ORIGINAL ARTICLE

Effects of Bupivacaine on the Development of the Heart and Kidney of Albino Mice

NAVEED AKHTER[1], MOHAMMAD TAHIR[2], SHAMSA IJAZ[3], *KHALID P LONE*[4]

ABSTRACT

Teratogenic effects of a local anesthetic bupivacaine were investigated in this study. Thirty mice were used; these comprised twenty four female and six male animals. Drug was injected intraperitoneally at a dose of 25 and 50 mg/kg on gestation day (GD) 6 to 14. Control group was injected equivalent volume of vehicle solution (normal saline) on GD 6 to14. Teratological assessment was carried out on GD18. The results showed that bupivacaine treatment during gestation resulted in reduced litter size and increased fetal resorptions. Groups of fetuses exposed to 25 and 50 mg/kg bupivacaine exhibited reduced birth weight and decreased crown rump length (CRL). Regarding anomalies of heart 10% of the fetuses revealed atrial septal defect in group C. Whereas 5% and 10% of the fetuses among group B and C respectively, were observed to have dextrocardia. None of the groups revealed gross anomalies of kidney.

MeSH words: Bupivacaine, local anesthetic, mic, heart, kidney

INTRODUCTION

Bupivacaine Hydrochloride (bupivacaine) pharmacologically and chemically belongs to the aminoacyl anesthetics having amino group and the aromatic nucleus bound to each other by amide linkage[1]. Bupivacaine inhibits in the generation and conduction of nerve impulses by increasing the threshold for electrical excitation in the nerves, by reducing the rate of rise of the action potential andby slowing the propagation of the nerve impulse[2].

Systemic absorption of bupivacaine yields effects on the central nervous systems (CNS) and cardiovascular systems. Plasma concentrations of bupivacaine after administration of normal therapeutic dose produce slightvariations in cardiacexcitability, refractoriness,conduction, contractility, and peripheral vascular resistance[3]. Even then, toxic plasma concentrations (4.5 mcg/mL) decrease cardiac excitability and conduction, which can cause ventricular arrhythmias, cardiac arrestand atrioventricular block, sometimes resulting in causalities[4].

The systemic absorption rate of bupivacaine isaffected by the route of administration, vascularity of the administered site and total dose and concentration of drug. The onset of anesthetic action of bupivacaine is quick and anesthetic action is sustainedfor longer duration when compared to other local anestheticsused commonly[5]. It has also been noted that analgesic effect of bupivacaine persists even after the return of sensation. This effects reduces the need for analgesics postoperatively.

Bupivacaine binds to plasma proteins in varying degrees and readily crosses the placenta by passive diffusion. The magnitude of placental transfer of bupivacaine depends upon the lipid solubility and degree of ionization[6]. After administration, drug is dispersed to all the tissues of body, with high concentrations markedly found in organs such as thebrain,liver, lungs, heart i.e. in highly vascularized tissues[7]. Peak plasma concentrations of bupivacaineare reached in 30 to 45 minutes after administration via caudal or epidural route[8], followed by a drop to trivial drug levelsin next 3-6 hours time[3]. The half life of bupivacaine in plasma is 2.7 hours in adults and 8.1 hoursin neonates[7].

Primarily metabolism of bupivacaine takes place in the liver by glucuronidation. Desbutyl bupivacaine, 4'-hydroxy bupivacaine, and 3'-hydroxy bupivacaine are three principal metabolites. Bupivacaine and their metabolites are primarily excreted by the kidneys[9]. Kidneys excrete only 6% of bupivacaine is unchanged in the urine.

Embryolethal effects of bupivacaine in rats and rabbits had been reported at nine and five times of the maximum human therapeutic dose respectively[10]. There are hardly any controlled studies during pregnancy in human or animal regarding the effect of bupivacaine on developing conceptus of the mother who received bupivacaine as an anesthetic agent[11,12]. It was, therefore, decided to design an experimental model in mice to evaluate the teratogenic effects of bupivacaine on kidney and heart.

[1]*Assistant Professor, Rai Medical College, Sargodha*
[2]*Professor and Chairman of Anatomy Department, UHS*
[3]*Assistant Professor Anatomy, Lahore Medical College, Lahore*
[4]*Professor and Head of Physiology Department, UHS*
Correspondence to Dr. Naveed Akhtar,
Email Address: naveed421@yahoo.com Cell: +923334252487

MATERIALS AND METHODS

Materials: Bupivacaine hydrochloride was obtained from Sigma-Aldrich, Germany. All the other chemicals were procured from standard commercial suppliers(Merck andBecton Dickinson) and were of analytical rank.

Methods: It was a randomized controlled trial.This study was conducted in the department of Anatomy of University of Health Sciences Lahore, Pakistan. This study was completed in one year.

Experimental animals and breeding procedure: BALB/cmice was procured form Veterinary Research Institute, Lahore (VRI). Mice were kept in stainless steel cages and were provided rodent laboratory chow and filtered tap water *ad libitum*. Animal house was maintained with temperature range of 22±1^{0}C and 12 hour light/dark cycleeah. Three nulliparous female mice were kept with one male in a cage. Finding of vaginal plug (evidence of mating) at 7:00 a.m. was used to label GD zero[13].

Eight pregnant mice were randomly assigned into groups A, B and C and kept individually in separate cages. The animals were housed according to the guidelines of the Ethical Review Committee of use of Experimental Animals, University of Health Sciences Lahore, Pakistan.

Experimental procedures: Group B received a single daily intraperitoneal injection of bupivacaine solution from GD 6 to 14 in a dose of 25mg/kg of body weight. Similarly group C received a single daily intraperitoneal injection of bupivacaine solution from GD 6 to 14 in a dose of 50mg/kg body weight. Group A received equivalent volume of 0.9% saline intraperitoneally from day 6 to 14 of gestational period[14]. With the purpose of minimizing exposure to the vehicle, a small injection volume (0.016 ml/g) was used[15]. The administration timing of the drugwas carefully chosen to intervene important stages of early organogenesis. Dose of the bupivacaine was translated from human to mice dose by normalization of the body surface area method[16]. Pregnant mice were daily observed for noticeable illness and food consumption. Mice weight was recorded daily to GD 18. Pregnancies were terminated near term, on GD 18 by euthanizing with choloroform[17]. The fetuses were considered to be viable if they breathedspontaneously or responded to tactile stimulation.

For detection of implantation sites, uterine horns were dissected and then were placed in phosphate-buffered saline for 10 minutes. The uterine horns were then stained with solution of 10% ammonium sulfide for 10 minutes. Implantation sites were observed as dark rings and were recorded[18].

The fetuses were euthanized with choloroform, decapitated and fixed in Bouin's solution and were subsequently examined for visceral anomalies, using the free-hand slicing method of Wilson[19]. The thorax and abdomen of the fetuses were opened by a midline incision and were observed under the dissecting microscope for any obvious gross anomalies. Position of heart and kidney was also observed[20].

Statistical analysis: The data was collected, entered and then was analyzed by using SPSS version 22.Sphapiro- Wilk test was applied to observe the normality of the data. One way ANOVA was used to detect group mean differences & Post Hoc Tukey test was applied to see which means differed. A p-value of <0.05 was considered statistically significant.

RESULTS

Maternal examination: Dams administered 25 or 50 mg/kg bupivacaine in groups B & C respectively, at different days of gestation showed no signs of disease or abnormal behavior during treatment period. No maternal mortality was noted in all groups. Maternal body weight gain in the groups B & C at GD 18 was significantly lower ($p<0.05$) than the vehicle group (Table1). There is no significant difference of number of implantation among control and groups B & C (Table 1). An increase in fetal resorption ($p<0.05$) was observed at both doses of bupivacaine used (Table 1).

Table 1: Maternal and fetal observations of mice treated with vehicle and bupivacaine from GD 6 to 14.

Observations	**Group A** (n=8)	**Group B** (n=8)	**Group C** (n=8)
Number of pregnant animals treated	8	8	8
No. of viable fetuses delivered per group	64	39	10
Maternal body weight (g ± SD) at term gestation	5.83±2.47	12.65±0.43	9.25±1.23*
No. of implantations per litter (mean ± SD)	8.0±0.71	6.88±0.39	6.63±0.42
Post- implantation loss/ fetal resorptions (mean ± SD)	ND	2.0±0.19	5.13±0.23*
No. of viable fetuses per litter (mean ± SD)	8.0±0.71	4.87±0.44*	1.25±0.45*+
No. of dead fetuses	0	0	0
Fetal Observations			
Mean fetal body weight per litter (g ± SD)	1.14±0.03	0.99±0.03*	0.85±0.07*
CRL (cm)	2.28±0.03	2.10±0..02*	1.94±0.09*

*statistically significant when compared to control group ($p<0.05$)
+statistically significant when compared to group B ($p<0.05$)
ND: not detected

Fetal examination: Bupivacaine administered to dams in groups B & C did not cause fetal mortality (Table 1). At 25 and 50mg/kg, bupivacaine administered during GD 6-14 produced significant changes ($p<0.05$) in CRL and fetal weight (Table1). Regarding anomalies of heart 10% of the fetuses revealed atrial septal defect in group C (table 2). Whereas 5% and 10% of the fetuses among group B and C were observed to have dextrocardia (table 2). None of the groups revealed gross anomalies of kidney.

Table 2: Observations for congenital anomalies of heart and kidney in mice treated with vehicle and bupivacaine from GD 6 to 14.

Observations	**Group A** (n=64)	**Group B** (n=39)	**Group C** (n=10)
Heart			
Atrial septal defect	0/64	0/39	1/10 (10%)
Ventricular septal defect	0/64	0/39	0/10
Transposition of great vessels	0/64	0/39	0/10
Patent ductus arteriosus	0/64	0/39	0/10
Pulmonary stenosis	0/64	0/39	0/10
Dextrocardia	0/64	2/39 (5.1%)	1/10 (10%)
Kidney			
Renal Agenesis	0/64	0/39	0/10
Cystic kidney	0/64	0/39	0/10
Renal ectopia	0/64	0/39	0/10
Horse shoe kidney	0/64	0/39	0/10

DISCUSSION

Bupivacaine is one of the most commonly used local and regional anesthetic in pregnancy and labour. The present study evaluated the effects of bupivacaine on heart and kidney of developing fetuses in mice when injected to their pregnant mothers during organogenesis.

The maternal weight gain in 25mg/kg bupivacaine group was reduced when compared with control group and this was not significant, whereas, the maternal weight gain was significantly reduced in 50mg/kg bupivacaine group when compared with control. Bupivacaine treatment of the pregnant mice had no effect on food intake however, the decrease in weight was probably due to the loss of fetuses on account of treatment with the bupivacaine. This points tothe dose related toxic effect on maternal weight gain. The same dose related effect on maternal weight was also reported when cocaine, a local anesthetic, was administered subcutaneously from GD 7-19 to Long-Evans rat[21]. The similar effect was seen in rats after chloroform inhalation on GD9, 11 and 14[22].

The litter size was reduced statistically without significant increase in fetal resorptions in 25mg/kg bupivacaine group when compared to the control. There was significant decrease in litter size with concomitant increase in fetal resorption when compared to control at higher dose level of bupivacaine; this is consistent with the information provided by the manufacturer on it[10]. Reduction in litter size had also been reported when cocaine was injected subcutaneously from GD 7-19 in rat[21] and when chloroform was administered by inhalation on GD 9, 11 and 14 to rat[22].

In this study bupivacaine harmfully affected the fetal growth. This was confirmed by reduction in fetal birth weight and CRL in both treatment groups B and C. Reduction of the fetal weight and CRL was also reported by Church et al (1988) when cocaine was injected subcutaneously from GD 7-19 in rat[21]. Regarding anomalies of heart 10% of the fetuses revealed atrial septal defect in group C. Whereas 5% and 10% of the fetuses among group B and C were observed to have dextrocardia. Similar findings were reported using lidocaine in rats[23].Lidocaine induced growth retardation and morphologic abnormalities (i.e., situs inversus) were seen at 375µM and 500µM concentration respectively, when given subcutaneously[24].

Delayed intrauterine growth is a component of developmental toxicity[25]. Growth retardation occurs in fetuses on treatment with toxic doses; in fact overall growth retardation is considered by some investigators to constitute a state of increased susceptibility to congenital anomalies[26].

CONCLUSION

Our results of treating mice with bupivacaine evidently showed that it produced deleterious effects in developing conceptus by way of delayed intrauterine growth, implantation loss/ fetal resorptions, atrial septal defect and dextrocardia, however it did not show any abnormality of kidney on gross observation.

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Conflict of interest statement: The authors declare that there are no conflicts of interest.

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