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Effects of 4(3H) Quinazlonones-2-Ethyl-2-Phenyl Ethyl on the Development of Balb/C Mice Embryonic Stomach and Heart

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Summary

Quinazolinones are heterocyclic components (able to form cyclized compounds) which have several medical effects. They belong to hyponic and potent anticonvulsant drugs, Act strongly, inhibiting human immunodeficiency virus (HIV) and cancer, enter circulatory system and pass through placenta barrier. In this study, for the first time different aspects of developmental effects of 4(3H) quinazlonones-2-ethyl-2-phenyl ethyl (QEPE) on stomach and heart of Balb/C mice embryos were investigated. Pregnant Balb/C mice were divided into 3 groups (n= 10) of control, receiving distilled water, sham, receiving 0.05% methyl cellulose (the solvent) and experimental group, receiving one of the most effective dose of 100 mg/kg/body weight of QEPE, by IP injections on day 8th to 15th of gestation. After anesthetizing mothers, stomachs and hearts of 5-day old newborn Balb/C mice were removed, fixed and stained with H & E for light microscopic and quantitative studies. Results showed symptoms of gastritis (hyperaemia and decrease in thickness of mucus layer) in newborn Balb/C mice of treated groups. 4(3H) quinazlonones-2-ethyl-2-phenyl ethyl (QEPE) also created necrotic cells and an increase in connective tissues of hearts of newborn mice of treated groups. In conclusion by being teratogens and toxins, these two new derivatives affected development of embryonic stomach and heart at histological level.

Keywords: 4(3H) quinazolinone-2- propyl-2-phenyl ethyl, Stomach, Heart, Mouse embryo.

Introduction

Different teratogens and toxins have different developmental effects on different species with different severity (1, 2). By passing through placenta (3, 4), treatments with teratogens such as alcohol, quinazolinones, etc create early death, malformations and irregularities in different parts of developing embryos. Quinazolinones are heterocyclic components with various characteristics; such as: anti-inflammation, anti-malaria, anti-spasm, anti-microbial, anti-hypertensive, anti-allergic, sedative, anti-tuberculosis, antihyperlipidemic, anxiolytic, analgesic, anticonvulsant, as well as hypnotic activities. They are also known for their fungicidal properties, inhibition of tyrosine-kinase (involved in tubulin and 8-hydroxy-2-methyl quinazolinone polymerization), DNA repair enzyme poly (ADP-ribose) polymerase (PARP), and hhs signalling pathways. They are also effective in treatment of osteoarthritis, cancer, diabetes and parkinsonism complications (5-8). Previous studies showed that 4(3H) quinazlonones-2-ethyl-2-phenyl ethyl (QEPE) can causes morphological, skeletal and histological abnormalities in Balb/C mice embryos (9-14). The mechanisms of the effects of quinazolinones on embryonic cells are not clear yet, but there are few reports showing its toxic characteristics.

Following our earlier demonstrations of their toxic effects at morphological and skeletal levels of Balb/C mice fetuses and embryos (15, 16),the pathological effects of QEPE have been investigated on the morphological and histological structures of newborn Balb/C mice internal organs, such as stomach, heart.

Materials and methods

Balb/C mice (8 to 12 weeks old) were purchased from Razi Institute,(Karaj, Iran), weighing 27-28 g were used in this study. Animals were maintained under a 12:12-hour light/dark photoperiod. Female mice were mated with males of the same strain (1:2) and isolated the following morning, upon finding the vaginal plug, day zero of the pregnancy was designated and mated animals were kept singly in cages, at ambient room temperature.
The new derivative of qunazolinones: 4(3H) quinazlonones-2-ethyl-2-phenyl ethyl (QEPE), synthesized at University of Shahid-Beheshti , Tehran , Iran (17) were used for IP injection. So, pregnant mice were divided into 3 groups (n=10) of control, sham, and experimental, received distilled water (10ml/kg), methyl cellulose %0.05 (10ml/kg) (the solvent of quinazolinones) and 100 mg/kg Balb/C body weight of QEPE (most effective dose), respectively, by IP injection, on days 8th to 15th of gestation. 5day old newborns were killed by cervical dislocation. Hearts and stomachs of 4-days old Balb/C mice were removed, fixed in %10 formaldehyde, stained with H and E, studied with light microscope. Parametric data were analyzed by statistical packages for social sciences (SPSS, version 9.0).

Results

No morphological abnormalities were observed in the stomachs of newborn Balb/C mice of experimental group in comparison with sham and control groups. There were no significant differences between control and sham groups. Injection of 100mg/kg/body weights of QEPE, resulted in the formation of normal (without hyperaemia) and abnormal (with hyperaemia) stomachs in newborn Balb/C mice. As statistical analysis revealed, significant decrease occurred in the thickness of mucosal layer of stomachs of newborn Balb/C mice of experimental group 1 (Figs. 1 & 2), comparing with stomachs of newborn Balb/C mice of sham and control groups. There were no significant differences between mucosal layers of stomachs of newborn Balb/C mice of QEPE group.

No significant differences were observed between morphological and histological structures of hearts of newborn Balb/C mice of control and sham groups. Injection of 100mg/kg/body weights of QEPE created abnormal hearts, hearts with necrotic cells and connective tissues between myocytes of newborn Balb/C mice comparing with newborn Balb/C mice hearts of sham and control groups (Figs. 3 & 4).

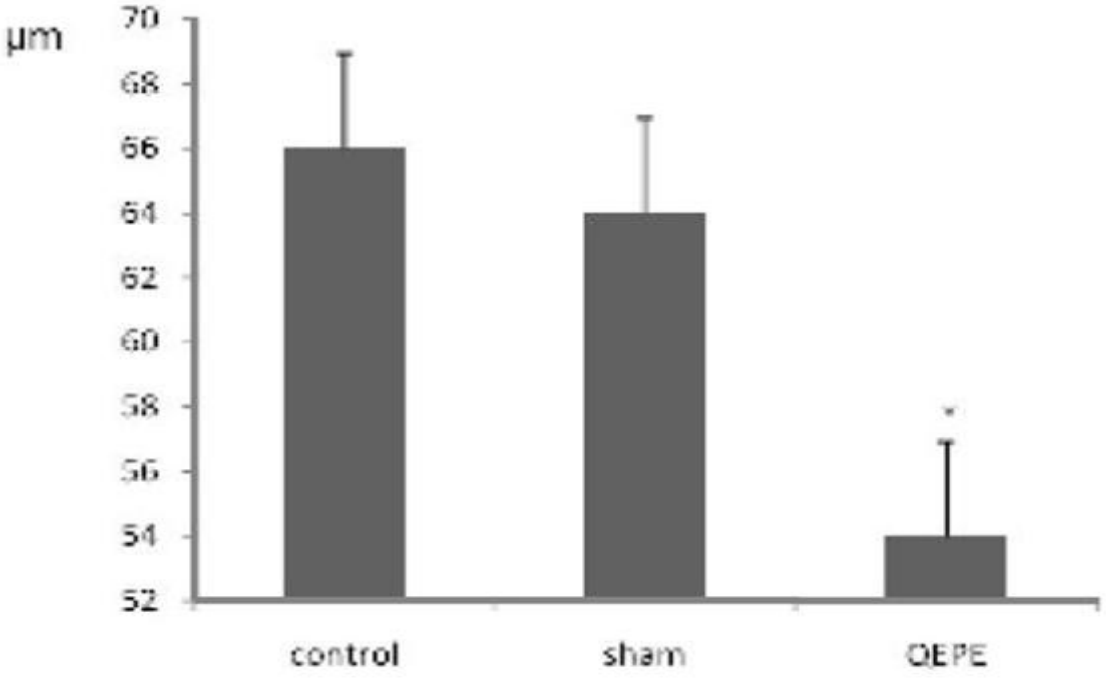

Figure 1. Comparison of the thickness (Pm)of mucus layer of stomachs of four different groups of newborn Balb/C mice. QEPE was more effective ($P<0.05$).

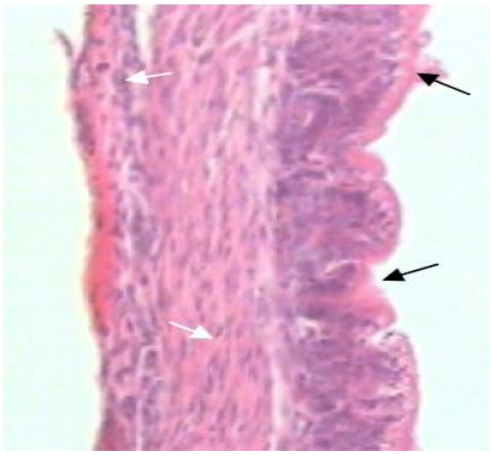

Figure 2. Hyperaemia (white arrows) and decrease in the thickness of mucus layers(black arrows) of stomachs of groups treated with QEPE(40X).

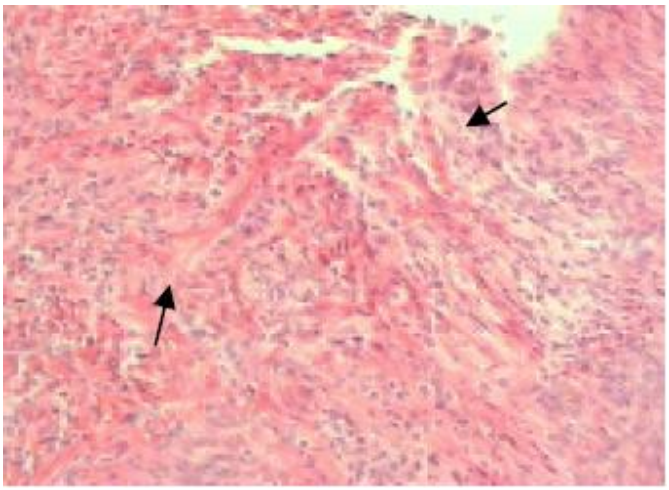

Figure 3. Necrosis (arrows) in hearts of newborn Balb/C mice of mother treated with QEPE.

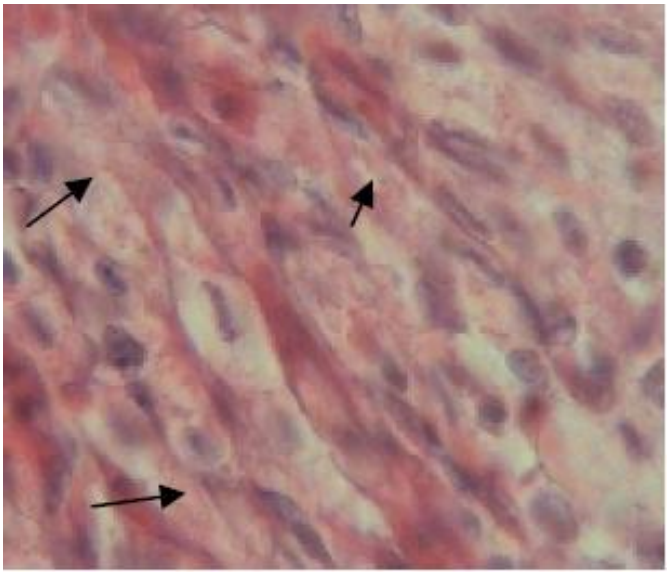

Figure 4. Connective tissues (arrows) in hearts of newborn Balb/C mice of mother treated with QEPE.

Discussion

Results of some pharmacokinetic studies suggested that quinazolinones pass through placenta barrier by simple diffusion, along a chemical gradient. Two possible explanations for this passage were considered: first possibility is related to some chemicals directly interacting with endogenous receptors for hormones, growth factors, cellsignalling molecules and other endogenous compounds. Second possibility shows that receptors can be broadly classified as cytosolic/nuclear or membrane bound. As results demonstrated, quinazolinones pass through

placenta barrier by simple diffusion, along a chemical gradient entering gastric tissues, causing inflammation (gastritis), because of atrophy in mucus layer, likely as the result of creating necrosis in stomach cells. QEPE is without active chemical groups but generate free radicals and active metabolites after metabolization leading to lipid peroxidation ,destroying cell membranes after releasing intracellular components such as lysosomal enzymes, causing further tissue damages. Oxygenderived free radicals play pathological roles in gastritis and radical scavengers such as alfa
tocopherol, carotenoids glutathione redox system play a significant role in protecting membranes from oxidative damages. Depletion of gastric mucus GSH may result in accumulation of free radicals, initiating membrane damages by lipid peroxidation, ultimately leading to necrosis in specially parietal cells ,because of their numerous surface receptors and pumps specializing for gastric acid production(18).Apoptosis is uncommon in normal mucosal layer ,but chronic inflammation is associated with increased apoptosis, happening mainly and only in mucus surface (19) . Appearance of connective tissues between myocytes is due to the stimulation of the multiplication of fibroblasts because of necrosis and their annihilation, so necrotice heart cells will be replaced by connective tissue (20).

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