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Evaluation of the effects of epidural bupivacaine on coagulation system by using thromboelastometry in women with gynecologic malignancies

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Summary

Purpose of investigation: In this study, epidural local anesthetic drugs are scheduled to examine their effects on the coagulation cascade. *Materials and Methods:* A total of 40 patients with gynecologic tumors were eligible. Epidural catheter was introduced into the epidural space of all patients. The patients were randomized into control and study groups. Before the induction of anesthesia, saline five cc, in control group and 25 mg bupivacaine was given via epidural catheter in study group. An epidural morphine infusion (0.2 mg/hour) was started in control group and an epidural infusion (0.2 mg/hour morphine + 0.75 mg/hour bupivacaine) was in study group. Before induction of anesthesia and postoperative 24 hours, thromboelastometry (TEG) analysis was performed. *Results:* Ellagic acid/phospholipid activation clotting time (INTEM CT), which was measured in the postoperative period, was significantly shorter in study group. Conclusion: Bupivacaine causes platelet dysfunction induced by various agonists, while neither this primary hemostatic abnormality nor other coagulation abnormalities can be detected by rotation thromboelastometry (ROTEM).

Key words: Thromboelastometry; Bupivacaine; Coagulation; Epidural analgesia.

Introduction

Abdominal surgery, general anesthesia, and malignancy are well known hyper-coagulable situations. Epidural analgesia, which is frequently used for the treatment of postoperative pain in patients undergoing abdominal cancer surgery, attenuates the hypercoagulable perioperative state and decreases the thromboembolic complications associated with surgery, by blunting the sympathetic response and improving lower extremity blood flow [1]. Tuman *et al.* [2] showed the negative effect of postoperative epidural analgesia on coagulation system by thromboelastography (TEG). Epidural analgesia provides better analgesia and earlier mobilization than intravenous analgesia. Local anesthetics, opioids or combined local anesthetic opioids are used in epidural [3]. Local anesthetic drugs have been shown to reduce the formation of a clot by inhibiting alpha granule release form platelets, thromboxane A2 signaling, and aggregation [4-8].

Bupivacaine is a local anesthetic frequently used in epidural analgesia. It has been shown to inhibit whole blood coagulation in vitro even in low concentrations [4, 9]. However studies performed in vivo have failed to show any effect of bupivacaine on TEG parameters [10].

There are conflicting and very few data about the effect of bupivacaine on coagulation system when it is given as epidural analgesic agent in the perioperative period. Therefore the present designed this study to show the possible independent effect of this agent on coagulation system in vivo.

Materials and Methods

A total of 40 patients with gynecologic tumors were eligible for the study, which was approved by the Ethics Committee, and informed consent was given. Two patients were excluded from the study because of breaches of the evaluation, and thus, a total of 38 patients' assessments were received. Patients with a history of hematological or coagulation disorders, those taking anticoagulant and antiplatelet therapy, those with renal or liver function, and history of recent anti-inflammatory drug (within two weeks of enrollment), were excluded.

Each patient received two separate intravenous catheters; one 18 gauge catheter in each hand. Standard monitoring of oxygen saturation, electrocardiogram, and blood pressure (non-invasively) were applied to all patients.

Before induction of anesthesia, the skin was infiltrated with 5% prilocaine and an 18-gauge Tuohy needle was introduced into the epidural space at the L4-5 level, after which the epidural catheter was advanced four cm into the epidural space. The patients were randomized into two groups with 19 patients per group; control and study groups. Before the induction of anesthesia, saline, five cc, was given via epidural catheter in control group. Upon arrival in the post-anesthesia recovery room, an epidural morphine infusion was started with a patient controlled analgesia device (PCA)

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at the rate of 0.2 mg/hour. In contrast, study group received 25 mg bupivacaine via epidural catheter before the induction of anesthesia. After arrival in the postanesthesia recovery room, an epidural morphine and bupivacaine infusion was started with a PCA at the rate of 0.2 mg/hour morphine + 0.75 mg/hour bupivacaine. Epidural infusions continued postoperatively for 24 hours.

All patients received sodium thiopental (4-5 mg/kg), fentanyl one µg/kg, and vecuronium 0.1 mg/kg to induce anesthesia. Endotracheal intubation was performed. Anesthesia maintenance was provided by 1-2% sevoflurane 50% N_2O_2.

Before induction of anesthesia and 24 hours after the operation, complete blood count, PTT, PT, platelet aggregation tests with ADP (adenozin diphoshate), epinephrine, collagen (COL), arachidonic acid (AA), and TEM analysis were performed on blood samples. The blood samples were taken from the right hand cannula, and drugs and intravenous fluid were administered through the left hand cannula. Blood was collected using a two-syringe technique. After discarding the initial two ml of blood to prevent tissue thromboplastin contamination, samples for TEG analysis were drawn into 4.5 ml vacutainers containing 3.2% trisodium citrate with a citrate/blood ratio of 1:9. Thrombelastometric analysis was performed with the rotation thromboelastometry (ROTEM) coagulation analyzer. The method and the parameters of ROTEM have been previously described in detail [11]. The following ellagic acid/phospholipid activation (INTEM) and tissue factor activation (EXTEM) ROTEM parameters were recorded: clotting time (CT), clot formation time (CFT), and maximum clot firmness (MCF). Aggregation studies were carried out in platelet-rich plasma using a lumi-aggregometer with the optical method. The whole blood specimen was centrifuged for ten minutes at 200 g to obtain platelet rich plasma (PRP). Platelet poor plasma was obtained on the remaining specimen by recentrifugation at 200 g for 15 minutes. A platelet count was performed on the platelet rich plasma and was adjusted to 300 ×103/µL with platelet poor plasma; 450 µL of this platelet rich plasma were transferred into cuvettes each containing a disposable siliconized bar. After agonist addition, platelet aggregation was measured over six minutes and expressed as a percentage of the maximal amplitude in PRP. The agonist used and their final concentrations were; ADP five µM, collagen two µg/ml, ristocetin 1.25 mg/ml, and epinephrine five µM.

During the operation and the postoperative 24-hour period, the amount of blood originating from the drains was recorded. Patients were followed in terms of nausea, vomiting, and itching in the postoperative period.

Statistical analysis was carried out using the SPSS Statistics 20. Power analysis determined the number of patients to be 19 per group for 90% power. Normally distributed, continuous dependent variables were analyzed using the paired *t*-test and presented as the mean and standard deviation. Non-normally distributed variables were compared with the Wilcoxon test for two dependent groups and presented as median (25^{th} to 75^{th} percentile). A *p*-value less than 0.05 ($p < 0.05$) was accepted as significant.

Results

Nineteen patients each for the study and control groups were enrolled in the study. Thirteen (68.4%) were diagnosed with endometrial cancer, four (21.1%) were diagnosed with ovarian cancer, one (5.3%) was diagnosed with cervical cancer, and one (5.3%) was diagnosed with peritoneal cancer. Six patients (31.6%) had hypertension, two (10.5%) had diabetes mellitus, and three had (15.8%) hypothyroidism. The primary diagnosis and comorbid diseases were not different between control and study groups.

Table 1. — *Demographic data and preoperative-postoperative loss of blood.*

	Control group	Study group	*p* value
Age (years)[a]	56.58±11.06	57.11±10.24	0.880
Body weight(kg)[b]	70 (68-88)	72 (67-87)	0.781
Perioperative loss of blood (ml)[a]	232.6±82.9	255±114	0.490
Loss of blood in postoperative first 24 hours (ml)[b]	50 (0-100)	40 (0-100)	0.840

[a] Values are mean ± SD. [b] Values are median (quartiles);

Table 2. — *Hemoglobin, platelet, coagulation, and aggregation test results of groups.*

	Preoperative	Postoperative	*p* value
Hemoglobin (g/dl)[a]			
Group C	11.9±1.5	11.368±1.1	0.048*
Group S	12.4±1.4	10.9±1.1	0.001*
Platelet count (/mm^3)[b]			
Group C	240 (219-325)x10^3	215 (182-281)x10^3	0.001*
Group S	302 (233-361)x10^3	225 (194-277)x10^3	0.001*
Epinephrine (%)[a]			
Group C	87 ± 31.3	75.9 ± 32.5	0.217
Group S	97.89±30.09	70.95±34.1	0.014*
ADP (%)[b]			
Group C	112 (110-113)	111.5 (110-113)	0.682
Group S	113 (112-114)	112 (111-113)	0.044*
Arachidonic acid (%)[b]			
Group C	113 (111-115)	78.5 (50-112)	0,001*
Group S	112 (109-113)	111 (56-112)	0.013*
COL (%)[b]			
Group C	113 (106-115)	112.5 (102.2-114.2)	0,649
Group S	114 (109-115)	113 (107-114)	0.962
PT (sec)[a]			
Group C	11±0.6	11.2±0.4	0.112
Group S	11.2±0.6	11.5±0.6	0.136
PTT (sec)[b]			
Group C	28.2 (25.1-29.2)	27.9 (25.3-28.4)	0.123
Group S	28.6 (27.1-30.3)	28 (27.4-29.4)	0.778

*$p < 0.05$. Normal values of aggregation parameters: EPI (63-90%), ADP (64-89%), AA (64-90%), and COL (50-80).
[a] Values are mean ± SD; [b] Values are median (quartiles).

The control and the study groups were not significantly different regarding age and body weight (Table 1). Both the control and study groups' postoperative platelet and hemoglobin levels were lower than the preoperative levels (Table 2). The amount of blood loss during and after the surgery was not different between the control and study groups. Additionally, although platelet and hemoglobin levels were lower in the postoperative period, they were not different between the two groups (Table 2). None of the patients received plasma or erythrocyte suspension.

PT and PTT results were not different before and after

Table 3. — *ROTEM parameters of groups.*

	Preoperative	Postoperative	*p* value
IntemCT			
Group C	185 (164-212)	175 (158-186)	0.198
Group S	187 (157-208)	148 (136-172)	0.002*
Intem CFT			
Group C	78 (59-89)	73 (57-88)	0.636
Group S	66 (56-79)	64 (57-76)	0.388
Intem MCF			
Group C	65 (62-70)	66 (63-69)	0.110
Group S	66 (65-70)	68 (65-70)	0.705
Extem CT			
Group C	89 (77-97)	83 (69-92)	0.41
Group S	83 (75-99)	77 (65-91)	0.263
Extem CFT			
Group C	82 (64-105)	83 (62-113)	0.96
Group S	82 (64-92)	76 (70-84)	0.382
Extem MCF			
Group C	66 (61-71)	66 (65-70)	0.270
Group S	68 (66-70)	69 (65-71)	0.774

*$p < 0.05$. Normal values of ROTEM parameters: EXTEM CT (79-38 sec), EXTEM CFT (159-34 sec), and EXTEM MCF (72-51 mm), INTEM CT (240-100 sec), INTEM CFT (110-30 sec), and INTEM MCF (65-50 mm). Values are median (quartiles)

surgery in both groups and there were no significant differences between study and control groups. The platelet aggregation test results can be seen in Table 2. Among the platelet aggregation studies, aggregation induced by arachidonic acid (AA) was significantly lower in the postoperative period in both the study and control groups. In the study group, aggregation induced by epinephrine and ADP were significantly diminished in the postoperative period. Aggregation induced by collagen was not different between groups. When the authors compared the study and control groups regarding the aggregation tests, they could not find any significant difference between the groups. Although there were some alterations in the aggregation test after the surgery, none of the median values of the test results were out of normal ranges.

ROTEM test results can be seen in Table 3. Postoperative INTEM CT [148 sec (136-172)] was shorter than preoperative INTEM CT [187 (157-208)] in study group. Also, postoperative INTEM CT was shorter in study group [148 sec (136-172)] than control group [med 175 sec (158-186)].

Discussion

The hypercoagulable state of patients with gynecological cancer has been clearly demonstrated by TEG [12]. During the time patients with gynecological tumors spend in surgical operations with positive pressure ventilation, venous stasis, neuromuscular blockage, and sympathetic activation, all contribute to the thrombotic situation. The present study population included patients with gynecologic malignancies undergoing abdominal surgery. These patients need precautionary measures to reduce the thrombotic risk.

Epidural anesthesia and analgesia have the potential to reduce the perioperative physiologic stress responses to surgery and blunt the sympathetic response. During and after, epidural anesthesia and analgesia of the sympathetic system leads to changes in the system of coagulation as a result of the normalization of factors VIII and VIII-related protein, decreases in plasminojen activator inhibitor (PAI-1), and an increased antithrombin III. Rodgers *et al.* [13] reported that epidural anesthesia and general anesthesia compared in 141 clinical trials with epidural anesthesia and/or analgesia. Ultimately, they found that the 44% reduction in deep venous thrombosis and the 55% reduction in pulmonary embolism.

Local anesthetic drugs (especially bupivacaine) are preferred during epidural anesthesia or analgesia. There are studies suggesting that bupivacaine itself can have an independent effect on coagulation besides the antithrombotic effect of epidural analgesia procedure [4, 9]. Kohrs *et al.* [4] reported that after incubation of whole blood with bupivacaine, the TEG activated clotting time (ACT) increased significantly and maximal amplitude decrease. In contrast, Gibbs and Sear [14] reported that bupivacaine has no obvious effects on TEG variables in vitro and also no clinically significant effects on overall coagulation in vitro. However these studies are in vitro. There are very few in vivo studies about the subject.

Benzon *et al.* [10] reported on abdominal and genitourinary surgery and divided epidural catheter implant patients into four groups; Group I: epidural fentanyl, Group II: epidural fentanyl with 0.1% bupivacaine, Group III: epidural fentanyl with 0.15% bupivacaine, Group IV:epidural fentanyl with 0.2% bupivacaine. There were no significant differences between groups in the TEG values. In another study, ten patients underwent epidural block for cesarean section with 150 mg of 0.5% bupivacaine and two mg of morphine; ten underwent subarachnoid block for cesarean section with 15 mg of 0.5% hyperbaric bupivacaine and 0.2 mg of morphine and ten underwent pudendal block for vaginal delivery with up to 100 mg of 0.5% bupivacaine without epinephrine. In these patients, prothrombin time, activated partial thromboplastin time, and TEG were performed. Consequently, the authors did not find a difference between these techniques [15].

In this in vivo study, the authors did not find any changes of ROTEM parameters. Indeed, the INTEM CT was shorter in the bupivacaine group than in the control group in the postoperative period. CT is the time from the start of the measurement until initiation of clotting. CT provides information about the initiation of clotting, thrombin formation, and start of clot polymerization. Shortening CT and CFT along with increased MCF indi-

cate hypercoagulability. However, only a reduced CT value does not necessarily mean that there is a hypercoagulable state in patients treated with bupivacaine. Since the other ROTEM parameters Intem CFT, Intem MCF, Extem CT, Extem CFT, and Extem MCF were not different among study and control groups in the present study, shortening of Intem CT alone was not thought to indicate hypercoagulability.

Both hemoglobin and platelet values were lower after the surgery. This finding is most likely due to the inevitable blood loss during surgery. Although the platelet count was lower in the postoperative period in both groups, the counts were within normal limits; thus, this reduction should not affect the aggregation test results. Aggregation studies with epinephrine, ADP, and AA showed a reduction in platelet functions in the bupivacaine group. Local anesthetic drugs cause membrane stabilization via their connection to nerve axon-specific receptors in the membrane and thereby prevent depolarization. As a result of the release of alpha granules from platelets, thromboxan A2 signaling and aggregation are inhibited. There was no significant difference in the aggregation test results between the study and control groups. Interestingly, the aggregation response to AA was reduced in both the bupivacaine group and control groups. These groups are similar in that they both received morphine after the operation. It has been suggested that low levels of local anesthetics in circulation after epidural anesthesia could be responsible for the anti-thrombotic effects of local anesthetics [4]. The present authors can assume that low levels of morphine could cause the same effect. An in vitro study by Hisao *et al.* reached the opposite conclusion, namely, that morphine was a potentiator for platelet aggregation [16]. The present authors do not have sufficient data to draw the opposite conclusion, but this point can be the subject of a future study.

Studies on hemostasis are difficult to interpret because there are many parameters, like vascular endothelium, hormonal status, and diurnal variation that are involved in the process; however, the present authors attempted to analyze bupivacaine's effect on coagulation system in thrombophilic patients. Their findings suggest that bupivacaine causes platelet dysfunction induced by various agonists, while neither this primary hemostatic abnormality nor other coagulation abnormalities can be detected by ROTEM.

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