Title: BUPIVACAINE ARRHYTHMOGENICITY IN HEART CELL CULTURE: EFFECT OF PROGESTERONE

Author: V.C. Baum, M.D., D.J. Miletich, Ph.D., M.J. Curran, M.D.

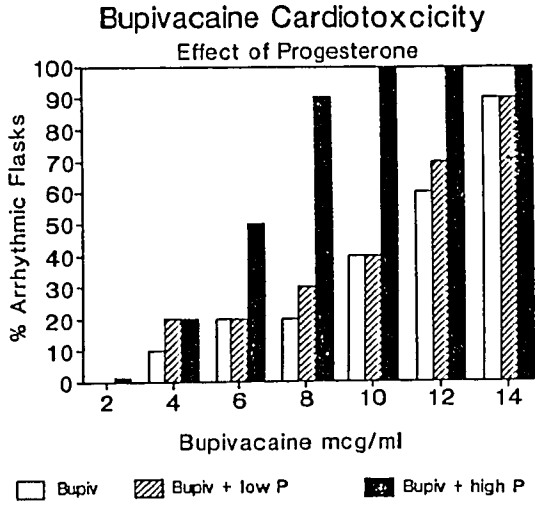
Affiliation: Department of Anesthesiology, Michael Reese Hospital & Medical Center, Chicago, IL 60616

Introduction: Bupivacaine cardiotoxicity has been well documented in a variety of experimental settings as well as in clinical practice. In addition, bupivacaine cardiotoxicity has been a particular concern in the obstetric population. The presence of myocardial progesterone receptors suggested to us that progesterone, a major hormone of pregnancy, might potentiate bupivacaine arrhythmogenicity. We evaluated the arrhythmogenic dose of bupivacaine in beating rat heart cell cultures grown either in normal medium or in medium supplemented with progesterone, at levels found in the human at term gestation (200 ng/ml).

Methods: Beating rat heart cell cultures were prepared as described in a previous report.[1] Briefly, three day old rat pups were sacrificed by cervical dislocation. The hearts were quickly removed, minced and pooled in iced phosphate buffered saline solution. Following this the pooled hearts were transferred to a digestion flask containing 0.1% trypsin. After a period of seven minutes the digestion mixture was centrifuged and the supernatant discarded. The remaining pellet was resuspended with trypsin once again. The digestion process was repeated five more times after which two final digestion steps were carried out with 0.05% collagenase. Once the heart cells were digested apart they were suspended in culture medium and pipetted into culture flasks at a concentration of 5×10^6 cells per flask. After three days incubation, the cells attached to the bottom of the flask and began to beat in normal rhythm. The arrhythmogenicity of bupivacaine in heart cells cultures was determined as previously reported.[1] A series of culture flasks (usually 20) were exposed to increasing dosages of bupivacaine until at least half of the test flasks became arrhythmic. The dose at which 50% of the flasks became arrhythmic is termed the AD_{50}. After determining the AD_{50} for bupivacaine alone, series of culture flasks were incubated with either a low dose of progesterone (100 ng/ml of culture medium) or high dose (200 ng/ml) for 48 hours and the AD_{50} for bupivacaine determined once again in the presence of progesterone.

Results: As shown in figure bupivacaine showed increasing arrhythmogenicity at increasing concentrations. The AD_{50} for bupivacaine was 11 mcg/ml. Culturing cells in the presence of progesterone 200 ng/ml decreased the AD_{50} to 6 mcg/ml. The effect of progesterone appears to be dose related as there was no significant effect of 100 ng/ml progesterone. Progesterone had positive a chronotropic effect on heart cells in culture. The contraction rate of non-progesterone treated cultures was 235 ± 11 mean ± SE beats per minute while culture treated with progesterone (100 ng/ml) for 48 hrs had a mean contraction rate of 258 ± 13 ($P<0.05$). Bupivacaine (with or without progesterone) in increasing dosages caused a dose related pronounced fall in rhythmic cell contraction rates until arrhythmia was reached.

Discussion Bupivacaine cardiotoxicity has been previously studied in a variety of experimental preparations. Bupivacaine cardiotoxicity has been shown to be more pronounced in pregnant vs non-pregnant ewes. At present the mechanism of bupivacaine toxicity is not clear. We elected to evaluate bupivacaine arrhythmogenicity in beating heart cell cultures, a preparation free of potentially confounding neurohumeral influences and which presents a clear end-point, i.e. arrhythmia. In this study, progesterone significantly increased the arrhythmic potential of bupivacaine. Bupivacaine was 100% arrhythmogenic in progesterone treated rat heart cell cultures at a dose level which allowed 60% normal rhythm in cultures treated with bupivacaine alone. The arrhythmogenic dose of bupivacaine which we have observed in heart cell culture is similar to cardiotoxic doses found in "in vivo" animal studies, and the level in our progesterone treated cells is similar to doses required in pregnant ewes. We conclude that heart cell culture is an applicable method for studying bupivacaine cardiac toxicity. Based on these preliminary data, bupivacaine cardiotoxicity in heart cell culture appears to be potentiated in the presence of progesterone at the cellular level at levels of progesterone found in the human at term gestation.



Reference:
1) Miletich DJ, et al: Use of heart cell cultures as a tool for the evaluation of halothane arrhythmia. Toxicol Appl Pharmacol 70:181-187, 1983.