Assessment of Teratogenic Effects of Lidocaine in Rat Embryos Cultured In Vitro

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Background: Lidocaine has been reported to cause neural tube closure defects *in vitro* in mice at clinically relevant concentrations. However, no studies have been conducted to further investigate this potentially hazardous effect of lidocaine. This study was aimed to reassess teratogenic effects of lidocaine *in vitro* in rats.

Methods: Sprague-Dawley rat embryos were explanted at 8:00 AM on gestational day 9 and were cultured in medium containing various concentrations of lidocaine. (Embryos in the control group were cultured without lidocaine). After 50 h of culture, they were evaluated for growth size and morphology, including the neural tube closure.

Results: In the presence of 250 μM of lidocaine, embryos showed a increased incidence of *situs inversus* compared with control group but were otherwise normal. At 375 μM, embryos showed slight growth retardation but no significant morphologic abnormalities. At 500 μM, all viable embryos showed severe morphologic abnormalities. However, morphologic abnormalities were so-called nonspecific types and neural tube closure defects were not observed.

Conclusions: Results from the current study indicate that lidocaine causes teratogenic effects *in vitro* in rats only at concentrations much higher than clinically relevant concentrations. Furthermore, lidocaine did not cause neural tube closure defects at any concentrations evaluated. (Key words: Local anesthetics; reproductive toxicity; *situs inversus*.)

APPROXIMATELY 0.5 to 2% of pregnant women are estimated to undergo surgery during pregnancy,[1-3] and approximately 40% of operations are performed during the first trimester when the embryo/fetus is most susceptible to teratogens.[3] Regional anesthesia is often chosen for such patients. The primary reason is the belief that exposing the embryo/fetus to the fewest number of drugs and the lowest possible drug mass is advantageous. However, regional anesthesia would only be an advantage if it were known that local anesthetics were safe for the embryo/fetus.

Teratogenic effects of local anesthetics have not been well investigated. Furthermore, no attempt has been made to reconcile conflicting results with lidocaine.[4] Although *in vivo* studies have not shown teratogenic effects in rats[5,6] and in mice,[7] *in vitro* studies in mice[8] and in chicks[9,10] have shown lidocaine to cause neural tube closure defects. Particularly, the study of O'Shea and Kaufman[8] reported that lidocaine caused a 90% incidence of neural tube defects in mice at 0.24 μg/ml concentration (approximately 1 μM).[8] It has been reported that peak plasma lidocaine levels reach approximately 1 to 5 μg/ml with epidural anesthesia[11-18] and approximately 1 μg/ml with spinal anesthesia.[19] Lidocaine is known to easily cross the placenta,[20] and the mean fetal/maternal drug concentration gradient at term has been estimated to be between 0.5 and 0.7.[21] Assuming that lidocaine is transferred to the embryo/fetus during organogenesis to the same degree as at term, lidocaine concentrations achieved in the embryo/fetus could be in the same range that induces neural tube defects in mice.[8] The current study was aimed primarily to reassess teratogenic effects of lidocaine in rat embryos cultured *in vitro*, particularly the effect on neural tube closure.

Material and Methods

In Vitro Whole-Embryo Culture Experiments

Sprague-Dawley rats purchased from B&K Universal (Fremont, CA) were used for the study. Timed-pregnant rats were obtained by mating rats for 2 h starting at 8:00 AM.[22] A copulatory plug was sought immediately after mating, and the day it was found was defined as gestational day 0. Rats were housed in the animal research facility and cared for according to standard operating procedures. The experimental protocol was approved by the Animal Care and Use Committee at the VA Palo Alto Health Care System.

Embryos were cultured based on the methods originally developed by New.[23,24] At 8:00 AM on gestational day 9, a timed-pregnant rat was anesthetized with isoflurane-oxygen-nitrous oxide, and its blood was collected from the abdominal aorta. The uterus was excised immediately from the carcass and individual implantation sites were harvested into a sterile petri dish containing Hank's balanced salt solution. Egg cylinders were dissected from deciduae under a dissecting microscope, and Reichert's membranes were removed from the egg cylinder starting from the side opposite the embryonic disc after the ectoplacental cone and roof of the ectoplacental cavity were excised. Embryos were then separated based on stages of development, and only those at stage 11b or 11a by a modified Theiler's staging system were selected.[25] (Stage 11a corresponds to the initial stage of neurulation process, namely, neural groove becomes visible in the midline in frontal view, but neural folds are not yet visible in lateral view. At stage 11b, neural folds become clearly visible in lateral view. The developmental time difference between stage 11a and 11b is approximately 5-6 h.) Three to five embryos were placed in a single glass culture bottle (60 ml volume) that contained 1.5 ml/embryo of culture medium consisting of 30% pregnant rat serum, 50% male rat serum, and 20% Hank's balanced salt solution; penicillin (100 units/ml) and streptomycin (50 μg/ml) were added to each bottle to prevent bacterial growth.[23] Rat serum was obtained from blood that was centrifuged immediately after collection and was heat inactivated (56°C for 30 min) before use.

Lidocaine hydrochloride (2-diethylamino-N-[2,6-dimethylphenyl]-acetamide, Lot No. 60H0865; Sigma Chemical Company, St. Louis, MO) was dissolved in Hank's balanced salt solution, and a stock solution of 10 mM concentration was prepared. This stock solution was diluted with Hank's balanced salt solution as appropriate and was added to the culture medium as 1% of the total volume to achieve the desired concentrations. Bottles were flushed for 1 min with a gas mixture of 5% oxygen/5% carbon dioxide/90% nitrogen, and were capped with rubber stopcocks and rotated at 30 rpm in a 37-38°C incubator. In keeping with the increased oxygen requirement of embryos as they develop, bottles were reflushed with a gas mixture of 20% oxygen/5% carbon dioxide/75% nitrogen at 3:00 PM on gestational day 10 and with 95% oxygen/5% carbon dioxide at 6:00 AM on gestational day 11. Oxygen and carbon dioxide concentrations were monitored before and after each change of atmosphere using a Datex 254 airway gas monitor (Datex Medical Instrumentation, Tewksbury, MA) and were recorded on a strip-chart recorder. This insured that the appropriate concentrations (± 2%) of gasses were delivered and maintained.

At 10:00 AM on gestational day 11, culture was terminated. Embryos were transferred to a petri dish containing Hank's balanced salt solution and were examined morphologically. First, the presence or absence of yolk sac circulation and heart beat were noted. If they were found to be absent, embryos were classified as nonviable. Crown-rump length and number of somite pairs were measured for viable embryos, and various standard morphologic structures were examined, including the neural tube. In addition, sidedness of three asymmetric structures were determined, namely, the chorioallantoic placenta (normally on the right side of the embryo), bulboventricular loop (normally "C" shaped from the frontal view) and lower part of the body (so-called tail, normally flexes to the right side of the body). Embryos were designated as having *situs inversus* when at least one of the three structures was inverted.

Measurement of Lidocaine and Its Metabolites in Culture Medium

Stock lidocaine solution was added to culture bottles (60 ml volume) to obtain a 100 μM concentration in 4.5 ml of (1) culture medium in which there were three embryos at stage 11b, (2) culture medium alone, or (3) 100% Hank's balanced salt solution. Three bottles were evaluated in each group. After 50 h of culture, as described, 1 ml of medium was sampled from each bottle, immediately frozen on dry ice, and stored at −80°C for subsequent assays. Concentrations of lidocaine and the most active metabolite of lidocaine, monoethylglycylxyline, were measured using a capillary-column gas chromatography with a nitrogen–phosphorus detector with a quantitation limit of approximately 1 μg/ml.

Statistical Analysis

Nonparametric data, such as the incidences of abnormalities, from treated and control groups were analyzed using contingency tables, and chi-square analyses were used as an *a posteriori* test. Parametric data, such as crown-rump length and number of somite pairs, were analyzed by one-way analysis of variance, and Scheffe's F-test was used as an *a posteriori* test. A P value less than 0.05 was considered significant.

Table 1. Outcomes of Embryos Cultured from Stage 11b (on Gestational Day 9) for 50 h under Various Concentrations of Lidocaine

	Number of Embryos Studied	Number of Viable Embryos (%)	Number of Malformed Embryos (%)	Number of Embryos with Situs Inversus (%)	Crown–Rump Length (mm, mean ± SD)	Number of Somites (mean ± SD)
Control	34	34	0	0	3.6 ± 0.2	24.9 ± 1.0
Lidocaine (μM)						
1	27	27	0	3 (11.1)	3.5 ± 0.1	25.2 ± 0.6
10	25	25	0	2 (8.0)	3.6 ± 0.2	25.9 ± 1.1
100	25	25	0	4* (16.0)	3.5 ± 0.2	25.6 ± 1.0
250	26	26	0	9* (34.6)	3.4 ± 0.1	25.2 ± 0.9
375	25	25	0	8* (32.0)	3.0 ± 0.2*	23.7 ± 1.5*
500	25	21 (84.0)	21* (100.0)	5* (23.8)	2.8 ± 0.2*	22.0 ± 1.8*
1,000	3	0	—	—		

* $P < 0.05$ versus control.

Results

In Vitro *Whole-Embryo Culture Experiments*

All 34 embryos in the control group developed normally (table 1 and fig. 1A). All embryos exposed to less than 375 μM lidocaine were viable, whereas only 84% of embryos (n = 25) were viable when exposed to 500 μM lidocaine (table 1). At 375 μM, embryos showed growth retardation indicated by decreased crown-rump length and somite numbers. Nevertheless they showed no morphologic abnormalities. At 500 μM, all viable embryos showed severe morphologic abnormalities (figs. 1B and 1C). Although there were no specific patterns of abnormalities, none of the embryos showed any neural tube closure defects.

Interestingly, the embryos exposed to more than 100 μM showed high incidences of *situs inversus* ranging

Fig. 1. Embryos at stage 15 after 50 h of culture from stage 11b (bar = 1 mm). (A) Normal embryo. (B,C) Embryos treated with 500 μM lidocaine showing growth retardation and severe non-specific morphologic abnormalities. Neural tube closure defects were not observed.

from 16.0% and 34.6% (table 1). In a subsequent experiment, when the embryos were cultured from stage 11a, the stage when α1-adrenergic stimulation causes a high incidence of *situs inversus*,[26] they showed a 60% (n = 25) incidence of *situs inversus* with 250 μM of lidocaine (table 2).

Measurement of Lidocaine and Its Metabolites in Culture Medium

Approximately 80% of the initial concentrations of lidocaine remained in the culture medium after 50 h of incubation (table 3). Monoethylglycylxyline, a principal metabolite of lidocaine, was not detected in any group.

Discussion

It is commonly thought that any agent can be shown to be teratogenic in animals, provided enough is administered at the right time: so-called "Karnofsky's principle".[27] Therefore, dose–response data are essential for animal teratology studies, and most animal studies are designed to maximize the likelihood of obtaining positive results. In the current *in vitro* rat study, lidocaine caused growth retardation and morphologic abnormalities at 375 and 500 μM, respectively. Types of morphologic abnormalities caused at 500 μM were so-called nonspecific malformations, but neural tube closure was not affected (fig. 1). These results are inconsistent with the results of the study by O'Shea and Kaufman[8] who reported that 0.24 μg/ml lidocaine (approximately 1 μM) caused a 90% (n = 22) incidence of neural tube closure defects in an *in vitro* mouse study using a similar system as the one used here. As one possible explanation for not observing neural tube closure defects in the current study, degradation of lidocaine in the culture medium
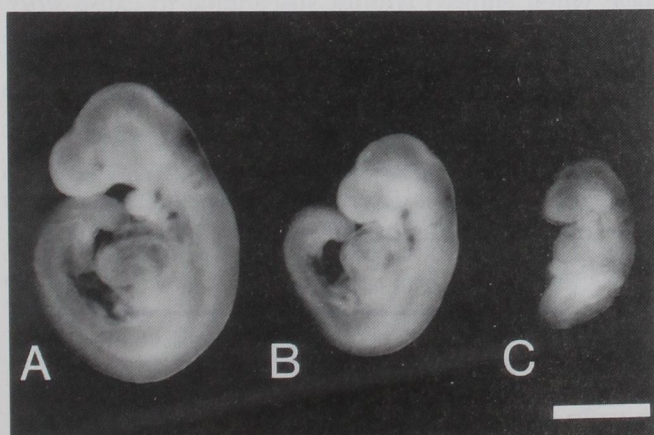
Table 2. Outcomes of Embryos Cultured from Stage 11a (Gestational Day 9) for 50 h with 250 μM Lidocaine

	Number of Embryos Studied	Number of Viable Embryos	Number of Malformed Embryos	Number of Embryos with Situs Inversus (%)	Crown–Rump Length (mm, mean ± SD)	Number of Somites (mean ± SD)
Control	52	52	0	7 (13.5)	3.3 ± 0.2	23.2 ± 1.0
Lidocaine (μM) 250	25	25	0	15* (60.0)	3.1 ± 0.2	23.6 ± 1.0

* P < 0.05 *versus* control.

was first suspected. However, this possibility was excluded by another experiment that showed that approximately 80% of initial lidocaine remained unmetabolized during the culture in the presence of embryos (table 3).

Another possible explanation for the inconsistent results between the two studies is species or strain difference in response to lidocaine. The results from the current *in vitro* study are consistent with *in vivo* studies in rats[5,6] that have shown no teratogenic effects at clinically relevant or higher concentrations. In addition, another *in vivo* mouse study using an inbred strain (*i.e.*, JBT/Jd strain) also showed no teratogenic effects at higher than clinically relevant concentrations.[7] Although other investigators reported that lidocaine caused neural tube closure defects in chick embryos,[9,10] the concentrations that caused neural tube closure defects were 100 μg/ml (approximately 400 μM; a 50% incidence, n = 20) and 300 μg/ml (approximately 1.2 mM; a 90% incidence, n = 24). These concentrations are at least several hundred times higher than the lidocaine concentration reported to cause neural tube defects in mice by O'Shea and Kaufman.[8] Therefore, it is likely that the strain of mice that was used by O'Shea and Kaufman[8] (*i.e.*, CFLP; Anglia Labs, United Kingdom) was perhaps extremely sensitive to lidocaine effect to cause neural tube closure defects.

In addition to the inconsistent results of lidocaine regarding its teratogenic effects, a similar inconsistency is seen in the reports of the effects of prenatal lidocaine exposure on postnatal behavioral effects. This inconsistency also may be explained by strain differences. For example, investigators who used Long-Evans rats reported adverse effects of lidocaine on postnatal behavioral effects,[28,29] whereas those who used Sprague-Dawley rats reported negative results.[30] A similar inconsistency also is seen in the effects of lidocaine during the preimplantation period. For example, those investigators who reported adverse effects of lidocaine during the preimplantation stage in mice used Swiss-family strains of mice.[31-33] To the contrary, several investigators reported a lack of such adverse effects in other strains of mice.[4] Therefore, it is likely that lidocaine toxicity is highly dependent on strains and species. Although this raises a possibility that certain groups of patients may be highly susceptible to lidocaine to cause teratogenic effects, further investigations are needed to clarify this issue.

An interesting finding in the current study was that lidocaine caused *situs inversus*, (*i.e.*, interfered with normal development of left-/right-sidedness) at the concentration that did not cause growth retardation or other morphologic abnormalities. (It must be emphasized, however, that the concentrations necessary to produce *situs inversus* are far in excess of those clinically achieved.) For example, 250 μM lidocaine caused a 34.6% (n = 26) incidence of *situs inversus* in embryos cultured from stage 11b. In a subsequent experiment, when embryos were cultured from stage 11a (*i.e.*, the stage when embryo is most susceptible to cause *situs inversus* by α1-adrenergic stimulation),[26] the incidence of *situs inversus* increased to approximately 50% (*i.e.*, 60.0% (n = 25). It has been suggested that exposure of embryos to certain chemicals, including α1-adrenoceptor agonist, at the critical time of development when left-/right-sidedness is determined, results in interference with normal determination of left-/right-sidedness.[34]

Table 3. Lidocaine (Free Base) Concentrations in the Culture Medium

	Beginning of culture [lidocaine (μg/ml)]	After 50 h of Incubation	
		Lidocaine (μg/ml)	MEGX
Culture medium with embryos	20.0 ± 0.7 (100%)	15.5 ± 0.7 (77.9%)	Negative
Culture medium alone	19.9 ± 0.3 (100%)	15.8 ± 1.2 (79.3%)	Negative
HBSS alone	20.9 ± 0.3 (100%)	19.1 ± 0.3 (91.4%)	Negative

MEGX = monoethylglycylxyline; HBSS = Hank's balanced salt solution.

Values are mean ± SD; n = 3 each.

This leads to a random determination of left-/right-sidedness. As a result, approximately 50% of embryos may have *situs inversus*. The results from the current study suggest that lidocaine also falls into this group of chemicals. In a previous study,[35] we suggested that changes in intracellular Ca^{2+} concentration at the critical time of development may be involved in causing *situs inversus*. Interestingly, lidocaine is well known to have an effect on Ca^{2+} mobilization in relation to the effects on muscle contraction. Although understanding the precise mechanism for this action necessitates further investigations, the finding from the current study adds to earlier evidence for involvement of changes in intracellular Ca^{2+} concentration in causing *situs inversus*.

In summary, in the current *in vitro* rat study, lidocaine showed teratogenic effects only at much higher than clinically relevant concentrations. Furthermore, lidocaine did not cause neural tube closure defects at any concentrations evaluated. These results are consistent with the results of most other studies that reported negative teratogenic effects of lidocaine at clinically relevant or higher concentrations. Therefore, the previous *in vitro* mouse study by O'Shea and Kaufman,[8] in which lidocaine caused neural closure defects at the clinically relevant concentration, seems likely to be a strain-specific finding.

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