Natural Science Research Univ. Tokushima (Peer-Reviewed Paper)
Vol. 25, No.2 (2011) p.7-13

Tetracaine decreases intracellular Zn^{2+} concentration by inhibiting Zn^{2+} influx in rat thymocytes

Kaori Kimura[1] • Yumiko Nishimura[2] • Keisuke Oyama[3] • Takuya Kawanai[4] • Erika Hashimoto[4] • Yasuo Oyama[4]

[1] Faculty of Dentistry, The University of Tokushima, Tokushima 770-8504, Japan.
[2] Faculty of Dentistry, Okayama University, Okayama 700-8525, Japan
[3] Faculty of Medicine, Saga University, Saga 849-8501, Japan
[4] Faculty of Integrated Arts and Sciences, The University of Tokushima, Tokushima 770-8502, Japan

Corresponding author: Yasuo Oyama, Ph.D. Email: oyama@ias.tokushima-u.ac.jp

Abstract In this study to examine the cytotoxic property of tetracaine, we cytometrically examined the effect of tetracaine on intracellular Zn^{2+} concentration by the use of FluoZin-3, a fluorescent indicator of intracellular Zn^{2+}. Lidocaine was used as a reference drug. The incubation of rat thymocytes with tetracaine decreased the intensity of FluoZin-3 fluorescence while that with lidocaine increased the intensity. The incubation with 10 μM DTPA, a chelator for extracellular Zn^{2+}, attenuated the tetracaine-induced decrease in fluorescence intensity. The application of $ZnCl_2$ augmented FluoZin-3 fluorescence. The augmentation by $ZnCl_2$ was a temperature-sensitive. Tetracaine attenuated the $ZnCl_2$-induced augmentation of FluoZin-3 fluorescence. Taken together, the results suggest that tetracaine attenuates membrane Zn^{2+} influx, resulting in a decrease in intracellular Zn^{2+} concentration in rat thymocytes. Although the cells in this study are not targets for actions of local anesthetics, the result may give one clue to explain the difference between the cytotoxicity of local anesthetics since the action of tetracaine on FluoZin-3 fluorescence was opposite to that of lidocaine.

Keywords: Tetracaine • Lidocaine • Zinc • Cytotoxicity

1. Introduction

Local anesthetics such as lidocaine and tetracaine increase intracellular Ca^{2+} concentration by increasing Ca^{2+} release and membrane Ca^{2+} permeability (Johnson et al., 2002; Nishimura et al., 2006). The increase in intracellular Ca^{2+} concentration may induce cell injury and death (Trump and Berezesky, 1995; Orrenius et al., 2003). There is a little information on the effects of local anesthetics on intracellular Zn^{2+} concentration although zinc possesses many physiological roles (Frederickson et al, 2005). Zinc is the second most prevalent trace element and it is involved in the structure and function of over 300 enzymes (Prasad, 1995). Furthermore, it stimulates the activity of approximately 100 enzymes (Sandstead, 1994). Therefore, abnormal increase in intracellular Zn^{2+} concentration may cause some cytotoxic phenomena. Lidocaine at sublethal concentrations increases intracellular Zn^{2+} concentration in absence of extracellular Zn^{2+} (Nishimura and Oyama, 2010), suggesting intracellular Zn^{2+} release by lidocaine. Since the toxicity of tetracaine is more potent than that of lidocaine (Lambert et al., 1994; Johnson et al., 2002; Yamashita et al., 2003; Werdehausen et al., 2009) and the mixture of lidocaine and tetracaine is used for dermatologic procedures (Alster, 2007 for review), we examined the effect of tetracaine on intracellular Zn^{2+} concentration of rat thymocytes by the use of a flow cytometer with FluoZin-3. Here we describe that the action of tetracaine on intracellular Zn^{2+} concentration may be opposite to that of lidocaine.

Thymocyte is used for a model experiment because of following reasons. (1) Since thymocytes are prepared without any enzymatic treatment for dissociation, cell membranes are remained intact. Intact membranes are important to examine the effect of test compound on membrane transport in single cells. (2) The process of cell death, apoptosis and necrosis, in thymocytes is well defined (McConkey et

al., 1994; Rinner et al., 1996; Winoto, 1997) and Zn^{2+} is one of factors affecting the process of cell death (Raqib et al., 2007; Someya et al., 2009; Haase and Rink, 2009; Wong et al., 2009). (3) Several types of biological substances and chemical compounds (including lidocaine) induce cell death in thymocytes under *in vivo* and *in vitro* conditions (Choyke et al., 1987; Quaglino and Ronchetti, 2001; Nishimura et al., 2006; Gruver and Sempowski, 2008).

2. Materials and methods

2.1. Materials

Tetracaine HCl, lidocaine HCl, NaCl, $MgCl_2$, KCl, $ZnCl_2$, NaOH, glucose, dimethyl sulfoxide (DMSO), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HPES) were purchased from Wako Pure Chemicals (Osaka, Japan). Diethylenetriamine-N,N,N',N",N"-pentaacetic acid (DTPA) was obtained from Dojin Chemical Laboratory (Kumamoto, Japan). Propidium iodide and FluoZin-3 pentaacetoxymethyl ester (FluoZin-3-AM) were products of Molecular Probes Inc. (Eugene, Oregon, USA). Final concentration of DMSO as a solvent in cell suspension was 0.1 % or less. Incubation with DMSO did not affect cell viability during present experiments.

2.2. Animals and cell preparation

This study was approved by the Committee for Animal Experiments in the University of Tokushima (No.05279). Procedure to prepare cell suspension was similar to that previously described (Chikahisa et al., 1996). Thymus glands dissected from ether-anesthetized rats (Wistar strain, Charles River Laboratories, Yokohama, Japan) were sliced at a thickness of about 1 mm with razor under an ice-cold condition. To dissociate single cells, the slices of thymus glands were triturated by gently shaking in chilled normal Tyrode's solution (in mM: NaCl 150, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, glucose 5, HEPES 10, appropriate amount of NaOH to adjust pH at 7.3-7.4) or Ca^{2+}-free Tyrode's solution (in mM: NaCl 150, KCl 5, $MgCl_2$ 3, glucose 5, HEPES 10, appropriate amount of NaOH to adjust pH at 7.3-7.4). Thereafter, Tyrode's solution containing single cells was passed through a mesh (a diameter of 10 μm) to prepare cell suspension (about 5×10^5 cells/ml). The cell suspension was incubated at 36-37ºC for 1 h at least before the experiment. Sixteen rats were used in this study. Each result was obtained from four experiments at least.

2.3. Fluorescence measurements of cellular and membrane parameters

The methods for measurements of cellular and membrane parameters by a flow cytometer (CytoACE-150, JASCO, Tokyo, Japan) and fluorescent probes were similar to those previously described (Chikahisa et al., 1996; Matsui et al., 2008). The fluorescence was analyzed by JASCO software (Ver.3XX, JASCO). Chemicals, except for fluorescent probes, used in this study exerted no fluorescence under our experimental condition.

Propidium iodide at 5 μM was added to cell suspension to assess cell viability. Since propidium stains dead cells, the measurement of propidium fluorescence from cells provides a clue to estimate cell lethality. The fluorescence was measured at 2 min after the application of propidium iodide. Excitation wavelength for propidium was 488 nm and emission was detected at 600 ± 20 nm.

FluoZin-3-AM (Gee et al., 2002) was used as a fluorescent indicator for intracellular Zn^{2+}. The cells were incubated with 500 nM FluoZin-3-AM for 60 min before any fluorescence measurements (Hashimoto et al., 2009; Kawanai et al., 2009). FluoZin-3 fluorescence was measured from the cells that were not stained with propidium iodide (Matsui et al., 2008). Excitation wavelength for FluoZin-3 was 488 nm and emission was detected at 530 ± 20 nm.

2.4. Statistics

Values were expressed as mean ± standard deviation of 4 experiments. Statistical analysis was performed by using Tukey's multivariate analysis. A P value of < 0.05 was considered significant.

3. Results

3.1. Effects of tetracaine and lidocaine on FluoZin-3 fluorescence of rat thymocytes

As shown in Fig. 1A, tetracaine at 1 mM shifted the histogram of FluoZin-3 fluorescence to a direction of lower intensity, suggesting tetracaine-induced decrease in intracellular Zn^{2+} concentration. Lidocaine at 10 mM increased the intensity of FluoZin-3 fluorescence (Fig. 1A), suggesting lidocaine-induced increase in intracellular Zn^{2+} concentration. The effects of lidocaine and

tetracaine on FluoZin-3 fluorescence were time-dependent. However, their effects attained steady state levels within 60-90 min after the start of application. Therefore, the effects were tested at 90-120 min after the start of drug application. Tetracaine at 0.1 mM or less and lidocaine at 1 mM or less did not affect FluoZin-3 fluorescence. The effect of tetracaine at 0.3-1 mM on FluoZin-3 fluorescence was opposite to that of lidocaine at 3-10 mM (Fig. 1B).

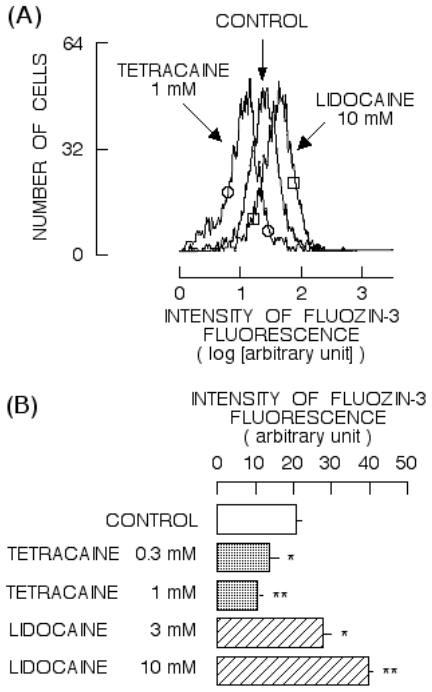

Fig. 1.
Effects of tetracaine and lidocaine on FluoZin-3 fluorescence. (A) Histograms of FluoZin-3 fluorescence monitored from control cells (center histogram) and the cells incubated with 1 mM tetracaine (left with circles) or 10 mM lidocaine (right with squares). Each histogram was constructed with 2000 cells. (B) Mean intensity of FluoZin-3 fluorescence monitored from the cells incubated with 0.3-1 mM tetracaine or 3-10 mM lidocaine. Column and bar indicate mean value and standard deviation of four experiments. Symbols (*, **) show significant changes ($P < 0.05$, $P < 0.01$) to the control.

The concentrations of tetracaine and lidocaine to affect FluoZin-3 fluorescence (Fig. 1B) were sublethal since tetracaine at 3 mM (Fig. 2A) and lidocaine at 30 mM (Nishimura et al., 2006) started to increase cell lethality, respectively. Dose-dependent action of tetracaine on mean intensity of FluoZin-3 fluorescence is summarized in Fig. 2B. Tetracaine at 0.1 mM or more seems to decrease intracellular Zn^{2+} concentration.

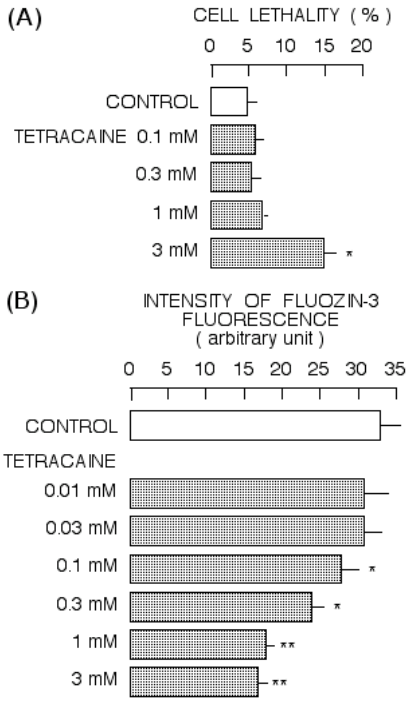

Fig. 2.
Concentration-dependent effects of tetracaine on cell viability and FluoZin-3 fluorescence. (A) Cell lethality in the cells incubated with 0.1-3 mM tetracaine. (B) Concentration-dependent attenuation of mean FluoZin-3 fluorescence intensity by tetracaine. Column and bar indicate mean value and standard deviation of four experiments. Symbols (*, **) show significant changes ($P < 0.05$, $P < 0.01$) to the control.

3.2. Effect of DTPA on tetracaine-induced decrease in intensity of FluoZin-3 fluorescence

The cell suspension contained 200-250 nM zinc derived from cell preparation (Sakanashi et al., 2009). To see if the decrease in Zn^{2+} influx is involved in the tetracaine-induced attenuation of fluoZin-3 florescence, the effect of tetracaine was examined in presence of DTPA, a chelator for extracellular Zn^{2+}. The cells were incubated with 10 μM DTPA for 60 min before applying tetracaine. The incubation of cells with DTPA decreased control level of FluoZin-3 fluorescence and tetracaine at 1 mM further decreased the intensity of FluoZin-3 fluorescence (Fig. 3A). However, the tetracaine-induced decrease in the intensity of FluoZin-3 fluorescence in the presence of DTPA (-27.6 %) was smaller than that under control condition (-43.4 %).

Ca^{2+} competes with Zn^{2+} in some ion transports of membranes (Oyama et al., 1982; Zhuang and Ahearn,

1996; Sensi et al., 1997). Further experiments were performed under nominal Ca^{2+}-free condition where $CaCl_2$ in Tyrode's solution was replaced with equimolar $MgCl_2$. As shown in Fig. 3B, the removal of extracellular Ca^{2+} significantly increased the intensity of FluoZin-3 fluorescence. The incubation with 1 mM tetracaine also attenuated the FluoZin-3 fluorescence under nominal Ca^{2+}-free condition. The incubation with DTPA significantly reduced control level of FluoZin-3 fluorescence (-63.8 %). In the presence of DTPA, tetracaine did not decrease the intensity of FluoZin-3 fluorescence under nominal Ca^{2+}-free condition (Fig. 3B). Results suggest the possibility that tetracaine inhibits Zn^{2+} influx.

3.3. Effect of tetracaine on $ZnCl_2$-induced augmentation of FluoZin-3 fluorescence

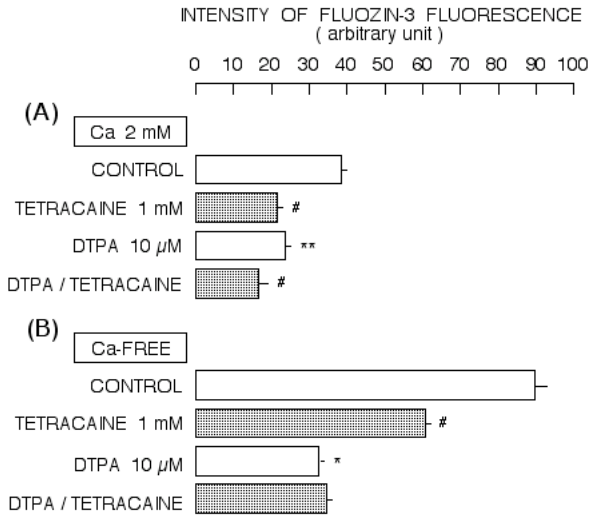

Fig. 3.
Effect of DTPA on tetracaine-induced attenuation of FluoZin-3 fluorescence. (A) The effect under normal Ca^{2+} condition. (B) The effect under nominal Ca^{2+}-free condition. Column and bar indicate mean value and standard deviation of four experiments. Symbols (*, **) show significant changes ($P < 0.05$, $P < 0.01$) to respective control. Symbol (#) indicates significant change between pared columns.

To test the possibility, the effect of tetracaine on $ZnCl_2$-induced augmentation of FluoZin-3 fluorescence was examined. Before the experiments, we examined the effect of $ZnCl_2$ on the intensity of FluoZin-3 fluorescence under cold condition to see if Zn^{2+} influx is passive. As shown in Fig. 4, the addition of 3 μM $ZnCl_2$ significantly increased the intensity of FluoZin-3 fluorescence under control condition (36-37ºC) while it was not the case under cold condition (3-4ºC). Thus, the augmentation of FluoZin-3 fluorescence by adding $ZnCl_2$ was not passive.

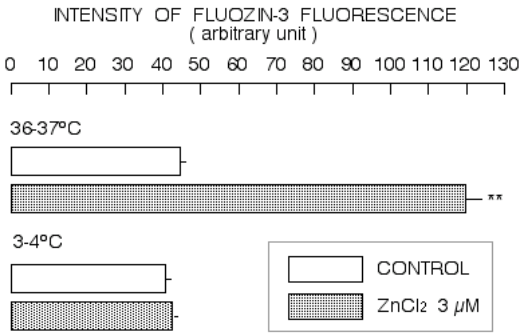

Fig. 4.
Effect of low temperature on the augmentation of FluoZin-3 fluorescence by adding $ZnCl_2$ into the cell suspension. (Upper panel) The augmentation of FluoZin-3 fluorescence under normal temperature (36-37ºC). (Lower panel) Complete attenuation of $ZnCl_2$-induced augmentation of FluoZin-3 fluorescence under low temperature condition (3-4ºC). Column and bar indicate mean value and standard deviation of four experiments. Symbol (**) shows significant change ($P < 0.01$) to the control.

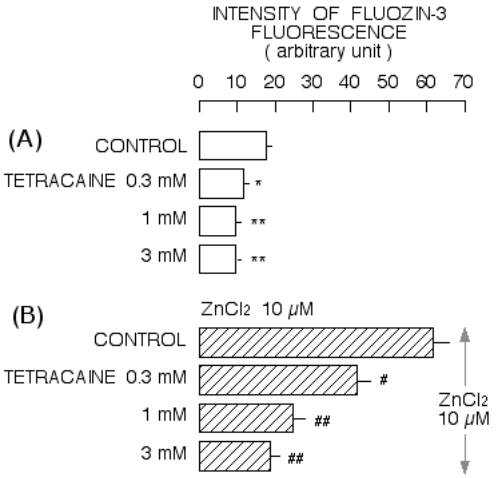

Fig. 5.
Concentration-dependent effect of tetracaine on the augmentation of FluoZin-3 fluorescence by $ZnCl_2$. (A) The effect of tetracaine on control FluoZin-3 fluorescence. (B) The effect of tetracaine on the augmentation of FluoZin-3 fluorescence by $ZnCl_2$. Symbols (*, **) show significant changes ($P < 0.05$, $P < 0.01$) to the control without adding $ZaCl_2$. Symbols (#, ##) indicate significant changes ($P < 0.05$, $P < 0.01$) to the control with adding $ZnCl_2$.

The cells were incubated with 0.3-3 mM tetracaine for 60 min before adding 10 μM $ZnCl_2$ (Fig. 5A). Addition of $ZnCl_2$ significantly increased the intensity of FluoZin-3 fluorescence under control condition. Tetracaine at 0.3-3 mM attenuated the $ZnCl_2$-induced augmentation of FluoZin-3 fluorescence in a concentration-dependent manner (Fig. 5B).

4. Discussion

4.1. Tetracaine-induced decrease in intensity of FluoZin-3 fluorescence

The incubation of cells with tetracaine at 0.1 mM or more (up to 1 mM) decreased the intensity of FluoZin-3 (Fig. 2B), suggesting tetracaine-induced decrease in intracellular Zn^{2+} concentration. The incubation with DTPA reduced control level of FluoZin-3 fluorescence and attenuated the tetracaine-induced decrease in FluoZin-3 fluorescence intensity (Fig. 3), indicating the involvement of extracellular Zn^{2+}. The cell suspension contained 200-250 nM Zn^{2+} derived from cell preparation (Sakanashi et al., 2009). Zn^{2+} influx seems to maintain intracellular Zn^{2+} level in this preparation. Tetracaine attenuated the $ZnCl_2$-induced augmentation of FluoZin-3 fluorescence (Fig. 5B). It is reminiscent of the possibility that tetracaine attenuates Zn^{2+} influx, resulting in the decrease in intracellular Zn^{2+} concentration in rat thymocytes. This Zn^{2+} influx seems to be temperature-sensitive because the $ZnCl_2$-induced augmentation of FluoZin-3 fluorescence was not observed under cold condition (Fig. 4). Such Zn^{2+} influxes were shown in divalent metal transporter 1 (Andrews, 1999; Olivi et al., 2001; Forbes and Gros, 2003; Knöpfel et al., 2005). It may be suggested that tetracaine inhibits Zn^{2+} influx *via* divalent metal transporter 1.

4.2. Toxicological implication

Lidocaine increased the intensity of FluoZin-3 fluorescence (Nishimura and Oyama, 2010) while tetracaine decreased the intensity (Fig. 1). Thus, the action of tetracaine on intracellular Zn^{2+} concentration seems to be opposite to that of lidocaine. Zinc is required in the maintenance and functioning of many proteins/metalloenzymes (Coleman, 1992; Choi and Koh, 1998) and enzymes involved in apoptosis such as endonuclease and caspase are abrogated by physiological concentrations of Zn^{2+} (Gunshin et al., 1997; Giannakis et al., 1991; Perry et al., 1997). If tetracaine decreases intracellular Zn^{2+} concentration, it would increase cell vulnerability to apoptosis (McCabe at al., 1993). It was reported that tetracaine, but not lidocaine, induced apoptosis in rat cortical astrocytes (Lee et al., 2009). Such a difference may be due to the effect on intracellular Zn^{2+} concentration. The mixture of tetracaine and lidocaine is used for dermatologic procedures (Alster, 2007 for review). Such a combination may minimize the change in intracellular Zn^{2+} concentration induced by respective local anesthetics under clinical condition. In preliminary experiment using FluoZin-3 and rat thymocytes, the change in FluoZin-3 fluorescence by the mixture of lidocaine and tetracaine was significantly smaller than those respectively induced by tetracaine and lidocaine (unpublished observation). The clinical efficacy of tetracaine was compared with that of lidocaine by several investigators (Noorily et al., 1995; Stevens et al., 1997; Amiel and Koch, 2007; Bourolias et al., 2009). Furthermore, toxic effects of local anesthetics were also compared in clinical and animal studies (Lambert et al., 1994; Yamashita et al., 2003; McGee and Fraunfelder, 2007; Werdehausen et al., 2009). Although this study may give one insight for such comparisons of local anesthetics, the data would be potentially interesting if the results are supported by the experiments that are relevant for effects of local anesthetics in further study.

Conflict of interest statement

All authors declare that there are no conflicts of interest in this study.

References

Alster TS (2007) The lidocaine/tetracaine peel: a novel topical anesthetic fordermatologic procedures in adult patients. Dermatol Surg 33:1073-1081

Amiel H, Koch PS (2007) Tetracaine hydrochloride 0.5% versus lidocaine 2% jelly as a topical anesthetic agent in cataract surgery: comparative clinical trial. J Cataract Refract Surg 33:98-100

Andrew CN (1999) The iron transporter DMT1. Internat J Biochem Cell Biol 31:991-994

Bourolias C, Gkotsis A, Kontaxakis A, Tsoukarelis P (2009) Lidocaine spray vs tetracaine solution for transnasal fiber-optic laryngoscopy. Am J Otolaryngol 31:114-116

Chikahisa L, Oyama Y, Okazaki E, Noda K (1996) Fluorescent estimation of H_2O_2-induced changes in cell viability and cellular nonprotein thiol level of dissociated rat thymocytes. Jpn J Pharmacol 71:299-305

Choi DW, Koh JY (1998) Zinc and brain injury. Annu Rev Neurosci 21:347-75

Choyke PL, Zeman RK, Gootenberg JE, Greenberg JN, Hoffer F, Frank JA (1987) Thymic atrophy and regrowth in response to chemotherapy: CT evaluation. Am J Roentgenol 149:269-272

Coleman JE (1992) Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. Annu Rev Biochem 61:897-946

Forbes JR, Gros P (2003) Iron, manganese, and cobalt transport by Nramp1 (Slc11a1) and Nramp2 (Slc11a2) expressed at the plasma membrane. Blood 102:1884-1892

Frederickson CJ, Koh JY, Bush AI (2005) The neurobiology of zinc in health and disease. Nat Rev Neurosci 6:449-462

Gee KR, Zhou ZL, Qian WJ, Kennedy R (2002) Detection and imaging of zinc secretion from pancreatic beta-cells using a new fluorescent zinc indicator. J Amer Chem Soc 124:776-778

Giannakis C, Forbes IJ, Zalewski PD (1991) Ca^{2+}/Mg^{2+}-dependent nuclease: Tissue distribution, relationship to inter-nucleosomal DNA fragmentation and inhibition by Zn^{2+}. Biochem Biophys Res Comm 181:915-920

Gruver AL, Sempowski GD (2008) Cytokines, leptin, and stress-induced thymic atrophy. J Leukoc Biol 84:915-923

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388:482-488

Haase H, Rink L (2009) The immune system and the impact of zinc during aging. Immun Ageing 6:9(1-17)

Hashimoto E, Oyama TB, Oyama K, Nishimura Y, Oyama TM, Ueha-Ishibashi T, Okano Y, Oyama Y (2009) Increase in intracellular Zn^{2+} concentration by thimerosal in rat thymocytes: intracellular Zn^{2+} release induced by oxidative stress. Toxicol In Vitro 23:1092-1099

Johnson ME, Saenz JA, DaSilva AD, Uhl CB, Gore GJ (2002) Effect of local anesthetic on neuronal cytoplasmic calcium and plasma membrane lysis (necrosis) in a cell culture model. Anesthesiol 97:1466–1476

Kawanai T, Satoh M, Murao K, Oyama Y 2009 Methylmercury elicits intracellular Zn^{2+} release in rat thymocytes: Its relation to methylmercury-induced decrease in cellular thiol content. Toxicol Lett 191 231–235

Knöpfel M, Smith C, Solioz M (2005) ATP-driven copper transport across the intestinal brush border membrane. Biochem Biophys Res Commun 330:645-652

Lambert LA, Lambert DH, Strichartz GR (1994) Irreversible conduction block in isolated nerve by high concentrations of local anesthetics. Anesthesiol 80:1082-1093

Lee WY, Park CJ, Shin TJ, Yum KW, Yoon TG, Seo KS, Kim HJ (2009) Only tetracaine and not other local anaesthetics induce apoptosis in rat cortical astrocytes. Br J Anaesth 103:719-725

Matsui H, Sakanashi Y, Oyama TM, Oyama Y, Yokota SI, Ishida S, Okano Y, Oyama TM, Nishimura Y (2008) Imidazole antifungals, but not triazole antifungals, increase membrane Zn^{2+} permeability in rat thymocytes: Possible contribution to their cytotoxicity. Toxicol 248:142-150

McCabe MJJr, Jiang SA, Orrenius S (1993) Chelation of intracellular zinc triggers apoptosis in mature thymocytes. Lab Investi 69:101-110

McConkey DJ, Jondal M, Orrenius S (1994) The regulation of apoptosis in thymocytes. Biochem Soc Transac 22:606-610

McGee HT, Fraunfelder FW (2007) Toxicities of topical ophthalmic anesthetics. Expert Opin Drug Saf 6:637-640

Nishimura Y, Kanada A, Yamaguchi JY, Horimoto K, Kobayashi M, Tatsuishi T, Kanemaru K, Ueno S, Oyama Y (2006) Cytometric analysis of lidocaine-induced cytotoxicity: a model experiment using rat thymocytes. Toxicol 218:48–57

Nishimura Y, Oyama Y (2010) Cytotoxic actions of lidocaine at sublethal concentrations: A model in vitro experiment using rat thymocytes. Nat Sci Res Univ Tokushima 24 :1-5 http://ias-www.ias.tokushima-u.ac.jp/bulletin/nat.html

Noorily AD, Noorily SH, Otto RA (1995) Cocaine, lidocaine, tetracaine: which is best for topical nasal anesthesia? Anesth Analg 81:724-727

Olivi L, Sisk J, Bressler J (2001) Involvement of DMT1 in uptake of Cd in MDCK cells: role of protein kinase C. Am J Physiol Cell Physiol 281:C793-800

Orrenius S, Zhivotovsky B, Nicotera P (2003) Regulation of cell death: the calcium–apoptosis link. Nature Rev Mol Cell Biol 4:552-565

Oyama Y, Nishi K, Yatani A, Akaike N (1982) Zinc current in Helix soma membrane. Comp Biochem Physiol 72:403-410

Perry DK, Smyth MJ, Stennicke HR, Salvesen GS, Duriez P, Poirier GG, Hannun YA (1997) Zinc is a potent inhibitor of the apoptotic protease, caspase-3. A novel target for zinc in the inhibition of apoptosis. J Biol Chem 272:18530–18533

Prasad AS (1995) Zinc: an overview. Nutri 11:93-99

Quaglino D, Ronchetti IP (2001) Cell death in the rat thymus: a minireview. Apoptosis 6:389-401

Raqib R, Hossain MB, Kelleher SL, Stephensen CB, Lönnerdal B (2007) Zinc supplementation of pregnant rats with adequate zinc nutriture suppresses immune functions in their offspring. J Nutr 137:1037-1042

Rinner I, Felsner P, Hofer D, Globerson A, Schauenstein K (1996) Chracterization of the spontaneous apoptosis of rat thymocytes in vitro. Int Arch Allergy Immunol 111:230-237

Sakanashi Y, Oyama TM, Matsuo Y, Oyama TB, Nishimura Y, Ishida S, Imai S, Okano Y, Oyama Y (2009) Zn^{2+}, derived from cell preparation, partly attenuates Ca^{2+}-dependent cell death induced by A23187, calcium ionophore, in rat thymocytes. Toxicol In Vitro 23:338–345

Sandstead HH (1994) Understanding zinc: recent observations and interpretations. J Lab Clin Med 124:322-327

Sensi SL, Canzoniero LM, Yu SP, Ying HS, Koh JY, Kerchner GA, Choi DW (1997) Measurement of intracellular free zinc in living cortical neurons: routes of entry. J Neurosci 17:9554-9564

Someya Y, Tanihata J, Sato S, Kawano F, Shirato K, Sugiyama M, Kawashima Y, Nomura S, Tachiyashiki K, Imaizumi K (2009) Zinc-deficiency induced changes in thedistribution of rat white blood cells. J Nutr Sci Vitaminol (Tokyo) 55:162-169

Stevens RA, Frey K, Liu SS, Kao TC, Mikat-Stevens M, Beardsley D, Holman S, White JL (1997) Sympathetic block during spinal anesthesia in volunteers using lidocaine, tetracaine, and bupivacaine. Reg Anesth 22:3253-31

Trump BF, Berezesky IK (1995) Calcium-mediated cell injury and cell death. FABES J 9:219-228

Werdehausen R, Fazeli S, Braun S, Hermanns H, Essmann F, Hollmann MW, Bauer I, Stevens MF (2009) Apoptosis induction by different local anaesthetics in a neuroblastoma cell line. Br J Anaesth 103:711-718

Winoto A (1997) Genes involved in T-cell receptor-mediated apoptosis of thymocytes and T-cell hybridomas. Semin Immunol 9:51-58

Wong CP, Song Y, Elias VD, Magnusson KR, Ho E (2009) Zinc supplementation increases zinc status and thymopoiesis in aged mice. J Nutr 139:1393-1397

Yamashita A, Matsumoto M, Matsumoto S, Itoh M, Kawai K, Sakabe T (2003) A comparisonof the neurotoxic effects on the spinal cord of tetracaine, lidocaine, bupivacaine, and ropivacaine administered intrathecally in rabbits. Anesth Analg 97:512-519

Zhuang Z, Ahearn G (1996) Ca^{2+} transport processes of lobster hepatopancreatic brush-border membrane vesicles. J Exp Biol 199:1195-1208

Article History:
Received MS – 1 April 2011
Received revised MS – 21 April 2011
Accepted MS – 21 April 2011