

Original Paper

Effects of aqueous *Echinacea purpurea* extract on immunogenicity of DNA vaccine encoding M2 gene of Influenza virus

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Abstract

Background and Objective: Continuous antigenic variation of Influenza A viruses causes a major concern to develop Influenza vaccine. Conserved antigens are suitable candidates for vaccine production due to its non-requirement to match the designed strains with circulating strains. The M2 gene is conserved among Influenza A viruses and has potential to be considered as a universal vaccine. This study was designed to evaluate the effects of aqueous *Echinacea purpurea* extract on immunogenicity of DNA vaccine encoding M2 gene of Influenza virus.

Materials and Methods: This interventional study was carried out on female BALB/c mice with 3-4 week age (250-300 gr). Plasmid DNA encoding M2 gene (pcDNA-M2) of Influenza virus A/New Caledonia/20/99 (H1N1) was transformed into *E.coli* top10 f' and cultured in LB broth media. Large scale plasmid preparation was done and the concentration was measured by spectrophotometric method. Mice were divided into eight groups and immunized three times with fifteen days apart. Vaccine groups received inactivated Influenza virus or pcDNA-M2, alone or in combination with Echinacea extract. Control groups were injected pcDNA, Echinacea extract, and phosphate buffer. All animals were left to bleed before immunization and at 21 days after the last vaccination and specific anti-M2 antibodies were measured by indirect ELISA. Then the mice were intranasally challenged under an anesthesia with mouse-adapted PR8 Influenza virus and monitored for 3 weeks to evaluate the vaccine regimen efficacy in reduction of mortality rate compared to control groups. Data were analyzed using SPSS-16, One-way ANOVA and Kaplan–Meier tests.

Results: The highest specific immune response was obtained in mice received inactivated virus plus extract (P<0.05). Immune responses in mice inoculated with pcDNA-M2 were significantly higher compared to all control groups mice (P<0.05). In addition the specific immune responses in group inoculated with pcDNA-M2 and aqueous extract was higher compared to the group receiving only pcDNA-M2 (P<0.001). The highest survival rate was observed in mice injected with inactivated virus or pcDNA-M2 plus extract.

Conclusion: This study showed that pcDNA-M2 induced specific immunity and protected mice against lethal challenge with PR8 Influenza virus. Furthermore, application of Echinacea extract with M2 gene vaccine increased vaccine efficacy.

Keywords: DNA vaccine, *Echinacea purpurea* extract, Influenza, M2 gene of Influenza virus

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