

Evaluation of the Teratogenicity of Morphine Sulfate by Oral Administration in Balb/C Mice

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Abstract

The teratogenic effects of morphine sulfate exposure, during pregnancy, were studied in Balb/C mice, in three different concentrations of 0.01, 0.05 and 0.1 mg/ml, using oral administration. For addition, the drug was provided in increasing concentrations for 3 weeks (n=26-63); one control and seven experimental groups, for every concentration were used, which included non-administered (untreated) and administered (treated) females mated with treated and untreated males. For the first time, cesarean sections were performed on the end of gestation; number of fetuses and resorption sites were recorded. Fetuses and placentas were examined externally and preserved for subsequent visceral and skeletal examinations. Fetal morphine sulfate exposure was associated with significant reduction in size of litters ($P<0.05$), fetal weight ($P<0.001$), length of crown-rump ($P<0.001$, $P<0.005$) and placenta weight ($P<0.001$, $P<0.005$) and diameter ($P<0.001$), in two doses of 0.01 and 0.05 mg/ml. With using 0.1 mg/ml, only some experimental groups showed significant decrease ($P<0.05$). All exposed-groups showed an increase in the percentage of abnormalities compared to control groups ($P<0.001$). Staining the skeletal structures showed an extra rib in fetuses. Results revealed that morphine possess: 1) teratogenic potential on developing mice fetuses, in doses of 0.01, 0.05 and 0.1 mg/ml; 2) male - exposure to morphine has lower disruptive effects on embryos than female exposure, and 3) teratogenic effects of morphine increase with lower doses.

Keywords: *Mouse embryo, Teratogenicity, Morphine sulfate.*

Introduction

Most of the few investigators, studying the teratogenic effects of morphine, administered the drug peritoneally by either repeated injections or implanting the pellets on specific days of pregnancy (Vathy, 1992; Siddigi *et al.*, 1997; Zagon & McLaughlin, 1977; Ray *et al.*, 1977). Because of the stressful procedures, oral administration of morphine sulfate in drinking water was used; this type of drug dosing is easy and has minimal teratogenic and toxic side effects. Some of the authors reported teratogenic effects of morphine in high doses (Zagon & McLaughlin, 1977; Arcuri & Gautieri, 1973; Geber & Schramm, 1975), but Ciociola *et al.*, (1983) provided evidences (with pumping morphine in CF₁ mice) that the number of abnormal embryos was higher, as well as mean fetal weight being lower, in lowest doses of morphine sulfate (0.04% or 0.4 mg/ml).

For this reason, three different doses of 0.1, 0.05 and 0.01 mg/ml of morphine sulfate were used, and the effects of its oral administration on Balb/C mice fetuses were investigated; effects of male exposure to morphine were also considered (Shams Lahijani & Sokhanvar, 1998; Ramezani, 1999).

The Specific aims of present study were: 1) to determine whether oral administration of lower doses of morphine has any/or more teratogenic effects, comparing to very few previous studies; 2) how different are the abnormalities induced by oral administration, and 3) its effects on skeletal structures.

Materials and Methods

Balb/C mice (25-30 g, obtained from Razi Institute, Karaj, Tehran, Iran) were fed on laboratory food (pellets) and housed at 21 ± 3°C room temperature, under 12-hr light-dark cycle. Morphine sulfate was administered in drinking water (500 ml/d), ad libitum (Shams Lahijani & Sokhanvar, 1998; Badaway *et al.*, 1982), chronically. Three doses of 0.01, 0.05 and 0.1 mg/ml were used. The drug was provided in an increasing concentrations (48 hr apart) of 0.01, 0.02, 0.03 and 0.04 mg/ml for 3 weeks of experiment. One untreated and four treated groups (0.01, 0.05 and 0.1 mg/ml) were mated with treated and untreated males, respectively (n=26-63), after which, female and male mice were placed in a cage at 4 PM up to 8 AM the following morning.

Females (with vaginal plugs) were regarded as being gravid and at day zero of gestation.

Gravid females were transferred to single cages (identical to those occupied by males) and sacrificed at the end of treatment period. After opening the abdominal wall and exposing the entire peritoneal cavity, the number of fetuses and resorption sites (identified by presence of small dark nodules) were recorded. The fetuses and placentas were bolted dry and weighed to the nearest 0.01g, on a torsion balancer. Length of crown-rump (CR) and weights and diameters of placentas were measured, then, they were fixed in Bouin's solution for soft tissue examinations. Some of the normal and abnormal fetuses were processed for alizarin red S and alcian blue 8GX staining, to study the bones and cartilages.

Statistical analysis

Nonparametric data (weight of embryos, crown-rump length, weight and diameter of placentas) were analyzed by Kruskal-Wallis analysis of variance (KWANOVA). Dunnett's multiple comparison test was used as a postriori test, when differences were found with KWANOVA.

For comparing the litter size of treated groups with control groups, odds ratio, followed by chi-square (χ^2) test, were used.

The percentage of normal and abnormal fetuses were compared in control and treated groups, using chi square (χ^2) test; $P < 0.05$ was considered significant.

Results

The mice drunk morphine sulfate solution (0.01, 0.05 and 0.1 mg/ml) as the only drinking liquid in their cages.

Administration of morphine sulfate decreased size of litters, in all experimental groups ($P < 0.05$), and mean fetal body weight was also significantly diminished in all treated groups ($P < 0.001$) except in FbNMA (Fig. 1). Length of crown-rump lessened significantly ($P < 0.01$, $P < 0.05$) in all treated groups (Fig. 2); Significant decrease in dose 0.1 mg/ml happened in FbNMA ($P < 0.05$). The number of birth given by three experimental groups and control is shown in Table 1.

Table 1- Comparing number of birth given in three experimental groups (0.01 (F), 0.05 (F_a) and 0.1(F_b) mg/ml of morphine), with control (CTRL) groups.

P	Odds Ratio	<u>No. of embryos</u> dame	Groups
-	1	10.5	CTRL
P<0.01	0.55**	5.83	FAMA
P<0.05	0.82*	78.6	FAMN
P<0.01	0.6**	8	FNMA
P<0.001	0.43***	4.77	F _a AMA
P<0.05	0.8*	8.33	F _a AMN
p>0.05	0.7*	7.2	F _a NMA
P<0.01	0.6**	6.2	F _b AMA
P<0.05	0.72*	7.6	F _b AMN
P<0.05	0.78*	8	F _b NMA

A = Administered (treated)

n= 26-63

F = Female

M= Male

N=Non-administered (untreated)

Lessening of placenta weight was observed in 0.01 and 0.05 mg/ml (P<0.05, P<0.001), in all three experimental groups, but it was not significant in 0.1 mg/ml (Fig. 3). Average diameter of placenta decreased significantly (P<0.001) in all three treated groups, in concentrations of 0.01 and 0.05 mg/ml, except in FNMA (Fig. 4). In concentration of 0.1 mg/ml, diameter of placenta decreased only in F_bAMA (P<0.001).

Figure 1 – Comparison of weight of embryo in three experimental groups (F, Fa & Fb) with control (CTRL).

Figure 2 – Comparison of length of crown-rump of embryo in three experimental groups (F, Fa & Fb) with control (CTRL).

Figure 3 – Comparison of weight of placenta of embryo in three experimental groups (F, Fa & Fb) with control (CTRL).

Figure 4 – Comparison of diameter of placenta of embryo in three experimental groups (F, Fa & Fb) with control (CTRL).

Significant occurrence of abnormalities ($P < 0.001$), compared to those of control, was observed in each concentrations (Fig. 5). The most frequent malformations, which are reported for the first time, were: resorption of embryos (Fig. 6), growth retardation, C-shaped embryos, abnormal tails (Fig.7), abnormal polarity in fore and hindlimbs (Fig.8), small extra digit in one or both forepaw, umbilical herniation, open eyelid, hematomas (subdermal hemorrhage), small placenta, giant placenta, fused placenta (Fig. 9) and light and sever placenta hemorrhage.

Figure 5 – Percentage of nonadministered (normal) and administered (abnormal) embryo in three experimental groups (F, Fa & Fb) with control (CTRL).

Examination of fetuses skeletal structures showed an extra rib in thoracic region (Fig.10), but no cartilages /or bones were observed in the fetuses with apparently small extra digits.

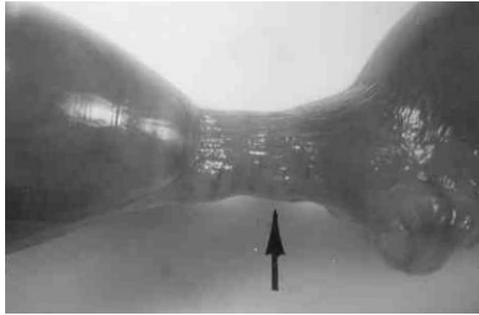


Figure 6 - Resorption of embryo in the uterus of treated mouse (arrow) (x 10).



Figure 7- C-Shaped embryo and abnormal tail in embryo of treated mouse (x 10).

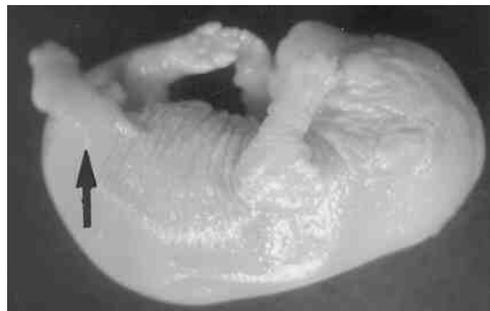


Figure 8 - Abnormal polarity in fore and hind-limbs of embryo of treated mouse (arrow) (x 10).

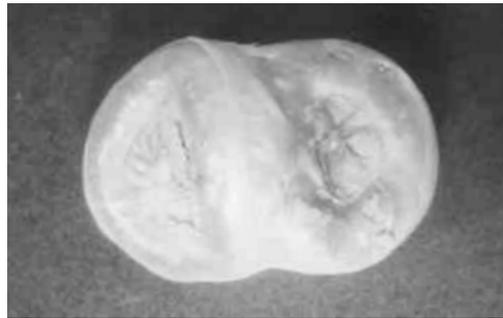


Figure 9 - Fused placentas in treated mouse (x 10).

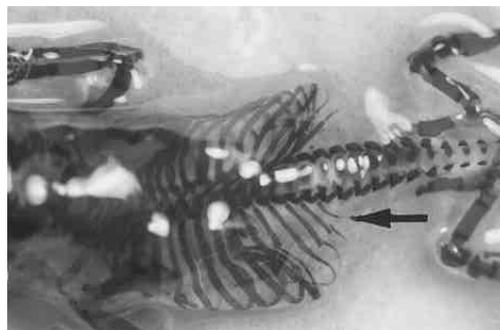


Figure 10 - Extra rib in the thoracic region of embryo of treated mouse (arrow) (alcian blue 8GX, alizarin red S staining) (x 10).

Discussion

Mice could consume morphine sulfate in drinking water ad libitum, in the absence of taste making chemicals. The success of this method depended on the initial provision of 0.1 mg/ml solution of morphine sulfate and lower.

Administered morphine sulfate produced very small litters, because of induced irregular estrus cycles, and reduced plasma and ovarian estradiol and progesterone levels (Ciociola & Ronald, 1983; Salo *et al.*, 1996). Morphine sulfate also blocks ovulation (Roloff *et al.*, 1975; Johnson & Rosecrans, 1980) and spermatogenesis (Siddigi *et al.*, 1995).

Fetal morphine treatment was associated with significant diminishes in fetal weight, crown - rump length, placenta weight and diameter, in the group teated with 0.05 mg/ml, but it was not significant in 0.1 mg/ml. Significant reduction in average fetal weight is in agreement with the results of previous data (Zagon & McLaughlin, 1977; Ciociola & Ronald, 1983; Nehlig & Derby, 1994; Roloff *et al.*, 1975).

The abnormal fetuses produced insignificant percentage, in three experimental groups, as a result of morphine sulfate administration; fetal resorption (Lehman, 1976; Williams *et al.*, 1985), sever growth retardation (Ray *et al.*, 1977), abnormal tail (Ciociola & Ronald, 1983), with codeine or morphine sulfate, and subdermal hemorrhage with alcohol, were noted previously (Salo *et al.*, 1996); also, incidence of small extra digit with codeine (Zeller & Gautieri, 1977) and extra rib in cocaine - treated mice, were reported previously (Mahalik *et al.*, 1980).

Normally, fetuses at this stage, do not have open eyelid and umbilical herniation, therefore, creating these abnormal fetuses display retardation of body growth in morphine-treated offsprings. Growth retardation is due, mainly, to a decrease in both size and number of cells in many organs (Arcuri & Gantieri, 1973; Sorbian, 1977). Other mentioned abnormalities (in the results) were seen for the first time, with morphine sulfate.

Drugs, such as cocaine and morphine, that increase catecholamine level, cause placenta vasoconstriction which probably create fetal malformations through reducing oxygen availability (Mahalik *et al.*, 1980). Because, these parameters were not significant in some groups, it was concluded that teratogenic potential of morphine sulfate was quite strong in pregnancy period. Also, administration of morphine sulfate to females had more teratogenic effects on fetuses than when it was administered to males.

As the results show, the lowest concentration of morphine sulfate (0.01 mg/ml) created more abnormalities, lower average fetal weights and lessening of crown-rump length, compared with high concentration of 0.1 mg/ml, which supports reports of Ciociola *et al.*, (1983).

If, as it is believed, the teratogenic effects of morphine sulfate are expressed through opiate receptor activities, the inhibition of receptors,

by chronic administration of morphine, may result in lower incidence of fetal anomalies at higher concentration of 0.1 mg/ml. There is a possibility that constant administration of lowest concentration of morphine (0.01 mg/ml) may not be sufficient to inhibit the receptor activity, but, is large enough to cause fetal abnormalities (Sorbian, 1977).

In fact, teratogenic agents can be classified into two categories: 1) some of them have teratogenic effects only when a threshold of tissue or plasma concentration has been exceeded, whereas, for others, 2) the effects depend on the concentration accumulated over a period of time. The absence of teratogenic action of morphine sulfate (when it is administered in large quantities), that is divided up during day, shows that morphine belongs to the first category (Nehlig & Derby, 1984). However, induction of fetal defects, by morphine sulfate, is probably not due to single cause, but to multiple and presently unknown factors.

The results of this research demonstrated that these three concentrations of morphine sulfate possess teratogenic potentials on mice fetuses.

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