Antiviral Activity of Tilmicosin on Highly Pathogenic PRRSV Isolated from North-Eastern Part of Thailand during 2010

ฤทธิ์การยับยั้งเชื้อไวรัสพีอาร์อาร์เอสสายพันธ์รุนแรงที่แยกได้จากพื้นที่ภาคตะวันออกเฉียงเหนือของไทยในปีค.ศ. 2010 ของทิลไมโคซิน

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ABSTRACT

Highly Pathogenic Porcine Reproductive and Respiratory Syndrome (HP-PRRS) cause economic losses to the pig production in China as well as other Asian countries. Consequently, immense challenge to control and elimination efforts could not be avoid. Biosecurity and vaccine have been used extensively. Moreover, tilmicosin, a macrolide antibiotic, was found to accumulate in high concentrations in Porcine Alveolar Macrophages (PAMs) and could inhibit PRRS virus (PRRSV). This could benefit to PRRS control. In this study, HP-PRRSV and PRRSV were tested with four brands of tilmicosin in the market (A, B, C and D). The results demonstrated that all of four brands of tilmicosin in the market could inhibit the infection of both PRRSV and HP-PRRSV in cell culture, however, with different concentrations. Furthermore, we also confirm that genetic variation of PRRSV could not escape from the efficacy of tilmicosin.

บทคัดย่อ

เชื้อไวรัสพีอาร์อาร์เอสสายพันธ์รุนแรงก่อให้เกิดความเสียหายแก่อุตสาหกรรมการผลิตสุกรในประเทศจีนและประเทศอื่นๆในเอเชียเป็นอย่างมากผลที่ตามมาก็คือประเทศเหล่านี้ต้องทุ่มเทความพยายามในการควบคุมและกำจัดโรคนี้อย่างหลีกเลี่ยงไม่ได้ทั้งการใช้วิธีการความปลอดภัยทางชีวภาพและการใช้วัคซีนได้ถูกนำมาใช้อย่างกว้างขวางนอกจากนี้ยังมีการใช้ยาปฏิชีวนะทิลไมโคซินซึ่งมีรายงานว่าสามารถสะสมในเซลเม็ดเลือดขาวในถุงลมและยับยั้งเชื้อไวรัสพีอาร์อาร์เอสได้ซึ่งทำให้น่าจะเป็นประโยชน์ต่อการควบคุมโรคพีอาร์อาร์เอสในการทดลองนี้ทำการทดสอบเชื้อเชื้อไวรัสพีอาร์อาร์เอสสายพันธ์รุนแรงและเชื้อไวรัสพีอาร์อาร์เอสสายพันธ์ปกติกับทิลไมโคซินที่มีขายในท้องตลาด4 ยี่ห้อซึ่งพบว่าทิลไมโคซินทั้ง4 ยี่ห้อสามารถยับยั้งเชื้อพีอาร์อาร์เอสทั้งสองสายพันธ์ในเซลเพาะเลี้ยงได้ที่ความเข้มข้นแตกต่างกันในแต่ละยี่ห้อนอกจากนี้การทดลองนี้ยังยืนยันว่าความแตกต่างของสายพันธ์ของเชื้อไวรัสพีอาร์อาร์เอสไม่มีผลต่อการทำงานของทิลไมโคซิน

Key words: HP-PRRSV, Antiviral, Tilmicosin, Cell culture.

คำสำคัญ: พีอาร์อาร์เอสสายพันธ์รุนแรงยาต้านไวรัสทิลไมโคซินเซลเพาะเลี้ยง

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is an infectious disease of pigs which brings a significant economic loss for the global commercial pig production (McOrist et al., 2011; Nieuwenhuis et al., 2012). The first appearance of PRRS was reported in North America in 1987 (Keffaber, 1989) and causedsevere reproductive losses, respiratory disease, reduction of growth rate, and increased mortality of pigs. In Europe, the clinically similar outbreak was found in Germany in November 1990 (OIE, 1992). However, it was not until 1991 that the etiological agent of the disease was first isolated in Europe and shortly thereafter in the United States and Canada (Collins et al., 1992; Wensvoort et al., 1991). Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) grows in alveolar macrophage cells in the lungs of infected pigs, and leads to acute symptomatic infections (Beyer et al., 2000).

Recently, the emerging of Highly Pathogenic PRRS (HP-PRRS) was reported in China in 2006, and characterized by high fever, high illness rates, and high death rates for pigs of all ages (Tong et al., 2007). The virus belongs to type 2 of PRRSV and is currently widespread in China and several Southeast Asian countries (Amonsin et al., 2009; An et al., 2010; Metwally et al., 2010**).** Moreover, previous studies shown that the HP-PRRSV caused more severe clinical signs and pathological lesions in swine than PRRSV, and has the ability to cause a severe disease even without co-infection with other diseases (Brockmeier, 2012; Lager, 2012; Lili Zhang, 2013). Pathogenicity studies showed that HP-PRRS viruses isolated from 2006 to 2010 maintain a similar level of high pathogenicity. In addition, the HP-PRRSV isolates with a unique discontinuous deletion of 30 amino acids in Nsp2 are still the predominant viruses (Yu et al., 2013). In fact, management practices and elimination programs such as bio-security, sanitation, depopulation, test and removal were applied to reduce and eliminate PRRS challenge. Nevertheless, not all of pig farm could adopt this valuable measure due to the high investment cost. Vaccination could not be used as an alternative measure because the efficacy of ML-vaccine against all the genetic variation of PRRSV are less effective and its efficiency might not consistent (Charerntantanakul, 2012; Cho and Dee, 2006; Corzo et al., 2010).

Due to the weak points of ML-vaccine and the high cost of other methods, an alternative approaches are needed to control or inhibit infection and transmission of PRRSV in pig farm. The best option to control PRRS is to eliminate PRRSV spread from swine farms. Under such scenario, novel antiviral chemotherapies are welcome options to complement other strategies for PRRS prevention and control. One of these options would be to use anti-PRRSV drugs such as tilmicosin which is a macrolide antibiotic. A few studies have indicated that tilmicosin mixed with foods and drinking water can reduce the damages caused by PRRS in pigs (Batista et al, 2009; Benfield et al, 2002; Misener et al, 2006). Additionally, tilmicosin also has ability to accumulate at high concentration in the Porcine Alveolar Macrophages (PAMs) and African green monkey kidney (MARC-145) cells, which are known to support PRRSV replication (Blais et al, 1994; Du et al, 2011; Therrien et al., 2000).

Due to the lack of information about the efficacy of tilmicosin to HP-PRRS, this study aimed to

study antiviralactivity of 4 brand of tilmicosin in the market on HP-PRRSV isolated from North-eastern part of Thailand during 2010.

Materials and Methods

Cells, viruses and tilmicosin

The PAMs were collected by alveolar lavage from the lung of 6_week_old piglets which were free from PRRSV and grown in RPMI-1640 medium (Rose-Peake Memorial Institute) containing 10% heat-inactivated Fetal Bovine Serum (FBS) and antibiotics. MARC-145 cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with a 10% FBS. During the experiments, all cells were incubated in a humidified incubator at 37ºC with 5% CO_2. 24h after adherence process, non-adherent cells were decanted or washed by Phosphate Buffer Saline (PBS) or Hank's Balance Salt Solution (HBSS). Cells were then refilled with medium containing FBS solution and antibiotics. The viruses used in this study were am isolated strain of HP-PRRSV isolated from the infected pigs in the Northeastern part of Thailand during 2010 and PRRSV which were adapted to grow in MARC-145 cells. Four different brands of tilmicosin samples were obtained from the market which were sold in powder form and had to be dissolved in RPMI-1640 and DMEM medium before testing in PAMs and MARC-145 cells, respectively. The dissolved drugs were sterilized by 0.2 µm filter (Minisart®).

In vitro cytotoxicity of tilmicosin on MARC-145 cells and PAMs

2.10^4 MARC-145 cells/well and 10^5 PAM cells/well were seeded in 96-well micro-titer plates, then incubated at 37°C, 5% CO_2 for 24 h. Two fold dilutions of tilmicosin were prepared starting with 1000 µg/ml to find the maximum concentrations that have no effect on cells. Each dilution was performed in triplicate and transferred to cells. Cytopathic effects (CPEs) were daily observed.

Determination of the antiviral efficacy of tilmicosin on PRRSV in MARC-145 cells and PAMs

Pre-infection experiments, $2x10^4$ MARC-145 cells/well and 10^5 PAM cells/well were seeded in 96-well micro-titer plates, and treated with serial two fold dilution of tilmicosin from different company overnight. The first dilution of tilmicosin was 80 µg/ml. Then the cells were infected with 100 $TCID_{50}$ of PRRSV for MARC-145 cells and 100 $TCID_{50}$ of HP-PRRSV for PAMs in the presence of the drugs. The CPEs was daily assessed using inverted light microscope.

The minimum concentration of tilmicosin which protected cells to produce fifty percent of CPE were recorded and calculated using Reech and Muech's formula. (Reed and Muench, 1938)

Results

Cytotoxicity

Morphology of cells was observed via microscopic monitoring to confirm substantial deformation, shape changes and cell destruction after treated with serial two fold dilution of tilmosin from four different brands in the market. The results are expressed in Table 1.

Table 1 The maximum concentrations of tilmicosin samples that had no cytotoxicity on PAMs and MARC-145 cells

Cultured cells	Medium	Sample A (µg/ml)	Sample B (µg/ml)	Sample C (µg/ml)	Sample D (µg/ml)
PAMs	RPMI-1640	62.5	31.25	15.265	62.5
MARC-145 cells	DMEM	62.5	62.5	31.25	125

Antiviral efficacy of tilmicosin on HP-PRRSV and PRRSV in PAMs and MARC-145 cells

In pre-infection experiments, the data in PAMs indicated that the effective concentrations of four samples A, B, C, D against HP-PRRSV were 11.5µg/ml, 11 µg/ml, 21µg/ml, 21.5µg/mL respectively. The results in MARC-145 cells showed the similar effective concentrations against PRRSV of sample A, B and D at 11.5 µg/ml, whereas that of C was 20.5 µg/ml and 21.5µg/ml. (Figure 1)

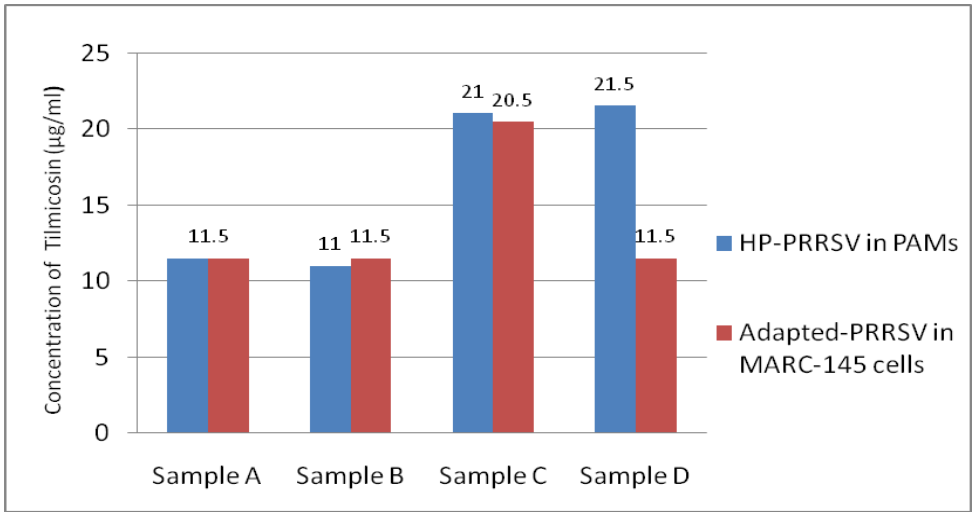

Figure 1 Effective concentrations of tilmicosin brand A, B, C, D which could protect 50% of cells from the infection of PRRSV

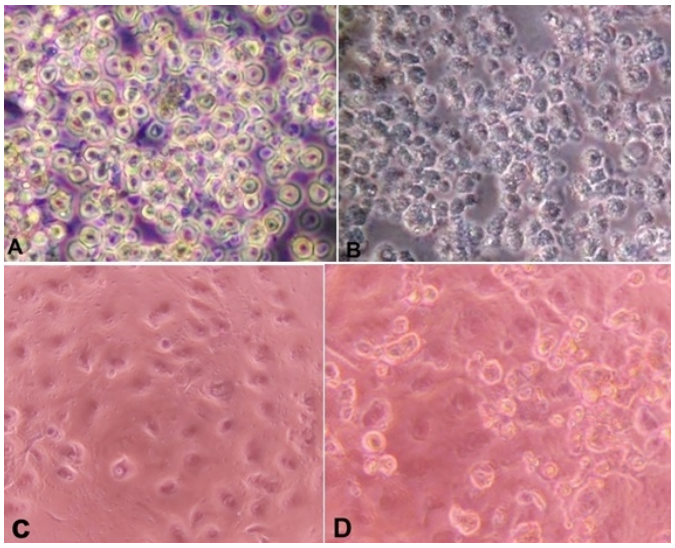

Figure 2 Cytopathic effect of HP-PRRSV on PAMs (A: control cells; B: infected cells). Cytopathic effect of PRRSV on MARC-145 cells (C: control cells; D: infected cells)

Discussion and Conclusions

The result showed that four brands of tilmicosin in the market have different cytotoxicity. Sample C was more cytotoxic to both PAMs and MARC-145 cells than others. The concentrations of tilmicosin that protected the cells from PRRSV infection were not cytotoxic and did not interfere the study. This study also showed that tilmicosin available in the market had different efficacy to inhibit the infection of PRRSV (Figure 1). Sample A and B were the most effective tilmicosin which inhibited infections of both PRRSV and HP-PRRSV inb MARC-145 cells and PAMs, respectively. In contrast, sample C needed more concentration to inhibit PRRSV. Interestingly, sample D showed different concentrations to inhibit PRRS and HP-PRRS. These results demonstrated that all of tilmicosin in the market could inhibit the infection of both PRRS and HP-PRRS in cell culture, however, with different concentration. Furthermore, we also confirm that genetic variation of PRRSv could not escape from the efficacy of tilmicosin.

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