Available online at www.sciencedirect.com
ScienceDirect
Neuroscience Letters 424 (2007) 22–26
Neuroscience Letters
www.elsevier.com/locate/neulet

Different mechanisms of lysophosphatidylcholine-induced Ca^{2+} mobilization in N2a mouse and SH-SY5Y human neuroblastoma cells

Xiao-Hua Li [a,b], Ding-Xin Long [a,b], Wei Li [a], Yi-Jun Wu [a,*]

[a] *Laboratory of Molecular Toxicology, State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Datunlu Road, Beijing 100101, PR China*
[b] *Graduate School of the Chinese Academy of Sciences, Beijing 100039, PR China*

Received 27 April 2007; received in revised form 6 July 2007; accepted 7 July 2007

Abstract

In mice, lysophosphatidylcholine (LPC) was found to be a physiological substrate of neuropathy target esterase, which is also bound by organophosphates that cause a delayed neuropathy in human and some animals. However, the mechanism responsible for causing the different symptoms in mice and humans that are exposed to neuropathic organophosphates still remains unknown. In the present study, we examined and compared the effect of exogenous LPC on intracellular Ca^{2+} overload in mouse N2a and human SH-SY5Y neuroblastoma cells. LPC caused an intracellular Ca^{2+} level ($[Ca^{2+}]_i$) increase in both N2a and SH-SY5Y cells; moreover, the amplitude was higher in N2a cells than that in SH-SY5Y cells. Preincubation of the cells with verapamil, an L-type Ca^{2+} channel blocker, did not affect the LPC-induced Ca^{2+} increase in N2a cells, verapamil inhibited the response by 23% in SH-SY5Y cells. In Ca^{2+}-free medium, LPC produced a significant $[Ca^{2+}]_i$ decrease in N2a cells, while it caused 64% of total $[Ca^{2+}]_i$ increase in SH-SY5Y cells. The results of a cell viability test suggest that N2a cells were more sensitive to LPC than were SH-SY5Y cells. These data suggested that the LPC-induced $[Ca^{2+}]_i$ increase was produced in each cell line through different mechanisms. In particular, the $[Ca^{2+}]_i$ increase occurred via entry through a permeabilized membrane in N2a cells, but through L-type Ca^{2+} channels as well as by Ca^{2+} release from intracellular Ca^{2+} stores in SH-SY5Y cells. Thus, the symptomatic differences of organophosphate-induced neurotoxicity between mice and humans are probably not related to the diverse amplitudes of intracellular Ca^{2+} overload produced by LPC. Moreover, the demyelination effect induced by LPC in mice may be a consequence of its detergent effect on membranes.

Keywords: Delayed neurotoxicity; Lysophosphatidylcholine; Membrane detergent action; Extracellular Ca^{2+} influx; Intracellular Ca^{2+} level; Neuroblastoma cell

Lysophosphatidylcholine (LPC), also called lysolecithin, is an important lipid molecule in mammalian tissues. Accumulation of LPC is associated with a host of diseases such as atherosclerosis, myocardial ischemia, and inflammatory diseases [22,12,18]. LPC is also a major lipid constituent and exhibits several types of neuroactivity in nervous system [23], and induces neuronal sheaths demyelination, together with a variable degree of axonal degeneration [5,7], which is a common pathological characteristic of neurodegenerative diseases [9,6].

Neuropathy target esterase (NTE) is a neuronal membrane protein in vertebrates, and was originally identified as the primary action site of those organophosphorus compounds (OPs) that induce a delayed neuropathy characterized by demyelination and degeneration of long nerve axons [8,2]. Recently, it was demonstrated that NTE may be a novel lysophospholipase (NTE-LysoPLA) that uses LPC as its physiological substrate [13]. At the same time, a significant elevation of LPC level in spinal cord but not brain was detected following OPs treatment [14]. Owing to LPC having adverse effects of demyelination and axonal lesion, which is somewhat similar to the pathological changes of organophosphate-induced delayed neuropathy (OPIDN), it was supposed that a localized increase in LPC levels as a result of NTE-LysoPLA inhibition may induce a series of cell signaling cascades, which ultimately may lead to demyelination and neurodegradation of nerve axons.

An increase in cytosolic Ca^{2+} level ($[Ca^{2+}]_i$) has been implicated in the chain of events leading to delayed neuropathies resulting from OPs [1,3]. Increased $[Ca^{2+}]_i$ may cause the break-

* Corresponding author. Tel.: +86 10 64807251; fax: +86 10 64807099.
E-mail address: wuyj@ioz.ac.cn (Y.-J. Wu).

0304-3940/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2007.07.020

down of cytoskeletal elements in various neurodegenerative states [16,17]. Moreover, the calcium ionophore ionomycin or calcium itself has been observed to induce central demyelination and axonal degeneration *in vivo* and the appearance and progression of lesions was strikingly similar to those seen after the intraneural injection of LPC [21,20]. Furthermore, as a bioactive lipid, LPC has been shown to enhance intracellular Ca^{2+} level in many cell types [11,26]. As such, it was postulated that LPC-induced demyelination and axonal degeneration, which might be involved in the pathogenesis of OP-induced delayed neuropathy, might be attributed to its effect of Ca^{2+} overload.

The mouse is considered to be insensitive and the hen sensitive to clinical expression of OPIDN, which is associated with inhibition of NTE. Also, mice respond differently than hens and humans to OP delayed neurotoxicants, with more rapid action (in 3–5 versus 10–14 days), higher incidence of fatality and less pronounced neuropathy (only some pathological changes), i.e., delayed toxicity rather than OPIDN [1,25].

In the present study, we examined and compared the effect of exogenous LPC on intracellular Ca^{2+} overload in mouse N2a neuroblastoma cells and human SH-SY5Y neuroblastoma cells. Using these two cell lines as models of sensitive and insensitive sources, we may postulate the possible significance of LPC-induced the Ca^{2+} changes in the process of OPIDN, as well as the cause of diverse clinical symptoms to OP delayed neurotoxicants in humans and mice.

SH-SY5Y and N2a cells were obtained from the Cell Center of Chinese Academy of Medical Sciences (Beijing, China). Dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco (Grand Island, NY, USA). Heat-inactivated fetal bovine serum (FBS) was purchased from Chinese Academy of Medical Sciences (Beijing, China) and trypsin was the product of Amresco (Solon, Ohio, USA). Fura-2/AM, L-a-lysophosphatidylcholine (primary palmitate and stearate esters) and verapamil were purchased from Sigma (St. Louis, MO, USA). All other chemicals were reagent grade and were purchased from Beijing Chemical Co. (Beijing, China).

SH-SY5Y and N2a neuroblastoma cells were grown in DMEM supplemented with 10% FBS, 100 units/ml of penicillin and 100 μg/ml of streptomycin, at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were passaged once a week and used in confluent culture in all the subsequent experiments.

$[Ca^{2+}]_i$ was measured using Ca^{2+}-sensitive fluorescent indicator Fura-2/AM. Cells were washed, suspended in Hepes-buffered medium (140 mM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mg/ml glucose and 20 mM Hepes, pH 7.4) at 1×10^7 cells/ml, and then were incubated with 2.5 μM Fura-2/AM for 30 min at 37 °C. After being washed twice, the cells were resuspended in Hepes buffer at 1×10^6 cells/ml. Fluorescence intensity was monitored at 510 nm (5 nm slit) with alternated excitation at 340 nm and 380 nm (5 nm slit) using a dual wavelength fluorescence spectrophotometer (F4500; Hitachi, Tokyo, Japan).

$[Ca^{2+}]_i$ was calculated by the FL Solutions 2.0 Intracellular Cation Scan software based on the formula described by Grynkiewicz et al. [4], assuming a dissociation constant k_d of 224 nM. Maximal and minimal fluorescence values were obtained at the end of experiment by the sequential addition of 0.1% Triton X-100 and 5 mM EGTA. In some experiments, Fura-2 loaded cells were washed and suspended in normally Ca^{2+}-free medium that contained similar components as Hepes-buffered medium except that $CaCl_2$ was omitted. In Ca^{2+} restoration experiments, Ca^{2+} was initially absent from the medium, and was restored to 1 mM after 250 s of LPC stimulation. In other experiments, Fura-2 loaded cells were permeabilized with 5 μM digitonin in Ca^{2+}-free medium for 5 min at 25 °C, and then cells were washed twice and resuspended in Ca^{2+}-free medium without digitonin.

Fura-2 loaded SH-SY5Y and N2a cell were adjusted to the same density as 1×10^6/ml, and then challenged with same concentrations of LPC.

Cell viability was determined using the dye exclusion assay. After the cells were challenged with different concentrations of LPC for 1 min, 0.02% trypan blue (final concentration) was added to the cell suspensions, and the percentage of viable cells was recorded within 3 min with an inverted microscope.

Comparison between different groups was performed by Student's two-tailed unpaired *t*-test or one-way analysis of variance (Dunnett's test). $P < 0.05$ were considered to be significant difference. All values are given as means ± S.D.

We first examined the effect of LPC on Ca^{2+} mobilization in N2a mouse and SH-SY5Y human neuroblastoma cells. As shown in Fig. 1, LPC dose-dependently caused an increase in

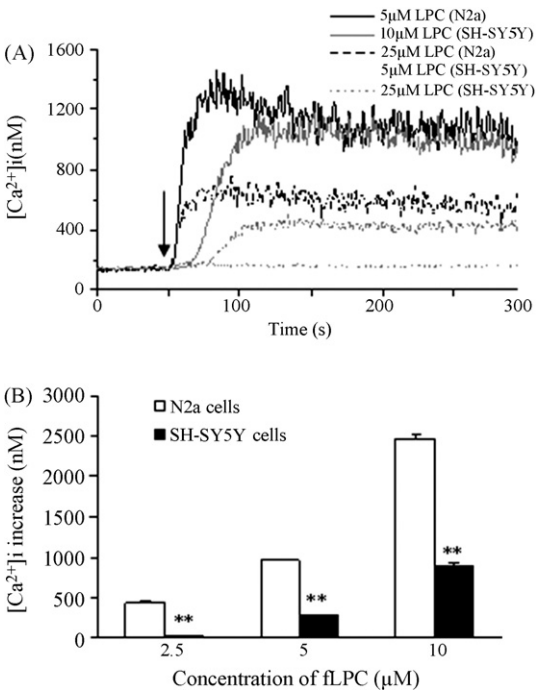

Fig. 1. Ca^{2+} influx is dose-dependent on LPC concentration in N2a and SH-SY5Y cells. Fura-2 loaded N2a and SH-SY5Y cells were stimulated by the addition of LPC in Ca^{2+}-containing medium. Arrow indicates the time of LPC addition. Traces are representative of at least 4 experiments. (A) Comparison of Ca^{2+} responses to LPC in N2a cells (black) and SH-SY5Y cells (grey). (B) Average $[Ca^{2+}]_i$ increase above basal level to different concentrations of LPC as indicated in N2a (□) and SH-SY5Y (■) cells. Data are means ± S.D. from at least four separate experiments. $^{**}P < 0.01$, compared with values of $[Ca^{2+}]_i$ increase in N2a cells.

$[Ca^{2+}]_i$, which remained elevated in Ca^{2+}-containing medium in both N2a and SH-SY5Y cells. The $[Ca^{2+}]_i$ increase in N2a cells was more rapid than seen in SH-SY5Y cells, since the increase appeared almost as soon as LPC was added to the suspension. In contrast, in SH-SY5Y cells the levels of Ca^{2+} rose slowly and plateaued (Fig. 1A). At the same time, the response in N2a cells was stronger than that in SH-SY5Y cells (Fig. 1A and B). Although a 2.5 μM LPC did not cause a $[Ca^{2+}]_i$ increase in SH-SY5Y cells, this dose of LPC produced a 466 ± 13 nM of $[Ca^{2+}]_i$ increase over the baseline in N2a cells. Similarly, while a 5 μM dose of LPC caused only 274 ± 12 nM of $[Ca^{2+}]_i$ increase in SH-SY5Y cells, it yielded a $[Ca^{2+}]_i$ increase to 892 ± 69 nM over the baseline in N2a cells. In SH-SY5Y cells, 10 μM of LPC was needed to achieve this level of response (Fig. 1B).

To assess whether LPC induced lesions within cell membranes, we assessed cell viability using the trypan blue assay, which is indicated as cell viability percentage. As shown in Fig. 2, at 1 μM LPC, few N2a cells were stained with trypan blue, although at 5 μM LPC there was an increase to 70%, and at 10 μM, all N2a cells appeared dead. In contrast, with concentrations up to 10 μM LPC showed no detergent effect on cell membrane under the same experimental conditions in SH-SY5Y cells. As such, a 10 μM dose of LPC could induce a large Ca^{2+} response, which is potent enough to stimulate downstream Ca^{2+}-sensitive processes in SH-SY5Y cells Therefore, since this concentration showed no detergent effect on cell membrane in SH-SY5Y cells, a 10 μM dose of LPC was used in SH-SY5Y cells. In N2a cells, because of the strong detergent action of LPC on cell membranes, we used an LPC concentration of 2.5 μM

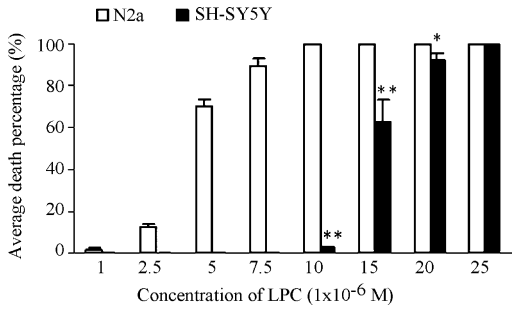

Fig. 2. Adverse effect of LPC on cell membrane integrity in N2a and SH-SY5Y neuroblastoma cells. N2a (grey) or SH-SY5Y (black) cells were challenged with different concentrations of LPC as indicated for 1 min, then stained with trypan blue dye (0.02% final concentration), and the percentage of viable cells was recorded in 3 min. Data are means ± S.D. from at least four separate experiments. Student's *t*-test was performed: $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with the data of N2a cells challenged by LPC at the identical concentration of LPC.

to produce a comparatively low detergent effect but yield a high Ca^{2+} increase response.

To assess whether the $[Ca^{2+}]_i$ increase induced by LPC was dependent on Ca^{2+} influx from either the external medium or from Ca^{2+} that is released from internal stores, we measured the effect of 10 μM LPC on Ca^{2+} response in the presence or absence of extracellular Ca^{2+} (Ca_e^{2+}). Our results demonstrate that in N2a cells, LPC did not induce any $[Ca^{2+}]_i$ increase but yielded a significant decrease in Ca^{2+}-free medium (Fig. 3A and C). These results are similar to the effect of 0.02% Triton X-100, a well-known detergent (Fig. 3D). However in SH-SY5Y cells, the amplitude was decreased by 36% in the absence of Ca_e^{2+},

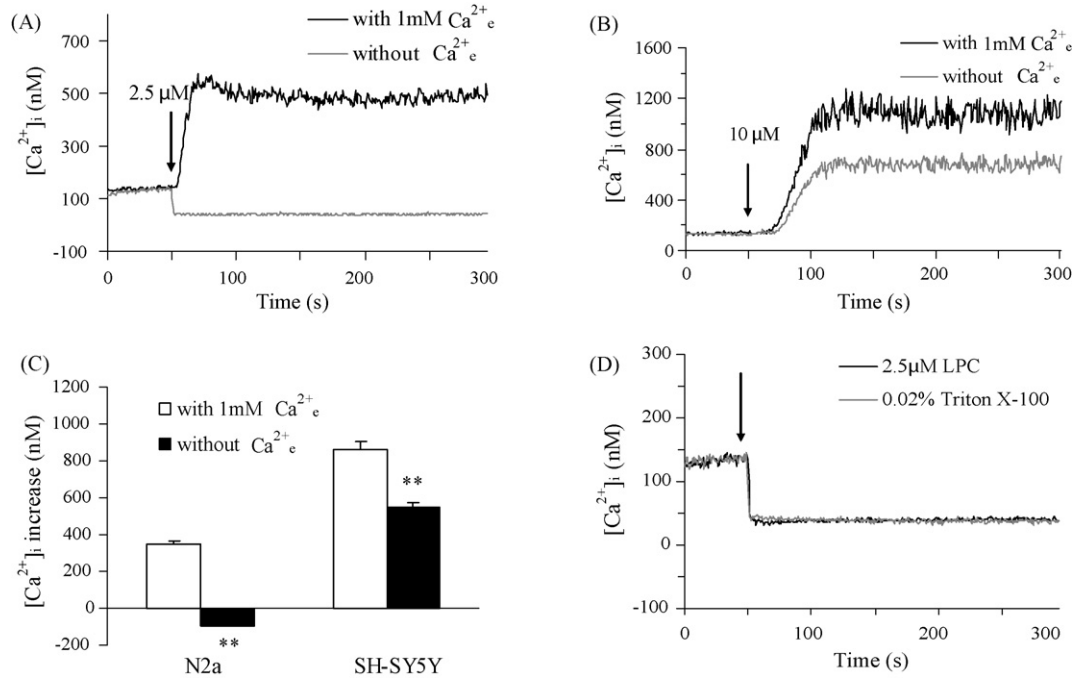

Fig. 3. Effect of external Ca^{2+} on LPC-induced Ca^{2+} response in SH-SY5Y cells and N2a cells. (A and B) Typical $[Ca^{2+}]_i$ responses of N2a (A) and SH-SY5Y (B) cells to 2.5 μM LPC and 10 μM LPC, respectively in the presence (black) or absence (grey) of 1 mM Ca_e^{2+} containing-medium. Traces are representative of at least 3–4 experiments. (C) Average $[Ca^{2+}]_i$ increase above basal level in the presence (□) or absence (■) of Ca_e^{2+}. Data are means ± S.D. from at least four separate experiments. $^{**}P < 0.01$, compared respectively with the values of corresponding group in the presence of 1 mM Ca_e^{2+} containing-medium. (D) Comparison of Ca^{2+} responses to 2.5 μM LPC (black) or 0.02% Triton X-100 (grey) in N2a cells. Arrow indicates the time of LPC or Triton X-100 addition. Traces are representative of at least 3 experiments.

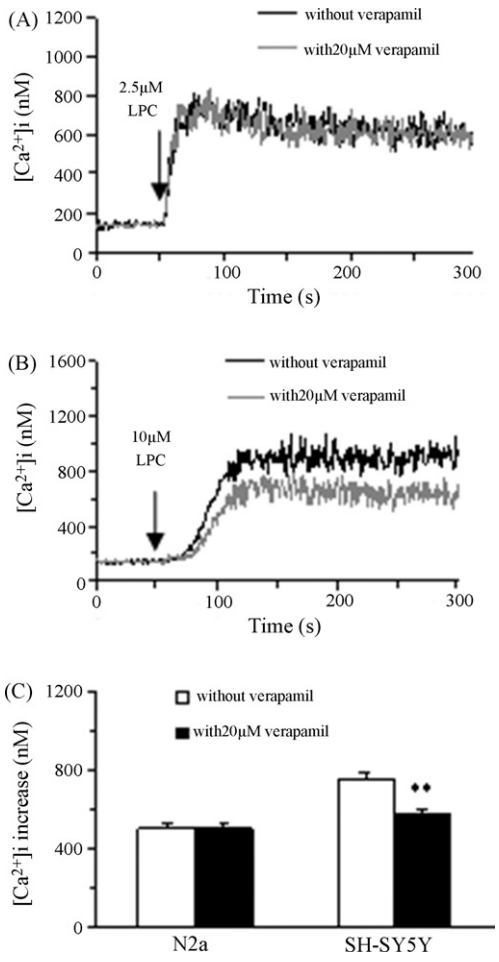

Fig. 4. Effect of verapamil on LPC-induced Ca^{2+} response. (A and B) Fura-2 loaded N2a cells (A) or SH-SY5Y cells (B) were pretreated with vehicle (black) or with 20 μM verapamil (grey) for 15 min, then stimulated with 2.5 μM or 10 μM LPC in Ca^{2+}-containing medium. Traces are representative of at least four experiments. (C) Average $[Ca^{2+}]_i$ increase above basal level in the presence (□) or absence (■) of Ca_e^{2+}. Data are means ± S.D. from at least four separate experiments. $^{**}P < 0.01$, compared respectively with the values of corresponding group treated with 0 μM verapamil in the medium.

compared with the response in the presence of Ca_e^{2+} (Fig. 3B and C).

Pretreatment of the cells with 20 μM verapamil (15 min), an L-type Ca^{2+} channel blocker, did not affect LPC-induced Ca^{2+} increase in N2a cells (Fig. 4A and C), but inhibited the response by 23% in SH-SY5Y cells compared with the response in the absence of verapamil in Ca^{2+}-containing medium (Fig. 4B and C). No further inhibitory effect was observed with higher concentrations of verapamil on N2a or SH-SYS5Y cells.

The present study examined and compared the effect of LPC on intracellular Ca^{2+} overload in mouse N2a neuroblastoma cells and human SH-SY5Y neuroblastoma cells. LPC caused a $[Ca^{2+}]_i$ increase in both N2a and SH-SY5Y cells in a dose-dependent manner. The $[Ca^{2+}]_i$ increase in N2a cells was more rapid than that in SH-SY5Y cells indicating that LPC produced the Ca^{2+} increase using different mechanisms in these two kinds of cells. Moreover, the amplitude in N2a cells was more potent than that in SH-SY5Y cells, which suggested that N2a cells were more sensitive to LPC than SH-SY5Y cells. Conversely, cell viability tested by trypan blue exclusion showed that even 1 μM LPC caused some of cells to stain blue under the experimental conditions, indicating that under this concentration, LPC may act as a detergent on the membranes of N2a cells. However, SH-SY5Y incubated with a dose of LPC up to 10 μM showed no detergent effect, although almost all of the N2a cells stained blue under these same conditions.

Preincubation of the cells with verapamil, an L-type Ca^{2+} channel blocker, did not affect the LPC-induced Ca^{2+} increase in N2a cells but inhibited the response by 23% in SH-SY5Y cells in Ca^{2+}-containing medium. In Ca^{2+}-free medium, LPC could not cause any $[Ca^{2+}]_i$ increase but produced a significant decrease in N2a cells, which was similar to the results seen with 0.02% of Triton X-100. However, in SH-SY5Y cells, there was still a 64% of total $[Ca^{2+}]_i$ increase. Considering that 10 μM of LPC in SH-SY5Y cells showed no detergent effect while 2.5 μM of LPC caused some N2a cells to stain positive with trypan blue, the increase in $[Ca^{2+}]_i$ in SH-SY5Y cells may be through a signaling transduction process involving a Ca^{2+} signaling protein, but N2a cells may utilize simple diffusion through a damaged membrane. Taken together, LPC-induced $[Ca^{2+}]_i$ mobilization was induced by different mechanisms in these two kinds of cells: in SH-SY5Y cells, it was probably by an external Ca^{2+} influx mainly through L-type Ca^{2+} channels, and Ca^{2+} release from intracellular Ca^{2+} stores; in N2a cells by Ca^{2+} entry from an external space through membranes permeabilized by the detergent effect of LPC. We hypothesize that LPC was unable to activated L-type Ca^{2+} channels in N2a cells since the LPC may have caused lysis of the cell membrane at that concentration, which was not sufficient to active the channels. More importantly, the amplitude of $[Ca^{2+}]_i$ increase in N2a cells was more potent than that in SH-SY5Y cells, and the membrane of N2a cells was more sensitive to LPC than that of SH-SY5Y cells.

The LPC molecule is wedge shaped consisting of one long hydrophobic fatty acyl chain and one large hydrophilic polar choline headgroup, attached to a glycerol backbone. The amphipathic nature of LPC gives it surfactant and detergent-like properties. At low concentrations, LPC exists as single molecules in solution. However, when the concentration of LPC exceeds the critical micelle concentration, LPC can form small micelles composed of approximately 180 molecules [15]. Although single LPC molecules can insert readily into the outer layer of the cell membrane, they do not seem to flip quickly into the inner layer of the cell membrane [10]. However, micelles formed from LPC may fuse with cell membranes to disturb membrane conformation, and perhaps lyse cells [24,19]. As such, the detergent effect of LPC mostly results LPC produced micelles. Regarding the different sensitivity of these two cell lines to LPC, we can speculate that this may be due to differences in lipid composition of their cell membranes, which ultimately influences interactions between LPC and the cell membrane.

The mouse is considered to be insensitive to clinical expression of OPIDN, with more rapid action, higher incidence of fatality and less pronounced neuropathy [1,25]. In the present study, we examined and compared the effect of LPC on

intracellular Ca^{2+} overload in mouse N2a cells and human SH-SY5Y cells, which represent sensitive and insensitive sources, respectively. Our results highlight a possible significance of LPC-induced Ca^{2+} overload in the process of OPIDN. First, the amplitude of $[Ca^{2+}]_i$ overload in mouse N2a cells was much stronger than that in human SH-SY5Y cells and indicates that the differences of OPIDN syndrome between mouse and human probably are not related to the diverse amplitudes of intracellular Ca^{2+} overload produced by LPC. Second, the demyelination effect of LPC to mouse may be related to its detergent effect on membranes, and that the Ca^{2+} overload that follows may accelerate the process of demyelination. Finally, whether the more rapid action and higher incidence of fatality in mice due to OP delayed neurotoxicants are attributable to increased sensitivity of murine membranes to LPC needs to be further investigated.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (30470228) and 863 Program (no. 2006AA06Z423) and the National Key Technologies R&D Program (2006BAK02A02). The authors would like to thank Dr. Susan Nozell for polishing the English.

References

[1] M.B. Abou-Donia, D.M. Lapadula, Mechanisms of organophosphorus ester-induced delayed neurotoxicity: type I and type II, Annu. Rev. Pharmacol. Toxicol. 30 (1990) 405–440.
[2] T.W. Bouldin, J.B. Cavanagh, Organophosphorous neuropathy. I. A teased-fiber study of the spatio-temporal spread of axonal degeneration, Am. J. Pathol. 94 (1979) 241–252.
[3] S. Choudhary, K.D. Gill, Protective effect of nimodipine on dichlorvos-induced delayed neurotoxicity in rat brain, Biochem. Pharmacol. 62 (2001) 1265–1272.
[4] G. Grynkiewicz, M. Peonie, R.Y. Tsien, A new generation of Ca^{2+} indicators with greatly improved fluorescence properties, J. Biol. Chem. 260 (1985) 3440–3450.
[5] S.M. Hall, The effect of injections of lysophosphatidyl choline into white matter of the adult mouse spinal cord, J. Cell Sci. 10 (1972) 535–546.
[6] K. Iqbal, C. Alonso Adel, E. El-Akkad, C.X. Gong, N. Haque, S. Khatoon, J.J. Pei, H. Tanimukai, I. Tsujio, J.Z. Wang, I. Grundke-Iqba, Alzheimer neurofibrillary degeneration: therapeutic targets and high-throughput assays, J. Mol. Neurosci. 20 (2003) 425–429.
[7] I. Jean, C. Allamargot, A. Barthelaix-Pouplard, C. Fressinaud, Axonal lesions and PDGF-enhanced remyelination in the rat corpus callosum after lysolecithin demyelination, Neuroreport 13 (2002) 627–631.
[8] M.K. Johnson, The delayed neurotoxic effect of some organophosphorus compounds. Identification of the phosphorylation site as an esterase, Biochem. J. 114 (1969) 711–714.
[9] R.M. Kostrzewa, J. Segura-Aguilar, Novel mechanisms and approaches in the study of neurodegeneration and neuroprotection, a review, Neurotox. Res. 5 (2003) 375–383.
[10] N. Mohandas, J. Wyatt, S.F. Mel, M.E. Rossi, S.B. Shohet, Lipid translocation across the human erythrocyte membrane, J. Biol. Chem. 257 (1982) 6537–6543.
[11] F. Okajima, K. Sato, H. Tomura, A. Kuwabara, H. Nochi, K. Tamoto, Y. Kondo, Y. Tokumitsu, M. Ui, Stimulatory and inhibitory actions of lysophosphatidylcholine, depending on its fatty acid residue, on the phospholipase C/Ca^{2+} system in HL-60 leukaemia cells, Biochem. J. 336 (1998) 491–500.
[12] M.T. Quinn, S. Parthasarathy, D. Steinberg, Lysophosphatidylcholine: a chemotactic factor for human monocytes and its potential role in atherogenesis, Proc. Natl. Acad. Sci. USA 85 (1988) 2805–2809.
[13] G.B. Quistad, C. Barlow, C.J. Winrow, S.E. Sparks, J.E. Casida, Evidence that mouse brain neuropathy target esterase is a lysophospholipase, Proc. Natl. Acad. Sci. USA 100 (2003) 7983–7987.
[14] G.B. Quistad, J.E. Casida, Lysophospholipase inhibition by organophosphorus toxicants, Toxicol. Appl. Pharmacol. 196 (2004) 319–326.
[15] L. Saunders, Molecular aggregation in aqueous dispersion of phosphatidyl and lysophosphatidyl cholines, Biochem. Biophys. Acta 125 (1996) 70–74.
[16] W.W. Schlaepfer, Neurofilaments: structure, metabolism and implication in disease, J. Neuropathol. Exp. Neurol. 46 (1987) 117–129.
[17] W.W. Schlaepfer, M.B. Hasler, Characterization of the calcium-induced disruption of neurofilaments in rat peripheral nerve, Brain Res. 168 (1979) 299–309.
[18] S.P. Sedlis, M. Hom, J.M. Sequeira, R. Esposito, Lysophosphatidylcholine accumulation in ischemic human myocardium, J. Lab. Clin. Med. 121 (1993) 111–117.
[19] B.A. Silverman, P.F. Weller, M.L. Shin, Effect of erythrocyte membrane modulation by lysolecithin on complement-mediated lysis, J. Immunol. 132 (1984) 381–391.
[20] K.J. Smith, S.M. Hall, Central demyelination induced in vivo by the calcium ionophore ionomycin, Brain 117 (1994) 1351–1356.
[21] K.J. Smith, S.M. Hall, Peripheral demyelination and remyelination initiated by the calcium-selective ionophore ionomycin: in vivo observations, J. Neurol. Sci. 83 (1988) 37–53.
[22] G.A. Vidaver, A. Ting, J.W. Lee, Evidence that lysolecithin is an important causal agent of atherosclerosis, J. Theor. Biol. 115 (1985) 27–41.
[23] A. Wang, R.A. Deems, E.A. Dennis, Cloning, expression, and catalytic mechanism of murine lysophospholipase I, J. Biol. Chem. 272 (1997) 12723–12729.
[24] H.U. Weltzien, Cytolytic and membrane-perturbing properties of lysophosphatidylcholine, Biochim. Biophys. Acta 559 (1979) 259–287.
[25] S.Y. Wu, J.E. Casida, Subacute neurotoxicity induced in mice by potent organophosphorus neuropathy target esterase inhibitors, Toxicol. Appl. Pharmacol. 139 (1996) 195–202.
[26] L. Yu, T. Netticadan, Y.J. Xu, V. Panagia, N.S. Dhalla, Mechanisms of lysophosphatidylcholine-induced increase in intracellular calcium in rat cardiomyocytes, J. Pharmacol. Exp. Ther. 286 (1998) 1–8.