J Anesth (2007) 21:538–539
DOI 10.1007/s00540-007-0555-5

Journal of
Anesthesia

Neurotoxicity of local anesthetics shown by morphological changes and changes in intracellular Ca^{2+} concentration in cultured neurons of *Lymnaea stagnalis*

Toshiharu Kasaba

Department of Anesthesiology and Intensive Care, University of Miyazaki, 5200 Kiyotake, Miyazaki 889-1692, Japan

T. Kasaba

Clinical profiles of neurotoxicity caused by local anesthetics have been based on many reports of cauda equina or transient neurologic symptoms following spinal anesthesia [1]. Covino and Wildsmith [2] have rated the relative systemic toxicity of commonly used local anesthetics, claiming that bupivacaine is more toxic than lidocaine or procaine. However, the toxicity of local anesthetics is different in different sites of organs or tissues. Although bupivacaine is more cardiotoxic than lidocaine, bupivacaine is less neurotoxic than lidocaine or tetracaine. The relative potential neurotoxicity of clinically used local anesthetics is not known, and the neurotoxicities of local anesthetics are difficult to compare clinically.

The mechanism of local anesthetic neurotoxicity has not been clearly demonstrated. One of the possible mechanisms is an extreme increase in intracellular Ca^{2+} concentration produced by lidocaine [3]. Another suggestion to explain lidocaine-induced neurotoxicity is that lidocaine directly disrupts the nerve membrane and produces nerve injury [4,5].

Address correspondence to: T. Kasaba
Received: June 15, 2007

Comparative neurotoxicities of clinically used local anesthetics

Cultured neurons from the freshwater snail, *Lymnaea stagnalis,* were used to determine the toxicity of drugs. Morphological changes in growth cones and neurites exposed to seven local anesthetics were assessed systematically. The median concentrations yielding a score showing moderate morphological changes were: 5×10^{-4} M for procaine, 5×10^{-4} M for mepivacaine, 2×10^{-4} M for ropivacaine, 2×10^{-4} M for bupivacaine, 1×10^{-4} M for lidocaine, 5×10^{-5} M for tetracaine, and 2×10^{-5} M for dibucaine. If procaine and mepivacaine were given a toxicity score of 1, the scores for ropivacaine and bupivacaine were 2.5, that for lidocaine was 5; for tetracaine, 10; and for dibucaine, 25.

Many local anesthetics are available commercially and they differ in both clinical profiles and potential for toxicity. The clinically used concentrations of local anesthetics in spinal anesthesia are: mepivacaine 4%, procaine 10%, bupivacaine 0.75%, ropivacaine 2%, lidocaine 5%, tetracaine 0.5%, and dibucaine 0.5%. If procaine was given a potency score of 1, mepivacaine was 0.4 (1*4/10), ropivacaine was 0.5 (2.5*2/10), bupivacaine was 0.19 (2.5*0.75/10), lidocaine was 2.5 (5*5/10), tetracaine was 0.5 (10*0.5/10), and dibucaine was 1.25 (25*0.5/10) [6] (Fig. 1).

Mechanism of local anesthetic neurotoxicity

To clarify the mechanism of local anesthetic neurotoxicity, we examined the relationship between the morpho-

The relative order of neurotoxicity

Bupivacaine < Mepivacaine < Ropivacaine = Tetracaine
0.19 0.4 0.5 0.5

< Procaine < Dibucaine < Lidocaine
1 1.25 2.5

Fig. 1. Of the seven local anesthetics tested, procaine is the safest; however, procaine is used at a higher concentration than other local anesthetics in clinical use. If procaine was given a potency score of 1, bupivacaine at 0.19 was the safest, and lidocaine at 2.5, was the most toxic of these seven local anesthetics

	Ca^{2+} concentration	Cell damage
Lidocaine	↑↑↑	+++
Lidocaine (Ca^{2+} -free)	↑↑↑	+++
Lidocaine (BAPTA-AM)	↑	+++
Ca^{2+} ionophore	↑↑↑	+

Fig. 2. Intracellular Ca^{2+} concentration and morphological changes after the addition of lidocaine, or lidocaine following Ca^{2+}-free, 1,2-bis-(2-aminophenoxy) ethane-N,N,N′N′-tetraacetic acid acetoxymethyl ester (BAPTA-AM) loading or Ca^{2+} ionophore loading. Although increasing the intracellular Ca^{2+} concentration was toxic to the cells, the morphological damage was not always correlated with the intracellular Ca^{2+} concentration

logical changes and elevated Ca^{2+} concentrations in the cells. The morphological changes in the cells, such as shrinkage or the formation of rugged surfaces, were considered toxic to the cells and were assessed in relation to the intracellular Ca^{2+} concentration. To determine the effects of Ca^{2+} concentrations outside the cells, we compared the effects in saline and in a Ca^{2+}-free medium. The neuronal Ca^{2+} concentrations in a cell were changed using a Ca^{2+} chelator, 1,2-bis-(2-aminophenoxy) ethane-N,N,N′N′-tetraacetic acid acetoxymethyl ester (BAPTA-AM), to decrease the effects of lidocaine on the Ca^{2+} outflow inside the cell, as well as the neurotoxicity. Furthermore, we used a Ca^{2+} ionophore to identify the relationship between the elevated Ca^{2+} concentration without lidocaine and neuronal lysis, using fura-2 imaging from a cultured neuron of *Lymnaea stagnalis*.

Lidocaine significantly increased the Ca^{2+} concentration dose-dependently in the normal saline and Ca^{2+}-free medium. Increasing the dose of lidocaine increased the intracellular Ca^{2+} concentration and produced cell damage both in saline and in the Ca^{2+}-free medium. Increasing doses of lidocaine increased the intracellular Ca^{2+} concentration and the damage to the cells; however, the intracellular calcium chelator, BAPTA-AM, which prevented an increase in intracellular Ca^{2+} concentration, did not prevent the lidocaine-induced morphological damage (Fig. 2). We also found in our experiments that the use of a Ca^{2+} ionophore without lidocaine resulted in an elevation of the Ca^{2+} concentration to more than 1000 nM, and cytotoxic levels that were revealed following a few hours' observation. However, the cells showed only slight swelling and no morphological damage in the short time period during the lidocaine loading experiments. These results suggested that the morphological damage was not always correlated with the intracellular Ca^{2+} concentration [7].

Although increasing the intracellular Ca^{2+} concentration was toxic to the cells, a high concentration of lidocaine had another mechanism of neurotoxicity. These results indicated that controlling increases in the intracellular Ca^{2+} concentration is insufficient to prevent the neurotoxicity of lidocaine.

References

1. Hodgson PS, Neal JM, Pollock JE, Liu SS (1999) The neurotoxicity of drugs given intrathecally (spinal). Anesth Analg 88:797–809
2. Covino BG, Wildsmith JAW (1998) Clinical pharmacology of local anesthetics agents. In: Cousins MJ, Bridenbaugh PO (eds) Neural blockade in clinical anesthesia and management of pain. Lippincott-Raven, Philadelphia, pp 97–128
3. Gold MS, Reiching DB, Hampl KF, Drasner K, Levine JD (1998) Lidocaine toxicity in primary afferent neurons from the rat. J Pharmacol Exp Ther 285:413–421
4. Kanai Y, Katsuki H, Takasaki M (2000) Lidocaine disrupts axonal membrane of rat sciatic nerve in vitro. Anesth Analg 91:944–948
5. Kitagawa N, Oda M, Totoki T (2004) Possible mechanism of irreversible nerve injury caused by local anesthetics. Anesthesiology 100:962–967
6. Kasaba T, Onizuka S, Takasaki M (2003) Procaine and mepivacaine have less toxicity in vitro than other clinically used local anesthetics. Anesth Analg 97:85–90
7. Kasaba T, Onizuka S, Kashiwada M, Takasaki M (2006) Increase in intracellular Ca^{2+} concentration is not the only cause of lidocaine-induced cell damage in the cultured neurons of *Lymnaea stagnalis*. J Anesth 20:196–201