INVESTIGATION OF THE IMMUNOPATHOLOGIC EFFECTS OF 4(3H)-QUINAZOLINONE-2-PROPYL-2-PHENYLETHYL (QPPE) IN NEWBORN BALB/C MICE

Estakhr Jasem[a*], Sanchooli Naser[a], Hatami Leili[a], Shams Lahijani Maryam[b]

[a]Department of Biology, Faculty of Sciences, Zabol University, Zabol, Iran.
[b]Department of Animal Sciences, Faculty of Biological Sciences, Shahid-Behshti University, Tehran, Iran.

***Corresponding author details:**
Estakhr Jasem, Department of Biology, Faculty of Sciences, Zabol University, Zabol, Iran.
Telfax: +98542-2232112. Mobile: +989179283966
Email: j.estakhr@yahoo.com

Summary

Water insoluble heterocyclic compounds such as quinazolinones have variety of biological and pharmacological properties. This study aim to investigate effects of 4(3H)-quinazolinone-2-propyl-2-phenylethyl (QPPE) as a new quinazolinone on spleen and immunocompetent cells of newborn Balb/C mice. 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 QPPE, by IP injections on day 8^{th} to 15^{th} of gestation. Blood samples from heart of newborn Balb/C mice were analyzed for immunocompetent cells and spleens were removed, fixed and stained with H&E, for qualitative and quantitative studies. Data indicated an increase in weight of spleen in experimental group. Pathological studies showed increase in capsule thickness and number of macrophage cells of experimental group. Statistical analysis showed significant differences in morphological studies between expimental, sham and control groups. The statistical data on capsule thickness and number of macrophage cells indicated significance difference between experimental, sham and control groups. Detailed observations showed increase in the volume of monocyte, neutrophile and eosinophile, in response to QPPE but the volume of lymphocyte and basophile were same in experimental, control and sham groups. The damages observed in the liver, intestine, kidney, heart, stomach and brain (in progress), could have been the reason for the increase in the number of immunocompetent and macrophage cells. Some studies showed damages to the organs such as liver and heart would lead to the increase in the thickness of spleen capsule, consequently, increase in its weight and creation of splenomegaly. So, QPPE can not be an appropriate candidate for drugs development.

Key Words: Balb/C newborn mice, Immunocompetent cells, Quinazolinones, Spleen.

Introduction

During the past few decades, it has become increasingly evident that human and animal embryos are subjected to a variety of environmental influences and drugs that could have deleterious effects on their development (1-3). Since the thalidomide tragedy, attention has been focused on drugs or chemicals as potential teratogen, to which pregnant women might be exposed (4-6).

Quinazolinones are heterocyclic and water insoluble compounds (7), with various pharmacological; antimicrobial, antifungal, antitumor, anticonvulsant, anti-inflammatory, antiallergy, antimalaria (8-14). They are more efficient than other chemicals in inhibiting HIV and cancer (15, 16).

The mechanism of the effects of quinazolinones on the embryonic cells is not clear yet, but there are quite a few reports showing its toxic characteristics. They inhibit polymerization of tubulin (17) and pass through placental barriers (18), so there is a possibility that it has some sort of toxic and teratogenic effects on embryos. Pervious studies at the Department of Zoology, Faculty of Biological Science, University of Shahid-Beheshti, 4(3H)-quinazolinone-2-propyl-2-phenylethyl (QPPE) can causes morphological, skeletal and histological abnormalities in Balb/C mice embryos (21-25).

In this regard spleen is involved in removed of pathogenic agents and some external components by phagositic cells (macrophage cells) , and some experiments have shown that , alfatoxin , 2-metoxyacetic , 2-metoxyethanol , 2-etoxyethanol , deoxynivalenol , dioxins , ethanol , parachloronitro-benzene and malaria toxins can cause damages and abnormalities in the spleen (26-32). Immunocompetence is vital in maintaining the overall health of an organism and is extremely sensitive to pathogens and toxins (33). Measurement of spleen weight and immunocompetent cells allow evaluation immunopathologic condition of some animals in response to chemical exposure (33-35). We interested that whether treatments pregnant mice with QPPE would affect the spleen morphology and histology and immunocompetent cells of Balb/C mouse fetuses.

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)-quinazolinone-2-propyl-2-phenylethyl (QPPE), synthesized at Department of Chemistry , Faculty of Science , University of Shahid-Beheshti , Tehran , Iran (19) 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 QPPE (most effective dose), respectively, by IP injection , on days 8^{th} to 15^{th} of gestation. 5day old newborns were killed by cervical dislocation.

The spleen was excised from each mouse and measured in weight. Then they were fixed in formalin %10, stained with H&E (Hematoxilin & Eosine) for histological and pathological studies under compound microscope. For counting of immunocompetent cells, blood samples from the heart were collected by using heparinized tubes. Counting was carried out manually by Neubaur chamber and using Turky's solution (36).

Data were analyzed with statistical packages for social sciences (SPSS,version 12.0).Mean and standard error of mean [SEM] were calculated and the significance of difference was analyzed by applying One-Way ANOVA. Level of significance difference was $P<0.05$.

Results

All investigated spleens and immunocompetent cells data are recorded in table 1 and 2. Treatment of mice with 100mg/kg of QPPE can increase weight of spleens (Figure 1-3). There was highly significant increase in spleen weight in experimental group compared the control and sham groups. Treatment of mice with 100mg/kg QPPE has increased the number of macrophage cells and capsule thickness in spleens (Figure 4-7). There were not significant differences between control and sham groups about number of macrophage cells and capsule thickness, but there are significant differences between experimental group and control and sham groups. Detailed observations showed significant increase in the volume of monocyte, neutrophile and eosinophile, in response to QPPE. Statistical results about the volume of lymphocyte and basophile cells showed no changes in experimental group in compared with control and sham groups.

Table 1: Effects of 100 mg/kg of QPPE on the spleen of newborn Balb/C mice

Parameters	Control	Sham	Experimental
Spleen Weight	0.04178 ± 0.0002	0.04185 ± 0.0001	0.05375 ± 0.0005*
Macrophage Cells	159.03 ± 2.53	158.8 ± 1.56	648.84 ± 5.78*
Capsule Thickness	2.98 ± 0.061	2.98 ± 0.03	4.81 ± 0.042*

Values are mean ± SEM (n=10); *P < 0.05 (significantly different) vs. control and sham groups

Table 2: Effects of 100 mg/kg of QPPE on the immunocompetent cells of newborn Balb/C mice

Parameters	Control	Sham	Experimental
Lymphocyte	67.47 ± 0.184	67.48 ± 0.189	67.51 ± 0.183
Monocyte	2.35 ± 0.022	2.36 ± 0.013	2.84 ± 0.016*
Neutrophile	29.64 ± 0.049	29.64 ± 0.046	30.21 ± 0.089*
Eosinophile	2.45 ± 0.012	2.44 ± 0.012	2.56 ± 0.012*
Basophile	0.36 ± 0.0057	0.36 ± 0.0078	0.36 ± 0.0066

Values are mean ± SEM (n=10); *P < 0.05 (significantly different) vs. control and sham groups

Figure 1: Normal spleen of control group that treated with 10 ml/kg body weight of distilled water (6X).

Figure 2: Normal spleen of sham group that treated with 10 ml/kg body weight of methyl cellulose %0.05 (6X).

Figure 3: The spleen of experimental group that treated with 100 mg/kg body weight of QPPE, in which splenomegaly were observed (6X).

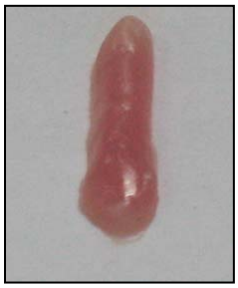


Figure 4: Normal spleen capsule of control group that treated with 10 ml/kg body weight of distilled water (H&E, 400X).

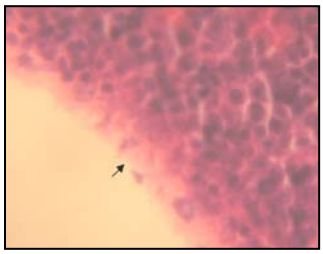


Figure 5: Normal spleen capsule of sham group that treated with 10 ml/kg body weight of methyl cellulose %0.05 (H&E, 400X).

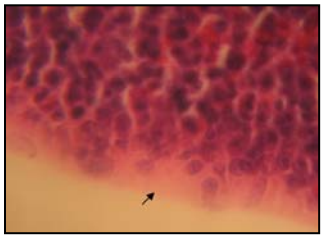


Figure 6: Increase of spleen capsule of experimental group that received with 100 mg/kg body weight of QPPE (H&E, 400X).

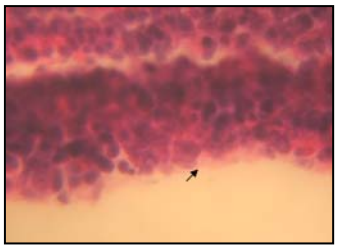
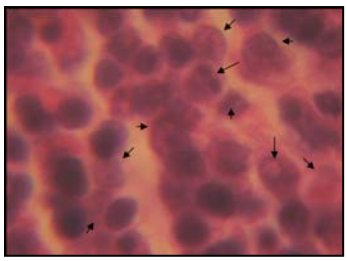


Figure 7: Cross section of spleen of treated group with 100 mg/kg body weight of QPPE, having large number of macrophage cells (arrows, H&E, 1000X).



Discussion

Quinazolinones are heterocyclic, water insoluble and lipophilic compounds, with various pharmacological characteristics; (antimicrobial, antifungal, antiswelling, Parkinson and etc.) (7-14). They are more efficient than other chemicals in inhibiting HIV and cancer (15-17). Xia displayed quinazolinone as a potent inhibitor of tubulin polymerization (20). They enter circulatory system and passes through placental barrier (18).

With due attention to results of earlier researches and to observe morphological abnormalities, skeletal malformation (21, 22) and damage in liver, intestine and kidney (23-25), brain, heart and stomach [in progress], we investigate effects of quinazolinones on the spleen development and immunucompetent cells in mouse Balb/C.

Results of present study showed that treatment with quinazolinones induce splenomegaly and increase capsule thickness. Measurements of immune-related hematological parameters in mouse blood show that QPPE causes monocytosis, neutrophilia and eosinophilia.

Splenomegaly is often seen in hypersplinism, increase of phagocitic cells (especially macrophage cells) and external articles in billroth's cords, patients with hepatic cirrhosis or portal hypertension and damage in liver and heart (30-32 and 38, 39). Most causes of splenomegaly in this research including: 1-Damage in liver, heart and other organs. 2-Increase of macrophage and immunocompetent cells. Monocytes circulate in the blood stream and differentiate into specific tissue macrophages which are actively phagocytic cells capable of ingesting and digesting exogenous such as the whole bacterial cells, virus particles, and injured or dead host cells (36).Treatments of mice with these components

lead to damages in liver, intestine, kidney, heart, stomach and brain. These damages can cause increase in the number of monocyte and macrophage cells. Some experiment have shown that, hyperlipidemia increase the number of macrophage cells in spleen. These macrophage cells interfere in removal of lipid from circulatory system. QPPE causes the increase and fullness of lipid in hepatic hepatocytes (25). It appears that QPPE disorders the metabolism of lipids and create hyperlipidemia. Also, these tow compound can cause neurophilia and eosinophilia. It is possible that, QPPE can increases migration of eosinophils and neutrophils from bone marrow to the blood stream. Eosinophils, like neutrophils, are motile, phagocytic cells that can migrate from the blood into the tissue spaces. Their phagocytic role is less important than that of neutrophils, and it is thought that their major role consists in defense against parasitic organisms (37). However, neutrophis are the first cells that arrive at a site of inflammation during response to many types of infection. Before investigations had shown that these two compounds can cause necrosis in some organs such as; intestine, kidney (23, 24) heart and stomach [in progress]. While creation of necrosis increase white blood cells, macrophage cells and defensive factors.

Increase of splenic capsule thickness is often seen in patients with hepatic cirrhosis or portal hypertension from other causes and in liver and heart (38). Other causes of splenic capsular thickening are increase of matrix contents and increase of blood cells and macrophage cells (39). In this study it is probable that most cause of splenic capsular thickening are caused by damages in liver and heart, and increase of macrophage cells.
We concluded that Treatments of mice with these components lead to damages in liver, intestine, kidney, heart, stomach and brain. These damages can cause increase in the number of immonucompetent and macrophage cells. Some studies have shown that damage in liver and heart lead to increase of capsule thickness. Indeed this factor causes increase in weight and leads to splenomegaly. Therefor, QPPE can not be an appropriate candidate for drugs development.

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