The Effect Of Combination Of Octadecanoic Acid, Methyl Ester And Ribavirin Against Measles Virus

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Abstract: Ribavirin is a broad spectrum antiviral drug and has been used to treat various diseases. It has been used as a treatment for subacute sclerosis panencephalitis (SSPE) caused by measles virus infection. However, there were several adverse effects when receiving ribavirin treatment. Other than ribavirin and vaccine, there is no cure for the disease thus medicinal plants being studied for their potential active compounds to be used as either mono or combined treatment with drugs. The objective of this study was to test antiviral activity of octadecanoic acid, methyl ester (OA), extracted from *Cymbopogon nardus* (sweet lemon grass) against measles virus in both mono and combination with ribavirin. The cytotoxicity and antiviral activity were tested at low concentration for the compound (25, 12.5 and 2.5 µg/ml) and ribavirin (0.1, 0.05 and 0.01 CC_{50}). The cytotoxicity result showed that the low concentrations of both compounds have low cytotoxicity on Vero cell although there was slight increment of toxicity when they were combined. However, the combined treatment showed higher antiviral activity ($p < 0.05$) compared to single treatment of both compounds (OA12.5 µg/ml + RBV0.05CC_{50}: 94.38 ± 1.5%, OA12.5 µg/ml: 67.09 ± 0.2%, RBV0.05CC_{50}: 51.12 ± 2.1%). The result has also shown that decreasing of the concentration of the combination could still maintained the antiviral activity comparable to single treatment and less cytotoxicity toward Vero cell. This study has proven that OA can be combined with commercial drug such as ribavirin to produce higher antiviral activity at lower concentration for combination of both compounds.

Index terms: Antiviral, *Cymbopogon nardus*, cytotoxicity, measles, octadecanoic acid, methyl ester, ribavirin

1 Introduction

Stearic acid, methyl ester or stearate is a saturated 19 carbon-chained compound and it is also known as octadecanoic acid methyl ester (OA). There are various antiviral activities from fatty acids against viruses. Fatty acid was able to inhibit the replication of HCV and synergistic effect with IFN-α was observed by Leu *et al*. [1]. Measles virus (MV) belongs to paramyxoviridae family along with the parainfluenza, mumps, Newcastle disease virus and several other viruses. The MV replicates first in the respiratory tract and moved to lymphoid tissue for further viral processes [2]. The MV causes high death rates annually and has claimed as much as 13 million lives of children less than 6 years of age [3]. Although introduction of vaccine has been for 50 years, the disease is still could not be eradicated due to difficulty of vaccine distribution especially in rural or undeveloped areas such as Africa [4]. Ribavirin is an antiviral drug used to treat respiratory syncytial virus, RSV and strains of influenza A and B [5]. It has also shown antiviral activity against RNA viruses such as influenza A and B, measles and parainfluenza [6]. The ribavirin has the ability to incorporate itself into viral RNA and the reaction caused mutation that interferes with the normal viral replication processes [7],[8].

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Although ribavirin showed antiviral activity against HCV, there was adverse effect to recipients. SARS patient that was treated with ribavirin developed hypoxemia, a state of low healthy hemoglobin to carry oxygen [9]. The advantage of synergistic effect between drugs in combination therapy is the additive effect or diminished resistance of viral infection. However, multiple therapies may incur higher cost, increase in toxicity and could lead to treatment failure compared to monotherapy using single drug [10]. Combination of drug with herbs may increase or decrease the activity of either component [11] and the combination could also cause various adverse effects such as bleeding when patient mixed warfarin with *Ginkgo biloba* and mild serotonin syndrome when serotonin-reuptake inhibitors mixed with *Herpericum perforatum* [12].

2 Materials and Methods

2.1 Octadecanoic acid, methyl ester preparation

The octadecanoic acid, methyl ester (OA) was isolated from *Cymbopogon nardus* with confirmation via gas chromatography mass spectrometry, GCMS. It was prepared at different concentrations (25, 12.5 and 2.5 µg/ml) by diluting in Dulbecco's Modified Eagle Medium, DMEM. The control drug, ribavirin was also prepared at 0.1, 0.05 and 0.01 CC_{50}.

2.2 Cell culture

The Vero cells were cultured and maintained in T-25 flask with HyClone DMEM/ low glucose 5% FBS (HyClone) added with penicillin streptomycin (AMRESCO tissue culture grade). The Vero cells were subcultured once it reached 80-90% confluent. The Vero cells were also given geneticin to maintain the Signaling Lymphocyte Activating Molecule (SLAM).

2.3 Vaccine

The vaccine used was the live attenuated Edmonston Strain of the MMR vaccine (Serum Institute of India Ltd.).