Cui R, Xu SY, Wang L, Lei HY, Cai QX, Zhang HF, Wang DM. Extracts from rabbit skin inflamed by the vaccinia virus attenuate bupivacaine-induced spinal neurotoxicity in pregnant rats. Neural Regen Res. 2013;8(11):983-990.

Extracts from rabbit skin inflamed by the vaccinia virus attenuate bupivacaine-induced spinal neurotoxicity in pregnant rats☆

Rui Cui[1, 2], Shiyuan Xu[1], Liang Wang[2], Hongyi Lei[1], Qingxiang Cai[1], Hongfei Zhang[1], Dongmei Wang[1]

1 Department of Anesthesiology, Zhujiang Hospital of Southern Medical University, Guangzhou 510280, Guangdong Province, China
2 Department of Anesthesiology, Shenzhen Maternity & Child Healthcare Hospital, Southern Medical University, Shenzhen 518028, Guangdong Province, China

Abstract

Extracts from rabbit skin inflamed by the vaccinia virus can relieve pain and promote repair of nerve injury. The present study intraperitoneally injected extracts from rabbit skin inflamed by the vaccinia virus for 3 and 4 days prior to and following intrathecal injection of bupivacaine into pregnant rats. The pain threshold test after bupivacaine injection showed that the maximum possible effect of tail-flick latency peaked 1 day after intrathecal injection of bupivacaine in the extract-pretreatment group, and gradually decreased, while the maximum possible effect in the bupivacaine group continued to increase after intrathecal injection of bupivacaine. Histological observation showed that after 4 days of intrathecal injection of bupivacaine, the number of shrunken, vacuolated, apoptotic and caspase-9-positive cells in the dorsal root ganglion in the extract-pretreatment group was significantly reduced compared with the bupivacaine group. These findings indicate that extracts from rabbit skin inflamed by the vaccinia virus can attenuate neurotoxicity induced by intrathecal injection of bupivacaine in pregnant rats, possibly by inhibiting caspase-9 protein expression and suppressing nerve cell apoptosis.

Key Words

neural regeneration; peripheral nerve injury; vaccinia virus; extract; bupivacaine; neurotoxicity; caspase-9; apoptosis; local anesthetic; grants-supported paper; neuroregeneration

Research Highlights

This study aimed to elucidate the neuroprotective properties of extracts from rabbit skin inflamed by the vaccinia virus on nerve injury. Our results indicated that extracts could inhibit caspase-9 protein expression, reduce nerve cell apoptosis, and attenuate neurotoxicity induced by intrathecal injection of bupivacaine in pregnant rats.

Rui Cui☆, M.D., Associate chief physician.

Corresponding author: Shiyuan Xu, Master, Professor, Doctoral supervisor, Department of Anesthesiology, Zhujiang Hospital of Southern Medical University, Guangzhou 510280, Guangdong Province, China, xushiyuan355@ yahoo.com.cn.

INTRODUCTION

Intraspinal anesthesia has been increasingly used to administer analgesics for cesarean delivery, normal delivery and for postoperative analgesia[1-2]. However, some parturient women presented sensory and motor dysfunction following intraspinal anesthesia[3-4]. Recent studies have reported that the incidence of severe nervous system complications from intraspinal anesthesia in parturient women was 0.58–0.92%[5-6], which may contribute

to nerve cell apoptosis resulting from local anesthetic-induced spinal neurotoxicity. Local anesthetic administration in the epidural and subarachnoid space directly exerts effects on nerve cells[7], and damages phospholipids and the protein structure of nerve fiber membranes. This damage leads to irreversible membrane rupture and disruption to oxidative phosphorylation, which affects mitochondrial transmembrane potential and promotes neuronal apoptosis[8-11].

Studies have shown that the mechanism of local anesthetic-induced spinal neurotoxicity highly correlates with nerve cell apoptosis and mitochondrial dysfunction[12-13], however, prevention and treatment of local anesthetic-induced spinal neurotoxicity remains difficult to treat[14-17].

Extracts from inflamed rabbit skin induced by the vaccinia virus contain components such as amino acids and peptides, but not protein[18], which have been shown to relieve pain and promote nerve injury repair[19].

The present study sought to verify whether injection of extracts from inflamed rabbit skin induced by the vaccinia virus could relieve local anesthetic-induced spinal neurotoxicity, and to investigate the possible neuroprotective mechanism of action.

RESULTS

Quantitative analysis of experimental animals

Intrathecal catheterization (subarachnoid space) was conducted in pregnant rats for 17 days, and 18 rats with successful intrathecal catheterization were equally and randomly assigned to three groups: control (intrathecal and intraperitoneal injection of normal saline, 10 mL/kg); bupivacaine (intrathecal injection of bupivacaine, followed by intraperitoneal injection of normal saline, 10 mL/kg); and analgecine pretreatment (intrathecal injection of bupivacaine and intraperitoneal injection of analgecine 3 days prior to intrathecal injection and 4 days after intrathecal injection). All 18 rats were included in the result analysis.

Influence of analgecine pretreatment on pain threshold in pregnant rats

There was no significant difference in tail-flick latency prior to and following intrathecal catheterization among the three groups ($P > 0.05$; Table 1).

Table 1 Effect of analgecine pretreatment on tail-flick latency (second) in pregnant rats

Group	Prior to catheterization	After catheterization	t	P
Control	5.10±1.08	4.99±1.13	1.576	0.176
Bupivacaine	4.53±1.12	4.72±0.71	−0.904	0.407
Analgecine pretreatment	4.67±0.90	4.61±0.54	0.284	0.788
F	0.492	0.322		
P	0.621	0.730		

Data are expressed as mean ± SD of six rats in each group. Intergroup comparisons were analyzed using one-way analysis of variance, and comparisons of tail-flick latency prior to and following intrathecal catheterization were analyzed using the paired *t*-test.

Analysis of variance of repeated measurement data showed a significant difference in the maximum possible effect of tail-flick latency among groups ($F = 22.329$, $P = 0.000$). Maximum possible effect remained unchanged with increasing time following administration ($F = 1.569$, $P = 0.229$), but exhibited an interaction between grouping and time ($F = 3.182$, $P = 0.038$), indicating that the difference among groups was different at different time points. Therefore, it was necessary to compare differences at each time point. Further analysis revealed that at 1 day, maximum possible effect was significantly higher in the bupivacaine and analgecine pretreatment groups when compared with the control group ($P < 0.05$); at 2–5 days, maximum possible effect was significantly higher in the bupivacaine group when compared with analgecine pretreatment and control groups ($P < 0.05$); at 2 and 3 days, maximum possible effect was significantly higher in the analgecine pretreatment group when compared with the control group ($P < 0.05$). Intragroup comparison showed that maximum possible effect peaked 1 day after intrathecal injection of bupivacaine in the analgecine pretreatment group, and gradually decreased thereafter; while maximum possible effect continued to increase after intrathecal injection of bupivacaine in the bupivacaine group (Table 2).

Influence of analgecine pretreatment and bupivacaine treatment on motor function in pregnant rats

The motor function score of bupivacaine and analgecine group rats was 0 following intrathecal injection of bupivacaine, indicating that motor function of pregnant rats was not affected.

Cell morphology in the dorsal root ganglion of pregnant rats following intrathecal injection of bupivacaine

At 4 days after intrathecal injection of bupivacaine, shrunken and vacuolated nerve cells were found in the dorsal root ganglion of pregnant rats.

Table 2 Influence of analgecine pretreatment on the maximum possible effect in pregnant rats

Group	Time after intrathecal injection of bupivacaine (day)					Sum	F	P
	1	2	3	4	5			
Control	0.04±0.01	0.05±0.02	0.05±0.04	0.04±0.03	0.08±0.09	0.05±0.05	0.838	0.517
Bupivacaine	0.39±0.35[c]	0.72±0.38[cd]	0.75±0.40[cd]	0.75±0.39[cd]	0.84±0.39[cd]	0.69±0.39	2.641	0.140
Analgecine pretreatment	0.24±0.72[c]	0.20±0.04[c]	0.17±0.09[c]	0.14±0.10	0.11±0.23	0.17±0.02	3.397	0.028
Sum	0.22±0.24	0.32±0.36	0.32±0.39	0.31±0.39	0.34±0.42	–	1.569[a]	0.229[a]
F	4.303	15.856	14.645	16.558	20.786	22.329[a]	(F = 3.182,	
P	0.033	0.000	0.000	0.000	0.000	0.000[a]	P = 0.038)[b]	

a: F and P of main effect; b: F and P of interaction; [c] $P < 0.05$, *vs.* control group; [d] $P < 0.05$, *vs.* analgecine pretreatment. Measurement data were expressed as mean ± SD of six rats in each group. Comparison of the maximum possible effect was analyzed using analysis of variance of repeated measurement data.

The number of vacuolated nerve cells was significantly reduced in the analgecine pretreatment group when compared with the bupivacaine group (Figure 1).
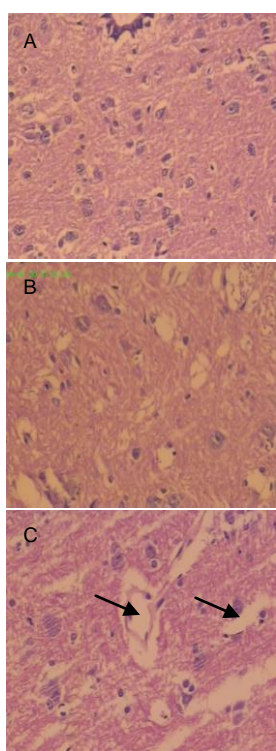

Figure 1 Morphology of cells in the dorsal root ganglion of pregnant rats (hematoxylin-eosin staining, × 400).

(A) Cell morphology was normal in the control group; (B) some shrunken and vacuolated nerve cells were observed in the analgecine pretreatment group; (C) the number of shrunken and vacuolated nerve cells was significantly increased in the bupivacaine group. Arrows represent shrunken and vacuolated nerve cells.

Cell apoptosis and caspase-9 expression in the dorsal root ganglion of pregnant rats following intrathecal injection of bupivacaine

Following 4 days of intrathecal injection of bupivacaine, the number of apoptotic cells and caspase-9-positive cells significantly decreased in the analgecine pretreatment group when compared with the bupivacaine group ($P < 0.05$; Table 3, Figures 2, 3).

Table 3 Influence of analgecine pretreatment on cell apoptosis and caspase-9 expression (cells/400-fold field of view) in the dorsal root ganglion of pregnant rats

Group	TUNEL	Caspase-9
Control	8.3±2.2	14.2±5.3
Bupivacaine	33.7±10.0[ab]	43.2±10.2[ab]
Analgecine pretreatment	12.0±7.5	20.0±6.7
F	17.198	23.909
P	0.002	0.000

[a] $P < 0.05$, *vs.* control group; [b] $P < 0.05$, *vs.* analgecine pretreatment. Measurement data are expressed as mean ± SD of six rats in each group. Comparison of the numbers of apoptotic cells and caspase-9-positive cells were analyzed using one-way analysis of variance; multiple comparisons were conducted using the Bonferroni (homogeneic variance) or Dunnett T3 test (heterogeneic variance). TUNEL: Terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate-biotin nick end labeling.

DISCUSSION

Clinical use of local anesthetics, such as lidocaine, bupivacaine, levobupivacaine, ropivacaine and mepivacaine, has the potential to cause spinal neurotoxicity[15, 20-29]. After intrathecal injection of local anesthetics, the agent diffuses along the obersteiner-redlich zone to the spinal cord. The degree of neurological impairment is dependent on the concentration and dose of medication, and the duration of exposure. However, studies have shown that administration of local anesthetics at commonly used concentrations can induce nerve cell swelling, an imbalance in ion concentrations in and out of membranes, disrupt membrane action potentials, and excessive release of excitatory amino acids, resulting in nerve motor and sensory dysfunction for several hours or days.
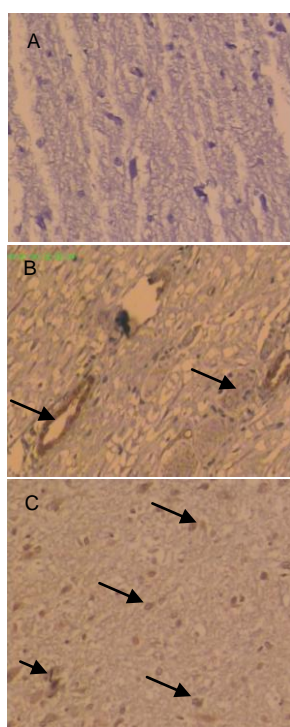
Figure 2   Apoptotic cell morphology in pregnant rats at 4 days following intrathecal injection of bupivacaine (TUNEL, × 400).

(A) No apoptotic cells were observed in the control group; (B) a few apoptotic cells with brown yellow particles in the nuclei were found in the dorsal root ganglion of the Analgecine pretreatment group; (C) the number of apoptotic cells with brown yellow particles in the nuclei were significantly greater in the bupivacaine group when compared with the analgecine pretreatment group. TUNEL: Terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate-biotin nick end labeling. Arrows indicate apoptotic cells.
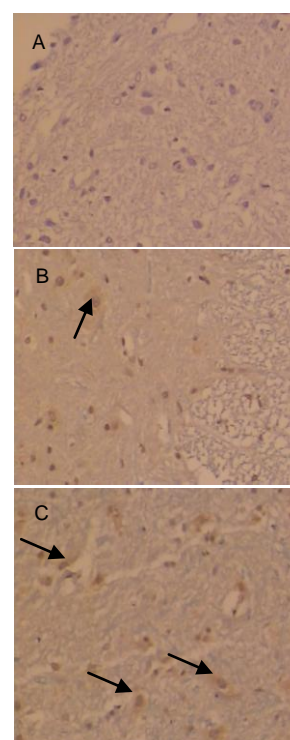

Figure 3   Caspase-9-positive cell morphology in pregnant rats at 4 days following intrathecal injection of bupivacaine (immunohistochemical staining, × 400).

(A) No caspase-9-positive cells were observed in the control group; (B) a few caspase-9-positive cells with a brown cytoplasm and cell membrane were found in the dorsal root ganglion of the analgecine pretreatment group; (C) the number of caspase-9-positive cells with a brown cytoplasm and cell membrane was significantly greater in the bupivacaine group when compared with the analgecine pretreatment group. Arrows indicate caspase-9-positive cells.

These effects may not be restored for long periods of time, and in some cases may be irreversible. For example, transient neurological syndrome is clinically characterized by lower limb pain and/or dysesthesia or hypersensitivity following subarachnoid block, which is accompanied by lower limb weakness, numbness, paraesthesia or urine retention. These effects have been shown to last for 24 hours after subarachnoid block is completely restored, and can sometimes last for 2, 5 and even 10 days[30]. Although the pathogenesis of these effects remains controversial, local anesthetic-induced neurotoxicity is one cause. Caspases are important proteases involved in cell apoptosis. Two main signal transduction pathways have been shown to activate caspases. The death receptor pathway involves death receptors on the cell surface, which bind to corresponding death ligands. These death ligands transfer extracellular apoptosis signals to cells, which activate caspase-8 and caspase-3 to trigger cell apoptosis.

Another pathway is the mitochondrial pathway, in which oxygen free radicals, calcium overload and other apoptosis-stimulating factors increase mitochondrial permeability to reduce mitochondrial transmembrane potential, and release cytochrome c. Cytochrome c activates caspase-9 and caspase-3 to induce cell apoptosis. The death receptor pathway and mitochondrial pathway are characterized by the activation of caspase-8 and caspase-9, respectively[31]. Werdehausen et al [7] reported that lidocaine-induced Jurkat cell apoptosis involves the mitochondrion-mediated endogenous apoptosis pathway, inducing Bcl-2 overexpression and caspase-9 activation. A previous study from our group also showed that single intrathecal injection of 2 or 4% (w/v) bupivacaine (30 μL) can induce cell apoptosis via the mitochondrial pathway in the dorsal root ganglion of rats by activating caspase-9[8]. In the present study, following multiple intrathecal injections of bupivacaine, sensory dysfunction

of pregnant rats was significantly prolonged, and maximum possible effect of tail-flick latency was significantly higher in the bupivacaine group when compared with the control group. Moreover, the number of apoptotic cells increased, and some cells were swollen or shrunken, consistent with previous results. The significant sensory dysfunction of pregnant rats following multiple intrathecal injections of bupivacaine highly correlated with caspase-9 activation and cell apoptosis in the dorsal root ganglion.

Prevention and treatment of local anesthetic-induced neurotoxicity remains difficult. Currently, prevention and treatment of local anesthetic-induced neurotoxicity includes four main methods. (1) Neurotrophic factor: experiments in *in vitro* cell cultures have shown certain neuroprotective effects. However, the effects of neurotrophic factor *in vivo* remain uncertain. (2) Excitatory amino acid receptor antagonist: *in vivo* and *in vitro* studies have demonstrated that glutamic acid receptor antagonists exhibit neuroprotective effects mainly through α-amino-3-hydroxy-5-methylisoxazole-propionic acid, but cannot completely eliminate neurotoxicity-induced injury. (3) Mitogen-activated protein kinase: a member of the serine-threonine protein kinase family, including extracellular signal-regulated kinases, c-Jun N-terminal kinases, and p38 kinase. Studies focusing on the relationship between local anesthetic-induced peripheral nerve toxicity injury and signal transduction have shown that local anesthetics can induce nerve cell apoptosis through the mitogen-activated protein kinase-related signal transduction system, and that p38 kinase inhibition has a neuroprotective effect on lidocaine-induced neurotoxicity injury and can reduce cell apoptosis[14-15]. (4) Chinese herbal medicine: studies have shown that pharmaceutical components of some Chinese herbal medicines, such as astragalus polysaccharides, baicalin, bilobalide, pelargidenon 1449, panax notoginseng saponins, ligustrazine, tanshinone, tanshinol and panaxoside, have protective effects on nerve cell injury[17]. Extracts from rabbit skin inflamed by the vaccinia virus is a biological product composed of multiple components, which have analgesic and immunoregulatory effects, improve autonomic nerve function, promote axon growth and Schwann cell proliferation, and antagonize hypoxia/ischemia- or glutamic acid-induced nerve cell injury[18].
Results from the present study showed that the number of apoptotic cells and caspase-9 protein expression significantly increased in the bupivacaine group. However, in the analgecine pretreatment group, the

duration of sensory dysfunction was not significantly prolonged, cell morphology in the dorsal root ganglion was nearly normal, and the number of apoptotic cells and caspase-9-positive cells did not significantly increase. Extracts from rabbit skin inflamed by the vaccinia virus have been shown to inhibit oxidation, reduce reactive oxygen species production and regulate thioredoxin expression, thereby decreasing apoptotic cell death[19-20]. In addition, these extracts can inhibit apoptosis and protect spinal cord neurons by influencing endogenous Bcl-2 expression. Following end-to-end anastomosis of the sciatic nerve, early application of extracts from rabbit skin inflamed by the vaccinia virus increased the ratio of Bcl-2/Bax and protected spinal cord anterior horn motor neurons against apoptosis[21]. In addition, extracts have also been used to treat intraspinal anesthesia-induced peripheral nerve injury, resident symptoms following surgery for lumbar spinal stenosis and tumor-related peripheral nerve disease[22-24]. Moreover, they are significantly effective in preventing pain and improving paraesthesia and numbness[22-24]. Analgecine can restore synaptic transmission and promote axon formation by regulating the cell immune system, activating nerve cells and promoting repair of nerve injury[25-27].

In summary, analgecine pretreatment can alleviate spinal neurotoxicity induced by intrathecal injection of bupivacaine in pregnant rats, possibly by inhibiting caspase-9 protein expression and reducing nerve cell apoptosis.

MATERIALS AND METHODS

Design
A randomized, controlled animal study.

Time and setting
The experiments were performed in the Laboratory of Neurosurgery, Zhujiang Hospital of Southern Medical University, China from June 2009 to February 2010.

Materials
Healthy female Sprague-Dawley rats, weighing 200–220 g and healthy male Sprague-Dawley rats, weighing 180–200 g, were provided by the Laboratory Animal Center of Guangdong Province in China (license No. SCXK (Yue) 2008-0002). Animals were separately housed in a barrier system at 18–28°C, 40–70% humidity, and allowed free access to food and water. During the heat period, female rats and male rats were housed in one cage at a ratio of 5:1. A vaginal smear was

conducted daily, and the day that sperm was observed was regarded as the first day of pregnancy. A total of 18 rats that were pregnant for 17 days, weighing 350–400 g, were selected.

Methods

Intrathecal catheterization

Rats were anesthetized by intraperitoneal injection of 10% (w/v) chloral hydrate (250 mg/kg; Guangzhou Zhujiang Hospital, Guangzhou, China). A blunt protuberance at the lower segment of the spinal column, hip nodule, was located at L_6[28], and a PE-10 catheter (AniLab Software & Instruments Co., Ltd., Ningbo, Jiangsu Province, China), 2 cm, was inserted headward through the L_{5-6} intervertebral space. The catheter was fixed and embedded subcutaneously. During catheterization, clear cerebrospinal fluid slowly flowed from the catheter, indicating the site of the subarachnoid space. Following catheterization, the rats were intramuscularly injected with benzylpenicillin sodium, and the temperature was maintained for 1–2 hours by table lamp irradiation. Rats were separately housed for 2 days, and those with limb motor dysfunction were excluded.

Preparation of analgecine dilution

Analgecine stock solution (batch No. 20070107; potency: 26.2 U/mL; Weishi Pharmaceutical, Hong Kong, China), 4 500 U, was mixed with water for injection to 450 mL (10 U/mL), desalinized until the sample osmotic pressure ratio was isoosmotic (compared with 0.9% (w/v) NaCl solution), filtered through a 0.22 μm filtration membrane in a sterile room, packaged in nine 50-mL sample bottles, filled with nitrogen, sealed and stored in the dark.

Medication

In the control group, 30 μL normal saline was intrathecally injected, followed by intraperitoneal injection of normal saline, 10 mL/kg. In the bupivacaine group, 30, 20 and 10 μL bupivacaine (2% (w/v)) was intrathecally injected three times, every 1 hour, followed by intraperitoneal injection of normal saline, 10 mL/kg. In the analgecine pretreatment group, 30, 20 and 10 μL bupivacaine (2% (w/v)) was intrathecally injected three times, every 1 hour, followed by intraperitoneal injection of analgecine (10 mL/kg). Following intrathecal injection, the catheter was washed using 10 μL normal saline. A dose of 2% (w/v) bupivacaine for intrathecal injection was used according to previous studies[9, 29].

Analgecine administration method

Analgecine dilution (10 U/mL) was injected for 3 days prior to intrathecal administration and 4 days after intrathecal administration, 100 U/kg, once a day, according to the manufacturer's instructions. The bupivacaine and control groups were intraperitoneally injected with equal volumes of normal saline (10 mL/kg), once a day.

Pain threshold detection following intrathecal injection of bupivacaine

Tail-flick latency of pregnant rats was detected at 1, 2, 3, 4 and 5 days following intrathecal injection of bupivacaine[9]. Briefly, different sites at the middle and lower 1/3 of the tail were irradiated using a high-intensity light beam of Tail Flick Analgesia Meter (YLS-12A, Shandong Academy of Medical Sciences, Shandong Province, China). Time from irradiation to tail flick reaction was regarded as the tail-flick latency. If rats did not flick their tail after 10 seconds of irradiation, tail-flick latency was regarded as 10 seconds. Determination was performed three times, at 15 second intervals, and the mean value was calculated. Maximum possible effect of tail-flick latency = (tail-flick latency after administration – baseline tail-flick latency)/(10 – baseline tail-flick latency) ×100%.

Lower limb motor function

Motor function was assessed at 1, 2, 3, 4 and 5 days following intrathecal injection of bupivacaine. Scores were represented by 0, no block; 1, partial block; 2, complete block. The addition of both lower limbs was the result value[9].

Isolation of the dorsal root ganglion

Pregnant rats were sacrificed at 4 days following intrathecal injection of bupivacaine and placed on ice. A median incision was made at the back, the skin and subcutaneous tissue were cut open, and muscle around the spinal column was blunt isolated to expose the vertebral spinous process and transverse process of the lower segment of the lumbar vertebra. The spinous process, vertebral plate and bilateral transverse process were removed, and the intervertebral foramen was clamped using a mosquito clamp. The trunk of the spinal nerve at the two sides of the dorsal root ganglion was removed, and intumescentia was maintained, *i.e.* dorsal root ganglion.

Pathohistological observation of the dorsal root ganglion

Rats were anesthetized by intraperitoneal injection of 10% (w/v) chloral hydrate (250 mg/kg) at 4 days after administration. The thoracic cavity was opened, and left ventricle intubation was conducted from the aorta; the right auricle was cut, perfused with 300 mL normal saline,

followed by 4% paraformaldehyde (400 mL) at 4°C. The dorsal root ganglion was harvested, paraffin embedded, sectioned (4 μm thick), stained with hematoxylin-eosin, and observed under a 100 and 400× magnification light microscope (BX50, Olympus, Tokyo, Japan).

TdT-mediated dUTP-biotin nick end labeling detection (TUNEL) for cell apoptosis in the dorsal root ganglion

The tissues were dehydrated at 4 days following intrathecal injection of bupivacaine, embedded using paraffin, sectioned, dewaxed and rehydrated. Sections were incubated in 3% (v/v) H_2O_2 at room temperature, washed with distilled water, digested in freshly diluted protease at 37°C, mixed with blocking solution at room temperature for 30 minutes, incubated in diluted biotinylated digibind (500 μL per pill; Boster, Wuhan, China) at 37°C for 30 minutes, followed by streptavidin biotin peroxidase complex (1:100; Boster) at 37°C for 30 minutes. The sections were visualized using diaminobenzidine, washed with distilled water, mildly counterstained with hematoxylin-eosin, and observed using light microscopy (Olympus). Cells with brown yellow particles in the nuclei were regarded as positive cells. Four nonoverlapping visual fields were randomly selected from each section to quantify apoptotic cells using a microimaging analysis system (Meta Morph/DD10/BX51, Universal Imaging Corporation, Sunnyvale, CA, USA).

Immunohistochemistry for caspase-9 protein expression in the dorsal root ganglion

Tissues were dehydrated at 4 days following intrathecal injection of bupivacaine, embedded using paraffin, sectioned, dewaxed and rehydrated, reacted with 3% (v/v) H_2O_2 at room temperature for 5 minutes, washed with distilled water, immersed in PBS for 5 minutes, and subjected to antigen retrieval using a microwave oven (98–100°C). Sections were then incubated with normal goat serum at room temperature for 10 minutes, rabbit anti-rat caspase-9 polyclonal antibody (batch No. F00037626; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 37°C overnight, washed with PBS three times, 5 minutes each, incubated with biotin labeled goat anti-rabbit IgG (Boster) at 37°C for 20 minutes, washed with PBS three times, 5 minutes each, incubated with horseradish peroxidase-labeled streptavidin (Fuzhou Maixin Bio, Fujian, China) at 37°C for 20 minutes and washed with PBS three times, 5 minutes each. Sections were visualized using diaminobenzidine, washed with distilled water, counterstained and mounted. PBS rather than primary antibody was used as a control. Coloration results were semiquantiatively determined using immunohistochemistry. Cells with a brown cytoplasm and membrane were regarded as positive cells, and photos were captured using a 400 magnification light microscope under the same light intensity (Olympus). Five fields of view were selected from each section, and positive cells were quantified using a microimaging analysis system (Meta Morph/DD10/BX51, Universal Imaging Corporation).

Statistical analysis

Measurement data were expressed as mean ± SD and analyzed using SPSS 13.0 software (SPSS, Chicago, IL, USA). Intergroup comparison of tail-flick latency prior to and following intrathecal catheterization was analyzed using one-way analysis of variance, and comparison of tail-flick latency prior to and following intrathecal catheterization was analyzed using the paired *t*-test. Maximum possible effect comparison was conducted using repeated measures analysis of variance. Comparison of the number of apoptotic cells and caspase-9-positive cells was analyzed using one-way analysis of variance; multiple comparisons were conducted using the Bonferroni (homogeneic variance) or Dunnett T3 test (heterogeneic variance). A value of $P < 0.05$ was considered statistically significant.

REFERENCES

[1] Rorarius M, Suominen P, Haanpaa M, et al. Neurologic sequelae after caesarean section. Acta Anaesthesiol Scand. 2001;45(1):34-41.

[2] Levy DM. Emergency caesarean section: best practice. Anaesthesia. 2006;61(8):786-791.

[3] Wan XH, Huang QQ, Su MX, et al. The investigation in application and security of continuous epidural analgesia. Zhonghua Mazuixue Zazhi. 2004;2(2):156-157.

[4] Ikeya K. Neurological deficit following lumbar epidural anesthesia with ropivacaine. Masui. 2007;56(8):945-948.

[5] Dar AQ, Robinson AP, Lyons G. Postpartum neurologic symptoms following regional blockade: a prospective study with case controls. Int J Obstet Anesth. 2002;11(2):85-90.

[6] Wong CA, Scavone BM, Dugan S, et al. Incidence of postpartum lumbosacral spine and lower extremity nerve injuries. Obstet Gynecol. 2003;101(2):279-288.

[7] Werdehausen R, Braun S, Essmann F, et al. Lidocaine induces apoptosis via the mitochondrial pathway independently of death receptor signaling. Anesthesiology. 2007;107(1):136-143.

[8] Cui Ru, Xu SY, Lei HY, et al. Effect of pregnancy of bupivacaine for spinal anesthesia in rats. Zhonghua Mazuixue Zazhi. 2010;30(9):1136-1138.

[9] Johnson ME, Uhl CB, Spittler KH, et al. Mitochondrial injury and caspase activation by the local anesthetic lidocaine. Anesthesiology. 2004;101(5):1184-1194.

[10] Neal JM. Effets of epinephrine in local anesthetic on the central and peripheral nervous system: neurotoxicity and neural blood flow. Reg Anesth Pain Med. 2003;28(2):124-134.

[11] Polley LS, Columb MO, Naughton NN, et al. Effect of epidural epinephrine on the minimum local analgesic concentration of epidural bupivacaine in labor. Anesthesiology. 2002;96(5):1123-1128.

[12] Park CJ, Park SA, Yoon TG, et al. Bupivacaine induces apoptosis via ROS in the Schwann cell line. J Dent Res. 2005;84(9):852-857.

[13] Lee HT, Xu H, Siegel CD, et al. Local anesthetics induce human renal cell apoptosis. Am J Nephrol. 2003;23(3):129-139.

[14] Tan Z, Dohi S, Chen J, et al. Involvement of the mitogen-activated protein kinase family in tetracaine induced PC12 cell death. Anesthesiology. 2002;96(5):1191-1201.

[15] Radwan I, Satio S, Goto F. Growth cone collapsing effect of lidocaine on DRG neuronsis partially reversed by several neurotrophic factors. Anesthesiology. 2002;97(3):630-635.

[16] Jia RZ, Jiang L, Qiao LX. Study on effect of radix astragali on injury of cerebral cortex in neonatal rats after hypoxia/ischemia brain damage. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2005;25(1):54-57.

[17] Li J, Wei YZ, Fan ZG. Present research on neuroprotective effects of Chinese medicinal herb. Zhongguo Linchuang Kangfu. 2005;41(11):108-110.

[18] Kudo T, Kushikata T, Kudo M, et al. Antinociceptive effects of neurotropin in a rat model of central neuropathic pain: DSP-4 induced noradrenergic lesion. Neurosci Lett. 2011;503(1):20-22.

[19] Hoshino Y, Nakamura T, Sato A, et al. Neurotropin demonstrates cytoprotective effects in lung cells through the induction of thioredoxin-1. Am J Respir Cell Mol Biol. 2007;37(4):438-446.

[20] Chen Z, Che J, Hou Y, et al. The extract of inflamed rabbit skin induced by inoculation of vaccinia virus possesses antioxidant and neuroprotective effects in acute ischemic stroke. J Stroke Cerebrovasc Dis. 2009;18(6):475-481.

[21] Qi W, Cheng AG, Liu H, et al. Possible protective effect and mechanism of neurotropin on spinal cord motoneuron after sciatic nerve injury in rats. Zhongguo Kangfu Lilun yu Shijian. 2008;14(8):703-704.

[22] Zhan WJ, Fang K. Clinical observation of treatment peripheral neuropathy after spinal anesthesia with neurotropin. Zhongguo Tengtong Yixue Zazhi. 2009;15(4):254-255.

[23] Gao BL, Yang Y, Zhao JM, et al. Investigation of the management of neurotropin for remnant symptom after post operation of lumbar spinal canal stenosis. Zhongguo Tengtong Yixue Zazhi. 2010;16(6):331-333.

[24] Lan PL, Zhu JC, Meng LX. Efficacy of neurotropin combined with nerve block for patients with tumor-associated peripheral neuropathy. Zhongguo Tengtong Yixue Zazhi. 2011;17(10):607-609.

[25] Li YH, Chen J, Xu X. Clinical observation of treatment diabetic peripheral neuropathy with analgecine. Zhongguo Yiyao Zhinan. 2009;7(13):35-37.

[26] Wang XH, Zhang DH, Lu SM, et al. Effect of mecobalamin combined with Honghua on refractory type 2 diabetic peripheral neuropathy. Lanzhou Daxue Xuebao: Yixue Ban. 2011;37(3):58-60.

[27] Yang XM, He RG. Effect of extracts from rabbit skin inflamed by vaccinia virus in the management of postherpetic neuralgia and on serum interleukin-6 level in aged patients. Nan Fang Yi Ke Da Xue Xue Bao. 2007;27(12):1941-1943.

[28] Yang AF, Wang P. The Anatomy of the Rats and Organization. Bejing: Science Press, China. 1985.

[29] Yamashita A, Matsumoto M, Matsumoto S, et al. A comparison of the neurotoxic effects on the spinal cord of tetracaine, lidocaine, bupivacaine, and ropivacaine administered intrathecally in rabbits. Anesth Analg. 2003;97(2):512-519.

[30] Pollock JE. Transient neurologic symptoms: etiology, risk factors, and management. Reg Anesth Pain Med. 2002;27(6):581-586.

[31] Faubel S, Edelstein CL. Caspases as drug targets in ischemic organ injury. Curt Drug Targets Immune Endocr Metabol Disord. 2005;5(3):269-287.

(Reviewed by Diwakarla S, Raye W, Zheng XY, Xiu B)
(Edited by Wang J, Su LL, Li CH, Song LP)