Egyptian Journal of Anaesthesia (2012) **28**, 3–8

Egyptian Society of Anesthesiologists

Egyptian Journal of Anaesthesia

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Research Article

Antiaggregatory effect of midazolam on human platelets during monitored anesthesia care for trans-vaginal oocyte retrieval

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Received 31 July 2011; revised 31 August 2011; accepted 4 September 2011
Available online 17 October 2011

KEYWORDS

Midazolam;
Platelet-aggregation;
Glycoprotein IIb–IIIa complex;
Thromboxane A2;
Thromboxane B2

Abstract *Background:* Midazolam plays a major role in sedation and large doses might be used under certain circumstances. Trans-vaginal oocyte retrieval is one of the most painful procedures in which monitored anesthesia care (MAC) is needed. The mechanism by which midazolam exerts anti-aggregation of human platelets is still unclear.
This work aimed to investigate the *in vivo* suppressing effect of midazolam on human platelet aggregation.
Methods: Sixty adult females ASA I–II, scheduled for trans-vaginal ultrasound oocyte retrieval by combined intravenous midazolam sedation with paracervical local nerve block were included. At the end of the procedure, it was found that the range of total midazolam consumption was 4.9–9 mg. Thereby, patients were divided into three groups according to the total consumption of midazolam: Group I (4.9–6.2 mg), Group II (6.3–7.6 mg), Group III (7.7–9 mg). Patients who needed other drugs for sedation were excluded. Pre- and post-operative Bleeding Time (BT) were recorded. Two blood samples were collected from each patient to test the inhibitory effect of midazolam on platelet aggregation. Platelet aggregation was tested using platelet-aggregometer. ELISA was used to measure thromboxane B2 formation. Flowcytometry was used to evaluate whether

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Peer review under responsibility of Egyptian Society of Anesthesiologists.
doi:10.1016/j.egja.2011.09.001

glycoprotein IIb–IIIa complex is the receptor site for the antiplatelet action of midazolam.

Results: Only 51 patients completed the study: Group I ($n = 16$), Group II ($n = 24$), and Group III ($n = 11$). Bleeding Time (BT) showed significant prolongation in group III compared to basal levels. Midazolam suppressed platelet aggregation in a dose-dependent manner as detected by aggregometer. Glycoprotein IIb–IIIa complex is not its site of action as shown by flowcytometric analysis. Lastly, thromboxane B2 was significantly inhibited by midazolam.

Conclusion: This study revealed that midazolam dose-dependently inhibits platelet aggregation by a mechanism not involving the binding of glycoprotein IIb–IIIa complex (fibrinogen receptor). It was also found that midazolam inhibits thromboxane A2 formation.

1. Introduction

Midazolam is a benzodiazepine derivative that is widely used in anesthetic practice. It is a familiar agent during monitored anesthesia care (MAC) [1].

Despite that the anti-aggregatory effect of benzodiazepine derivatives remains unclear [2], many precious studies explained the inhibitory effect of benzodiazepines on platelet aggregation via inhibiting the binding of platelet-activating factor to platelets (triazolam and alprazolam) [3] or interfering with arachidonate-induced aggregation (clonazepam, diazepam, and flumazenil) [4]. Other studies explained the antiaggregatory effect of benzodiazepines derivatives by inhibition of lipid peroxidation (gidazepam and phenazepam) [5]. The mechanism by which midazolam inhibits platelet aggregation is still unclear [6].

Trans-vaginal ultrasound-guided oocyte retrieval represents one of the most painful (despite vital) maneuvers during the process of *in vitro* fertilization (IVF) [7]. Different sedative and anesthetic techniques have been described for calming the patient and controlling her pain during oocyte retrieval [8]. One of these techniques is the MAC, which is a technique that is explained by the American Society of Anesthesiologists (ASA) as a planned procedure during which the patient undergoes local anesthesia together with sedation and analgesia [9]. The level of sedation during this procedure varies from light (patient just feels very relaxed), to heavy (patient is unaware and only rouses to significant stimulation). The commonest anesthetic agents to be used for providing MAC are propofol, midazolam, and/or remifentanyl due to its favorable pharmacodynamics and pharmacokinetics [9].

The present study was designed to use midazolam as a sole intravenous drug combined with paracervical local nerve block in patients under monitored anesthesia care for ultrasound guided trans-vaginal oocyte retrieval. The aim of our work was to study the *in vivo* anti-aggregatory effect of midazolam on human platelets.

2. Patients and methods

After local ethics committee approval and an informed written patient consent, 60 adult females classified according to American Society of anesthesiologists as ASA I–II (aged between 20 and 40 years), scheduled for trans-vaginal ultrasound oocyte retrieval (via combined intravenous midazolam sedation with paracervical local nerve block), have been included in the current study. Exclusion criteria included: refusal of the patient, respiratory impairment where arterial O_2 saturation ($SaO_2 < 92\%$) on room air, impaired renal (creatinine > 1.5 mg/dl) or hepatic function (serum bilirubin > 1.5 mg/dl or doubled AST), coagulopathies (platelet count $< 100 \times 10^3/\mu l$, BT < 3 min), and those who had taken any medications during the preceding 2 weeks (as the presence of chylomicra or drugs may disturb aggregation battern).

For all patients, pre-operative and post-operative Bleeding Time (BT normal levels = 1–3 min) were recorded.

A dose of 0.06–0.1 mg/kg midazolam was administered to achieve a Ramsay sedation score [11] (RSS) of 5. RSS has six levels according to patient response: (1) anxious and/or agitated; (2) cooperative, oriented and tranquil; (3) responds to commands only; (4) brisk response to light, glabellar tap or loud auditory stimulus; (5) sluggish response to light, glabellar tap or loud auditory stimulus and (6) no response.

Patients who reached the maximum dose of sedation of midazolam without achieving the targeted RSS were excluded from the study and were given 1 mg/kg propofol.

Paracervical local nerve block was performed by the gynecologist to block the inferior hypogastric plexus and ganglia by injecting of the local anesthetic (lidocaine 0.5 mg/kg in 20 ml saline) into the lateral fornices of the vagina after performing syringe aspiration to exclude inadvertent intravascular injection [7].

All patients were allowed to breath O_2 100% spontaneously via transparent face mask and were closely monitored by attendant anesthesiologist, ECG, non-invasive blood pressure measurement, and pulse oximetry.

Only 51 patients completed the study (nine were excluded due to inadequate overall intraoperative RSS and propofol injection was needed).

At the end of the procedure, it was found that the range of total midazolam consumption was 4.9–9 mg (the lowest dose of midazolam used was 4.9 mg, while the highest dose was 9 mg). Patients were then divided into three groups according to the total consumption of midazolam: Group I (4.9–6.2 mg); Group II (6.3–7.6 mg) and Group III (7.7–9 mg). It was then found that Group I included 16 patients, Group II included 24 patients, while Group III included 11 patients.

2.1. Preparation of human platelet suspensions

Two blood samples were collected from the patient in the same way pre-operatively [before giving midazolam (basal)] and post-operatively [3 min after injecting the last dose of midazolam].

Ten mililiter of venous blood sample was collected without stasis and anticoagulated with 1 volume of sodium citrate, 38 g/L, pH 7.4, to 9 volumes of blood.

Platelet-rich plasma (PRP) was obtained from citrated whole blood by 20-min centrifugation at 100 g at room temperature; platelet-poor plasma (PPP) was prepared by further centrifugation at 2000 g for 10 min.

2.2. Platelet aggregation

Platelet-aggregometer (PA-200 instrument, Japan) was used to detect platelet aggregation. Pre-warming of (0.4 ml) of platelet suspensions was done at 37 °C for 2 min. A platelet-aggregation inducer (collagen 2 μg/ml) was added (Sigma Chemical Co.). The reaction was allowed to stand for at least 6 min. The extent of aggregation of platelets at the end of the operation was expressed as a percentage of the basal aggregation.

2.3. Analysis of the platelet surface glycoprotein IIb–IIIa complex using flow cytometry

Fluorescein isothiocyanate conjugated triflavin (FITC-triflavin) is a specific fibrinogen receptor antagonist [10]. This technique is based upon the idea that if midazolam binds to fibrinogen receptor (glycoprotein IIb–IIIa complex), the FITC-conjugated triflavin will not be able to bind to the receptor. This will result in no increase in fluorescence intensity. While if midazolam does not bind to the receptor (i.e., it is not the site of antiplatelet effect of midazolam), the fluorescence intensity will rise as a result of binding of FITC-conjugated triflavin to the fibrinogen receptor.

The final concentration of FITC- triflavin (US Biological, Swampscott, Massachuse-tts, USA) was adjusted to 1 mg/ml. 2 μl of FITC-triflavin was added to aliquots of platelet suspensions 200 μg (4×10^8/ml). The suspensions were incubated for 5 min, and the volume was adjusted to 1 ml/tube with phosphate buffered saline solution. The suspensions were then assayed using a flow cytometer (FACscan Calibur; Bectopn Dickinson, San Jose, CA) for fluorescein-labeled platelets. Data were collected from 50.000 platelets for each patients group.

2.4. Detecting thromboxane B_2 formation

Thromboxane B2 is the stable metabolite of T × A2, released during aggregation. Collagen (2 μg/ml) was added to human platelet suspensions (4×10^8/ml) for all groups. 6 min later, 2 mM EDTA and 50 μM indomethacin were added to the reaction suspensions. The vials were then centrifuged for 3 min at 14,000 rpm. The thromboxane B2 concentrations of the supernatants were measured using an enzyme immunoassay kit (R&D Minopllas, USA).

2.5. Statistical analysis

Sample size was calculated ($n = 60$) by using a pilot study to determine patients scheduled for trans-vaginal ultrasound oocyte retrieval in 1 month then the whole patients (whole population) for 1 year was calculated. Power of the study was 80%, confidence interval was 95%, and level of significance was determined at 5% ($P < 0.05$).

Data were checked, entered and analyzed using SPSS version 11. Data were expressed as mean ± standard deviation for parametric results, as number for qualitative one, or as percentage. ANOVA, paired *t*-test, chi-square (χ^2) or Krunskal–Wallis test were used when appropriate. $P < 0.05$ was considered significant.

3. Results

There were no statistical significant differences in demographic data (age and weight), or the duration of the procedure between the three groups (Table 1).

Bleeding Time (BT) showed significant prolongation in group III compared to basal levels (Table 2).

3.1. Effect of midazolam on platelet aggregation in human platelet suspensions

Midazolam suppressed collagen-induced platelet aggregation in a dose-dependent manner. Midazolam at the concentration of 7.2 mg inhibited platelet aggregation by 50%, i.e. the inhibitory concentration values of midazolam to 50% (IC_{50}) for collagen induced platelet aggregation, was about 7.2 (Fig. 1).

3.2. Results of flow cytometry analysis of the platelet surface glycoprotein IIb–IIIa complex

Triflavin is a substance that is purified from snake venom and has antiplatelet activity. It is a specific antagonist to fibrinogen receptor that exerts its anti-platelet effect through direct interference with binding of fibrinogen to its receptor (glycoprotein IIb–IIIa complex) [10]. The binding of fibrinogen to the glycoprotein IIb–IIIa complex is the final destination for agonist-induced platelet aggregation [12]. In the present study we were interested to find out if midazolam binds directly to the fibrinogen receptors on platelet membrane, leading to inhibition of platelet aggregation induced by agonist (collagen 2 μg/ml).

The mean fluorescence intensity of (FITC)-triflavin (2 μg/ml) bound directly to collagen (2 μg/ml) activated platelets was 90 ± 11, and it was significantly reduced in the presence of 5 mM EDTA (negative control 70 ± 23). Effect of midazolam was studied at two doses and it did not significantly inhibit FITC-triflavin binding to fibrinogen receptor in platelet suspensions at any dose: (a) at a concentration of 7 mg (94.6 ± 16.4) and (b) at a higher concentration of 9 mg (92.3 ± 13.5). These results suggest that midazolam's inhibitory effect on platelet aggregation is not through binding to fibrinogen receptor (glycoprotein IIb–IIIa complex) (Fig. 2).

3.3. Effect of midazolam on thromboxane B_2 formation

Resting platelets produced relatively little thromboxane B_2 (47.3 ± 11.6) compared with collagen activated platelets (259.2 ± 39.4).

Results obtained using various doses of midazolam indicated that midazolam (4.9–9 mg) dose-dependetly inhibited thromboxane B_2 formation in platelets stimulated by collagen (2 μg/ml) (Table 3). Significant inhibition of thromboxane B_2 formation occurred with group I (172.8 ± 23.5), while highly significant inhibition occurred with groups II (126.4 ± 19.7) and III (71 ± 11.2). These results indicate that midazolam has an inhibitory effect on thromboxane A_2 formation.

4. Discussion

The results obtained in present study showed that midazolam inhibited collagen-induced human platelet aggregation and attenuated thromboxane A_2 formation in a dose-dependent

Table 1 Demographic data and duration of the procedure in the studied groups.

	Age (years)	Weight (kg)	Duration of procedure (min)
Group I (n = 16) [midazolam = 4.9–6.2 mg]	26.3 ± 7.0	85.8 ± 4.1	17.5 ± 3.2
Group II (n = 24) [midazolam = 6.3–7.6 mg]	25.8 ± 5.9	86.7 ± 3.3	15.7 ± 4.7
Group III (n = 11) [midazolam = 7.7–9 mg]	26.1 ± 6.3	85.4 ± 3.7	16.3 ± 5.1

Data were presented as mean ± SD.

Table 2 Bleeding time before and after the procedure.

	Bleeding time (min)
Basal	1.73 ± 0.02
Group I (n = 16) [midazolam = 4.9–6.2 mg]	1.91 ± 0.08
Group II (n = 24) [midazolam = 6.3–7.6 mg]	2.07 ± 0.03
Group III (n = 11) [midazolam = 7.7–9 mg]	2.6 ± 0.05*

Data were presented as mean ± SD.
* $P < 0.05$ considered significant as compared to basal levels.

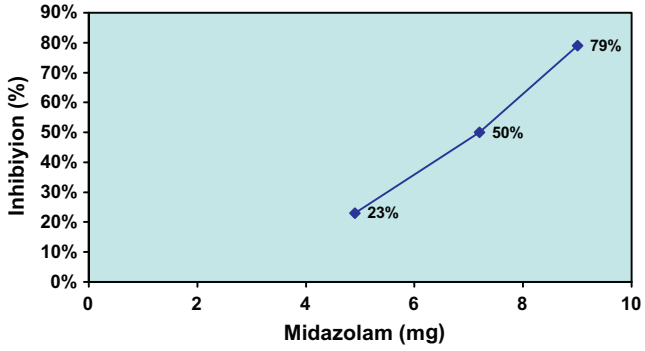

Figure 1 The inhibitory concentration curve of midazolam for collagen-induced platelet aggregation. Aggregation of collagen-induced platelet was suppressed by 50% at the dose of 7.2 mg of midazolam (IC_{50} = 7.2).

manner in patients under monitored anesthesia care for ultrasound guided trans-vaginal oocyte retrieval. It was also shown that midazolam does not act through fibrinogen receptor (glycoprotein IIb–IIIa complex) on the platelets surface membrane.

Platelet aggregation may occur by at least two independent but closely linked pathways. The first pathway involves arachidonic acid metabolism. Activation of phospholipase enzymes (PLA_2) releases free arachidonic acid from membrane phospholipids. About 50% of free arachidonic acid is converted by lipooxygenase enzyme to a series of products which are important chemo-attractants of white cells. The remaining 50% of arachidonic acid is converted by the enzyme cyclo-oxygenase into labile cyclic endoperoxides, most of which are in then converted by thromboxane synthetase into thromboxane A_2 [13].

The second pathway of platelet aggregation can proceed completely independently from the first one: various platelet agonists including thrombin, thromboxane A_2 and collagen bind to receptors and via a G-protein mechanism activates phospholipase C. That leads to activation of different regulatory proteins and also for the liberation of arachidonic acid from membrane phospholipids and the generation of thromboxane A_2. Thromboxane A_2 is very labile with a half-life of less than 1 min before it is degraded into the inactive thromboxane metabolite B_2 and malonyldialdehyde [13].

Thromboxane A2 (TXA2) is synthesised by activated platelets and it induces platelet aggregation by expressing the glycoprotein complex GP IIb/IIIa in the cell membrane of platelets. Fibrinogen attaches to these receptors resulting in further strengthening the clot. Some of the dugs that possess antiplatelet properties like aspirin irreversibly inhibit platelets by inducing inhibition of thromboxane A2 formation. Because of his very short half life, TXA2 primarily functions as a mediator in the tissues close to the site of its production. In human studies, measuring thromboxane B2 is used to indirectly measure TXA2 production [14,15]. In the present study, midazolam was found to dose-dependently inhibit thromboxane A_2 formation.

In the current study, Bleeding Time (BT) was significantly prolonged in group III compared to basal levels. However, such prolongation did not exceed the high normal level of BT. These results supports the previously obtained results by Rodgers and Levin [16] which stated that there is no correlation between the BT and defects in platelet adhesions; thus, BT is not useful for detecting clinically significant abnormalities in platelet adhesion.

Glycoprotein IIb–IIIa is the principal receptor on the platelet plasma membrane. Fibrinogen and other agonists bind to glycoprotein IIb–IIIa [17]. Bound fibrinogen links platelets together in an aggregate [18].

Midazolam sedation dose range between 0.01 and 0.1 mg/kg [1]. Midazolam is a member of a generation of benzodiazepines, called imadodiazepines. Adding imidazole ring to the benzodiazepine nucleus resulted in its unique pharmacokinetic properties [19].

The present study showed that midazolam has a dose-dependent inhibitory effect on human platelet aggregation. The inhibitory concentration values of midazolam to 50% (IC_{50}) for collagen induced platelet aggregation, was achieved at a dose of 7.2 mg in the current study. Results obtained by other studies indicated that concentrations of (6–26 μM) midazolam used to inhibit platelet aggregation *in vitro* had an (IC_{50} = 16.1 μM) and claimed that these concentrations are close to those of blood concentrations obtained during midazolam-induced sedation *in vivo* [12]. The results obtained in that *in vitro* study was in agreement with the results obtained in the current *in vivo* study.

Another study by Hsiao and his colleagues [2] (performed both *in vitro* and *in vivo*) demonstrated that midazolam possesses anti-aggregatory effects that results in delayed latent period of platelet formation which may significantly inhibit thrombus formation *in vivo*. This was in consistence with the results obtained in the current study. In their study, Hsiao and his colleagues [2] explained the antiplatelet activity of midazolam by its inhibition of phospholipase C activation and inhibition of intracellular Ca^{+2} mobilization in platelets.

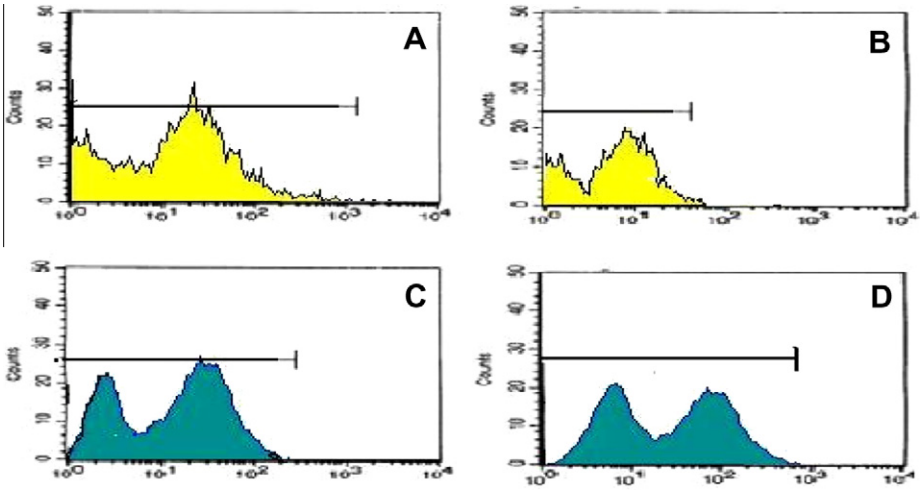

Figure 2 Flowcytometric analysis of triflavin binding to human platelets in the absence or presence of different concentrations of midazolam (7 and 9 mg): (A) the histogram represents the fluorescence profiles of triflavin (2 μg/ml) in the absence of midazolam as a positive control; (B) in the presence of EDTA (5 mM) as the negative control, (C) in the presence of a total given dose 7 mg and (D) 9 mg midazolam respectively. Midazolam at the two studied doses did not inhibit FITC-triflavin binding to fibrinogen receptor in platelet suspensions suggesting that midazolam's inhibitory effect on platelet aggregation is not through binding to fibrinogen receptor.

Table 3 Effect of midazolam on thromboxane B_2 formation induced by collagen in washed human platelets in the three studied groups.

	Thromboxane B_2 (ng/ml)
Resting	47.3 ± 11.6
Collagen (2 μg/ml)	259.2 ± 39.4*
Samples of Group I (n = 16) + Collagen (2 μg/ml)	172.8 ± 23.5†
Samples of Group II (n = 24) + Collagen (2 μg/ml)	126.4 ± 19.7•
Samples of Group III (n = 11) + Collagen (2 μg/ml)	71 ± 11.2•

Data were presented as mean ± SD.
* $P < 0.001$ as compared with the resting group.
† $P < 0.05$ as compared with the collagen group.
• $P < 0.001$ as compared with the collagen group.

The current study attributed the antiplatelet effect of midazolam to inhibition of thromboxane A_2 formation.

In the current study, midazolam did not significantly inhibit the binding of FITC-triflavin to fibrinogen receptor, signifying that the antiplatelet effect of midazolam might not be caused by preventing fibrinogen from binding to its specific receptor on the platelet membrane.

A Limitation to our study is that we did not repeat the tests few hours or few days later to detect the remote effect of drug on platelets.

5. Recommendations

We recommend that if the total dose of midazolam used during sedation or anesthesia exceeded 7.2 mg, and/or if the patient was having a problem with his platelets (e.g. on chronic antiplatelet therapy or bleeding tendencies); then, investigations should be performed to detect performance of midazolam on platelets adhesions to ensure patient safety.

6. Conclusion

The present study shows that midazolam dose-dependently inhibits collagen-induced human platelet aggregation and inhibits thromboxane A_2 formation. It was also concluded that fibrinogen receptor (glycoprotein IIb–IIIa complex) on the platelet surface membrane is not the site of action of midazolam.

References

[1] Heinrich M, Wetzstein V, Muensterer OJ, Till H. Conscious sedation: Off-label use of rectal S(+)-ketamine and midazolam for wound dressing changes in paediatric heat injuries. Eur J Pediatr Surg 2004;14(4):235–9.
[2] Hsiao G, Shen MY, Chou DS, Chang Y, Lee W, Lin CH, et al. Mechanisms of antiplatelet and antithrombotic activity of midazolam in vitro and in vivo studies. Eur J Pharmacol 2004;487(1–3):159–66.
[3] Kornecki E, Ehrlich YH. Lenox RH: Platelet-activating factor-induced aggregation of human platelets specifically inhibited by triazolobenzodiazepines. Science 1984;226:1454–6.
[4] Romstedt K, Huzoor A. Benzodiazepines inhibit human platelet activation: comparison of the mechanism of anti-platelet actions of flurazepam and diazepam. Thromb Res 1985;38:361–74.
[5] Karaseva TL, Belikova MV, Pavlovskii VI, Andronati KS, Kahanova TA. In vitro effect of 1.2-dihydro-3H-1, 4-benzo diazepine-2-one derivatives on the platelet aggregation and lipid peroxidation in rats. Ukr Biokhim Zh 1998;70:81–5.
[6] Lingjaerde O. Effects of the benzodiazepine receptor ligands midazolam. RO15-1788, and RO 5-4864, alone or in combi nations, on platelet serotonin uptake. Pharmacopsychiatry 1986;19:15–8.
[7] Ng EH, Chui DK, Tang OS, Pak Chung Ho PC. Paracervical block with and without conscious sedation: a comparison of the pain levels during egg collection and the postoperative side effects. Fertil Steril 2001;75(4):711–7.
[8] Ben-Shlomo I, Moskovich R, Katz Y, Shalev E. Midazolam/ketamine sedative combination compared with fentanyl/propfol/isoflurane anaesthesia for oocyte retrieval. Human Reprod 1999;14(7):1757–9.

[9] Ghisi D, Fanelli A, Tosi M, Nuzzi M, Fanelli G. Monitored anesthesia care. Minerva Anestesiol 2005;71(9):533–8.
[10] Sheu JR, Teng CM, Huang TE. Ttiflavin, an RGD – containing antiplatelet peptide, binds to GP IIIa of ADP-stimulated platelets. Biochem Biophys Res Commun 1992;189:1236–42.
[11] Ramsay MA, Savege TM, Simpson BR, Goodwin R. Controlled sedation with alphaxolone-alphadalone. BMJ 1974;2: 656–9.
[12] Sheu JR, Hsiao G, Luk HN, Chen YW, Chen TL, Lee LW, et al. Mechanisms involved in the antiplatelet activity of midazolam in human platelets. Anesthesiology 2002;96:651–8.
[13] Lassan MA, Manning RA. In: Practical Haematology, vol. 16. Harcout Publishers Limited; 2001. p. 339–91.
[14] Catella F, Healy D, Lawson JA, FitzGerald GA. 11-Dehydrothromboxane B2: a quantitative index of thromboxane A2 formation in the human circulation. PNAS 1986;83(16): 5861–5.
[15] Lordkipanidzé M, Pharand C, Schampaert E, Turgeon J, Palisaitis DA, Diodati JG. A comparison of six major platelet function tests to determine the prevalence of aspirin resistance in patients with stable coronary artery disease. Eur Heart J 2007;28(14):1702–8.
[16] Rodgers RP, Levin J. A critical reappraisal of the bleeding time. Semin Thromb Hemost 1990;16:1–20.
[17] Cramer EM, Savidge GF, Vainchenker W, et al. Alpha-granule pool of glycoprotein IIb–IIIa in normal and pathologic platelets and megakaryocytes. Blood 1990;75:1220–7.
[18] Sims PJ, Ginsberg MH, Plow EF, Shattil SJ. Effect of platelet activation on the conformation of the plasma membrane glycoprotein IIb–IIIa complex. J Biol Chem 1991;266:7345–52.
[19] Yuan R, Flockhart DA, Balian JD. Pharmacokinetic and pharmacodynamic consequences of metabolism-based drug interactions with alprazolam, midazolam and triazolam. J Clin Pharmacol 1999;39:1109–25.