ORIGINAL RESEARCH

Adnan Tüfek MD¹
Savaş Kaya MD, PhD²
Orhan Tokgöz MD¹
Uğur Fırat MD³
Osman Evliyaoğlu MD⁴
Feyzi Çelik MD¹
Haktan Karaman MD¹

¹ Department of Anesthesiology, ² Department of Immunology, ³ Department of Pathology, and ⁴ Department of Biochemistry, School of Medicine, Dicle University, 21280 Diyarbakır, Turkey

The protective effect of dexmedetomidine on bupivacaine-induced sciatic nerve inflammation is mediated by mast cells

Abstract

Purpose: This study was designed to assess the correlation between the neuroprotective effect of dexmedetomidine and oxidative stress, neural inflammation and mast cell stability in rats with bupivacaine-induced sciatic nerve toxicity.

Methods: Forty adult Wistar Albino rats, eight rats per group, were used. Saline (0.3 ml of 0.9%), dexmedetomidine (20 µg/kg), 0.5% bupivacaine or 0.5% bupivacaine+dexmedetomidine (20 µg/kg) was injected into the sciatic nerve. A control group of rats received no injection. Fourteen days after injection, the sciatic nerves were harvested and total oxidant status, total anti-oxidant status, paraoxonase-1, galectin-3 and matrix metalloproteinase 2 and 9 levels were measured in the sciatic nerves. In addition, the presence and status of inflammation, edema, and mast cells were evaluated histopathologically.

Results: The combination of dexmedetomidine and bupivacaine alleviated oxidative stress. In addition, it decreased matrix metalloproteinase 9 and galectin-3 levels and increased matrix metalloproteinase 2 levels. Moreover, it stabilized recruited mast cells at the injury site; however, it did not significantly decrease inflammation or edema.

Conclusion: Dexmedetomidine may ameliorate bupivacaine-induced neurotoxicity by modulating mast cell degranulation. The neuroprotective effect of dexmedetomidine may make it a suitable adjuvant agent to local anesthetics in peripheral nerve blocks.

Manuscript submitted 14th November, 2012
Manuscript accepted 25th February, 2013

Clin Invest Med 2013; 36 (2): E1 -E8.

Correspondence to:

Adnan Tüfek,
Department of Anesthesiology, School of Medicine,
Dicle University, 21280 Diyarbakır, Turkey
e-mail: adnantufek@hotmail.com

Bupivacaine is a long-acting local anesthetic with severe toxicity [1]. It can trigger apoptosis in nerve cells, depending on exposure time and concentration [2,3]. Bupivacaine-induced apoptosis in the Schwann cell line is associated with reactive oxygen species (ROS) production [4]. Furthermore, in bupivacaine-induced injury, mast cells play an important role in the accumulation of neutrophils at the injury site [5].

Schwann cells in peripheral nerve injury and activated macrophages express galectin-3, a galactose-specific lectin formerly known as MAC-2 [6,7]. Galectin-3, present on activated macrophages, is also a marker of Schwann cell damage. Matrix metalloproteinase 9 (MMP-9), which is increased in early phases of inflammation, is expressed by macrophages and other inflammatory cells [8]. In addition, MMP-9 inhibition is correlated with amelioration of inflammation. Matrix metalloproteinase 2 (MMP-2), which is increased in the late phase of inflammation, is expressed by activated fibroblasts and is necessary for fibrosis [9,10]. In other words, galectin-3 is a useful marker of activated macrophages and injured Schwann cells. MMP-9 is also a marker of inflammatory cells and macrophages and MMP-2 is a marker of activated fibroblast and is seen during the late phase of inflammation.

Recently, a new role other than allergic reaction has been noted for mast cells in inflammation and wound healing processes [11-13]. Mast cells are crucial sensors of cell injury and initiators of proinflammatory responses [14]. In addition, early studies suggest that the alpha-2 adrenergic receptor agonist clonidine may modulate mast cell function [15-18].

Compared with the alpha-2 adrenoceptor agonist, clonidine, dexmedetomidine has an eight-times higher affinity for the alpha-2 adrenoceptor [19]. In addition, perineural administration of dexmedetomidine ameliorates bupivacaine-induced perineural inflammation in rats [20]. It has been suggested that the neuroprotective effects of dexmedetomidine depend on reduced ROS production in the hippocampus of rabbits after a subarachnoid hemorrhage [21].

In this study, the protective effect of dexmedetomidine in bupivacaine-induced sciatic nerve injury was investigated to determine if it was associated with mast cell modulation.

Materials and Methods

Ethical approval for this study (Ethical Committee N° 2010/39) was provided by the Local Animal Ethical Committee of Dicle University, Diyarbakir, Turkey (Chairperson Prof. M. Serdar Kemaloğlu) on 27 October 2010. In this study, rats were handled in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Local Animal Ethical Committee of Dicle University.

Forty adult female Wistar Albino rats, average weight 250 g, were obtained from the Laboratory Animals Facility of Dicle University.

Intraneural Injection into the Sciatic Nerve

The rats were divided randomly into five groups with eight rats per group. In rats of all groups, the sciatic nerve was surgically exposed. In groups II-V, rats received 0.3 ml intraneural injection into the right sciatic nerve. In group I (sham group), the rats received no injection. In group II (saline group), rats received an injection of 0.9% NaCl. In group III (bupivacaine group), rats received an injection of 0.2 ml of 0.5% bupivacaine hydrochloride (4 mg/kg; Marcaine™, produced by Eczacıbaşı under license from Astra Zeneca PLC, Lüleburgaz, Turkey) and 0.1 ml of 0.9% NaCl. In group IV (dexmedetomidine group), rats received an injection of dexmedetomidine hydrochloride (20 µg/kg; Precedex™, Hospira, North Rocky Mount, NC 27801, USA). In group V (bupivacaine+dexmedetomidine group), rats received an injection of 0.2 ml of 0.5% bupivacaine hydrochloride (4 mg/kg) and 0.1 ml dexmedetomidine hydrochloride (20 µg/kg). Before procedures, all rats were anesthetized with intraperitoneal ketamine hydrochloride (80 mg/kg). The surgical area was shaved and disinfected with povidone iodine. All surgical procedures were performed under aseptic conditions and under a microscope. Surgical incision was made through the gluteal muscles in the prone position, as described elsewhere [22]. The sciatic nerve was exposed in the trifurcation region. Injections were performed 5 mm proximal to this point with a 30 G needle, intraneurally, either intra- or extra-fascicular. Injections were confirmed by the appearance of a fusiform swelling of the sciatic nerve. All injections were performed by the same researcher. After injection, muscle and skin were closed and 14 days later the rats were sacrificed and sciatic nerve samples were collected for examination.

Measurement of Oxidative Stress

The excised sciatic nerve samples were weighed and immediately stored at -80°C. Assays were performed on the supernatant of the prepared homogenate at 14,000 rpm for 30 min at +4°C. The protein concentration of the tissues was measured by the Lowry method [41]. Using a modified version of the Eckerson method, serum paraoxonase-1 (PON-1) levels were measured spectrophotometrically [23]. The total antioxidant status (TAS) and total oxidant status (TOS) of supernatant fractions were evaluated using novel automated and colorimetric measurement methods developed by Erel [24,25]. The TAS results are expressed as nmol Trolox equivalent/mg protein.

TABLE 1. Sciatic nerve tissue paraoxanase-1 (PON-1), total antioxidant status (TAS), total oxidant status (TOS), galectin-3, matrix metalloproteinase 2 and 9 (MMP-2 and 9) levels

Groups	PON-1	TAS	TOS	Galectin-3	MMP-2	MMP-9
(I) Sham	0.95±0.05	0.43±0.025	10.28±0.25	11.62±0.54	618.6±0.52	828.58±0.52
(II) Saline	0.72±0.20	0.55±0.10	12.25±2.56	10.06±1.28	593.87±120.18	915.45±72.62
(III) Dexmedetomidine	0.82±0.12	0.41±0.14	13.36±2.52	9.07±0.67	571.2±23.56	710.6±161.97
(IV) Bupivacaine	0.38±0.11	0.15±0.02	27.99±10.84	14.77±1.81	298.86±76.66	1293.83±304.64
(V) Bupivacaine + Dexmedetomidine	0.84±0.23	0.56±0.20	14.50±5.92	10.40±0.91	516.23±96.62	799.85±267.71
<i>P-values</i>						
I-II	0.124	0.300	0.751	0.150	0.723	0.680
I-III	0.377	0.884	0.619	0.023	0.498	0.575
I-IV	0.000	0.005	0.001	0.001	0.000	0.010
I-V	0.363	0.149	0.392	0.155	0.072	0.863
III-IV	0.001	0.008	0.006	<0.001	<0.001	0.002
IV-V	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

TAS, TOS, PON-1, galectin-3, MMP-2 and MMP-9 measured in isolated rat sciatic nerve samples 14 days after injection; n=8 rats/group.

The TOS results are expressed as $\mu\text{mole H}_2\text{O}_2$ equivalent/g protein.

Assessment of Galectin-3, MMP-2 and MMP-9 Levels with ELISA

In the sciatic nerve homogenate, galectin-3, MMP-2, and MMP-9 levels were assayed according to the product manual with rat galectin-3 ELISA kit (Catalog no. E90303Ra, Uscn Life Science Inc., China), rat MMP-2 ELISA kit (Catalog no. E90100Ra, Uscn Life Science Inc., China), and rat MMP-9 ELISA kit (Catalog no. E90553Ra, Uscn Life Science Inc., China), respectively.

Histopathological Evaluation

Sciatic nerve tissue samples were stored in 10% formaldehyde solution for 48 hours then embedded in paraffin and sliced with microtome into $5\mu\text{m}$ sections for histopathological analyses. Hematoxylin and eosin staining was performed and all samples were evaluated using a light microscope (Nikon ECLIPSE, 80i, Japan) by an expert pathologist, blinded to the study groups. Histopathological changes were scored (0=no inflammation, 1=small focal mild edema, 2=moderate edema and inflammation, 3=extensive edema and marked inflammation) [20]. Tissue samples were also stained with toluidine blue for detection of mast cells and were scored (0=no mast cells, 1=1-2 mast cells, 2=3-4 mast cells, 3=5 or more mast

cells) by counting intraneural mast cells at x200 magnification fields.

Statistics

Data are expressed as mean $\pm$ standard deviation (S.D.). The one-way analysis of variance (ANOVA) and *post hoc* multiple comparison tests (LSD) were performed on the data of biochemical and histopathological variables to examine differences among groups. A P-value of <0.05 was considered statistically significant.

Results

Effects of Dexmedetomidine on Bupivacaine-induced Oxidative Stress in the Sciatic Nerve

TAS was almost equal in the sciatic nerve tissue of the sham, saline, dexmedetomidine and bupivacaine+dexmedetomidine groups, but was significantly lower in the bupivacaine group (Table 1). There was no difference in TOS between the sham, saline and dexmedetomidine groups. TOS increased significantly in the bupivacaine group but approached the sham level in the bupivacaine+dexmedetomidine group (Table 1). PON-1 levels in the sham, saline and dexmedetomidine groups were similar, but decreased significantly in the sciatic nerve tissue of the bupivacaine group. PON-1 level in the bupivacaine+dexmedetomidine group was as high as in the sham group (Table 1).

TABLE 2. Inflammation, edema and mast cells scores of all groups.

Groups	inflammation	edema	mast cells
(I) Sham	0	0	0
(II) Saline	0.25±0.46	0.38±0.74	0
(III) Dexmedetomidine	1.50±0.53	1.25±0.70	2.75±0.46
(IV) Bupivacaine	2.87±0.35	2.63±0.51	2.25±0.70
(V) Bupivacaine + Dexmedetomidine	2.50±0.75	2.25±0.88	2.87±0.35
<i>P values</i>			
I-II	0.314	0.256	
I-III	<0.001	<0.001	<0.001
I-IV	<0.001	<0.001	<0.001
I-V	<0.001	<0.001	<0.001
III-IV	<0.001	<0.001	0.020
III-V	<0.001	0.004	0.546
IV-V	0.134	0.256	0.004

Hematoxylin and eosin stained samples evaluated at 200 magnification fields.

Effects of Dexmedetomidine on Bupivacaine-induced Inflammation and Nerve Injury

The level of galectin-3, an indicator of Schwann cell injury and marker of activated macrophages, did not differ significantly in the sham, saline and dexmedetomidine groups; however, a markedly higher level of galectin-3 was observed in the bupivacaine group. Interestingly, in the bupivacaine+dexmedetomidine group, the galectin-3 level was at the level of the sham group (Table 1).

MMP-9, produced by macrophages and other inflammatory cells except T cells and mast cells, was detected in similar amounts in the sham, saline and dexmedetomidine groups. As expected, MMP-9 was produced in significantly higher amounts in the bupivacaine group; however, the MMP-9 level was similar to the sham group in the bupivacaine+dexmedetomidine group (Table 1).

The level of MMP-2, which is produced by fibrocytes, was almost the same in the sham, saline and dexmedetomidine group, but significantly lower in the bupivacaine group. Surprisingly, the MMP-2 level in the bupivacaine+dexmedetomidine group was comparable to the value of the sham group (Table 1).

Effects of Dexmedetomidine on Bupivacaine-induced Mast Cell Recruitment

The degrees of inflammation and edema were lowest and almost the same in sham and saline groups. In the dexmedetomidine group, mild to moderate edema and cell infiltrate were

detected in the sciatic nerve tissue. Edema and cell infiltrate in the sciatic nerve were marked in the bupivacaine group but less marked in the bupivacaine+dexmedetomidine group (Table 2).

Besides inflammation and edema, the presence and appearance of mast cells were evaluated. In the sham and saline groups, mast cells were absent at the injury site. In the dexmedetomidine and bupivacaine+dexmedetomidine groups, the number of mast cells was significantly higher than in bupivacaine group (Table 2). On the other hand, in the bupivacaine group, granulating and degranulated mast cells were evident than in the dexmedetomidine and bupivacaine+dexmedetomidine groups (Fig. 1). Figure 1B is a representative image of a large mast cell degranulation in the bupivacaine group. Stabilized mast cells were smaller in size in dexmedetomidine group than in the bupivacaine group (Fig. 1A and 1C).

Discussion

Our objective was to study the possible protective effects of dexmedetomidine in bupivacaine-induced sciatic nerve injury in rats. The therapeutic potential of dexmedetomidine, when it is combined with bupivacaine, may be associated with decreased oxidative stress (marked with increased TAS, decreased TOS and increased PON-1), decreased galectin-3, increased MMP-2 and decreased MMP-9. Inflammatory scores and edema scores of the dexmedetomidine-injected group and bupivacaine-injected group were not significantly different.

FIGURE 1. Morphology of representative mast cells in dexmedetomidine (A), bupivacaine (B) and dexmedetomidine+bupivacaine (C) groups in rat sciatic nerve samples. Panel B shows representative, degranulating mast cells (filled arrows) and free mast cell granules (outlined arrows). Panels A and B present representative intact mast cells (filled arrows). Samples are stained with toluidine blue and photographs are captured from 1000 magnification fields.

The existence of mast cells is clearly observed in all bupivacaine- and dexmedetomidine-injected groups; however, the mast cells in groups injected with dexmedetomidine are not degranulated.

Our results suggest that mast cells may play a prominent role in bupivacaine-induced nerve injury. New findings about mast cells show that they are crucial in the early phases of tissue injury and inflammation. Mast cells are the first cells recruited to the injury site and produce pro-inflammatory mediators selectively without degranulation for recruitment of neutrophils, macrophages and other monocytes [13]. The cytotoxic side effect of bupivacaine on nerves and the presence of mast cells in bupivacaine-induced injury site are reported elsewhere [4,5]. In parallel, this study is the first to reveal accumulation of mast cells in bupivacaine-induced injury in the sciatic nerve (Fig. 1B). In our study, an increased level of galectin-3 and high oxidative stress may be an indicator of the presence of macrophages in bupivacaine-induced sciatic nerve injury. High oxidative stress itself may be a reflection of the presence of neutrophils at the site. In addition to Schwann cells in peripheral nerve injury, it is well known that macrophages, particularly activated ones, express galectin-3 [6,7,26]. Moreover, during the phagocytosis process, phagocytes such as macrophages and neutrophils produce high levels of ROS; therefore, assessment of oxidative stress in tissue may supply information that is directly correlated to the number of phagocytes present at the injury region [27-29]. It has been suggested that mast cell density is strongly associated with expression of MMP-9 in chronic inflammation [30]. It has also been reported that MMP-9 plays

a critical role in the progression of inflammation in the early phases, specifically MMP-9 produced by macrophages and other inflammatory cells but not by T cells and mast cells [8]. MMP-2 is mostly produced by fibrocytes, which are known to be active in the late phases of the wound healing process [8, 31-33]. We show here that high MMP-9 and low MMP-2 levels are observed in a bupivacaine-injured sciatic nerve. These findings, along with histopathological results, suggest that bupivacaine induces intense inflammation, with infiltrated macrophages and other inflammatory cells, and with low levels of fibrocytes. In summary, these findings confirm that the attraction of mast cells to the injection site has a role in initiating intense inflammation in bupivacaine-induced neurotoxicity.

It has been reported that degranulation of mast cells exacerbates inflammation in bupivacaine-injected tissue. Nonetheless, sodium cromoglycate inhibits degranulation of mast cells; therefore, the number of neutrophils recruited to the bupivacaine-injected site is decreased [5]. Studies in animal models indicate that stabilized mast cells are present in the early stages improves wound healing [34]. Histamine is used as a marker of mast cell degranulation and this histamine release from the mast cells is inhibited by tryptase and chymase inhibitors [35, 36]. Both tryptase and chymase originate from mast cell granules have crucial roles in the recruitment of inflammatory cells to an injury [37-40]. It has also been reported that clonidine, an α -2 adrenoceptor agonist, inhibits histamine release and degranulation of mast cells [15, 16]. Another study reported that dexmedetomidine, a potent α -2 adrenoceptor agonist, has an eight times higher affinity for the α -2

adrenoceptor than clonidine [19]. All the literature presented so far suggests that dexmedetomidine may be a strong stabilizer of mast cells and may inhibit inflammation by blocking degranulation. Thus, the mast cell stabilizing potential of dexmedetomidine may explain its protective effect in bupivacaine-induced sciatic nerve injury.

Although bupivacaine-induced neurotoxicity is a rare adverse effect seen in peripheral nerve blocks, it may be very serious and frequent in patients with diseases such as diabetes mellitus and accompanying neuropathy. In these patients, dexmedetomidine may be the choice of drug as an adjuvant. In addition, the method presented here may be a good model for the study of neuropathy.

Limitations

This study focused on biochemical and histopathological findings. There was no neurobehavioral examination of the rats; therefore, it is hard to make a full assessment of clinical importance of these findings.

The dose of dexmedetomidine used in this study was adjusted according to previously published animal studies; however, it exceeds the dose appropriate for humans. These findings should be replicated in lower doses.

It is speculated that dexmedetomidine effect on mast cell may depend on alpha-2 adrenoceptor although this hypothesis was not tested with an antagonist agent.

Conclusion

Both bupivacaine and dexmedetomidine attract mast cells to the injection region of the sciatic nerve: bupivacaine results in inflammation and nerve injury, whereas dexmedetomidine does not. It seems that the neuroprotective effect of dexmedetomidine depends on its mast cell modulating potential. Thus, our findings suggest that dexmedetomidine, with its neuroprotective effect, may be a suitable adjuvant for regional anesthesia.

Acknowledgments

This work was supported by Dicle University Scientific Research Project Coordination Unit (DÜBAP), Diyarbakır, Turkey (Grant No 10-TF-152).

References

1. Casati A, Putzu M. Bupivacaine, levobupivacaine and ropivacaine: are they clinically different? *Best Pract Res Clin Anaesthesiol.* 2005 Jun;19(2):247-68. PubMed PMID: 15966496. Epub 2005/06/22. eng.
2. Perez-Castro R, Patel S, Garavito-Aguilar ZV, Rosenberg A, Recio-Pinto E, Zhang J, et al. Cytotoxicity of local anesthetics in human neuronal cells. *Anesth Analg.* 2009 Mar;108(3):997-1007. PubMed PMID: 19224816. Epub 2009/02/20. eng.
3. Selander D, Brattsand R, Lundborg G, Nordborg C, Olsson Y. Local anesthetics: importance of mode of application, concentration and adrenaline for the appearance of nerve lesions. An experimental study of axonal degeneration and barrier damage after intrafascicular injection or topical application of bupivacaine (Marcain). *Acta anaesthesiologica Scandinavica.* 1979 Apr;23(2):127-36. PubMed PMID: 442943.
4. Park CJ, Park SA, Yoon TG, Lee SJ, Yum KW, Kim HJ. Bupivacaine induces apoptosis via ROS in the Schwann cell line. *J Dent Res.* 2005 Sep;84(9):852-7. PubMed PMID: 16109997. Epub 2005/08/20. eng.
5. Cote CH, Tremblay MH, Duchesne E, Lapoite BM. Inflammation-induced leukocyte accumulation in injured skeletal muscle: role of mast cells. *Muscle Nerve.* 2008 Jun;37(6):754-63. PubMed PMID: 18506708. Epub 2008/05/29. eng.
6. Novak R, Dabelic S, Dumic J. Galectin-1 and galectin-3 expression profiles in classically and alternatively activated human macrophages. *Biochim Biophys Acta.* 2011 Dec 6. PubMed PMID: 22155450. Epub 2011/12/14. Eng.
7. Reichert F, Saada A, Rotshenker S. Peripheral nerve injury induces Schwann cells to express two macrophage phenotypes: phagocytosis and the galactose-specific lectin MAC-2. *J Neurosci.* 1994 May;14(5 Pt 2):3231-45. PubMed PMID: 8182468. Epub 1994/05/01. eng.
8. Matsumoto Y, Park IK, Kohyama K. Matrix metalloproteinase (MMP)-9, but not MMP-2, is involved in the development and progression of C protein-induced myocarditis and subsequent dilated cardiomyopathy. *J Immunol.* 2009 Oct 1;183(7):4773-81. PubMed PMID: 19734212. Epub 2009/09/08. eng.
9. Rodriguez D, Morrison CJ, Overall CM. Matrix metalloproteinases: what do they not do? New substrates and biological roles identified by murine models and proteomics. *Biochim Biophys Acta.* 2010 Jan;1803(1):39-54. PubMed PMID: 19800373. Epub 2009/10/06. eng.
10. Bergman MR, Teerlink JR, Mahimkar R, Li L, Zhu BQ, Nguyen A, et al. Cardiac matrix metalloproteinase-2 expression independently induces marked ventricular remodeling and systolic dysfunction. *Am J Physiol Heart Circ Physiol.* 2007 Apr;292(4):H1847-60. PubMed PMID: 17158653. Epub 2006/12/13. eng.
11. Kennelly R, Conneely JB, Bouchier-Hayes D, Winter DC. Mast cells in tissue healing: from skin to the gastrointestinal tract.

- Curr Pharm Des. 2011 Nov;17(34):3772-5. PubMed PMID: 22103847. Epub 2011/11/23. eng.
12. Younan GJ, Heit YI, Dastouri P, Kekhia H, Xing W, Gurish MF, et al. Mast cells are required in the proliferation and remodeling phases of microdeformational wound therapy. *Plast Reconstr Surg*. 2011 Dec;128(6):649e-58e. PubMed PMID: 22094766. Epub 2011/11/19. eng.
 13. Theoharides TC, Alysandratos KD, Angelidou A, Delivanis DA, Sismanopoulos N, Zhang B, et al. Mast cells and inflammation. *Biochim Biophys Acta*. 2012 Jan;1822(1):21-33. PubMed PMID: 21185371. Pubmed Central PMCID: 3318920. Epub 2010/12/28. eng.
 14. Enoksson M, Lyberg K, Moller-Westerberg C, Fallon PG, Nilsson G, Lunderius-Andersson C. Mast cells as sensors of cell injury through IL-33 recognition. *J Immunol*. 2011 Feb 15;186(4):2523-8. PubMed PMID: 21239713. Epub 2011/01/18. eng.
 15. Lindgren BR, Grundstrom N, Andersson RG. Comparison of the effects of clonidine and guanfacine on the histamine liberation from human mast cells and basophils and on the human bronchial smooth muscle activity. *Arzneimittelforschung*. 1987 May;37(5):551-3. PubMed PMID: 2441713. Epub 1987/05/01. eng.
 16. Anderson CD, Lindgren BR, Andersson RG. Effects of clonidine on the dermal inflammatory cell response of experimental toxic and allergic contact reactions and intradermal hypersensitivity. *Int Arch Allergy Appl Immunol*. 1987;83(4):371-6. PubMed PMID: 3610350. Epub 1987/01/01. eng.
 17. Lavand'homme PM, Eisenach JC. Perioperative administration of the alpha2-adrenoceptor agonist clonidine at the site of nerve injury reduces the development of mechanical hypersensitivity and modulates local cytokine expression. *Pain*. 2003 Sep;105(1-2):247-54. PubMed PMID: 14499442.
 18. Lavand'homme PM, Ma W, De Kock M, Eisenach JC. Perineural alpha(2A)-adrenoceptor activation inhibits spinal cord neuroplasticity and tactile allodynia after nerve injury. *Anesthesiology*. 2002 Oct;97(4):972-80. PubMed PMID: 12357167.
 19. Bhana N, Goa KL, McClellan KJ. Dexmedetomidine. *Drugs*. 2000 Feb;59(2):263-8; discussion 9-70. PubMed PMID: 10730549. Epub 2000/03/24. eng.
 20. Brummett CM, Norat MA, Palmisano JM, Lydic R. Perineural administration of dexmedetomidine in combination with bupivacaine enhances sensory and motor blockade in sciatic nerve block without inducing neurotoxicity in rat. *Anesthesiology*. 2008 Sep;109(3):502-11. PubMed PMID: 18719449. Pubmed Central PMCID: 2709835. Epub 2008/08/23. eng.
 21. Cosar M, Eser O, Fidan H, Sahin O, Buyukbas S, Ela Y, et al. The neuroprotective effect of dexmedetomidine in the hippocampus of rabbits after subarachnoid hemorrhage. *Surg Neurol*. 2009 Jan;71(1):54-9; discussion 9. PubMed PMID: 18207556. Epub 2008/01/22. eng.
 22. Whitlock EL, Brenner MJ, Fox IK, Moradzadeh A, Hunter DA, Mackinnon SE. Ropivacaine-induced peripheral nerve injection injury in the rodent model. *Anesth Analg*. 2010 Jul;111(1):214-20. PubMed PMID: 20442258. Epub 2010/05/06. eng.
 23. Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. *Am J Hum Genet*. 1983 Nov;35(6):1126-38. PubMed PMID: 6316781. Pubmed Central PMCID: 1685985. Epub 1983/11/01. eng.
 24. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem*. 2004 Feb;37(2):112-9. PubMed PMID: 14725941. Epub 2004/01/17. eng.
 25. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*. 2005 Dec;38(12):1103-11. PubMed PMID: 16214125. Epub 2005/10/11. eng.
 26. Rotshenker S. Wallerian degeneration: the innate-immune response to traumatic nerve injury. *J Neuroinflammation*. 2011;8:109. PubMed PMID: 21878125. Pubmed Central PMCID: 3179447. Epub 2011/09/01. eng.
 27. El-Benna J, Dang PM, Gougerot-Pocidalo MA, Elbim C. Phagocyte NADPH oxidase: a multicomponent enzyme essential for host defenses. *Arch Immunol Ther Exp (Warsz)*. 2005 May-Jun;53(3):199-206. PubMed PMID: 15995580. Epub 2005/07/05. eng.
 28. Brehm M, Schiller E, Zeller WJ. Quantification of reactive oxygen species generated by alveolar macrophages using lucigenin-enhanced chemiluminescence--methodical aspects. *Toxicol Lett*. 1996 Oct;87(2-3):131-8. PubMed PMID: 8914621. Epub 1996/10/01. eng.
 29. Park JB. Phagocytosis induces superoxide formation and apoptosis in macrophages. *Exp Mol Med*. 2003 Oct 31;35(5):325-35. PubMed PMID: 14646585. Epub 2003/12/04. eng.
 30. Souza Freitas V, de Andrade Santos PP, de Almeida Freitas R, Pereira Pinto L, de Souza LB. Mast cells and matrix metalloproteinase 9 expression in actinic cheilitis and lip squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2011 Sep;112(3):342-8. PubMed PMID: 21612954. Epub 2011/05/27. eng.
 31. Hanata K, Yamaguchi N, Yoshikawa K, Mezaki Y, Miura M, Suzuki S, et al. Soluble EMMPRIN (extra-cellular matrix metalloproteinase inducer) stimulates the migration of HEp-2 human laryngeal carcinoma cells, accompanied by increased MMP-2 production in fibroblasts. *Arch Histol Cytol*. 2007 Dec;70(5):267-77. PubMed PMID: 18431027. Epub 2008/04/24. eng.
 32. Wilkinson JM, Davidson RK, Swingler TE, Jones ER, Corps AN, Johnston P, et al. MMP-14 and MMP-2 are key metalloproteases in Dupuytren's disease fibroblast-mediated

- contraction. *Biochim Biophys Acta*. 2012 Jun;1822(6):897-905. PubMed PMID: 22342364. Epub 2012/02/22. eng.
33. Orsini G, Mazzoni A, Orciani M, Putignano A, Procaccini M, Falconi M, et al. Matrix metalloproteinase-2 expression induced by two different adhesive systems on human pulp fibroblasts. *J Endod*. 2011 Dec;37(12):1663-7. PubMed PMID: 22099901. Epub 2011/11/22. eng.
 34. Noli C, Miolo A. The role of mast cells in the early stages of wound healing. *Int Wound J*. 2010 Dec;7(6):540. PubMed PMID: 21073684. Epub 2010/11/16. eng.
 35. He S, Gaca MD, Walls AF. The activation of synovial mast cells: modulation of histamine release by tryptase and chymase and their inhibitors. *Eur J Pharmacol*. 2001 Feb 2;412(3):223-9. PubMed PMID: 11166285. Epub 2001/02/13. eng.
 36. He SH, Xie H, Zhang XJ, Wang XJ. Inhibition of histamine release from human mast cells by natural chymase inhibitors. *Acta Pharmacol Sin*. 2004 Jun;25(6):822-6. PubMed PMID: 15169639. Epub 2004/06/01. eng.
 37. Harvima IT, Nilsson G. Mast cells as regulators of skin inflammation and immunity. *Acta Derm Venereol*. 2011 Oct;91(6):644-50. PubMed PMID: 21879235. Epub 2011/09/01. eng.
 38. Iddamalgoda A, Le QT, Ito K, Tanaka K, Kojima H, Kido H. Mast cell tryptase and photoaging: possible involvement in the degradation of extra cellular matrix and basement membrane proteins. *Arch Dermatol Res*. 2008 Apr;300 Suppl 1:S69-76. PubMed PMID: 17968569. Epub 2007/10/31. eng.
 39. Tani K, Ogushi F, Kido H, Kawano T, Kunori Y, Kamimura T, et al. Chymase is a potent chemoattractant for human monocytes and neutrophils. *J Leukoc Biol*. 2000 Apr;67(4):585-9. PubMed PMID: 10770293. Epub 2000/04/19. eng.
 40. Terakawa M, Tomimori Y, Goto M, Fukuda Y. Mast cell chymase induces expression of chemokines for neutrophils in eosinophilic EoL-1 cells and mouse peritonitis eosinophils. *Eur J Pharmacol*. 2006 May 24;538(1-3):175-81. PubMed PMID: 16690053. Epub 2006/05/13. eng.
 41. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951 Nov;193(1):265-75. PubMed PMID: 14907713.