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Commentary

A comparison study of effects of *Echinacea* extract and levamisole on phenytoin-induced cleft palate in mice

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Abstract

There are many reports that the teratogenic effects of phenytoin, especially cleft palate can be decreased by stimulation of maternal immune system. Also, there is some evidence that *Echinacea* extract and levamisole are immunomodulator drugs. So, in this study, we compared the prophylactic effects of levamisole and *Echinacea* extract on teratogenic effects of phenytoin. This study was performed on 32 pregnant mice that were divided into four groups. The first group (control group) received normal saline intraperitoneally and the other groups (test groups) received phenytoin (65 mg/kg intraperitoneally) at 10th day of gestation. Levamisole and extract of *Echinacea purpurea* were administrated at dose of 10 and 360 mg/kg intraperitoneally, respectively, in along with and 12 h later after phenytoin injection, in two groups. Fetuses were carried out in 19th day of gestation and after determination of weight and length; they were stained by Alizarin red–Alcian blue method. Cleft palate incidence was 16, 5.3, and 3.2% in fetuses of mice that received only phenytoin, phenytoin with levamisole, and phenytoin with *Echinacea* extract, respectively. Mean weight and length of fetuses of animals that received levamisole and *Echinacea* extract were significantly greater than those received only phenytoin. It is concluded that *Echinacea* can stimulate immune system more than levamisole and has better prophylactic effect on incidence of phenytoin-induced cleft palate, but it is not significant.

Keywords: Phenytoin; Cleft palate; Levamisole; *Echinacea*; Mice

1. Introduction

Some chemical agents and drugs can induce teratogenic effects and abortion (Giavini and Menegola, 2004). Developmental defect belong the major problems of health, so as, in USA 3–5% of fetuses have congenital abnormality. Nearly 2–3% of developmental defects in the general population are related to teratogenic agents (Finell, 1999) which 1% of them are caused by use of drugs in pregnancy period (De santis et al., 2004). Although 40 agents are teratogenic for human fetuses, more agents are teratogenic in laboratory animals. Valperoic acid, cyclophosphamide, methylnitrous urea, and phenytoin are the best known teratogenic drugs in human and laboratory animals (De santis et al., 2004; Orup et al., 2003; Prater et al., 2004; Syska et al., 2004; Winn and Wells, 1999).

Phenytoin as hydantoin derivative is used for control of epilepsy. It is believed that phenytoin produces anomalies in 34% of fetuses which are exposed to it (Winn and Wells, 1999). Several studies show that, the stimulation of maternal immune system can decrease or prevent drug-induced embryonic abnormalities (Holladay et al., 2002, 2000; Prater et al., 2004). For example, in one study, macrophage activation decreases incidence of cleft palate and digital and tail anomalies in fetuses of mice that received urethane and methylnitrous urea (Holladay et al., 2000). In other

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study, interferon-γ reduced urethane-induced cleft palate and granulocyte-colony stimulating factor decreased cyclophosphamide-induced distal limb abnormalities in mice (Syska et al., 2004).

In the other hands, levamisole as antinematodal agent has immunomodulatory effect in human and animals. This drug stimulates T and B cells proliferation and antibodies production (Hardman and Limbird Lee, 2001). *Echinacea* potentiates immune system and is applied in the prevention or treatment of some diseases including influenza and common cold. *Echinacea* extract actives macrophage, polymorphonuclear leukocytes and natural killer cells (Barrett, 2003).

In present study, the prophylactic effect of levamisole and *Echinacea* extract was compared on phenytoin-induced cleft palate in mice.

2. Materials and methods

Dried aerial parts of *Echinacea purpurea* were purchased from Goldaruo Co. Isfahan, Iran. The plant was taxonomically identified at department of Botany, school of Agriculture, Shahid Chamran University, Ahvaz, Iran. Plant was powdered using a grinder (MSE, England). One hundred grams of this powder was placed in a beaker and 1000 ml of 70% ethanol was added. The mixture was left in room temperature for 3 days. The extract was separated and remaining plant was extracted by more ethanol after 2 days. The extract was filtered by Whattman (No. 1) filter paper and concentrated by vacuum evaporation and then concentrated extract was dried by oven at low temperature.

Male and female healthy mice of NMRI strain, 6–8 weeks of age, weighing 28–30 g were purchased (Razi Institute, Karadje, Iran) and housed individually (males) or at 10 per polycarbonate cage (female) for a 2-week acclimation period. Mice were feed *ad libitum* by standard laboratory pellet (Pars khurakdam, Shushtar, Iran) and tap water. A 12-h light:12-h dark cycle was maintained. Room temperature was at 23 ± 2 °C with a relative humidity of 45–55%.

Male and female mice were housed together. The day a vaginal plug was found was assumed as first day of gestation (GD1). Pregnant females were divided into four groups ($n = 8$) and treated as follow:

First group received normal saline (10 ml/kg), the second group received phenytoin (65 mg/kg) (Winn and Wells, 1999), the third group received phenytoin (65 mg/kg) and along with it and 12 h later levamisole (10 mg/kg) (Clarke et al., 1997), and the fourth group received phenytoin (65 mg/kg) and along with it and 12 h later extract of *E. purpurea* (360 mg/kg) (Mishima et al., 2004). All drugs were diluted in distilled water but for *Echinacea* extract was added Tween 80, then were administrated intraperitoneally.

The animals were sacrificed by cervical dislocation at 19th day of gestation and fetuses were collected and numbered, then weight and length of them were measured. Fetuses were stained by Alizarin red–Alcian blue method (Yolanda, 1993) and investigated by stereomicroscope for cleft palate. The incidence of cleft palate was determined and mean of weight and length of fetuses were compared in groups.

Statistical significance between groups was determined using SPSS program and compared by one factor analysis of variance (ANOVA) and Chi-square test. The minimum level of significance was $p < 0.05$.

3. Results

There were not any aborted or absorbed fetuses from the treated animals. Total number of collected fetuses from groups 1, 2, 3, and 4 were 64, 81, 77, and 61, respectively.

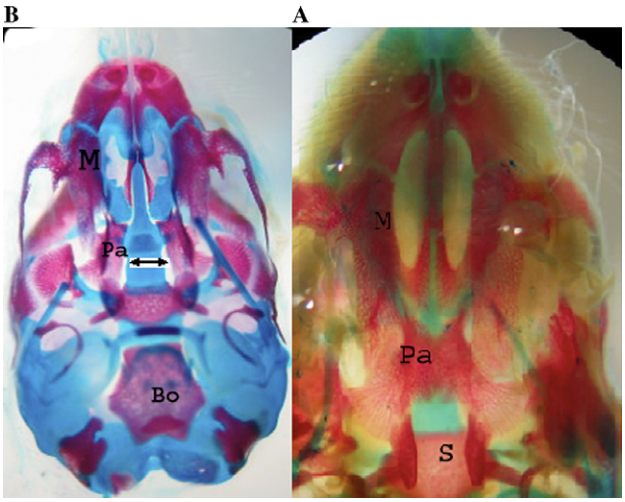

Fig. 1. Ventral view of skull of GD 19 fetal mice. (A) Normal palatine bone. (B) Cleft palate induced by phenytoin (arrow) which stained with Alizarine red–Alcian blue. M, maxilla; Pa, palatine; S, sphenoid; Bo, basioccipital.

In control group, palatal closures of fetuses were normal (Fig. 1A). Phenytoin-induced cleft palate at 16% incidence (Fig. 1B). Levamisole reduced incidence of pheytoin-induced cleft palate to 5.3%, but extract of *E. purpurea* reduced it to 3.2% (Fig. 2). Mean weight and length were significantly ($P < 0.001$) decreased in group which received only phenytoin. The means of weight and length in groups that received levamisole and *Echinacea* extract were greater than the group received only phenytoin (Figs. 3 and 4).

4. Discussion

Several studies have verified that the maternal immune stimulation can reduce teratogenic anomalies. Mechanisms of this effect remain unclear, but it is thought the fetal gene expression has been modulated (Holladay et al., 2002).

In the other hands, enhancing antioxidative effects can protect fetuses against phenytoin teratogenicity (Syska et al., 2004). Sharova et al. showed that interferon-γ and Freund's complete adjuvant reduced severity of the urethane-induced cleft palate in mice (Sharova et al., 2002). In the present study, the prophylactic effects of levamisole and *Echinacea* on phenytoin-induced cleft palate were compared in mice. Both levamisole and *Echinacea* reduced the severity of incidence of clefting. *Echinacea* was greater decreased incidence of cleft palate than levamisole, but it was not significant.

Levamisole is anthelmintic agent that also apparently enhances immune responsiveness. It is believed that levamisole mediates immune function of T-cells and stimulates phagocytosis by monocytes. Its immunostimulating effects are greater in immune-compromised animals. In addition, levamisole had antitumor effect in mice (Clarke et al., 1997).

In present study, levamisole probably increased immune response that lead to decreasing incidence of cleft palate.