抗流行性感冒病毒(H1N1)天然藥物之探索-訶子

Studies on the natural medicines for anti-influenza virus (H1N1) -Terminalia chebula

中文摘要

本研究為尋求抗流行性感冒病毒(H1N1)藥物，進行訶子成分之分離及抗病毒活性測試，追蹤其有效活性成分。研究內容包括：一、化學探討- 由含水丙酮萃取物，經由大孔吸附樹脂、多葡糖聚脂及逆相層析等管柱，分離出 [1] Phenolcarboxylic acid: gallic acid (1), protocatechuic acid (2), chebulic acid (3)。 [2] Glloylglucoses: 1,6-di-O-galloyl-β-D-glucopyranose (4), 1,3,6-tri-O-galloyl-β-D-glucopyranose (5), 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (6)。 [3] Galloylshikimic acids: 4-O-galloylshikimic acid (7), 5-O-galloyl shikimic acid (8)。 [4] Ellagitannins: casuariin (9), chebulanin (10), corilagin (11), chebulagic acid (12), chebulinic acid (13), castalagin (14) 等十四個化合物。 二、抗 H1N1 流感病毒活性 MTT 測試- [I] 藥物及流感病毒同時加入細胞培養測試，以 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose(6) 抗病毒活性最優，其細胞存活率為 144.5 %，高於 ribavirin (105.0 %)的效果，MIC 值與正對照組 ribavirin 同為 12.5 μg/ml，其次為 chebulinic acid (13), 131.8 % ，MIC 值為 25 μg/ml。 [II] 加入藥物 1 小時後，再加入流感病毒。 在沒有藥物的保護作用之下，其細胞受病毒感染後，細胞存活率為 8.5 %，而 1,2,3,4,6-penta-O-galloyl-?nβ-D-glucopyranose (6), 135.9 % 和 chebulinic acid (13), 134.1 %之抗病毒活性最好，其細胞存活率比正對照組 ribavirin, 112.6 %高。在細胞存活率的數據顯示，本組實驗之 MIC 以 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose 效果最好為 12.5 μg/ml，和 ribavirin 一樣。[III] 加入病毒一小時後，再加入藥物，評估治療效果。在沒有任何藥物的保護作用之下，其細胞受病毒感染後，細胞之存活率為 7.9 %，正對照組 ribavirin 為 111.6 %， 而所試驗之化合物中以 chebulagic acid (12), 126.9 % 和 chebulinic acid (13), 120.7 % 治療性抗病毒作用最好，高於 ribavirin 的作用，其次 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose(6)和 castalagin (14)的細胞存活率分別為 109.2 % , 110.8 % ，和 ribavirin 的效果一致。正對照組 ribavirin 之 MIC 為 12.5 μg/ml，而以上四個有效化合物之 MIC 均為 25 μg/ml。 結論：(1) 由化學結構與活性相互關係之探討得知，多酚性化合物對流行性感冒病毒(H1N1)之抑制、預防及治療作用，以 1,2,3,4,6-penta-O-galloyl-?nβ-D-glucopyranose (6) 及 chebulinic acid (13)呈現最顯著之抗病毒作用。同時，取代之 galloyl 或 chebuloyl 等 acyl group，總數目之多寡將影響其活性之大小。 (2) 依化學結構分類，小分子之 Phenolcarboxylic acid 類化合物僅以 protocatechuic acid (2)之作用較佳；Glloylglucoses 類化合物則以接五個 galloyl 基之 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (6) 抗 H1N1 病毒效果最好；Galloylshikimic acids 類之抑制 H1N1 病毒作用不明顯；Ellagitannins 類化合物呈現最佳之抗 H1N1 作用，但糖基上若有兩個以上之 OH 基未接取代基時，其作用顯著下降。

英文摘要

This study aimed to find out anti-H1N1 virus drugs from Terminaria chebula approached by constituents isolation of and anti-H1N1assay. The methods and results include: (1) Chemical investigation- From aqueous acetone extract, by high porous adsorbents resin, polydextran, and reverse column chromatography and fourteen compounds were isolated as following: [1] Phenolcarboxylic acid: gallic acid (1), protocatechuic acid (2), chebulic acid (3). [2] Glloylglucoses: 1,6-di-O-galloyl-β-D-glucopyranose (4), 1,3,6-tri-O-galloyl -β-D-glucopyranose (5), 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (6). [3] Galloylshikimic acids: 4-O-galloyl shikimic acid (7), 5-O-galloyl shikimic acid (8). [4] Ellagitannins: casuariin (9), chebulanin (10), corilagin (11), chebulagic acid (12), chebulinic acid (13), castalagin (14). (2) Antivirus activity tests against influenza H1N1 by MTT assay- [I] Drugs and virus were added to cell culture plate at the same time. The 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (6) showed the highest anti-H1N1 activity and the cell viability was 144.5 %, higher than the effect of ribavirin (105.0 %). The second is chebulinic acid(13), 131.8 % and 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (6), had equivalent activity as the positive control, ribavirin and the MIC was 12.5 μg/ml. [II] When treatments with or without tested drugs were added 1 hour pre-incubated with the cells before adding virus, the cells without any treatments were infected and the viability was 8.5 %. However, 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (6), 135.9 % and chebulinic acid (13), 134.1 % showed excellent protective activity and the cell viability was 144.5 %, which is higher than ribavirin (112.6 %), the positive control. The MIC of 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (6) was 12.5 μg/ml and similar to the one of ribavirin. [III] Add drugs to cell culture plate at 1 hr after virus added. After virus infection, if without any drug’s treatment, the cell viability is 7.9 %. Using ribavirin as positive control, the cell viability was 111.6 %. chebulagic acid (12), 126.9 % and chebulinic acid (13), 120.7 % possess the best therapeutic activity and higher activity than ribavirin for H1N1 virus infection. The second and the third are 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (6), castalagin(14) and their cell viabilities were 109.2 % and 110.8 %, respectively, the same as ribavirin. The MIC of ribavirin was 12.5 μg/ml, the MIC of four active polypenolics were 25 μg/ml. In conclusion: (1) Based on SAR discussion, the inhibition, prevention, and therapeutic effects of polyphenolics for H1N1 virus infection, both 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (6) and chebulinic acid (13) showed the most significant activity. The number of substituted galloyl or chebuloyl acyl group has impact on the activity. (2) According to chemical classification, small molecule’s phenolcarboxylic acid, protocatechuic acid (2) is the only one which show good activity; in glloylglucoses, five galloyl substituted

1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (6) exhibited excellently anti-H1N1 virus activity; the activity of galloylshikimic acids were not significant; ellagitannins showed the best, but if sugar moiety remains more than two free OH residues, the anti-H1N1 virus activity would decreased significantly.