Iranian Journal of Science & Technology, Transaction A, Vol. 30, No. A1
Printed in The Islamic Republic of Iran, 2006

TERATOGENIC EFFECTS OF A NEW QUINAZOLINONE DERIVATIVE ON THE DEVELOPMENT OF BALB/C MICE FETUSES ON DAYS 9 10 AND 11 OF GESTATION*

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Abstract – For the first time, a spectrum of morphological abnormalities have been produced in developing mouse embryos (N=50), by injecting (IP) 75 and 100 mg/kg of maternal body weight of a new quinazolinone derivative, 4(3H)quinazolinone-2-propyl-2-phenylethyl(QPPE), on days 9, 10 and 11 of gestation. Measurements of the length of the crown-rump (CR), weight of body of the embryos and placenta on day 17 showed significant growth retardation ($P<0.05$) with day 9 being the critical day. The severity of the changes was dependent on the concentrations of quinazolinone, as higher doses created more abnormal embryos (syndactily, scaphocephaly, exocephaly, microcephaly, acrocephaly, abnormal polarity in lower and upper limbs, deviation of body axis, growth retardation) and placentas. Apparently QPPE acts on organogenesis on a specific phase of the developmental stage by expressing genes regulating mitosis and cell proliferation.

Keywords – Quinazolinone, Balb/C mice embryos, abnormalities

1. INTRODUCTION

During the past few decades, it has become increasingly evident that human and animal embryos are subjected to a variety of environmental influences 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 extensively used in the treatment of prevalent diseases [7-10]; They belong to hyponic [11, 12] and potent anticonvulsant drugs [13-15] and act strongly to inhibit human immunodeficiency virus [16-18]; Anticancer and antimicrobial activities of these drugs have been well documented [19-21]. MCI-176, a quinazolinone derivative, acts as calcium antagonist [22] and can inhibit histamin dependent HCL secretion [23]. They are non-peptide antagonist cholecystokinin receptors (CCR-B, CCR-A) and are used for the treatment of several anxiety disorders [24, 25].

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 [26] and pass through placental barriers [27], so there is a possibility that it has some sort of toxic and teratogenic effects on embryos. Therefore, the present study was directed via IP administration of two known doses of a new quinazolinone derivative (QPPE) on Balb/C mice embryos. In addition,the survey aimed to correlate these changes to the critical day of drug consumption.

*Received by the editor September 27, 2004 and in final revised form December 26, 2005
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2. MATERIALS AND METHODS

Six to eight week old Balb/C mice were obtained from the Pasteur Animal Facility, Karaj, Iran. 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, with a controlled light and dark period of 12 hours. Then, they were then divided into three groups (control, sham, and experimental).

Experimental groups (N=30) were treated intraperitoneally (IP) with 75 and 100 mg/kg maternal body weight of QPPE, synthesized at the Department of Chemistry, Faculty of Science, University of Shahid Beheshti, Tehran ,Iran [28], on days 9, 10 and 11 of gestation, separately. Sham groups (N=15) received propylene glycol as a solvent of quinazolinones [29] in a volume of 0.1 ml (minimum volume of solvent that could solve 100mg of QPPE). Control mice (N=5) were injected with an equal volume of sterile saline.

The fetuses were taken out by killing the mothers under chloroform anaesthesia on day 17 and were fixed in a bouin fixative. Their weights, length of crown-rump (CR), weight and the diameter of the placenta were measured. The morphology and possible abnormalities were also examined.

The measurements of quantitative data were studied using an LSD test, and differences between normal and abnormal embryos were measured using a chi-square (χ^2) test. The level of significance was set at $p<0.05$.

3. RESULTS

The results showed no significant difference between sham and control groups (Table 1). Abnormalities produced by the administration of 75 and 100 mg/kg QPPE were higher on day 9 of gestation and had increased the rate of abnormalities to %75 and %91/6, respectively (Table 2).

Table 1. The effects of administration of propylene glycol on 9, 10 and 11 day old Balb/C mice fetuses

control	49	6	1.07±9.86E-02	20.99±1.09	0.15±1.20E-02	8.47±0.32
day 9	40	4	1.08±0.15	20.98±1.19	0.15±1.23E-02	8.41±0.3271
day 10	38	5	1.073±0.11	20.99±1.09	0.15±1.55E-02	8.51±0.36
day 11	41	6	1.07±7.7E-02	20.86±0.9	0.15±1.46E-02	8.59±0.34

Table 2. The result of the effects of QPPE on 17-day old Balb/C mice fetuses, using χ^2 test

days	no. of adults	QPPE(mg/kg)	total number	abnormalities (%)
9	5 5 5	0 75 100	49 40 48	12.24 75* 91.6*
10	5 5 5	0 75 100	49 38 38	12.24 71.05* 86.84*
11	5 5 5	0 75 100	49 40 39	12.12 62.5* 74.35*

* $P<0.05$

There was a significant decrease in the weight of live embryos of the experimental group

(0.93±1.33E-02, 0.92±1.58E-02), compared with the control groups (1.7±9.86E-02). The overall lengths of CR in treated embryos were less than those of controls, with a statistical difference ($P<0.05$) (Table 3).

Table 3. The effects of 75 and 100 mg/kg of QPPE on the weight of embryo and length of CR of 9, 10 and 11 day old Balb/C mice fetuses, using LSD test

days	doses	no. of fetuses	weight of embryo (gr)±SD	length of CR (mm) ±SD
9	0 75 100	49 40 48	1.07±9.86E-02 0.93±1.33E-02* 0.92±1.85E-02*	20.99±1.09 19.28±0.12* 19.26±0.17*
10	0 75 100	49 38 38	1.07±9.86E-02 0.98±2.20E-02* 0.96±1.91E-02*	20.99±1.09 19.66±0.21* 19.49±0.13*
11	0 75 100	49 40 39	1.07±9.86E-02 1.01±1.35E-02* 0.96±0.17*	20.99±1.09 20.20±0.28* 19.76±0.3*

* $P<0.05$

Mean placenta weights and diameters diminished in all QPPE treated groups, compared with control embryos (Table 4).Treatment of pregnant mice on days 10 and 11, with both 75 and 100 mg/kg of QPPE, also produced abnormalities, but their effects reduced with age (Tables 3 and 4).

Table 4. The effects of 75 and 100 mg/kg of QPPE on the weight of placenta and length of CR of 9, 10 and 11 day old Balb/C mice fetuses, using LSD test

days	doses	no. of fetuses	weight of placenta (gr)±SD	length of CR (mm)±SD
9	0 75 100	49 40 48	0.15±1.42E-02 0.12±1.1E-02* 0.11±8.6E-02*	8.47±0.32 8.07±0.22* 8.11±0.22*
10	0 75 100	49 38 38	0.15±1.42E-02 0.13±1.2E-02* 0.13±7.22E-02*	8.47±0.32 8.35±0.12* 8.24±0.11*
11	0 75 100	49 40 39	0.15±1.42E-02 0.14±6.60E-02* 0.13±6.7E-03*	8.47±0.32 8.45±0.11* 8.35±0.1*

* $P<0.05$

QPPE affected embryos and created: C-shaped embryos (Fig. 1), deviation of body axis (Fig. 2), curved tail,straight neck,abnormal polarity in upper and lower limbs (Fig. 3), short forelimb, cutaneous projections, exocephaly, acrocephaly, scaphocephaly (Fig. 4), furrow in neck and lumbar regions (Fig. 5), open eyelids, small placenta,fused placenta and several other abnormalities (Table 2), on days 10 and 11 of pregnancy. One case of a defect on the forelimb (syndactily) was observed on day 11 (Fig. 6).

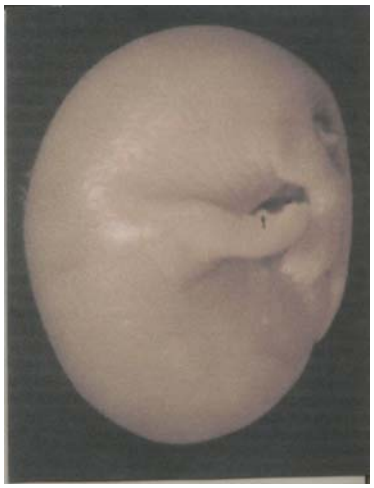

Fig. 1. Lateral view of C-shaped mouse embryo with extra digit (arrow), after injecting (IP) 100 mg/kg of QPPE, on day 10 of gestation

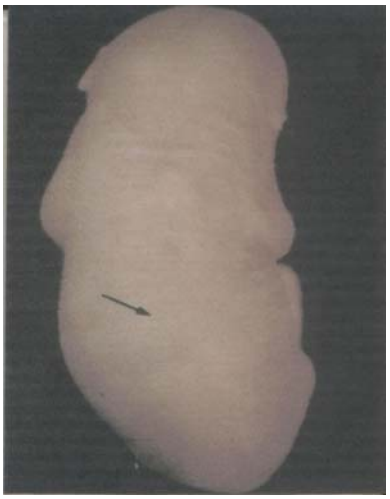

Fig. 2. Deviation of body axis of mouse embryo (arrow), after injecting (IP) 100 mg/kg of QPPE, on day 9 of gestation

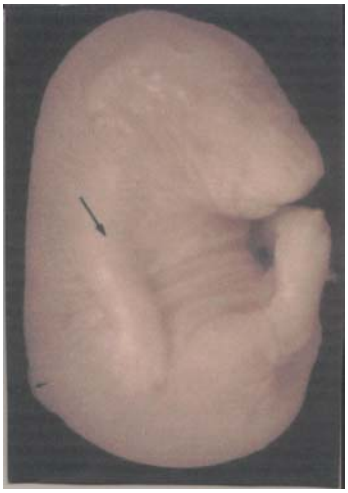

Fig. 3. Abnormal polarity in upper limbs of mouse embryo (arrow), after injecting (IP) 75 and 100 mg/kg of QPPE, on days 10 and 11 of gestation

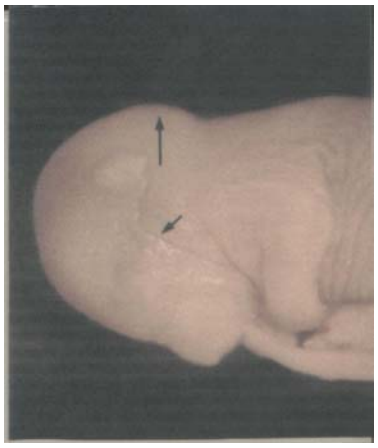

Fig. 4. Abnormal head (scaphocephaly) in mouse embryo (arrows), after injecting (IP) 75 and100mg/kg of QPPE, on days 9, 10 and 11 of gestation

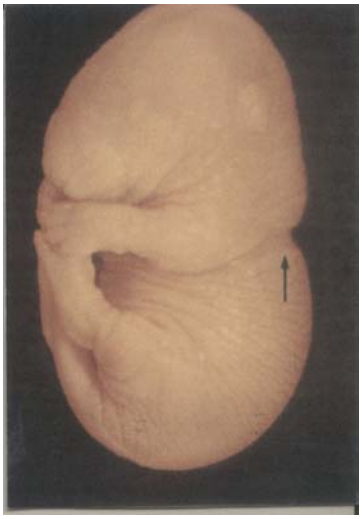

Fig. 5. Fissure in lumbar region of mouse embryo (arrow), after injecting (IP) 75 mg/kg of QPPE, on days10 and 11 of gestation

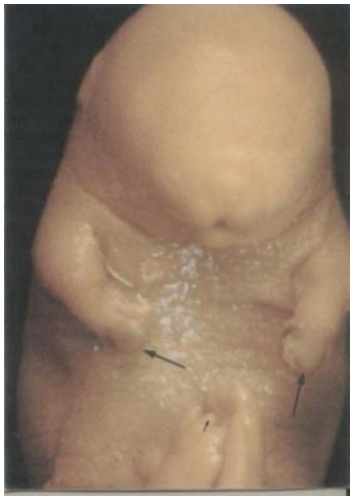

Fig. 6. Syndactily in mouse embryo (arrows), after injecting (IP) 100 mg/kg of QPPE, on day 11 of gestation

4. DISCUSSION

Before the basic phenomena (involving mammalian teratogenesis) can be adequately described, it is necessary to develop a model system which will permit a wide range of experimental manipulations [1]. So, it was attempted to develop such a model by using Balb/C mice, and the aromatic compound QPPE as a teratogen. Due to the fact that there is a high level of spontaneous malformations among mice, large numbers of control groups were studied in order to establish a reliable percentage of specific defects [2]. This research generated data concerning the optimal dosage of teratogen and time of administration needed to produce a high incidence of defects in developing embryos. It was found that day 9 of gestation and 100mg/kg of QPPE have maximum effects on developing Balb/C mice embryos. This emphasizes the fact that there are critical stages during the development in which teratogens may exert their effects. In addition, observation of syndactily on day 11 exhibited minimum effects on day 11, after using of 75 mg/kg of QPPE.

Quinazolinone derivatives probably exert their teratogenic effects via several mechanisms. They act as mitotic inhibitors; On the other hand, cell division is higher in developing mice embryos. It appears that mitosis stops in proliferating tissues and organs [18, 19]. In addition, quinazolinone disrupts the process of energy production [30], which is needed for growth. It may therefore be concluded that a decrease in energy can be the cause of malformations.

It is well known that quinzolinones are lipophilic agents and pass through the biological membranes quite easily [25]. Exogenous aromatic hydrocarbon, such as methaqualone, a member of the quinazolinone drug family is bound to the aromatic hydrocarbon receptor (AHR) [31]. The complex of AHR and aromatic hydrocarbon is transferred into nucleus and activates several genes, such as: CyPcA2 and CyP1A2 [32]. It appears that QPPE, as an exogeneous aromatic hydrocarbone, acts in this manner. Jeffery et al. suggested that AHR is coded before implantation and interacts with cell proliferation and differentiation in mice embryos [33].

Although the effects of these agents on Balb/C mice fetuses have also been investigated on days 6, 7 and 8, and at cellular level, and is in the process of publication, more investigation is needed to present reliable hypothesis for the teratogenic effects of quinazolinone derivatives [34, 35]. Using a Balb/C fetus as a model proves experimentally that quinazilinone is a teratogen.

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