

The effects of local anaesthesia with bupivacaine and botulinum toxin-A after thoracotomy on stress hormone levels

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Aim: To investigate the effects of local and systemic analgesics on stress hormone levels, which increase after thoracotomy.

Materials and methods: Sixty-three male rats were divided into 9 groups (n = 7). In the control animals, only a blood sample was taken. In the thoracotomy group, a retractor was inserted for 1 h. In the local anaesthesia group, intercostal nerve block was performed with bupivacaine or botulinum toxin-A. In the systemic analgesia group, blockade was performed with bupivacaine and diclofenac sodium was given intraperitoneally. Blood samples were taken twice 4 h and 24 h after thoracotomy.

Results: At 4 h, stress hormone levels were significantly higher in all groups compared to the control (P < 0.05). In the systemic analgesia group, cortisol level was lower than in the thoracotomy group (P < 0.05), and lactate dehydrogenase level was lower than in the thoracotomy and bupivacaine groups (P < 0.05).

At 24 h, all stress hormones except glucose were significantly higher in all groups compared to the control (P < 0.05). Norepinephrine level was significantly lower in the systemic analgesia group as compared with the thoracotomy group (P < 0.05).

Conclusion: Our study showed that analgesic therapy alone may be insufficient to lower stress hormone levels, which increase after thoracotomy.

Key words: Stress hormone, thoracotomy, intercostal nerve block, analgesia, botulinum toxin-A, bupivacaine

Torakotomi sonrası bupivakain ve botulinum toksin-A ile uygulanan lokal anestezinin stres hormon düzeylerine etkisi

Amaç: Lokal ve sistemik analjeziklerin torakotomi sonrası artan stres hormonları üzerine etkilerinin araştırılması.

Yöntem ve gereç: Altmış üç adet sıçan rastgele 9 gruba ayrıldı (n = 7). Kontrol gurubundan sadece kan örneği alındı. Torakotomi gurubunda 1 saat otomatik ekartör yerleştirildi. Lokal analjezi guruplarında, bupivakain veya botulinum toksin A ile interkostal sinir bloğu uygulandı. Sistemik analjezi gurubunda ise bupivakain ile interkostal sinir bloğu yapıldı ve periton içine diklofenak sodyum verildi. Torakotomiden 4 ve 24 saat sonra kan örnekleri alındı.

Bulgular: Dördüncü saatte kontrol grubuna kıyasla tüm guruplarda stres hormon düzeyleri anlamlı olarak artmıştı (P < 0,05). Sistemik analjezi grubunda kortizol düzeyleri torakotomi grubuna kıyasla, LDH düzeyleri ise torakotomi ve bupivakain grubuna kıyasla anlamlı olarak daha düşüktü (P < 0,05).

Yirmi dördüncü saatte, tüm guruplarda glukoz hariç diğer stres hormon düzeyleri kontrol grubuna kıyasla anlamlı olarak yüksekti (P < 0,05). Sistemik analjezi grubunda norepinefrin düzeyleri torakotomi grubuna kıyasla anlamlı olarak daha düşüktü (P < 0,05).

Sonuç: Çalışmamız göstermiştir ki, analjezik terapi torakotomi sonrası artan stres hormon seviyelerini düşürmekte tek başına yetersiz kalabilir.

Anahtar sözcükler: Stres hormonu, torakotomi, interkostal sinir blokajı, analjezi, botulinum toksin-A, bupivakain

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Introduction

Thoracotomy pain occurs in 22%-67% of patients and persists for 3-30 months (1-3). Chronic pain may originate from genetic factors, epigenetic factors, and others like comorbidity, familial factors, psychological status, and social support (3). Clinical symptoms of pain include allodynia, hyperalgesia, hypoesthesia, and unprovoked burning pain that may persist for several weeks and months (4).

Intercostal blockade is a useful technique for pain management; botulinum toxin-A and bupivacaine were used for blockade in our study. Botulinum toxin is the most potent biological toxin and there are 7 serotypes from A to G (5,6). Generally it is well tolerated despite the risk of local reactions (7).

Increased stress hormone levels may cause increases in heart rate and breathing, tightening of the muscles, constriction of the blood vessels, and initiate vasodilatation in muscle, brain, lung, and heart (3). The aim of this study was to investigate the effects of local and systemic analgesics on stress hormone levels, which increase after thoracotomy.

Materials and methods

Animals

Sixty-three male Wistar rats weighing between 280 and 320 g were used. This study was approved by the local ethics committee and financially supported by the scientific research board of the university. All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources (8). Sixty-three male rats were randomly divided into 9 groups including 7 rats in each group.

Procedure

The rats were anesthetized with intraperitoneal (ip) administration of 50 mg/kg thiopentone (Pental Sodyum, IE Ulagay, İstanbul, Turkey), and the left hemithorax was shaved. The shaved skin was then repeatedly swabbed with sterile Betadine wipes to sterilize the area. Animals were intubated using tongue retraction by an endotracheal catheter (14 gauge, PTFE IV catheter; Mediflon, Eastern Medikit, India). The catheter was connected to a rodent ventilator (Harvard Instrument, ASVP, USA). Animals were ventilated with room air (10 cm H₂O of

peak inspiratory pressure, 4 cm H₂O of positive end expiratory pressure and frequency of 60 breath/min).

All animals were kept supine for the duration of the experiment. A 3 cm incision was made in the skin of the lateral chest wall between the right 4th and 5th ribs. The deep and superficial muscles covering the ribs were retracted to expose the intercostal muscle. Intercostal muscle and pleura was incised 1.5 cm above the 5th rib. An automatic retractor was carefully placed and opened 1 cm. The retractor was inserted for 1 h and during this time the wound opening was covered by gauze soaked in normal saline. Afterwards, the retractor was removed. The deep muscles covering the ribs were sutured with 2-0 silk and the skin was closed with 4-0 nylon sutures. Intercostal nerves were injected under direct vision from inside the chest before thoracotomy closure in the groups of local anaesthesia and systemic analgesia. Blockage was made from 8 points per 0.1 mL of bupivacaine or botulinum toxin-A injected each region, being careful not to inject the dose intravascularly. The regions injected were the upper and lower layers of the wound, just below of the anterior and posterior intercostal bundle of the 4th, 5th, and 6th ribs. The endotracheal catheter was removed after the animal was spontaneously moved. Blood samples were taken 4 and 24 h after thoracotomy and were stored at -80 °C.

Groups

- 1- Control group (n = 7): Only blood samples were collected.
- 2- Four hour after thoracotomy group (n = 7): A left thoracotomy was performed under anaesthesia, an automatic retractor was inserted, kept 1 h, and then incision was closed. Blood samples were taken 4 h after thoracotomy.
- 3- Twenty-four hour after thoracotomy group (n = 7): After thoracotomy and retraction period, blood samples were taken 24 h after thoracotomy.
- 4- Four hour after thoracotomy - local anaesthesia with bupivacaine (n = 7): After thoracotomy, intercostal nerve block was performed with bupivacaine. Blood samples were taken 4 h after thoracotomy.

- 5- Twenty-four hour after thoracotomy - local anaesthesia with bupivacaine (n = 7): Blood samples were taken 24 h after thoracotomy and intercostal nerve block with bupivacaine.
- 6- Four hour after thoracotomy - local anaesthesia with botulinum toxin A (n = 7): After thoracotomy, intercostal nerve block was performed with botulinum toxin A (Botox). Blood samples were taken 4 h after thoracotomy.
- 7- Twenty-four hour after thoracotomy - local anaesthesia with botulinum toxin A (n = 7): Blood samples were taken 24 h after thoracotomy and intercostal nerve block with botulinum toxin A (Botox).
- 8- Four hour after thoracotomy - systemic analgesia with bupivacaine and diclofenac sodium (n = 7): After thoracotomy, intercostal nerve block was performed with bupivacaine and diclofenac sodium was given intraperitoneally. Twelve hours after thoracotomy, a dose of diclofenac sodium was repeated. Blood samples were taken 4 h after thoracotomy.
- 9- Twenty-four hour after thoracotomy - systemic analgesia bupivacaine and diclofenac sodium (n = 7): The procedure was the same as group 8. Only blood samples were taken 24 h after thoracotomy.

Stress hormone levels

Norepinephrine (NE), epinephrine, cortisol, glucose, and lactate dehydrogenase (LDH) were determined in the serum. Norepinephrine and epinephrine levels were detected with HPLC (high performance liquid chromatography) using electrochemical detection. Data of norepinephrine and epinephrine are expressed as pictogram per millilitre. Cortisol levels were determined by Electrochemiluminescence Immunoassay (ECLIA) method with a Roche Modular E 170 analyser (Roche Cobas kit) and activity is expressed as microgram per decilitre. Glucose levels were analysed by enzymatic reference method with a Hexokinase Roche Cobas Integra analyser (Roche Cobas kit) and expressed as milligrams per decilitre. LDH levels were measured with UV assay according to IFCC method and

expressed as international units per litre. All of the kits and instruments were produced by Roche Diagnostics GmbH, D-68298 Mannheim, Germany.

Statistical analysis

Statistical analyses were performed with SPSS for Windows version 15.0 (SPSS Inc, Chicago, IL, USA). Data were expressed as mean \pm standard deviation (SD). One-way ANOVA was used for nonparametric and parametric comparisons among the groups. Post hoc multiple comparisons were calculated with Tukey's test. P value less than 0.05 was accepted as statistically significant.

Results

At 4 h, stress hormone levels were significantly higher in all groups compared to control ($P < 0.05$). In the systemic analgesia group, cortisol level was significantly lower than in the thoracotomy group ($P < 0.05$), and LDH level was significantly lower than in the thoracotomy and bupivacaine groups ($P < 0.05$). There was no difference in other hormones among the analgesic treatment groups ($P > 0.05$).

At 24 h, all stress hormones except glucose were significantly higher in all groups compared to the control ($P < 0.05$). Glucose level was not significantly different in all analgesia groups than the control ($P > 0.05$), but was significantly higher in the thoracotomy group compared to all other groups ($P < 0.05$). Norepinephrine level was significantly lower in the systemic analgesia group as compared with the thoracotomy group ($P < 0.05$). Stress hormone levels of the groups and statistical analyses of intergroup comparisons are shown in Table 1 and P values of multiple comparisons are shown in Table 2.

Discussion

Our study showed that thoracotomy increased serum stress hormone levels although our local or systemic analgesic therapy after thoracotomy did not decrease serum stress hormone levels. We determined that all stress hormone levels were significantly higher in all groups compared with the control, and only glucose levels decreased 24 h after thoracotomy. Only a few parameters were changed by analgesic therapies but these were not sufficient to affect results.

Table 1. Stress hormone levels and statistical analyses of intergroup comparisons.

Stress hormones	Control	TT	LA Bupivacaine	LA Botulinum	SA	P value
Glucose 4 [§]	158 ± 12	310 ± 42	289 ± 12	288 ± 25	280 ± 32	<001*
Glucose 24	158 ± 12	232 ± 6	203 ± 9	173 ± 12	211 ± 26	013*
Cortisol 4 [‡]	1.9 ± 0.4	5.6 ± 0.2	4.6 ± 0.7	4.7 ± 0.3	4.2 ± 0.1	<001*
Cortisol 24	1.9 ± 0.4	5.7 ± 0.1	4.4 ± 0.2	4.6 ± 0.1	4.2 ± 0.1	<001*
LDH 4 [†]	231 ± 23	1881 ± 177	1725 ± 200	1403 ± 129	966 ± 40	<001*
LDH 24	231 ± 23	1080 ± 225	962 ± 180	888 ± 158	844 ± 41	<001*
Epinephrine 4 [¶]	321 ± 42	1661 ± 174	1243 ± 131	1231 ± 118	1574 ± 395	001*
Epinephrine 24	321 ± 42	1616 ± 236	1218 ± 259	1086 ± 66	1069 ± 124	<001*
Norepinephrine 4 [¶]	216 ± 25	1360 ± 249	1235 ± 131	1073 ± 116	828 ± 134	<001*
Norepinephrine 24	216 ± 25	1585 ± 166	1414 ± 277	905 ± 74	852 ± 29	003*

[*] Statistically significant,

TT: Thoracotomy group, SA: Systemic analgesia group, LA: Local anaesthesia group.

§: mg/dL; ‡: µg/dL; †: IU/L; ¶: pg/mL,

[Four and 24 h levels of all parameters are given separately in the table]

Table 2. P values of multiple comparisons.

Stress hormones	Control vs. TT	Control vs. LA bupivacaine	Control vs. LA botulinum	Control vs. SA	TT vs. LA bupivacaine	TT vs. LA botulinum	TT vs. SA	LA bupivacaine vs. LA botulinum	LA bupivacaine vs. SA	LA botulinum vs. SA
Glucose 4 [§]	0.000	0.001	0.002	0.003	0.959	0.951	0.853	1.000	0.998	0.999
Glucose 24	0.014	0.260	0.961	0.133	0.656	0.069	0.857	0.636	0.996	0.412
Cortisol 4 [‡]	0.000	0.000	0.000	0.000	0.082	0.133	0.030	0.999	0.992	0.960
Cortisol 24	0.000	0.000	0.000	0.000	0.016	0.047	0.004	0.990	0.982	0.852
LDH 4 [†]	0.000	0.000	0.000	0.005	0.922	0.115	0.000	0.454	0.003	0.172
LDH 24	0.003	0.012	0.029	0.047	0.979	0.890	0.795	0.997	0.980	1.000
Epinephrine 4 [¶]	0.001	0.032	0.035	0.002	0.631	0.605	0.998	1.000	0.801	0.778
Epinephrine 24	0.000	0.007	0.028	0.033	0.485	0.213	0.189	0.982	0.972	1.000
Norepinephrine 4 [¶]	0.000	0.000	0.003	0.017	0.975	0.659	0.271	0.939	0.602	0.958
Norepinephrine 24	0.0000	0.000	0.022	0.039	0.925	0.024	0.013	0.141	0.085	0.999

(*) Statistically significant,

TT: Thoracotomy group, SA: Systemic analgesia group, LA: Local anaesthesia group.

§: mg/dL; ‡: µg/dL; †: IU/L; ¶: pg/mL

[Four and 24 h levels of all parameters are given separately in the table]

Surgical stress causes upregulation of hypothalamic-pituitary-adrenal axis (HPA) and sympathetic system activity (7). Tissue trauma, pain perception, and psycho-emotive factors could be responsible for this activity.

The main hormones circulating by stress mechanisms are the catecholamines NE and epinephrine, which affect by binding to adrenergic receptors on the surface of target cells (3). NE is an indicator of sympathoneural activity and is mainly released from sympathetic nerve endings. Epinephrine is released mainly by the adrenal medulla and reflects the sympathomedullar activity (9). Cortisol is the primary agent of stress in human and a classic marker of HPA (3,9). Cortisol has complex effects throughout the body. Cortisol levels are high in the morning and decrease throughout the day during a normal circadian rhythm. Cortisol release can be modulated by circulating cytokines including interleukins-6 and 8, and tumour-necrosis factor (10). Determined developmental differences were also found for plasma lactate levels for the postoperative period (11).

Thoracotomy may affect the cortisol plasma level via an enhanced local production of proinflammatory factors and spillover the soluble mediators into the systemic circulation. Endocrine and metabolic responses to severe injury of tissues like thoracotomy consist of a hypermetabolic period. Despite this homeostatic disturbance, cellular dehydration, capillary leakage, and organ dysfunction may occur and important reductions in stress responses depend on the analgesic method (11). Therefore, depending on pain and elevation of stress hormone levels, higher morbidity and mortality rates could occur.

Especially in high risk patients, increased cortisol level may inhibit natural killer cells activity, wound healing processes, and platelets adhesion, which may all negatively affect both perioperative recovery and long-term outcomes (10). NE and epinephrine levels are less sensitive indicators of stress (9).

A 60-min rib retraction produced allodynia in rats (12). Rib retraction caused total conduction block in the intercostal nerves on both sides of the retractor and also blocks 50% of the intercostal nerves (12). Nara et al. (4) demonstrated that ligation of intercostal nerves yielded allodynia in 70% of

rats (4). Our limitation was that we did not check analgesia and allodynia as an acute marker of pain and so we could not evaluate the sufficiency of the present pain control in this study.

Good pain control in thoracotomy is crucial for the ability of effective coughing and breathing to protect the patient from hypoxia, ventilation-perfusion mismatch, atelectasis, mucous plugging, chest infection, and respiratory failure (13,15). The most common methods of pain management are systemic narcotics and epidural administrations. Intercostal nerve blockade techniques are used less often (13) and the simplest method is injection of local anaesthetics in multiple intercostal nerves before closure of the incision.

Blockade has generally provided better pain relief than narcotics alone, but comparing with epidural analgesia, neither technique is clearly superior (13). Cosmo et al. (16) showed that superior analgesia was associated with epidural techniques but there were not so many studies about thoracotomy. Fibla et al. (14) and Ju et al. (2) considered epidural anaesthesia as a gold standard for thoracic analgesia (17). There are some other methods like cryotherapy of intercostal nerves, or regional anaesthesia by thoracic paravertebral block, or interpleural or subpleural catheter insertion (13-15,17).

In a study involving 11,000 patients, with 2 mL bupivacaine injection ranging from 0.25% to 0.5% per intercostal nerve, no systemic toxicity was found (13,17). However, according to other authors, it sometimes resulted in fatal cardiovascular and/or central nervous system toxicity (14,16,18). Bupivacaine is a long-acting amide-type local anaesthetic and inhibits the conduction of the action potential in nerves involved in sensory-motor and sympathetic activity by the inhibition of voltage gated sodium channels (16,18). Wheatley et al. (16) suggested that the use of a mixture of local anaesthetics and opioid is significantly better for pain relief after thoracic surgery.

The use of botulinum toxin A in pain syndromes has been popular in recent years (19). It has been successfully used in the treatment of many diseases (5,7,19,20). Analgesia is provided before paralysis without any weakness, which has been demonstrated in rat and rabbit tissues (7). Effects of botulinum

toxin on pain can often be dissociated from changes induced in neuromuscular transmission and exerts the action by inhibiting the exocytosis of acetylcholine in cholinergic nerve endings (20). There is no paucity of possible mechanisms by which botulinum toxin could influence pain perception, including direct effects on muscle nociceptors, influence on sensitizing mediators, alteration of afferents derived from muscle spindles, physiologic changes in reflex and synergistic movements, direct and secondary

autonomic effects, and induced neuroplasticity in the nerve system (5,6).

In the present study there is no possible decrease in stress hormone levels. Also stress hormones could increase due to other factors except pain, such as tissue trauma, pain perception, and emotional factors.

In conclusion, our study suggests that the analgesic therapy used may be insufficient to lower stress hormone levels, which increase after thoracotomy.

References

1. Flatters SJL. Characterization of a model of persistent postoperative pain evoked by skin/muscle incision and retraction (SMIR). *Pain* 2008; 135: 119-30.
2. Ju H, Feng Y, Yang B, Wang J. Comparison of epidural and intercostal nerve cryoanalgesia for post-thoracotomy pain control. *Eur J Pain* 2008; 12: 378-84.
3. Chapman CR, Tuckett RP, Song CW. Pain and stress in a systems perspective: reciprocal neural, endocrine, and immune interactions. *J Pain* 2008; 9: 122-45.
4. Nara T, Saito S, Obata H, Goto F. A rat model of postthoracotomy pain: behavioural and spinal cord NK-1 receptor assessment. *Reg Anesth Pain Med* 2001; 48: 665-76.
5. Willis B, Eubanks LM, Dickerson TJ, Janda KD. The strange case of the botulinum neurotoxin: using chemistry and biology to modulate the most deadly poison. *Angew Chem Int Ed* 2008; 47: 8360-79.
6. Caleo M, Antonucci F, Restani L, Mazzocchio R. A reappraisal of the central effects of botulinum neurotoxin type A: by what mechanism? *J Neurochemistry* 2009; 109: 15-24.
7. Jeynes LC, Gauci CA. Evidence for the use of botulinum toxin in the chronic pain setting- a review of the literature. *World Institute of Pain* 2008; 8: 269-76.
8. Guide for the care and use of laboratory animals. Bethesda, MD: National Institutes of Health, 1985; Publication No. 85-23.
9. Mravec B, Tillinger A, Bodnar I, Nagy GM, Palkovits M, Kvetnansky R. The response of plasma catecholamines in rats simultaneously exposed to immobilization and pain stimuli. *Ann NY Acad Sci* 2008; 1148: 196-200.
10. Tacconi F, Pompeo E, Sellitri F, Mineo TC. Surgical stress hormones response is reduced after awake videothoracoscopy. *Interactive Card Thor Surg* 2010; 10: 666-71.
11. Bouwmeester NJ, Anand KJS, Dijk MV, Hop WCJ, Boomsma F, Tibboel D. Hormonal and metabolic stress responses after major surgery in children aged 0-3 years: a double blind, randomized trial comparing the effects of continuous versus intermittent morphine. *Br J Anaesth* 2001; 87: 390-9.
12. Buvanendran A, Kroin JS, Kerns JM, Nagalla SNK, Tuman KJ. Characterization of a new animal model for evaluation of persistent postthoracotomy pain. *Anesth Analg* 2004; 99: 1453-60.
13. Dettnerbeck FC. Efficacy of methods of intercostal nerve blockade for pain relief after thoracotomy. *Ann Thor Surg* 2005; 80: 1550-9.
14. Fibla JJ, Molins L, Mier JM, Sierra A, Vidal G. Comparative analysis of analgesic quality in the postoperative of thoracotomy: paravertebral block with bupivacaine 0.5% vs ropivacaine 0.2%. *Eur J Cardio-thorac Surg* 2008; 33: 430-4.
15. Dabir S, Parsa T, Radpay B. Interpleural morphine vs bupivacaine for postthoracotomy pain relief. *Asian Cardiovasc Thorac Ann* 2008; 16: 370-4.
16. Cosmo GD, Mascia A, Clemente A, Congedo E, Aceto P. Use of levobupivacaine for the treatment of postoperative pain after thoracotomies. *Minerva Anesthesiol* 2005; 71: 347-51.
17. Navlet GM, Garutti I, Olmedilla L, Pérez-Peña JM, San Joaquin MT, Martinez-Ragues G, et al. Paravertebral ropivacaine 0.3%, and bupivacaine 0.25%, provide similar pain relief after thoracotomy. *J Cardiothorac Vasc Anesth* 2006; 20: 644-7.
18. McLeod GA, Burke D. Levobupivacaine. *Anaesthesia* 2001; 56: 331-41.
19. Durmazlar SPK, Eskioğlu F. Botulinum toxin (Fundamentals before practice): Review. *Tur Klin Med J Med Sci* 2009; 29: 702-7.
20. Lu DW, Lippitz J. Complications of botulinum neurotoxin. *Dis Mon* 2009; 55: 198-211.