Mechanism by Which Rhubarb Suppresses Influenza Virus Replication at the Fusion/Uncoating Stage

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Background/Objective

Rhubarb is a conventional herbal medicine and has been widely used in Asia for managing fever and removing toxicity. It also has activities against microorganisms, inflammation, and viral infection.

Method

Antiviral activity of non-toxic Rhubarb ethanol extract (Rex) was determined by neutralization assay (inhibition of virus-induced cell death) using MDCK cell line. Time-of-addition assay is used to examine -Rex inhibitory effect at specific replication stages. Viral binding, attachment, penetration, and hemagglutination inhibition assays were used for detecting entry step inhibition by - -Rex. Viral RNA, protein expression, and protein distribution were analyzed by quantitative real-time PCR, immunoblotting, and immunofluorescence assay, respectively. To explore where -Rex ethanol extract affects fusion/uncoating step of life cycle, red blood cell (RBC) hemolysis inhibition and cell-cell fusion inhibition assays were performed.

Result

Rex inhibits influenza A viruses of H1N1 subtypes, including swine origin influenza virus strains and clinical oseltamivir-resistant strains in MDCK cells with EC50 in the range of 10 μg/ml and a selectivity index of 10-35. Time-of-addition assay revealed that Rex inhibited viral entry step during life cycle. We further confirmed that -Rex effectively inhibited viral attachment and penetration with EC50 of 49.41 ± 8.45 g/mL and 52.08 ± 3.12 g/mL, respectively. Rhubarb ethanol extract might target to HA by binding assay. Through hemagglutination inhibition assay Rhubarb could not inhibit HA1 of receptor binding activity. But RBC hemolysis inhibition and cell-cell fusion assay suggest that Rhubarb blocked HA2 of fusion activity (virus-endosome fusion) at fusion/uncoating step.

Conclusion

Our findings demonstrated that Rhubarb extract effectively suppressed influenza virus replication by inhibiting viral uncoating step during entry. Rhubarb shows its specificity against H1N1 influenza virus including clinical oseltamivir-resistant strains. Rhubarb could be potentially developed as an anti-influenza agent.