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 sclerosing 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 CC50). 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 + RBV0.05CC50: 94.38 ± 1.5%, OA12.5 µg/ml: 67.09 ± 0.2%, RBV0.05CC50: 51.12 ± 2.1%). The result has also shown that decreasing of the concentration of the combination could still maintain 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].

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 synergic 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 CC50.

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.).
2.4 Antiviral assay
The Vero cells with the cell counts of 1.0 x 105 cells/ml were plated onto the 96-well plate with DMEM 2% FBS and incubated overnight until the cells are confluent. The medium were discarded and the plates were washed with DMEM twice and 10 µl of the measles vaccine was added into the designated wells of the plate and incubated for 30 minutes to allow the adsorption of virus to the cells. Then, 100 µl of groups of ribavirin and octadecanoic acid, methyl ester at different concentrations were added separately and combined to each well and left in the 37 ºC 5% CO2 incubator for 48 hours.

2.5 Plate processing
After 48 hours, the medium were discarded and the cells were fixed with 125 µl of cold TCA for each well which then incubated for 1 hour at 40 ºC. The solution were removed and washed 5 times with distilled water and left in 40 ºC for 1 hour. About 100 µl of eosin were added and left for 1 hour at room temperature. The plates were washed 5 times with 300 µl of acetic acid to wash the excess dye and left for 1 day at room temperature. About 200 µl of 5mM NaOH were added and left for 20 minutes at room temperature. The optical density, OD readings were taken with the Elisa reader (Microplate reader Metertech Inc.) at 490nm wavelength absorbance.

2.6 Statistical assay
Each treatment of fractions/subfractions was carried out in 5 replicates. The data were analyzed with one-way ANOVA test to compare between groups of treatment and Student T-test between the highest activity of treated group with control group. The criteria for statistical significance were taken as p < 0.05.

3 Results
The combination of octadecanoic acid, methyl ester (OA) and ribavirin (RBV) would determine whether the combination treatment could exhibit higher antiviral activity. It was also vital that the combination used lower cytotoxicity and lower concentrations compared to individual treatment. In this study, both compounds (octadecanoic acid, methyl ester and ribavirin) were tested for their cytotoxicity against Vero cell prior to antiviral assay both individual and combined treatment (Fig. 1).

In this study, the inhibition of ribavirin ranged from 68.67 ± 0.4% to 40.59 ± 1.9% while the octadecanoic acid, methyl ester had inhibition activity ranging from 82.33 ± 1.0% to 58.79 ± 2.4% (Fig. 2). When OA combined with RBV at concentration of 12.5 µg/ml and 0.05CC50, respectively, there was an increase of inhibition activity observed. The individual treatment had lower activity (OA 12.5 µg/ml: 67.09 ± 0.2% and RBV 0.05CC50: 51.12 ± 2.1%) compared to combined treatment of the same concentration, 94.38 ± 1.5%. The combined treatment had higher (p <0.05) inhibition activity even when compared to individual treatments at double the concentrations (OA 25 µg/ml: 96.88 ± 0.3% and RBV 0.01CC50: 98.22 ± 1.7%).

Fig. 1: Cytotoxicity of octadecanoic acid, methyl ester (OA) and ribavirin (RBV) against Vero cell. The graph showed the percentage of cell viability after different treatments given.

Fig. 2: Antiviral activity of octadecanoic acid, methyl ester and ribavirin against measles disease virus. The graph was based on the viability of treated infected Vero compared to non-treated infected-Vero.
Anti-RBV concentration was decreased from 0.05 to 0.01CC50: 68.67 ± 0.4%). By alternating the concentration of ribavirin and octadecanoic acid, methyl ester, there was an influenced on the inhibition activity. When the OA concentration was decreased from 12.5 to 2.5 µg/ml while maintaining RBV concentration at 0.05CC50, there was 16.06% decrease of activity. Whereas when the RBV concentration was decreased from 0.05 to 0.01CC50 with fixed concentration of OA at 12.5 µg/ml, there was 11.41% decrease of activity.

4 Discussions
In this study, the octadecanoic acid, methyl ester (OA) and ribavirin (RBV) have shown their antiviral activity against measles disease virus in both combined and separate treatment. Before the antiviral assay was conducted, it is vital to test the cytotoxicity of both compounds. Both compounds showed very low cytotoxicity against the Vero cell line with more than 90% viability for all treatments. The cytotoxicity assay is important to determine the toxic level of tested compounds that causes cell death via necrosis or apoptosis [13]. The compound to be tested should be able to induce antiviral activity at low cytotoxicity toward cells because without carrying out cytotoxicity assay, the result of antiviral activity might not be important though it was tested at low concentration [14]. This would relate to the cytotoxicity of combined OA and RBV in this study that has showed slight increment but able to exert high antiviral activity. The concentration of RBV could be manipulated by lowering it further to decrease the cytotoxicity while maintaining concentration of OA to produce high antiviral activity in combined treatment. Although this study used measles attenuated vaccine, it could still carry out viral processes that caused cell to lyse and cytophytic effect such as multinucleated cells [2] as the effect could be observed during the study. The antiviral results were based on the viability of treated (with OA and RBV) measles infected Vero cells in comparison to the control, measles infected Vero cells. The combination treatment of OA and RBV in this study showed a promising enhanced antiviral activity of both compounds by inhibiting the viral processes that leads to cell lysis. The combination of both compounds showed higher antiviral activity compared to their separate treatment at double concentration. The control drug, ribavirin has been known as a broad spectrum antiviral drug which is able to inhibit inosine monophosphate dehydrogenase that reduces GTP hence restricting replication processes [6]. It has been used in combination with IFN-α for treatment against subacute sclerosing panencephalitis (SSPE) virus infection in hamsters and showed higher viral inhibition than individually while no significant toxicity increase when combined [15]. OA itself has been shown in a previous study by Reagan et al. [16] and found to possessed antiviral activity against measles disease. OA is classified as fatty acid methyl ester, FAMES and this group has been shown to have antimicrobial [17] and potential anticancer [18] properties.

5 Conclusion and recommendation
The outcome of this preliminary study showed that octadecanoic acid, methyl ester (OA) possessed important antiviral properties and it can be combined with drug such as ribavirin to exert higher activity. Further study is needed on cytotoxicity against different cells such as human normal and cancer cells together with testing for other bioactivities such antimicrobial, antioxidant and antifungus. OA might possess various bioactivities that are yet to be fully studied and could also provide based-structure for other derivatives that can be produce chemically.

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References


[17]. M. Chandrasekaran, A. Senthilkumar, and V. Venkatesalu, “Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of Sesuvium portulacastrum L.,” European Review or Medical and Pharmacological Sciences, 15(7), 775–80, 2011.