EVALUATION OF ANTIVIRAL PROPERTY OF CINNAMALDEHYDE-DERIVED BENZIMIDAZOLE AGAINST PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Abstract- Porcine reproductive and respiratory syndrome virus (PRRSV) causes an extensive morbidity and fatality of pigs resulting in significant economic loss of swine industry worldwide .Benzimidazole is n heterocyclic aromatic compound commonly used as anthelminthics in domestic animals .Its antagonistic properties on various viral and bacterial species have been widely documented, but the anti-PRRSV property has not yet been elucidated .Therefore,the antiviral effect of benzimidazole derivative against PRRSV grown in MARC-145 cells was assessed in this study .Benzimidazole was synthesized from the condensation between1,2-phenylene diamine and cinnamaldehydewhichextracted from the essential oil of CinnamomumverumJ .Presl.Treatment on pre-infection and post-infection of PRRSVwas evaluated .Results showed that benzimidazole derivative has little anti-PRRSV property with maximum only 5 %virus titer reduction in pre-virus entry, and hasno effect in post-infection study .These resultsindicate thatcinnamaldehyde-derived benzimidazole is of limited use for PRRSV protection.

Index Terms- Cinnamaldehyde, Benzimidazole, Pig, Porcine Reproductive and Respiratory Syndrome Virus

I. INTRODUCTION

Porcine reproductive and respiratory syndrome virus (PRRSV), a member of family Arteriviridae, is a positive single-stranded and enveloped RNA virus containing 10 open reading frames (ORFs). PRRSV infects sows and gilts resulting inabortion,stillbirth and weak piglets .Aside from this, development of interstitial pneumonia causes high illness and casualty for swine in all ages[1]. Alveolar macrophages are the primary susceptible cells for PRRSV replication as proven by in situ detection of PRRSV antigens or viral RNA .Therefore, the search foreffective strategy on anti-PRRSV infection has been carried out.

Essential oils and extracts from a wide variety of plants have long been used for medicinal purposes [2]. Essential oils usually consist of bioactive compound targeting the bacterial cells. Cinnamaldehyde is the major organic flavonoid constituent of essential oil found in cinnamon

isolated from Cinnamomum trees belonging to the family Laureaceae. As the name implies, it is the aldehyde responsible for the aromatic flavor of cinnamon occurring naturally as trans (E) isomer. Appeared as slightly yellow liquid, its chemical formula C_9H_8O (3-phenyl-2-propenal)has a molecular weight of 132.16 g/mol .The chemical stability of this organic compound is fair to poor .

Benzimidazole, an organic adsorption reagent, is among heterocyclic pharmacophores comprising benzene and imidazole. Commonly, benzimidazoles are a broad biochemical family used for nematode and trematode infestation treatments in domestic animals . Additionally, it was documented that benzidimazole has antimicrobial [3], antiviral [4], anticancer [5], and analgesic [6] properties. To improve the pharmacological activities, various derivatives of benzimidazole were synthesized although some of the readily synthesized compounds have found very robust application in medical treatments [7]. In the present study, the derivative benzimidazole were synthesized by the condensation between 1,2 phenylene diamine and cinnamaldehyde (Fig. 1). This affords the product without Schiff base compound as by product in order to improve antimicrobial activities.

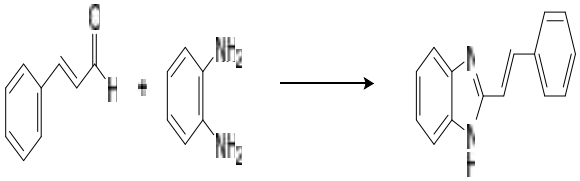


Fig .1 The reaction of 1,2 phenylene diamine and cinnamaldehyde.

Various benzimidazole derivatives have been evaluated for their antiviral activities .The antiviral properties of eighty-six benzimidazole derivatives against ten selected RNA and DNA viruses were screened [8]. Among those viruses, CVB-5 (coxsackie B-5), RSV (respiratory syncytial virus), BVDV (bovine viral diarrheal virus) and Sb-1 (poliovirus sabin strain 1) were significantly affected. Also derivatives of 2-phenylbendizimadole have been reported to have antiviral activities against CVB-2, BVDV, Sb-1, HSV-1 (herpes simplex virus) and YFV (yellow fever virus) while HIV-1 (human

immunodeficiency virus) and VSV (vesicular stomatitis virus) were not affected [9].

Despite several reports on the antiviral properties of cinnamaldehyde and benzimidazole against many viruses, study on their anti-PRRSV activity has not been elucidated .Therefore, the current study evaluates the antiviral activity of cinnamaldehyde-derived benzimidazole against PRRSV grown in vitro on MARC-145 cells.

II. METHODS

A. Essential Oil Extraction

The volatile oil of Cinnamomumverum J .Presl. bark was obtained by hydrodistillation [10]. One kg of each of Cinnamomumverum J .Presl. bark powders was mixed with 3 L of distilled water, followed by heating at 100 ºC for 8 h .The volatile products were extracted from the water phase three times using methylene dichloride [10].

B. Synthesis of Benzimidazole

The synthetic strategy leading to the target compounds is illustrated in Figure 1 .The benzimidazole derivative was synthesized by mixing 0.05 mole of 1,2-phenylenediamine and 0.10 mole of cinnamaldehyde in 30 mL ethanol and refluxed for 2-6 hr .The mixture was cooled for 1 d and filtered. The product was recrystallized with absolute alcohol [11].

C. Culture of MARC-145 cells and PRRSV

Cell line and virus were obtained from Veterinary Diagnostic Laboratory, Faculty of Veterinary Science, Chulalongkorn University .MARC-145 cells and PRRSVwere maintained in MEM++ comprising Minimum essential medium (Caisson Laboratories, UTAH, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Capricorn Scientific GmbH, Germany), antibiotics/anti-mycotic penicillin (100 IU/ml), streptomycin (100 µg/ml), and amphotericin B (250 ng/ml) (all from Gibco, New York USA). Virus was propagated in sub cultured MARC-145 cells grown in MEM++ at 37°C in a 5 % CO2incubator. After four days of incubation, the virus cultures were frozen overnight and thawed twice, centrifuged, and supernatant was harvested, filtered through 0.22 µM filter (Minisart®, Sartorius, France) and stored at -80°C .Titer was determined and adjusted to 10^6 $TCID_{50}$/ml..

D. Cell viability test by trypan blue dye exclusion assay and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method

MARC-145 cell cultures with approximately $1x10^5$ cells/ml were prepared in flat-bottomed 96-well plate (Nunc, Denmark). Serial 10-fold dilutions of extracts were added onto the wells and the cells were cultivated at 37°C in humidified 5 %CO_2 atmosphere for 96 h . Trypan blue dye aqueous solution was added in each cell suspension and viable cells in each well were counted using hemocytometer under the light compound microscope .Percentage of viable cells were calculated and compared to cell controls .On the other hand, another cell culture set up was prepared for MTT method .After 96h of incubation, MTT solution was added to each well and the plates were incubated for 4 h at room temperature .Then, MTT solution was carefully pipetted out and 100 µl of DMSO was added to dissolve formazan crystals .The plates were gently shaken for five minutes until the crystals were fully dissolved .The optical density (OD) was measured at 620 nm on a microplate reader .Percentage of cell viability was calculated using the formula [(A-B)/Ax100] where A and B are the OD of treated and control cells, respectively.

E. Antiviral activity against PRRSV

Using 50 %tissue culture infectious dose (TCID50)endpoint dilution assay,pre-treatment and post-treatment antiviral effect were investigated on this study .Three concentrations of the synthesized product were based on the results of cytotoxicity assay described above and activity of these compounds were tested against PRRSV .For pre-treatment effect, MARC-145 cells were cultured in 25-ml flask and incubated for 16 h .Then, the medium was removed and fresh MEM++ was added prior to the addition of the selected concentration of synthesized extracts .Five hundred µl of PRRSV titer was added onto the flask and the culture was incubated at 37°C in humidified 5% CO_2atmosphere for 96 h .Supernatant was collected and stored at -80°C .Subsequently, PRRSV titration was performed by the addition of serially 10-fold diluted supernatant in MEM++ and inoculated in 96 well-plate containing 100 µl of confluent MARC-145 cell .The cultures were incubated for 96 h at 37°C in a 5% CO_2incubator .TCID50 values were determined after uninfected and infected cells were observed under inverted microscope.

For post-treatment effect, the sub cultured MARC-145 cells in 25-ml flask was cultivated for 16 h .The medium was removed and 500 µl of PRRSV was added, followed by fresh MEM++. Then, the selected concentration of synthesized extract was added and incubation was carried outfor 16 h at 37°C in 5% CO_2incubator .Supernatant was collected and was 10-fold serially diluted .The diluted supernatant in MEM++ was added to the wells containing confluent MARC-145 cells .The cells were incubated for 96 h at 37°C in a 5% CO_2incubator .TCID50 values were determined under inverted microscope.

F. Percentage inhibition analysis

The calculation of virus inhibition percentage was based on the formula [100%x(A-B)/A] where A and B denotes to logarithmic number of virus titer in the absence and presence of test sample, respectively .

III. RESULTS AND DISCUSSION

The antiviral activity ofcinnamaldehyde-derived benzidimazole was summarized in Table 1 .In pre-virus entry study, benzimidazole 1, which was synthesized from cinnamaldehyde obtained from Cinnamomum trees in Indonesia, did not show PRRSV inhibitory effect. Benzimidazole 2 which was synthesized from cinnamaldehyde obtained from Cinnamomum trees in Thailand, showed little antiviral effect with maximum 5 %inhibition of PRRSV titer .In fact, benzimidazole 1 seemed to enhance PRRSV infection as the PRRSV titer was increased up to 15% after benzimidazole 1 treatment.

In post infection treatment, neither benzimidazole 1 nor benzimidazole 2 inhibited PRRSV replication. Instead, benzimidazole 1 did enhance PRRSV replication by up to 45%, while benzimidazole 2 enhanced PRRSV replication by up to 28% .

Extract	Concentration nl/ml	%Virus inhibition	
		Pre-infection	Post-infection
Benzimidazole 1	312.5	-10 %	-45 %
	31.25	-15 %	-28 %
	3.125	-5 %	-23 %
Benzimidazole 2	156	-45 %	-28 %
	15.6	5 %	-7 %
	1.56	-30 %	-17 %

Table I Antiviral activity ofcinnamaldehyde-derived benzimidazole

These results indicate that cinnamaldehyde-derived benzimidazole has low to no anti-PRRSV activity, and rather it enhances PRRSV replication in both pre-virus and post virus entry studies .Similar results have been reported showing the benzimidazole derivatives with no selective antiviral activity against selected RNA and DNA viruses including HIV-1, BVDV, YFV, DENV-2, WNV, HBV, HCV and human RSV [12]. In line with this, various benzimidazole derivatives such as 5-methoxy-l-methylbenzimidazole and 5-methyl-2-n-ribobenzimidazole enhances viral multiplication [13]. Our findings may imply that the use of benzimidazole for deworming in pigs might have positive effect on PRRSV replication. However, this possibility needs further investigation.

CONCLUSION

This study evaluates anti-PRRSV property of benzimidazole derivative synthesized from thecondensation between 1,2-phenylenediamine and cinnamaldehyde extracted from Cinnamomum essential oil .We detected low to no anti-PRRSV activity of this derivative, and unexpectedly observed its enhancing effect on PRRSV replication.

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