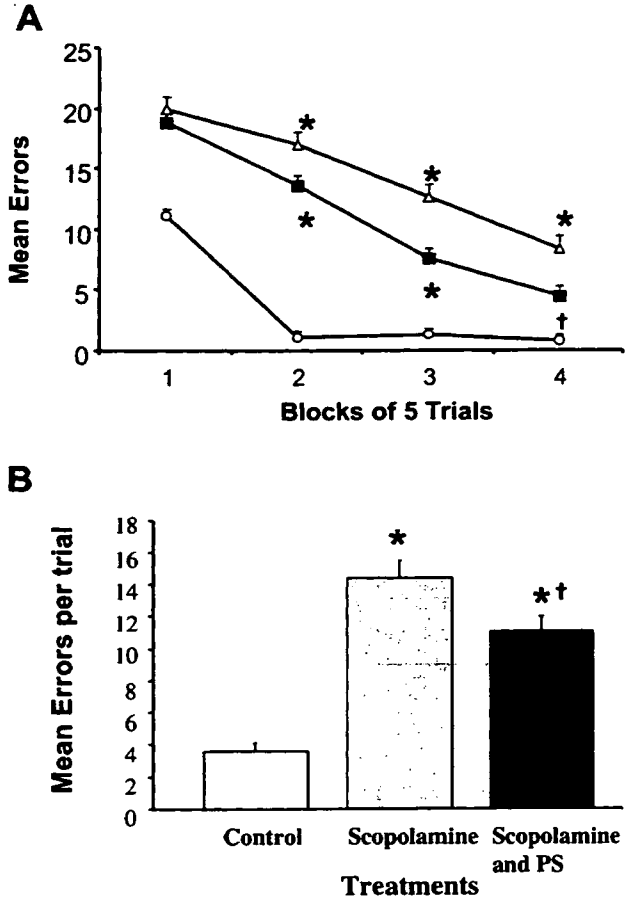

Fig. 8. Effects of transdermal administration of FAPG ointments containing PS [20% (w/w)], supplemented with HPE-101 [5% (w/w)], on scopolamine-induced cognitive impairment in rats. Errors assessed in 14-unit T-maze trials. ○, control; △, scopolamine; and ■, scopolamine + PS. *, $p < 0.01$ versus control; †, $P < 0.01$ versus scopolamine group. Each point represents mean ± S.E.M. (n = 7–8).

amine. Data indicate mean errors per block, across four blocks of five trials performed sequentially (Fig. 8A), and mean errors per block, across the total 20 trials for each group (Fig. 8B). One-way analysis of variance with repeated measures (four blocks of five trials) yielded a main effect of drug treatment [$F(2,19) = 51.77$; $p < 0.001$] and blocks of trials [$F(3,57) = 81.40$; $p < 0.001$]. An interaction of drug treatment and blocks of trials was also observed [$F(6, 57) = 4.38$; $p < 0.001$]. Post hoc comparisons (LSD test; $p < 0.05$) of the mean errors per trial for each group were performed to determine the locus of the main effect. These analyses indicated that in scopolamine-treated animals significantly more errors were observed for all blocks compared with the control group ($p < 0.05$). PS treatment significantly decreased the number of errors induced by scopolamine, at blocks 3 and 4 ($p < 0.05$). Furthermore, at block 4, the error score in the PS group was not statistically different from that of the control group. Across all 20 trials, PS treatment significantly reduced cognitive errors induced by scopolamine, thereby improving overall maze performance (Fig. 8B; Fisher, LSD test; $p < 0.01$).

Plasma and brain (cortex) AChE and BChE activities from the maze-tested animals are shown in Table 3. Scopolamine treatment did not affect cholinesterase activities in plasma or cortex, compared with the control group; thus, these groups were combined. In contrast, cotreatment with scopolamine and transdermal PS significantly ($p < 0.05$) reduced AChE activities in plasma and brain by 68 and 66%, respectively. PS treatment lowered BChE activity in plasma by 18% ($p < 0.05$), and it had no effect on that in brain ($p > 0.05$).

Discussion

Data from these investigations are supportive of the potential future use of PS as a transdermally applied therapeutic for AD and other conditions that feature a central cholinergic deficit. Initial in vitro studies indicated the suitability of PS for transdermal application, as would be predicted from its physicochemical characteristics (Ozawa et al., 1988; Yu et al., 2001).

We examined PS and PST permeabilities through both intact skin and tape-stripped skin isolated from hairless mice, and we demonstrated the suitability of LP and FAPG as vehicles in which the agent was formulated. For intact skin, in vitro total fluxes were greater for PS than PST, with release from FAPG being superior to that from LP. Furthermore, the time course profile of flux rates through intact skin (Fig. 2B) clearly shows a superior, more predictable release of PS from vehicle compared with PST. It would be expected that the free base (PS) would be readily released from vehicle compared with the more water-soluble tartrate salt (PST). In the stripped skin studies, the formulation vehicle seemed to have little impact. It has been widely reported that the stratum corneum represents a major barrier to transdermal permeability (Scheuplein and Blank, 1973; Smith et al., 1982; Madison et al., 1987), and as expected, transdermal fluxes of PS were greater through stripped skin than intact skin. Because the barrier integrity of the stratum corneum may be compromised with age and because AD is an age-associated disease, the former was assessed to provide an estimate of maximal permeability. However, the goal of developing a clinical application system, precluded the further investigation of a delivery system reliant on the absence of the stratum corneum. It has also been reported that the use of an albumin solution in the receptor phase of the diffusion cell, to

TABLE 3
AChE and BChE activities in plasma and cerebral cortex of rats treated with and without scopolamine (i.p.) and cotreated with PS ointment
Number of animals is shown in parentheses.

Treatment	Plasma		Cortex	
	AChE	BChE	AChE	BChE
	U/l ± S.E.M.		*U/g ± S.E.M.*	
Control (with and without scopolamine)	1754 ± 62 (14)	1006 ± 43 (14)	9687 ± 955 (14)	777 ± 60 (14)
PS	551 ± 65** (8)	822 ± 32** (8)	3242 ± 385** (8)	783 ± 77 (8)
Inhibition	68%	18%	66%	0%

** Dunnett's t test; $p < 0.01$.

better mimic subdermal physiological conditions, may increase drug permeabilities by greater than 5-fold, compared with flux into pH-adjusted saline solutions as in our studies (Surber et al., 1991). Therefore, it may have been possible to achieve enhanced permeabilities through the skin of the hairless mouse in vitro by modification of the experimental conditions.

Several studies have shown that penetration enhancers are effective to improve fluxes and subsequent bioavailability related to poor kinetic profiles of the transdermal administration route (Aungst et al., 1986; Tsuzuki et al., 1988). In these studies, we examined the effectiveness of HPE-101, a well studied penetration enhancer (Nakashima et al., 1996; Yasuno et al., 2001), to optimize the permeability of PS in vitro. HPE-101 seems to improve permeability through hydrophilic routes of the stratum corneum without significant toxicity (Yano et al., 1992, 1993). Data indicated that the optimal concentration of HPE-101 to increase PS flux, was in the range 3 to 5% (w/w).

Using the in vitro permeability findings as a basis for the design of in vivo efficacy studies, we modulated the area available for transdermal flux so that the in vitro model predicted the achievement of systemic, in vivo concentrations of PS within the calculated therapeutic window for efficacy in AChE inhibition. This optimization was also necessitated by reports of significant reductions of in vivo absorption rate (flux) in rats and humans compared with in vitro permeability data from hairless mice (Tregear, 1966; Iwasaki et al., 1999). Hence, a diffusion area of 5 cm^2 was used during the in vivo permeability and efficacy studies performed in adult Fischer-344 rats.

The in vivo studies used PS ointments with and without HPE-101, formulated in FAPG. Statistically significant reductions (30% decrease at 8 h) in plasma AChE activity were observed with PS [10% (w/w)] in the presence of HPE-101 [5% (w/w)]. In the absence of HPE-101, plasma AChE activities were also reduced, but less effectively than with the penetration enhancer. It has been reported that intravenous administration of 1.0 mg/kg PS inhibits AChE activity in plasma and cerebrospinal fluid of rats by 60 and 90%, respectively. This greater level of cerebrospinal fluid inhibition is almost certainly related to the observed brain/plasma partitioning of PS (10:1) (Greig et al., 2000). Indeed, the AChE activity was dramatically reduced in brain samples (60% inhibition) compared with inhibition in plasma (30% inhibition) in our in vivo studies when HPE-101 was included in the formulation. These results are suggestive of the requirement for incorporation of a permeability enhancer in the ointment formulation to facilitate PS movement through intact skin. Furthermore, the levels of biochemical markers of hepatic, renal, and muscle function remained within their biological ranges, indicating that administration of PS ointments, with and without HPE-101, were well tolerated. These results suggest that selective reductions in plasma and brain AChE activity may be achieved by the transdermal delivery of an agent such as PS, whereas avoiding some of the pitfalls associated with oral administration, including hepatic toxicity and clearance as a consequence of first-pass metabolism.

To assess the efficacy of transdermal PS treatment on cognition (or combined higher brain functions/learning), we used an accepted animal model of pharmacologically induced cognitive impairment (Ingram, 1988). In this model, animals trained to negotiate a 14-unit T-maze commit negotiation errors under the influence of the muscarinic antagonist, scopolamine. Scopolamine has been widely used to assist in the animal modeling of dementias involving a central cholinergic deficit (Ingram et al., 1994). Treatment with scopolamine induces rapid and significant reductions in rat brain cholinergic activity and causes learning impairment as assessed in the 14-unit T-maze (Spangler et al., 1986; Iijima et al., 1993).

In our studies, the transdermal administration of PS significantly reduced the number of cognitive errors induced by scopolamine. In a sequence of 20 trials (four blocks × five trials), significant improvements in cognitive performance of the PS treated animals were observed beginning at trial 13. This compares favorably with our previous systemic treatment with PS (1.5–10 mg/kg i.p.) using the same experimental paradigm, which resulted in significant effects occurring slightly earlier in the trials sequence; beginning at trial 8 (Iijima et al., 1993; Patel et al., 1998). The delay in onset of the PS effects in the transdermal model might be caused by the lag-time of PS release from the ointment and/or slow kinetics associated with transdermal absorption and systemic assimilation.

The improvements in maze performance were accompanied by selective inhibition of AChE activities in plasma and brain as measured in tissues collected at the end of the series of maze trials. BChE activities in plasma and brain were less affected by administration of transdermal PS. PS has previously been characterized as having a 70:1 preference for inhibition of AChE compared with BChE (Greig et al., 2005b). It has been reported in AD patients that improvements in cognition, and particularly short-term memory, correlate with the extent of plasma AChE inhibition, within the range of 0 to 50% inhibition (Becker et al., 1991; Greig et al., 1995). The data from this series of investigations clearly suggest that transdermal treatment with an appropriately formulated ointment of PS could have the potential to achieve levels of AChE inhibition that would provide similar clinical benefits.

In addition to a central cholinergic deficit that may be partly ameliorated by AChE inhibition with agents such as PS, AD is further characterized by the presence of central extracellular plaques, primarily composed of Aβ (Sambamurti et al., 2002). These deposits are considered to be intimately involved in the pathology of AD; although exact mechanisms remain to be elucidated (Selkoe, 2005). PS possesses additional noncholinergic actions to reduce Aβ levels through lowering the rate of APP synthesis (Shaw et al., 2001; Greig et al., 2005b). Therefore, it is likely that PS delivered via the transdermal route, as in this series of studies, would exert beneficial effects on APP processing in addition to anticholinesterase activity. Further studies will be undertaken to elucidate these probable effects of transdermal PS on Aβ kinetics in this animal model. The transdermal application of agents such as PS may also provide real benefits with respect to treatment compliance. In a disorder such as AD where the ability to accept oral medications often progressively declines, the option of a therapeutic patch for drug delivery is especially attractive.

We conclude that these investigations demonstrate that PS may be a suitable candidate for transdermal administration; achieving transdermal flux of sufficient magnitude to sup-

port cognitive efficacy in an accepted animal model of cognitive impairment. Furthermore, the transdermal route avoids concerns of immediate hepatic toxicity and other deleterious consequences of first-pass metabolism. The slow release of active agent from ointment vehicle in a transdermal (patch) setting may also prove beneficial in sustaining the duration of action of PS, which has been suggested to be a limiting factor for clinical efficacy. These findings allow us to speculate that PS thus may be formulated to provide clinical benefits when delivered transdermally that may help to reduce the cholinergic deficit of AD and possibly influence APP processing (Lahiri et al., 2007).

References

Addicks WJ, Flynn GL, and Weiner N (1987) Validation of a flow-through diffusion cell for use in transdermal research. *Pharm Res (NY)* **4**:337–341.

Aungst BJ, Rogers NJ, and Shefter E (1986) Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxide and amides. *Int J Pharm* **33**:225–234.

Becker RE, Moriearty P, and Unni L (1991) The second generation of cholinesterase inhibitors: clinical and pharmacological effects, in *Cholinergic Basis for Alzheimer's Therapy* (Becker RE and Giacobini E eds) pp 263–297, Birkhäuser, Boston, MA.

Doucett R, Fisman M, Hachinski VC, and Mersky H (1986) Cell loss from the nucleus basalis of Meynert in Alzheimer's disease. *Can J Neurol Sci* **13**:435–440.

Ellman GL, Courtney KD, Andres V Jnr and Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* **7**:88–95.

Ghadially R, Brown BE, Sequeira-Martin SM, Feingold KR, and Elias PM (1995) The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. *J Clin Investig* **95**:2281–2290.

Greig NH, DeMichel E, Holloway HW, Yu QS, Utsuki T, Perry T, Brossi A, Ingram DK, Deutsch J, Lahiri DK, et al. (2000) The experimental Alzheimer drug phenserine: preclinical pharmacokinetics and pharmacodynamics. *Acta Neurol Scand* **176**:74–84.

Greig NH, Pei XF, Soncrant TT, Ingram DK, and Brossi A (1995) Phenserine and ring C hetero-analogues, drug candidates for the treatment of Alzheimer's disease. *Med Res Rev* **15**:3–31.

Greig NH, Ruckle J, Comer P, Brownell L, Holloway HW, Flanagan DR Jr, Canfield CJ, and Burford RG (2005a) Anticholinesterase and pharmacokinetic profile of phenserine in healthy elderly human subjects. *Curr Alzheimer Res* **2**:483–492.

Greig NH, Sambamurti K, Yu Q-S, Brossi A, Bruinsma GB, and Lahari DK (2005b) An overview of phenserine tartrate, a novel acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. *Curr Alzheimer Res* **2**:281–290.

Iijima S, Greig NH, Garofalo P, Spangler EL, Heller B, Brossi A, and Ingram DK (1993) Phenserine, a physostigmine derivative that is a long-acting inhibitor of cholinesterase and demonstrates a wide dose range for attenuating scopolamine-induced learning impairment of rats in a 14-unit T-maze. *Psychopharmacology (Berl)* **112**:415–420.

Ingram DK (1988) Complex maze learning in rodents as a model of age-related memory impairment. *Neurobiol Aging* **9**:475–485.

Ingram DK, Spangler EL, Iijima S, Kuo H, Bresnahan EL, Greig NH, and London ED (1994) New pharmacological strategies for cognitive enhancement using a rat model of age-related memory impairment. *Ann NY Acad Sci* **717**:16–32.

Iwasaki K, Kawamura A, Teramura Y, Hata T, Ninomiya S, and Esumi Y (1999) Absorption, distribution and excretion of ^{14}C-labeled Tacrolimus (FK506) ointment after a single dermal application to the rat. *Xenobiol Metab Dispos* **14**:1–10.

Lahiri DK, Chen D, Maloney B, Holloway H, Yu QS, Utsuki T, Giordano T, Sambamurti K, and Greig NH (2007) The experimental Alzheimer's disease drug posiphen lowers amyloid-β peptide levels in cell culture and mice. *J Pharmacol Exp Ther* **320**:386–396.

Lahiri DK, Rogers JT, Greig NH, and Sambamurti K (2004) Rationale for the development of cholinesterase inhibitors as anti-Alzheimer agents. *Curr Pharm Des* **10**:3111–3119.

Leo A, Greenberg SM, and Growdon JH (2006) Current pharmacotherapy for Alzheimer's disease. *Annu Rev Med* **57**:513–533.

Madison KC, Swartzendruber DC, Wertz PW, and Dowing DT (1987) Presence of intact intercellular lipid lamellae in the upper layers of the stratum corneum. *J Investig Dermatol* **88**:714–718.

Malcolm R and Thomas NT (1995) *Clinical Pharmacokinetics Concepts and Applications, 8: Movement through Membranes*, pp 109–117, Lippincott Williams & Wilkins, Baltimore, MD.

Nakashima M, Zhao MF, Ohya H, Sakurai M, Sasaki H, Matsuyama K, and Ichikawa M (1996) Evaluation of in vivo transdermal absorption of cyclosporin with absorption enhancer using intradermal microdialysis in rats. *J Pharm Pharmacol* **11**:1143–1146.

Okuyama H, Iseda Y, Kasai S, Imamori K, Takayama K, and Nagai T (1999) Influence of diisopropyl adipate on percutaneous absorption and subcutaneous tissue penetration of diclofenac from alcoholic gel ointment. *J Pharm Sci Technol* **59**:75–83.

Ozawa Y, Yamashita T, Sunara H, and Nadai T (1988) Influence of fatty acid-alcohol ester on percutaneous absorption of hydrocortisone butyrate propionate. *Chem Pharm Bull* **36**:2145–2151.

Patel N, Spangler EL, Greig NH, Yu Q-S, Ingram DK, and Meyer RC (1998) Phenserine, a novel acetylcholinesterase inhibitor, attenuates impaired learning of rats in a 14-unit T-maze induced by blockade of the N-methyl-D-aspartate receptor. *Neuroreport* **9**:171–176.

Sambamurti K, Greig NH, and Lahiri DK (2002) Advances in the cellular and molecular biology of the beta-amyloid protein in Alzheimer's disease. *Neuromolecular Med* **1**:1–31.

Scheuplein RJ and Blank IH (1973) Mechanism of percutaneous absorption. IV. Penetration of nonelectrolytes (alcohols) from aqueous solution and from pure liquids. *J Investig Dermatol* **60**:286–296.

Sclafani J, Nightingale J, Liu P, and Kurihara-Bergstrom T (1993) Flow-through system effects on in vitro analysis of transdermal systems. *Pharm Res (NY)* **10**:1521–1526.

Selkoe DJ (2005) Defining molecular targets to prevent Alzheimer disease. *Arch Neurol* **62**:192–195.

Shaw KT, Utsuki T, Rogers J, Yu Q-S, Sambamurti K, Brossi A, Ge YW, Lahiri DK, and Greig NH (2001) Phenserine regulates translation of β-amyloid precursor protein mRNA by a putative interleukin-1 responsive element, a target for drug development. *Proc Natl Acad Sci USA* **98**:7605–7610.

Smith WP, Christensen MS, Nacht S, and Gans EH (1982) Effect of lipids on the aggregation and permeability of human stratum corneum. *J Investig Dermatol* **78**:7–11.

Spangler EL, Rigby P, and Ingram DK (1986) Scopolamine impairs learning performance of rats in a 14-unit T-maze. *Pharmacol Biochem Behav* **25**:673–679.

Surber C, Wilhelm K, and Maibach HI (1991) In-vitro skin pharmacokinetics of acitretin. Percutaneous absorption studies in intact and modified skin from three different species using different receptor solutions. *J Pharm Pharmacol* **43**:836–840.

Tanzi RE (2005) Tangles and neurodegenerative disease–a surprising twist. *N Engl J Med* **353**:1853–1855.

Tregear RT (1966) The permeability of mammalian skin to ions. *J Investig Dermatol* **46**:16–23.

Tsuzuki N, Wong O, and Higuchi T (1988) Effect of primary alcohols on percutaneous absorption. *Int J Pharm* **46**:225–234.

Whitehouse D, Price DL, and Struble R (1982) Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science (Wash DC)* **215**:1237–1239.

Yano T, Higo N, Furukawa K, Tsuji M, Noda K, and Otagiri M (1992) Evaluation of a new penetration enhancer 1-[2-(decylthio)ethyl]azacyclopentan-2-one (HPE-101). *J Pharmacobiodyn* **9**:527–533.

Yano T, Higo N, Fukuda K, Tsuji M, Noda K, and Otagiri M (1993) Further evaluation of a new penetration enhancer, HPE-101. *J Pharm Pharmacol* **9**:775–778.

Yasuno N, Obata Y, Tsuchiya M, Imai K, Arakawa Y, and Takayama K (2001) The effect of N-methyl-2-pyrrolidone on the percutaneous absorption of ethenzamide. *J Pharm Sci Technol* **61**:154–162.

Yu Q-S, Holloway HW, Flippen-Anderson JL, Hoffman B, Brossi A, and Greig NH (2001) Methyl analogues of the experimental Alzheimer's drug phenserine: synthesis and structure/activity relationships for acetyl- and butyrylcholinesterase inhibitory action. *J Med Chem* **44**:4062–4071.

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Prolongation of Intrathecal and Sciatic Nerve Blocks Using a Complex of Levobupivacaine with Maltosyl-β-Cyclodextrin in Rats

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BACKGROUND: We used a cyclodextrin-based drug delivery system, consisting of levobupivacaine complexed with maltosyl-β-cyclodextrin (G2-β-CD), in spinal and sciatic nerve blocks in rats to investigate prolongation of the local anesthetic effect.
METHODS: Rats were assigned to four groups (n = 6 in each) and received intrathecally 30 μL of 1% levobupivacaine complexed with 0 (control), 50, 100, or 200 mM of G2-β-CD. Muscle tone and tail flick latency were studied after intrathecal administration. Four more groups (n = 6) of rats received a sciatic nerve block with 0.5% or 1% levobupivacaine complexed with either 0 or 100 mM of G2-β-CD. The time course of changes in proprioception, motor function, and nociception after circumferential subcutaneous administration to the sciatic nerve was examined.
RESULTS: With the intrathecal block, all rats stopped tail movement immediately after injection of the local anesthetic. The mean time to recovery of muscle tone with 1% levobupivacaine complexed with 100 mM (80.0 ± 8.9 min) and 200 mM (91.7 ± 7.0 min) of G2-β-CD was significantly longer than that of the control group (38.3 ± 3.1 min), but tail flick latency was not prolonged. With the sciatic nerve block, all rats were temporarily immobilized after levobupivacaine injection. The anesthetic effects of 0.5% levobupivacaine with 100 mM of G2-β-CD were twice as long as those for 0.5% levobupivacaine alone, and those of 1% levobupivacaine with 100 mM of G2-β-CD were 1.4 times longer than those for 1% levobupivacaine alone.
CONCLUSIONS: The complex of levobupivacaine with G2-β-CD prolonged the anesthetic effect of levobupivacaine in both intrathecal and sciatic nerve blocks in rats.
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Local anesthetics have been used in acute and chronic pain management for 100 years. The use of continuous infusion or repeated injections not only increases the risk of infection and tissue damage but is also inconvenient for patients. Postoperative pain usually lasts several days, but the analgesic effect of local anesthetics is very short. Therefore, longer-acting local anesthetics would be useful for a nerve block. Previously, studies to prolong local anesthetic duration focused on structural alteration of local anesthetic molecules. Levobupivacaine is an S(−)-enantiomer of racemic bupivacaine and has less potential for producing toxicity in both the central nervous system and the cardiovascular system than R(+)-bupivacaine in animals (1,2) and humans (3).

Prolonged delivery systems for local anesthetics using biocompatible drug carriers, such as cyclodextrins and liposomes, have been studied (4). The cyclodextrin-based drug carrier system is a well established technology. More than 15 drug delivery systems, which contain cyclodextrins or their derivatives and are intended for oral and injectable use, have been approved by regulatory agencies in various countries (5). Cyclodextrins are cyclic oligosaccharides consisting of 6, 7, and 8 α-1,4 linked glucopyranose units, respectively, and are called α-, β-, and γ-cyclodextrins. They have a slightly apolar and hydrophobic internal cavity into which various guest molecules can be accommodated; thereby, they can act as suitable carrier materials to eliminate undesirable properties of drug molecules and can be used to prepare new and

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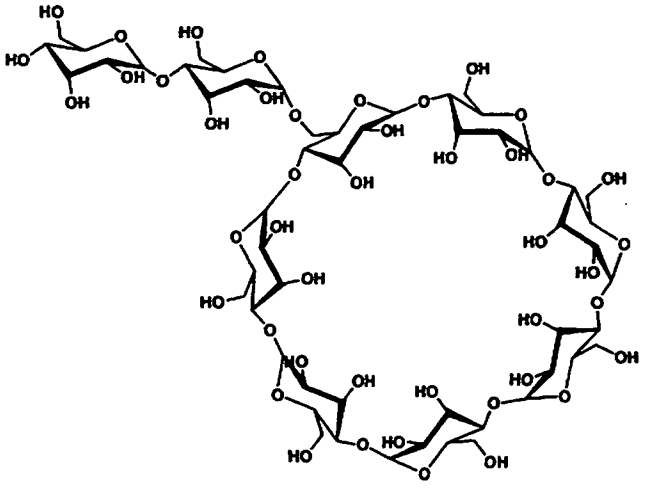

Figure 1. Chemical structure of maltosyl-β-cyclodextrin.

differentiated dosage forms of the drugs. Cyclodextrins have been reported to prolong the anesthetic effects of short-acting opioids after intrathecal administration through inclusion complex formation and to be compatible with spinal tissue (6,7).

In the present study we used the cyclodextrin derivatives, maltosyl-β-cyclodextrin (G2-β-CD), in which one of the primary hydroxyl groups of β-cyclodextrin is substituted by maltose through the α-1,6 glycosidic linkage (Fig. 1). Our preliminary study showed that G2-β-CD forms an inclusion complex with levobupivacaine in a 1:1 molar ratio in an aqueous solution, as assessed by nuclear magnetic resonance spectroscopy and solubility analysis. In addition, G2-β-CD has been shown to increase the solubility of levobupivacaine hydrochloride and levobupivacaine free base in aqueous media at pH ranging from neutral to alkaline and to decrease the surface tension-decreasing ability of the drug.

The present study was designed to determine whether the complex of levobupivacaine with G2-β-CD is effective to use in intrathecal block and to determine whether this complex extends the duration of local anesthetic effects in a sciatic nerve block.

METHODS

A levobupivacaine free base and its hydrochloride were supplied by Maruishi Phamaceutical Co. (Osaka, Japan). G2-β-CD was donated by Ensuiko Sugar Refining Co. (Yokohama, Japan). Other chemicals were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

This study was approved by both the Animal Research and the Use Committees of University of Miyazaki. Male Sprague-Dawley rats weighing 260–330 g were used. All rats were maintained at the Experimental Animal Center of the University of Miyazaki under a controlled ambient temperature of 23 ± 1°C and a humidity of 50% ± 10% with a 12-h light–dark cycle. Rats were allowed free access to food and water and were handled for 7–10 days before the commencement of this study, so that they would be familiar with the experimental environment and procedures.

Intrathecal Block

After obtaining baseline values for muscle tone and tail-flick latency, the rats were anesthetized by inhalation of a low concentration of sevoflurane (Sevofrane, Maruishi, Osaka), which allowed recovery within 2–3 min. The rats were held in a prone, elevated lumbar position, and their backs shaved and sterilized with 10% povidone iodine. The study anesthetic solutions were injected intrathecally with a 30-gauge needle on a 50-μL syringe (Hamilton, NV) at the L4–5 interspace with a positive indication of tail movement (8). This movement of the tail is "electrical shock-like" and is observed only when the needle is inserted into the intrathecal space. From immediately after injection until awakening from sevoflurane anesthesia, the rats were placed in a prone, horizontal position.

The rats were randomly divided into four groups (n = 6) to receive an intrathecal injection of one of the following doses of levobupivacaine complexed with G2-β-CD. The solutions were prepared aseptically and administered intrathecally in a blinded, randomized fashion at a dose of 1% levobupivacaine complexed with 0 mM (control group), 50, 100, and 200 mM of G2-β-CD. The pH and osmotic pressure of the solutions were adjusted to be pH 5.05–5.32 and 300 mOsm, respectively, by titration of hydrochloric acid and sodium chloride. All solutions were injected in a volume of 30 μL over 5 s. Animals were observed to identify the time to the onset of the nerve block. Muscle tone and tail-flick latency were assessed and were measured at 5-min intervals after injection in a blinded fashion.

General skeletal muscle tone was graded by manual manipulation and visual observation according to the scale proposed by Bromage et al. (9) for humans, which we modified for the rat model as follows: 3 = normal tone, free movement of the hindlimb; 2 = weak hypotonia of the hindlimbs/body posture; 1 = moderate hypotonia of the hindlimbs/body posture; and 0 = inability to support the body on the hindlimbs and flat body posture.

To determine the tail-flick latency, a heat stimulus (xenon bulb, 24 V, 150 W) was directed at a portion of the tail approximately 5 cm from the tip. The time between stimulus onset and withdrawal of the tail was defined as the response latency. A tail-flick testing device (model 33B, IITC, Inc., USA) was calibrated to give an average baseline latency of approximately 3–5 s. To prevent tissue damage, the heat stimulus was stopped if no response occurred within 10 s (cut-off time). Tail-flick testing began after confirmation of the

animal's ability to move its tail after an injection of a local anesthetic.

After the experimental series, rats had a laminectomy under deep pentobarbital anesthesia on the first day after injection. We confirmed the 30-gauge needle intrathecal injection hole in the dura. Rats were then perfused with formalin, and the spinal cords were removed from the 10th thoracic to the first sacral vertebra. Spinal cords were fixed in formalin and embedded in acrylic polymer (JB-4 TM plus embedding kit, Polysciences, Inc., USA). The spinal cords were then sliced in 1-μm transverse sections at the level of the cauda equina, stained with toluidine blue, and histologically evaluated under a light microscope. Examinations were performed on four slices near the level of the needle hole in a blind, randomized fashion. Sections were rated as severe (necrosis, axonal degeneration, large hemorrhage, and loss of myelin), mild (inflammatory cells, edema, and limited lesion of hemorrhage), or normal. Sections of a rat were evaluated if the hemorrhage was limited to the arachnoidea spinalis (subdural space) or to only one slice; in this case, a rat section was rated as mild. When there were many findings in a rat slice, the slice was classified as having the highest degree of damage.

Sciatic Nerve Block

Once baseline values were obtained, a unilateral sciatic nerve was blocked using brief sevoflurane anesthesia. A 30-gauge needle was inserted between the greater trochanter and ischial tuberosity with the bevel turned to the femoral head. When the needle encountered the body of the ischium, the local anesthetic solution was injected over a 5-s period (10).

The rats were randomly divided into four groups (n = 6) to receive the sciatic nerve block of one of the following doses of 0.5% or 1% levobupivacaine complexed with either 0 (control group) or 100 mM of G2-β-CD. The pH and osmotic pressure of the solutions were adjusted to be pH 5.05–5.32 and 300 mOsm, respectively, by titration of hydrochloric acid and sodium chloride. The solution was administered in a blinded, randomized fashion in a constant volume of 100 μL. The animals were observed over the period when the nerve block was detectable.

Proprioception, motor function, and nociception were measured before and at 5, 10, and 15 min postinjection and then at 15-min intervals until neurological deficit was no longer observed. The measurements were taken in the following order: (1) proprioception (tactile placing followed by hopping response), (2) motor function, and (3) nociception, within a 100-s timeframe. All tests were performed by an investigator blinded to the drugs administered.

Proprioception was based on the resting posture and the postural reaction, i.e., the tactile placing and the hopping response, respectively. For tactile placing, the rat was kept in a normal resting posture, and the hindpaw toes were flexed with their dorsi placed on the table; then, the rat's ability to reposition the toes was evaluated. To measure the hopping response, the rat was placed with the hindlimbs on a supporting surface, and the front half of the animal was held up. One hindlimb at a time was lifted off the ground, and the animal's body was moved laterally. The hopping response time was measured as the time at which the animal hopped normally with the weight-bearing limb in the direction of movement of the body to avoid falling over. With impaired proprioception, the animal would drag its leg on the table. This functional deficit was measured by the following scoring system: 3 = normal posture and gait with symmetrical postural reactions; 2 = toes are flat, a slight lameness is observed, and the postural reactions are slower on the affected side; 1 = toes are flat and ventroflexed and are not involved in walking, and the postural reactions are severely damaged; the rat does not correct the position of toes in tactile placing and cannot bear weight and follow in the hopping reaction; 0 = complete absence of postural reaction and dragging.

Motor function was evaluated by measuring the extensor postural thrust: the rat was held upright with the affected hindlimb extended so that the body's weight was supported by the distal metatarsus and toes, and the extensor thrust was measured as the force applied to a digital platform scale (model 1458N, TANITA, Japan). If complete blockade of sciatic nerve function was achieved, only passive force, or the lateral hindlimb's weight, was measured.

The withdrawal reflex to the mechanical stimulation of deep tissue was used to evaluate nociception. Forceps were used to apply pressure across the distal phalanx of the fifth toe. The force exerted by the forceps was decided with a protractor as a minimal force which all rats showed a withdrawal response to in our preliminary study. The withdrawal reflex was measured by the following scoring system: 4 = normal: general motor reaction, vocalization, and withdrawal of the hindlimb; 3 = slower withdrawal than that on the control side; 2 = not a complete response; no vocalization or general motor reaction, only a turn of the head; 1 = only a weak attempt to withdraw; 0 = no response.

Neurotoxicity of Cyclodextrin

We finally examined the neurotoxicity of intrathecal G2-β-CD alone. The rats were randomly divided into four groups (n = 4) to receive intrathecal injections of one of the following solutions that had been prepared aseptically and was administered intrathecally in a blinded and randomized fashion. The solutions used were saline (0.9% NaCl) and G2-β-CD at concentrations of 50, 125, and 250 mM. At all concentrations, the solution was injected in volumes of 30 μL over 5 s.

We watched for general neurological symptoms, such as somnolence, irritation, convulsion, paralysis, autotomy, or any other abnormal phenomenon. The

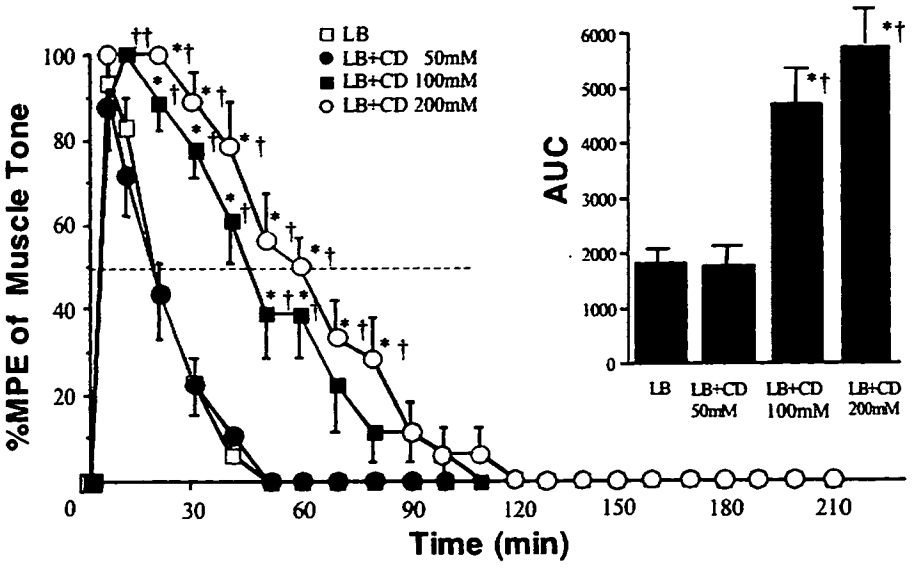

Figure 2. Time course effects and area under the time-effect curve (AUC) of the intrathecal administration of anesthetics for muscle tone, expressed as a percent maximum possible effect (%MPE) of 1% levobupivacaine and 1% levobupivacaine conjugated with 50, 100, and 200 mM of G2-β-CD; $n = 6$ for each group. Data are presented as mean ± SEM. *Significant difference when compared with 1% levobupivacaine. †Significant difference when compared with 1% levobupivacaine conjugated with 50 mM of G2-β-CD. LB represents levobupivacaine. CD represents G2-β-CD. The dashed line intersects the graphs where %MPE is 50%.

tail-flick latency was measured before, and at 5, 10, 30, 60, 120, 240, 360, 480 min and 24 h after injection in a blinded fashion.

After the experimental series, rats were perfused with formalin, and the spinal cord was removed from the 12th thoracic to the first sacral vertebra under deep pentobarbital anesthesia. The spinal cords were embedded in acrylic polymer and then sliced in 1-μm transverse sections at the level of cauda equina. The sections were stained with toluidine blue and histologically evaluated under a light microscope. Sections were rated as severe, mild, or normal, as in the intrathecal protocol.

Treatment of Data

To assess the effect of local anesthetics on muscle tone, tail-flick latency, proprioception, motor function, and nociception, the measured values were converted to a percent maximum possible effect (%MPE). The increase in %MPE means an increase of neural blocking effect with local anesthetics. Muscle tone %MPE was estimated by the following formula: [(control − score)/control] × 100, where the control was the muscle tone before injection and the score was the muscle tone after injection. Tail-flick latency %MPE was calculated by the following equation: %MPE = [(tail-flick latency − control)/(cut-off time − control)] × 100, where the control latency was the time of withdrawal before injection and tail-flick latency was the time of withdrawal after injection. Motor function %MPE was calculated by the equation: %MPE = [(control − experiment)/(control − residual)] × 100, where the control was the weight of the hindlimb before injection, the experiment was the weight of the hindlimb after injection, and the residual was the residual weight of the hindlimb in the absence of thrust. Proprioception and nociception %MPE were estimated by the formula: [(control − experiment)/control] × 100, where the control was the score before injection and the experiment was the score after injection.

The area under the time-effect curve (AUC) was calculated by integrating the %MPE × minutes using the trapezoidal integration method. To evaluate the duration of the effect of the local anesthetics, the time from the end of the intrathecal injection to the time of %MPE 50% (%MPE50T) was calculated from the time-effect curve.

To assess the neurotoxicity of G2-β-CD, the measured values of tail-flick latency were converted to %MPE, calculated as [(tail-flick latency − control)/(cut-off time − control)] × 100.

The values of %MPE, AUC, and %MPE50T with each local anesthetic solution are expressed as the mean ± SEM.

Statistics

The differences among four groups of the intrathecal or sciatic nerve block in %MPE, AUC, and %MPE50T were compared using the one-way analysis of variance followed by the Bonferroni (Dunn) test. The data from the neurotoxicity study were analyzed using Mann–Whitney *U*-test with Bonferroni correction. $P < 0.05$ was considered significant.

RESULTS

Intrathecal Block

All rats had tail movement at the time of intrathecal injection which stopped immediately after injection of the local anesthetic. The site of injection in the dura was observed in all rats at the completion of the experiment, and no neurological dysfunction was observed in the first days after the study. The recovery time of the motor block was significantly shorter than that of the sensory block with all preparations of anesthetics used.

The mean times to the recovery of muscle tone after an intrathecal injection of 1% levobupivacaine and 1% levobupivacaine with 50, 100, and 200 mM of G2-β-CD were 38.3 ± 3.1, 38.1 ± 4.8, 80.0 ± 8.9, and 91.7 ±

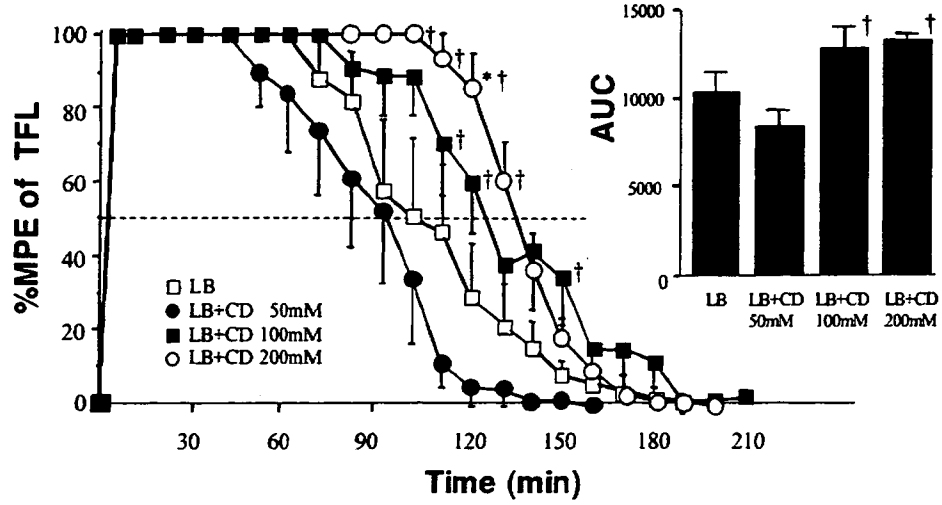

Figure 3. Time course effects and area under the time-effect curve (AUC) of the intrathecal administration of anesthetics for the tail-flick latency (TFL), expressed as a percent maximum possible effect (%MPE) of 1% levobupivacaine and 1% levobupivacaine conjugated with 50, 100, and 200 mM of G2-β-CD; $n = 6$ for each group. Data are presented as mean ± SEM. *Significant difference when compared with 1% levobupivacaine. †Significant difference when compared with 1% levobupivacaine conjugated with 50 mM of G2-β-CD. LB represents levobupivacaine. CD represents G2-β-CD. The dashed line intersects the graphs where %MPE is 50%.

7.0 min, respectively (Fig. 2). The mean time to the recovery of muscle tone and the mean AUC of 1% levobupivacaine complexed with 100 and 200 mM of G2-β-CD were significantly longer than those of 1% levobupivacaine alone.

The %MPE50Ts calculated from tail-flick latency after 1% levobupivacaine and 1% levobupivacaine complexed with 50, 100, and 200 mM of G2-β-CD were 102.0 ± 10.7, 82.8 ± 9.5, 125.5 ± 12.2, and 132.8 ± 4.9 min, respectively (Fig. 3). The mean AUC of tail-flick latency of 1% levobupivacaine with 100 and 200 mM of G2-β-CD was significantly longer than that of 1% levobupivacaine with 50 mM of G2-β-CD but not significantly different from that of 1% levobupivacaine alone. The time to the recovery of tail movement was shorter with the test solutions than the time to the recovery from the sensory block. No histological abnormalities were found in the spinal cord.

Sciatic Nerve Block

The hindlimb of the injected side in all rats was temporarily immobilized during this study and recovered with full neurological function at its completion. The time course effects for proprioception, motor function, and nociception after sciatic nerve block were similar (Fig. 4). However, the antinociceptive effect was slightly shorter than either the proprioception or motor function in individual rats.

The %MPE50T of motor block after 0.5% levobupivacaine, 0.5% levobupivacaine with 100 mM of G2-β-CD, 1% levobupivacaine, and 1% levobupivacaine with 100 mM of G2-β-CD was 113 ± 4, 221 ± 12, 182 ± 7, and 252 ± 15 min, respectively (Table 1). The anesthetic effect of 0.5% levobupivacaine with 100 mM of G2-β-CD was twice as long as that of 0.5% levobupivacaine, and that of 1% levobupivacaine with

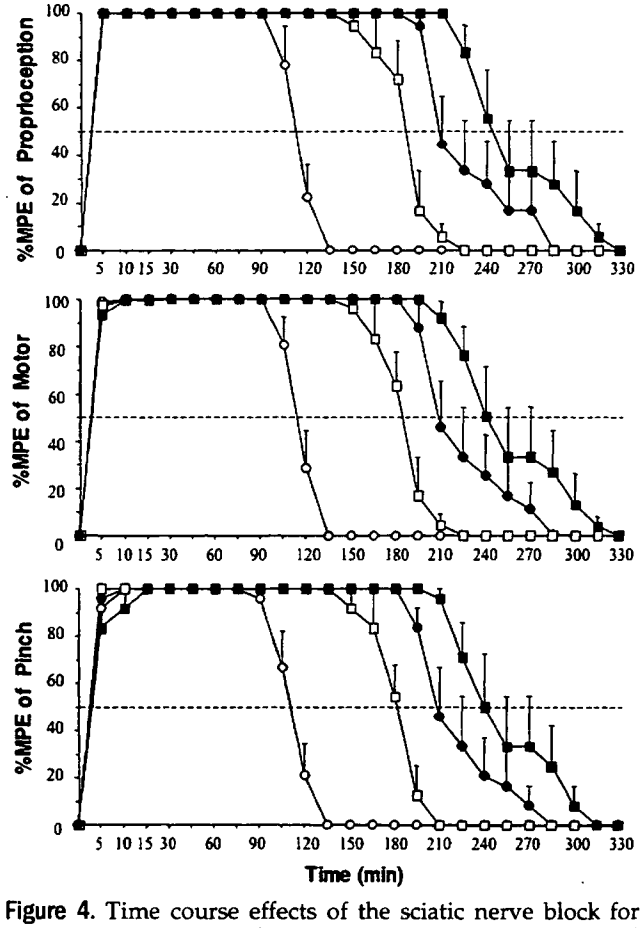

Figure 4. Time course effects of the sciatic nerve block for proprioception, motor function, and nociception expressed as the percent maximum possible effect (%MPE) of 0.5% levobupivacaine (open circle), 0.5% levobupivacaine with 100 mM of G2-β-CD (closed circle), 1% levobupivacaine (open square), and 1% levobupivacaine with 100 mM of G2-β-CD (closed square); $n = 6$ for each group. Data are presented as mean ± SEM. The dashed line intersects the graphs where %MPE is 50%.

Table 1. The duration (min) of %MPE ≥50% After Sciatic Nerve Block

	0.5% LB (n = 6)	0.5% LB+CD (n = 6)	1.0% LB (n = 6)	1.0% LB+CD (n = 6)
Proprioception	113 ± 4	223 ± 13*	183 ± 7*	253 ± 15*†
Motor function	113 ± 4	221 ± 12*	182 ± 7*	252 ± 15*†
Nociception	110 ± 4	218 ± 12*	179 ± 7*	250 ± 14*†

Values are mean ± SEM.

%MPE = percent maximum possible effect; LB = levobupivacaine; CD = maltosyl-β-cyclodextrin.

* $P < 0.05$ compared with 0.5% levobupivacaine.

† $P < 0.05$ compared with 1.0% levobupivacaine.

100 mM of G2-β-CD was 1.4 times longer than that of 1% levobupivacaine alone.

Neurotoxicity of Cyclodextrin

We observed no abnormal neurological symptoms in the saline and the three concentrations of G2-β-CD groups. The changes for %MPE of tail-flick latency were not different among the groups. However, two rats that received 250 mM of G2-β-CD showed a markedly increased tail-flick latency value (8.5–10 s) at 4 and 8 h after injection. The mean baseline value of tail-flick latency in all rats was 3.7 ± 0.08 s.

In a histological study, no abnormal lesion was seen in the saline group, and no significant findings were seen in rats that received G2-β-CD, except for two rats that received 250 mM of G2-β-CD, which had a small hemorrhage on the surface of the spinal cord.

DISCUSSION

We demonstrated that an intrathecal injection of a complex of levobupivacaine with G2-β-CD significantly prolonged motor block and that an injection of this complex around the sciatic nerve also provided significantly prolonged anesthetic effects, as evaluated by proprioception, motor function, and nociception, when compared with levobupivacaine alone. Furthermore, no neurological signs were evident after the intrathecal and sciatic nerve blocks. This complex with G2-β-CD prolonged the anesthetic effect of levobupivacaine.

Each cyclodextrin has the ability to form inclusion complexes with specific molecules; this depends on the correct fit of the molecule into the hydrophobic cyclodextrin cavity (5). However, natural cyclodextrins have relatively low solubility, both in water and organic solvents, which limits their use in pharmaceutical formulations. The introduction of a maltosyl group onto the hydroxyl group of cyclodextrins confers increased solubility with a slight temperature dependency, e.g., the solubility of G2-β-CD in water is move than 50% (w/v) at 25°C, (5,11) similar to that of hydroxypropyl-β-cyclodextrin. Such a property is particularly useful for designing aqueous injectable cyclodextrin solutions that are to be heat-sterilized. The metabolic fate of G2-β-CD is suggestive of less renal toxicity compared with that of β-cyclodextrin, as the nephrotoxicity of the parent β-cyclodextrin at higher doses is ascribed to the crystallization of a less soluble β-cyclodextrin or its cholesterol complex in renal tissue (12). In fact, G2-β-CD is excreted as soluble 6-O-glucosyl-β-CD, not as the less soluble β-cyclodextrin (5). In addition, natural cyclodextrins possess hemolytic ability at a higher concentration (13,14), but G2-β-CD shows no or only slight hemolytic action (15,16). Therefore, G2-β-CD may be useful as a solubilizer for parenteral preparations such as injections because of its high solubilization ability, weak hemolytic ability, and high bioadaptability. For example, some biological surface membrane constituents such as cholesterol and phospholipids and some drugs with hydrophobic moiety seem to be good candidates as the guest molecules. The binding constant of the complex of 1% levobupivacaine with 200 mM G2-β-CD is 60.2 M^{-1}, as determined by nuclear magnetic resonance spectroscopy. Based on the binding constant of the complex, more than 90% of levobupivacaine is assumed to exist as a complexed form with G2-β-CD in equilibrium immediately after injection. Subsequently, the complex is gradually dissociated into levobupivacaine and G2-β-CD, due to dilution with the biological fluid at the injection site and the competitive binding with other guest molecules.

There are a number of reasons why cyclodextrin drug carrier systems can extend the local anesthetic effect. As local anesthetics are small-size molecules, their rapid redistribution from the injected site limits the duration of effective analgesia. The molecular weight of the complex of levobupivacaine with G2-β-CD is estimated to be 1747 Da, which is approximately 6 times heavier than that of levobupivacaine alone. As large molecules diffuse more slowly from the injection site, the complex of local anesthetic and cyclodextrin is cleared more slowly from the effective site than the local anesthetic alone. The sustained release of local anesthetics from the carrier vesicle will prolong the anesthetic effect. In addition, excess cyclodextrin could potentially slow the redistribution of the drug further because the movement of the drug into the biophase is in competition with the free cyclodextrin molecules in the same space (7).

G2-β-CD lacks detergency ability, which lowers the surface tension of the solution. The surface tension-decreasing ability of the compound would be proportional to the tendency of the compound to move to the biological interface between biological fluid and cell

surfaces and to enter into the cells. Therefore, the surface tension-decreasing ability of the compound is used as a simple and reliable measure to predict the local tissue irritancy of the compound at nontargeted tissues in vivo. From the viewpoint of local tissue irritancy, the lack of surface tension-decreasing ability of G2-β-CD is desirable as a biocompatible drug carrier. Furthermore, G2-β-CD decreases the surface tension-decreasing ability of levobupivacaine; the surface tensions of 0.5% levobupivacaine solution were 59.1 and 62.9 dyn/cm^2 in the absence and presence of 200 mM G2-β-CD in water at 25°C. This indicates that G2-β-CD has the potential to reduce the local tissue irritation associated with prolonged stay of levobupivacaine at relatively high concentrations at the site of administration.

In the spinal and sciatic nerve block studies, the upper limit of the G2-β-CD concentration was restricted by the osmotic pressure setting of the solution and the safety margin of G2-β-CD. In our preliminary studies, we used the concentrations of G2-β-CD up to 200 mM both for spinal and sciatic blocks. In the case of the sciatic nerve block, G2-β-CD, even at the lower concentration (100 mM), prolonged the anesthetic effect of levobupivacaine. Therefore, we chose the concentration of G2-β-CD at 100 mM for the sciatic nerve block studies. In the neurotoxicity studies, we chose higher concentrations of the cyclodextrin up to 250 mM in order to confirm the safety margin of the cyclodextrin.

Our study showed that the complex of cyclodextrin and levobupivacaine prolonged the effects of the local anesthetic in the sciatic nerve block to a greater extent than in the spinal block. One reason for this difference would be the environmental differences in the injection sites. The release of levobupivacaine from its cyclodextrin complex depends on the concentration gradient of levobupivacaine; therefore, the release of levobupivacaine is expected to occur more rapidly in the fluid environment of the intrathecal space than in the solid environment of the circumference of the sciatic nerve.

Meert and Melis (6) showed that hydroxypropyl-β-cyclodextrin significantly increased the duration of analgesia after both epidural and intrathecal administration of sufentanil and bupivacaine in rats. However, the complex of bupivacaine and cyclodextrin had no anesthetic effect because of the small dose (80 μg) of bupivacaine. Fréville et al. (17) showed that epidurally administered complexes of bupivacaine and hydroxypropyl-β-cyclodextrin could prolong the duration of the action and the systemic uptake of bupivacaine in rabbits. However, sulfobutylether7-β-cyclodextrin delayed the onset of the motor block and did not modify the duration after bupivacaine administration near the sciatic nerve in rabbits (18). Overall, the anesthetic effects of the complex of local anesthetics with cyclodextrin have been controversial. Regarding analgesic opioids, Yaksh et al. (7) found that cyclodextrin could enhance the action of intrathecally administered opioids.

This study has two limitations. First, we did not compare G2-β-CD alone, as a control vehicle, with the G2-β-CD–levobupivacaine complex in the same protocol. In general, cyclodextrins are thought to be pharmacologically inactive. The tissue toxicity induced by cyclodextrin is very low in humans and animals (5,16). We also showed that there is no difference in the tail flick response between the cyclodextrin alone and reference control. However, two rats received G2-β-CD at a high concentration of 250 mM showed an increased tail-flick latency value and mild histological damage. Moreover, the number of models studied was small, and not all potential toxicities were evaluated. Our single study is not definitive for evaluating the safety of this complex. Second, sensory function was not easily distinguishable from motor function during the sciatic nerve block because the motor block affected sensory assessment. As shown in our study, the time course effects for proprioception, motor function, and nociception after the sciatic nerve block were similar. Although we adapted a well-established neurological evaluation for the rat (10), the interrelation of neurological function should not be completely ignored.

In conclusion, the complex of levobupivacaine with G2-β-CD prolonged the anesthetic effect of levobupivacaine in both intrathecal and sciatic nerve blocks in rats. This complex may be used to prolong the effect of a long-acting local anesthetic such as levobupivacaine 1.4–2.0 times through a conduction block and to supply adequate postoperative analgesia. Further studies are necessary to fully establish the safety and efficacy of this interesting complex for prolonging local anesthetic block.

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REFERENCES

1. Aberg G. Toxicological and local anaesthetic effect of optically active isomers of two local anaesthetic compounds. Acta Pharmacol Toxicol 1972;31:272–86.
2. Huang YF, Pryor ME, Mather LE, Veering BT. Cardiovascular and central nervous system effect of intravenous levobupivacaine and bupivacaine in sheep. Anesth Analg 1998;86:797–804.
3. Bardsley H, Gristwood R, Baker H, et al. A comparison of the cardiovascular effects of levobupivacaine and rac-bupivacaine following intravenous administration to healthy volunteers. Br J Clin Pharmacol 1998;46:245–9.
4. Grant GJ, Barenholz Y, Bolotin EM, et al. A novel liposomal bupivacaine formulation to produce ultralong-acting analgesia. Anesthesiology 2004;101:133–7.
5. Uekama K, Hirayama F, Irie T. Cyclodextrin drug carrier systems. Chem Rev 1998;98:2045–76.
6. Meert TF, Melis W. Interactions between epidurally and intrathecally administered sufentanil and bupivacaine in hydroxypropyl-β-cyclodextrin in the rat. Act Anaesthesiol Belg 1992;43:79–89.
7. Yaksh TL, Jang J, Nishiuchi Y, et al. The utility of

2-hydroxypropyl-β-cyclodextrin as a vesicle for the intracerebral and intrathecal administration of drugs. Life Sci 1991;48:623–33.
8. Hylden JL, Wilcox GL. Intrathecal morphine in mice: a new technique. Eur J pharmacol 1980;67:313–16.
9. Bromage PR, Burfoot MF, Crowell DE, Pettigrew RT. Quality of epidural blockade.I. Influence of physical factors. Br J Anaesth 1964;36:342–52.
10. Thalhammer JG, Vladimirova M, Bershadsky B, Strichartz GR. Neurologic evaluation of the rat during sciatic nerve block with lidocaine. Anesthesiology 1995;82:1013–25.
11. Okada Y, Kubota Y, Koizumi K, et al. Some properties and the inclusion behavior of branched cyclodextrins. Chem Pharm Bull 1988;36:2176–85.
12. Frank DW, Gray JE, Weaver RN. Cyclodextrin nephrosis in the rat. Am J Pathol 1976;83:367–82.
13. Ohtani Y, Irie T, Uekama K, et al. Differential effects of α-, β-, and γ-cyclodextrins on human erythrocytes. Eur J Biochem 1989;186:17–22.
14. Irie T, Otagiri M, Sunada M, et al. Cyclodextrin-induced hemolysis and shape change of human erythrocytes in vitro. J Phamacobiodyn 1982;5:741–4.
15. Yamamoto M, Yoshida A, Hirayama F, Uekama K. Some physicochemical properies of branched β-cyclodextrin and their inclusion characteristics. Int J Pharm 1989;49:163–71.
16. Irie T, Uekama K. Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation. J Pharm Sci 1997;86:147–62.
17. Fréville JC, Dollo G, Le Corre P, et al. Controlled systemic absorption and increased anesthetic effect of bupivacaine following epidural administration of bupivacaine-hydroxypropyl-β-cyclodextrin complex. Pharm Res 1996;13:1576–80.
18. Dollo G, Thompson DO, Le Corre P, et al. Inclusion complexation of amide-typed local anesthetics with β-cyclodextrin and its derivatives. III. Biopharmaceutics of bupivacaine-SBE7-βCD complex following percutaneous sciatic nerve administration in rabbits. Int J Pharm 1998;164:11–19.