 2,4-dichlorobenzoyl thiourea suspension. Treatment group II received 50 mg/kg of 2,4-dichlorobenzoyl thiourea suspension. The standard group received oral phenobarbital Na solution with ratio of 26 milligrams for each kilogram of the mice body weight. After 30 minutes or the duration of peak effect time, each group was then induced with *Maximum Electroshock Seizure*, and the duration of seizures of each group was observed (Siswandono, 2003)

3. Data Analysis

Data analysis method that was used in the experiment is One Way Anova by utilizing SPSS program with $\alpha = 5\ \%$ or mean degree of 0,05.

RESULTS AND DISCUSSION

Induction of seizure was carried out using *Maximum Electroshock Seizure* (MES) placed on the corneas of mice. The part of MES which was placed on the corneas was a probe. The probe is a blunt electrode made of metal that will deliver electric current. Electric induction was given through the corneas of mice because the eye is associated with the second and third cranial nerve which connects the eye to the brain. When the electrical impulses reach the brain, they will be released through the intralaminar nuclei of thalamus thus stimulating the cerebral cortex which is the functional area of the motor nervous system. Then, the impulses will be delivered to the motor organs, causing abnormal motor activity and results in seizure. Electric current of 50 miliAmpere for 0.2 seconds was used for seizure induction because it can induce a *maximal seizure* in mice while keeping the lethal effects as small as possible. This method was chosen because it was simple, easily conducted, the results were reproducible, and it was suitable for laboratory scale.

The duration of seizure of mice on each group and the diagram of duration of seizure of mice is presented in table 3.1, 3.2, 3.3, 3.4 and figure 3.1

Table 3.1 The duration of seizure of mice on control group (CMC-Na)

Subject (mice)	Duration of seizure			
	Tonic (s)	Clonic (s)	Tonic-clonic (s)	Total (s)
1	16.37	-	17.11	33.48
2	19.7	3.4	12.3	35.40
3	18	2	10.48	30.48
4	20	2	8.40	30.40
5	19.71	10.94	-	30.65
6	16.5	5	12.7	34.20
7	15	12	10	37
8	20.98	3	8.02	32
9	28.04	2	-	30.04
10	17	6.67	5.93	29.6
			Mean	±32.22
			SD	2.56

Tabel 3.2 The duration of seizure of mice on standard group (Phenobarbital -Na 26 mg/kg)

Subject (mice)	Duration of seizure			
	Tonic (s)	Clonic (s)	Tonic-clonic (s)	Total (s)
1	9.08	2	9.29	20.37
2	-	3.45	15.91	19.36
3	7.25	3	12.49	22.74
4	6.16	8.74	4.92	19.82
5	-	10.58	15.17	25.75
6	-	9.87	12.63	25.5
7	7.5	5.45	9.84	22.79
8	11.51	2.88	5.24	19.63
9	11.55	3	9.96	24.51
10	-	9.77	17.92	27.69
			Mean	± 22.82
			SD	2.97

Tabel 3.3 The duration of seizure of mice on treatment I group (2,4-dichlorobenzoyl thiourea 25 mg/kg)

Subject (mice)	Duration of seizure			
	Tonic (s)	Clonic (s)	Tonic-clonic (s)	Total (s)
1	18.04	2.01	5.05	25.1
2	14.95	2	3.93	20.88
3	16.25	2	3.3	21.55
4	16.10	2.03	3.67	21.8
5	13.02	3.36	4.09	20.47
6	15.53	1	7.29	23.82
7	14.18	2	7.06	23.24
8	14.16	2.03	3.97	20.16
9	16.94	2	6	24.94
10	15.91	1.15	7.18	24.24
			Mean	± 22.62
			SD	1.87

Tabel 3.4 The duration of seizure of mice on treatment II group (2,4-dichlorobenzoyl thiourea 50 mg/kg)

Subject (mice)	Duration of seizure			
	Tonic (s)	Clonic (s)	Tonic-clonic (s)	Total (s)
1	17.21	5.89	-	23.10
2	14.19	2	5.41	21.6
3	12.79	3	9.06	24.85
4	11.25	2	8.29	19.54
5	17	2.79	-	19.79
6	8.15	5.05	6.85	20.05
7	14.90	2	6.15	23.05
8	16.39	1	8.01	25.40
9	16	-	10.06	26.06
10	13.28	2	8.11	23.39
			Mean	± 22.68
			SD	2.37

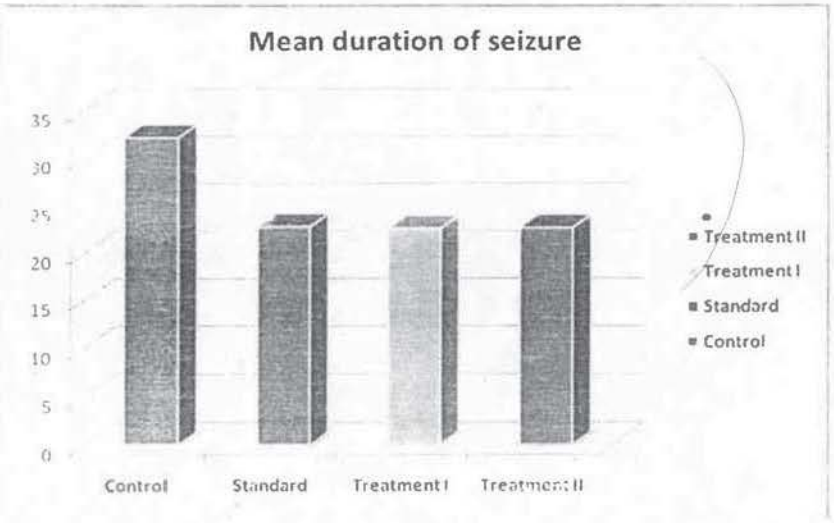

Figure 3.1 The chart of mean duration of control, treatment I, treatment II, and standard group comparison

Tabel 3.5 Summary of the statistical analysis (*post Hoc Test*) of duration of seizure on control, treatment, and standard group

(I) Group	(J) Group	Significance (α)
Control	Phenobarbital-Na	.000
	Treatment I	.000
	Treatment II	.000
Phenobarbital-Na	Control	.000
	Treatment I	.860
	Treatment II	.905
Treatment I	Control	.000
	Phenobarbital-Na	.860
	Treatment II	.955
Treatment II	Control	.000
	Phenobarbital-Na	.905
	Treatment I	.955

The mice used in this study should be naive or had never been used in any experiments. If the mice was not naive, it is possible that the mice had experienced trauma and would give a different response (Thompson, 1990).

Table 3.5 shows that there was a significant difference in seizure duration of treatment I and II group compared to control group. Therefore, it can be said that 2,4-dichlorobenzoyl thiourea provides an anticonvulsant activity. There was no significant difference among the standard group which received 26 mg/kg of Phenobarbital-Na and the treatment group, so that it can be said that 2,4-dichlorobenzoyl thiourea have the same activity with Phenobarbital-Na. There was no significant difference of effect between both of the treatment groups. The result means, dose enhancement was not accompanied by increased drug activity. The absence of increased activity was possibly caused by the saturation of drug receptor (pharmacodynamic phase). Although the systemic level of drug and the quantity at the site of action increase, the receptor saturation can occur if the number of receptors are less than the amount of drug, and not all of the drug are bound to the receptors (Shargel L, 1988). Therefore, the drugs which can not be bound to the receptor can not give effect, so a further research is required to confirm this.

CONCLUSION

Based on the results obtained in this study, it can be concluded that 2,4-dichlorobenzoyl thiourea has anticonvulsant activity and there is no increase in drug activity with increasing doses.

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INTERNATIONAL SEMINAR ON MEDICINAL CHEMISTRY AND TIMMERMAN AWARD 2011

PROGRAM AND ABSTRACT BOOK

SURABAYA, OCTOBER, 15, 2011

Table of Contents

WELCOME MESSAGE FROM THE CHAIRMAN OF ORGANIZING COMMITTEE

Distinguished Guests, Ladies and Gentleman

Welcome to Indonesia the lovely country, especially to Surabaya, the city of heroes, and to the Airlangga University which lies at east java, for all guests and the participant International Seminar on Medicinal Chemistry (ISMC) 2011.

It is the first time for us in Airlangga University to hold International Seminar on Medicinal Chemistry. This seminar is jointly organized by four Indonesian universities (Airlangga University, University of Indonesia, Bandung Institute of Technology, and Gajah Mada University), with Indonesian Institute of Science (LIPI) and Indonesian Medicinal Chemistry Association (PERAKMI).

At 2009, seminar medicinal chemistry was held with Timmerman Award. But now in 2011, the six collaborating institutes decided to develop this activity to become the International Seminar on Medicinal Chemistry (ISMC). The activities in ISMC are divided into three parts. The first is workshop on medicinal chemistry; the second part is Timmerman Award competition; and the third part is the seminar which consists of plenary lectures, poster session, and presentation of Timmerman Award to the winners.

The Workshop is kindly handled by Schrodinger Inc., USA. Twenty eight participants from Indonesia and Malaysia attended this intensive workshop on computer-aided drug design. The plenary lectures will be given by Professor Timmerman and other outstanding international scientists working in areas of medicinal chemistry. Sixty four posters will be presented during the poster session. We hope all participants can join together and discuss everything about recent scientific research and other academic activities

I would like to express my gratitude to Professor Timmerman, all the plenary lecturers, workshop trainers, juries of Timmerman Award, workshop participants, Timmerman Award participants, seminar participants, Organizing Committee members, and everyone who contributed to the success of this event. Finally, I would like to thank the six institutions which give trust to Faculty of Pharmacy Airlangga University to hold this ISMC 2011. For the future I hope the collaboration of the six institutions will maintain this activity which will give the advantage to the development of medicinal chemistry.

Best regards,

Dr.Bambang Tri Purwanto

Chairman of Organizing Committee

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SEMINAR PROGRAM

08.30 – 09.00 : Registration
09.00 – 09.10 : Opening Ceremony
09.10 – 09.30 : Coffee Break

08.30 – 15.00 : **POSTER DISPLAY**

Session I
Moderator : Prof. Dr. Umar Anggara Jenie (UGM)

09.30 – 10.10 : Plenary Lecture I
Prof. Dr. Henk Timmerman (Vrije Universiteit, Amsterdam)
"Receptor, from Concepts to Reality and Beyond"

10.10 – 10.50 : Plenary Lecture II
Prof. Dr. Masahiro Toyota (Osaka Prefecture University)
"Development of Two Different Types of Palladium-Catalyzed Cycloalkenylations and Application to Bioactive Natural Product Synthesis"

10.50 – 11.30 : Plenary Lecture III
Prof. Dr. Siswandono (PERAKMI and Airlangga University)
"Molecular Modeling and QSAR of Benzoylurea Derivates as CNS Depressant"

11.30 – 12.00 : Discussion

12.00 – 13.00 : Break for prayer and lunch

Poster Session
13.00 – 14.00 : Discussion.
Presenter should be on poster site for discussion.

Session II
Moderator : Prof. Dr. Daryono Hadi Tjahjono (ITB)

14.00 – 14.40 : Plenary Lecture IV
Prof. Dr. L. Broto S. Kardono (Indonesian Institute of Science)
"Search on Lead Compounds from Indonesian Natural Products and Their Prospect for Further Development"

14.40 – 15.20 : Plenary Lecture V
Dr. Raghu Rangaswamy (Senior Director, Schrodinger Inc.)
"Combinatorial Library Building and Lead Optimization"

15.20 – 16.00 : Plenary Lecture VI
Prof. Dr. Madhavi Shastry (Senior Application Scientist, Schrodinger Inc.)
"Prediction of Cytochrome P-450 Sites of Metabolism using Induced Fit Docking"

16.00 – 16.30 : Discussion

16.30 – 17.00 : Coffee break and prayer

17.00 – 17.15 : Timmerman Award Presentation.

17.15 – 17.30 : Closing Ceremony.

CERTIFICATE OF ATTENDANCE

presented to

AGUSLINA KIRTISHANTI

POSTER PRESENTER

INTERNATIONAL SEMINAR ON MEDICINAL CHEMISTRY
AND TIMMERMAN AWARD 2011

SURABAYA, OCTOBER, 15, 2011

Prof. Dr. Siswandono
President of Indonesian Medicinal Chemists Association

INTERNATIONAL SEMINAR ON MEDICINAL CHEMISTRY
SURABAYA INDONESIA
TIMMERMAN AWARD 2011

Dr. Bambang Tri Purwanto
Chairman of Organizing Committee