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SCIENTIFIC ARTICLE

In Vitro Effect of Dexmedetomidine on Platelet Aggregation

Emine Arzu Kose[a,*], Oral Nevruz[b], Mehtap Honca[c], Vedat Yildirim[d]

[a] Department of Anesthesiology and Reanimation, School of Medicine, Kirikkale University, Kirikkale, Turkey
[b] Department of Hematology, Gulhane Military Medical Academy, Ankara, Turkey
[c] Department of Anesthesiology and Reanimation, Kecioren Teaching and Medical Research Hospital, Ankara, Turkey
[d] Department of Anesthesiology and Reanimation, Gulhane Military Medical Academy, Ankara, Turkey

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Abstract
Background and objectives: Dexmedetomidine is a selective α_2-agonist. There are 250-300 α_2-adrenoceptor on the surface of each human platelet and ephedrine induces platelet aggregation by binding these receptors. This study was designed to study platelet function after incubation with therapeutic concentrations of dexmedetomidine.
Methods: The study was carried out on 18 healthy, non-smoking males, ages ranging 25 to 35 years old. Because of the recommended therapeutic concentration range of dexmedetomidine obtained by intravenous infusion is 0.4-1.2 ng.mL^{-1}, dexmedetomidine solutions were prepared in three different concentrations. The calculated value of dexmedetomidine solution and diluent without dexmedetomidine as control were added to the blood sample. Thus, we obtained 0, 0.4, 0.8 and 1.2 ng.mL^{-1} dexmedetomidine concentrations of plasma. Each concentration of dexmedetomidine was incubated with whole blood at 37°C during 15 minutes. Then blood samples were centrifugated to prepare platelet-rich plasma and platelet-poor plasma. The platelet-rich plasma was diluted with the platelet-poor plasma to yield test platelet-rich plasma with a final platelet count of 250 ± 50 X 10^9.L^{-1}.
Results: The platelet aggregation amplitudes and slopes were statistically similar among all groups by the aggregation test, which were performed with ADP, collagen or epinephrine.
Conclusion: Therapeutic concentrations of dexmedetomidine had no effect on the platelet functions in healthy individuals in vitro.

* *Corresponding author.* Department of Anesthesiology and Reanimation, School of Medicine, Kirikkale University, 71100-Kirikkale, Turkey.
E-mail: arzuhct@hotmail.com (E.A. Kose)

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Introduction

Dexmedetomidine is a selective α_2 agonist for which labeled indication is intensive care unit (ICU) sedation. It can produce cooperative sedation associated with minimal respiratory depression and analgesia.[1,2] Those properties make it useful in many clinical situations, including the sedation of ICU patients in whom the ability to perform frequent neurologic examination is of clinical importance. Additionally, because of the same properties, using this agent may be advantageous in central nervous system related procedures such as awake craniotomy, carotid endarterectomy with regional anesthesia, carotid angioplasty and stenting.[1,3] It can also be preferred as an adjuvant in general anesthesia because of the possibility of the early extubation and recovery of cognitive functions. In this way, it is possible to reveal the additional postoperative neurological deficits in early postoperative period.[4] The patients who are in ICU generally received concomitant medications such as vasopressors, vasodilators, digoxin, diuretics, beta blockers, aspirin, low molecular weight heparin that have all possible effects on platelet function. On the other hand, it is known that there are 250-300 α_2-adrenoceptor on the surface of each human platelet and ephedrine activates platelets and induces their aggregation by binding these receptors.[5] Ephedrine is considered a weak platelet agonist, the function which is mainly to sensitize platelets to other activating agents.[6-9] Due to the possibility of the increased risk of hemorrhage by drug interactions, it is important to clarify the effects of dexmedetomidine on platelet functions for other medications may have possible additive effects on platelet function. This study was designed to study platelet function after incubation with therapeutic concentrations of dexmedetomidine in healthy volunteers.

Materials and methods

After obtaining local ethic committee approval and informed consent, we carried out the study on 18 healthy, non-smoking males, ages ranging 25 to 35 years old (mean ± SD = 28.32 ± 4.35 years). We did not include subjects who had an abnormal platelet count, a history of thrombosis or abnormal bleeding, active neoplasia or active inflammatory disease. All studies were done in the morning with the volunteers fasting overnight and we ascertained that no drugs had been taken within 2 weeks before testing. The venous blood samples were drawn without a tourniquet with a 20-gauge needle from the antecubital vein. We anti-coagulated the samples with 3.8% 0.130 M sodium citrate solution (blood to anticoagulant ratio: 9/1). There was no hemolysis in samples. We kept them at room temperature and tested within 60 minutes of collection. We counted platelets using an automated cell counter device (Abbott Cell-Dyne 4000; Abbott Park, Chicago, IL, USA).

Given the recommended therapeutic concentration range of dexmedetomidine obtained by intravenous infusion is 0.4-1.2 $ng.mL^{-1}$ (Precedex® SPC; Abott Laboratories, Abbott Park, IL), we prepared dexmedetomidine solutions in three different concentrations which would yield 0.4 $ng.mL^{-1}$, 0.8 $ng.mL^{-1}$ and 1.2 $ng.mL^{-1}$ dexmedetomidine by using 0.9% sodium chloride as diluent. We used the diluent not including dexmedetomidine as control. After the determination of hematocrit values, we divided the blood samples into four equal parts. According to the amount of plasma (derived from the hematocrit), we added the calculated value of dexmedetomidine solution and diluent without dexmedetomidine as control to blood sample (1 µL solution for 1 mL of plasma) and obtained 0, 0.4, 0.8 and 1.2 $ng.mL^{-1}$ dexmedetomidine concentrations of plasma. Each concentration of dexmedetomidine was incubated with whole blood at 37°C. After incubation for 15 minutes, blood samples were centrifugated (100 g, 10 minutes) to isolate platelet-rich plasma (PRP) from supernatant. We centrifuged the remainder of blood again (2,400 g, 20 minutes) to prepare platelet-poor plasma (PPP). The PRP was diluted with the PPP to yield test PRP with a final platelet count of 250 ± 50 X $10^9.L^{-1}$. Aggregation was performed using a turbidometric method (Chrono-log Corporation, Model 560-Ca; Havertown, PA, USA) according to the protocol of Chrono-log Corporation. We randomly changed the sequence by which different dilutions were studied in the aggregation, which was unknown to the persons performing the studies. We evaluated platelet aggregation response with adenosine diphosphate (ADP) (5 and 10 µM final concentrations), collagen (3 $\mu g.mL^{-1}$ final concentrations) and epinephrine (10 µM final concentration). We obtained aggregating agents from Chrono-log Corporation. The device calculated dose-response cures automatically and evaluated them using amplitude and slope.

Statistical analysis

We analyzed data using the SPSS 11.5 (SPSS Inc. Software, Chicago, Illinois, USA) statistical software. Because the data were not normally distributed and the variations were not homogenous among all groups, we used Friedman repeated measure analysis of variance to compare platelet aggregation response to ADP, collagen or epinephrine between control and each dexmedetomidine solution. All data were presented as median and range. We considered $p < 0.05$ statistically significant.

Results

The platelet aggregation amplitudes and slopes were statistically similar among all groups by the aggregation test, performed with ADP, collagen or epinephrine (Table 1).

Discussion

Dexmedetomidine, the dextro enantiomer of medetomidine, is a potent and highly selective α_2-adrenoreceptor agonist.[1-3] It is 8 to 10-fold more potent than clonidine for the α_2-adrenoceptors. By virtue of this potency, dexmedetomidine is considered to be a full agonist of the α_2-adrenoceptors, which might permit its application in relatively high doses for sedation and analgesia without the unwanted vascular effects from activation of α_1-adrenoceptors.[1-3] In addition to the administration in ICU as a sedative-analgesic agent, there have been a number of studies of lower doses of dexmedetomidine as an adjunct to anesthesia in cardiac and noncardiac surgical operations.[10,11] On the other hand, human platelets have 250-300 α_2-adrenoreceptor on their surfaces. Ephedrine activates platelets and induces their aggregation by binding these receptors.[5] Alpha-adrenergic receptors of human platelets are exclusively α_{2A} subtype.[11] In vitro, stimulation of

Table 1 Platelet aggregation results and statistical comparison of four groups.

	Parameter	Control Mean ± SD	Dex-0.4 ng.mL^{-1} Mean ± SD	Dex-0.8 ng.mL^{-1} Mean ± SD	Dex-1.2 ng.mL^{-1} Mean ± SD	P value
Collagen	Amp (%)	59 ± 18	64 ± 19	66 ± 21	65 ± 23	0.070
3 µg.mL^{-1}	Slope	99.44 ± 34.14	96.72 ± 37.40	101.88 ± 43.26	97.66 ± 34.91	0.906
ADP	Amp (%)	61 ± 10	63 ± 12	62 ± 10	61 ± 10	0.776
5 µM	Slope	74.83 ± 26.76	85.33 ± 24.44	86.22 ± 18.67	83.50 ± 20.24	0.064
ADP	Amp (%)	62 ± 16	63 ± 11	63 ± 18	62 ± 14	0.633
10 µM	Slope	87.55 ± 20.26	85.72 ± 18.95	96.27 ± 44.94	82.55 ± 26.82	0.795
Epinephrine	Amp (%)	60 ± 09	63 ± 12	63 ± 12	60 ± 12	0.737
10 µM	Slope	86.55 ± 36.59	89.16 ± 39.77	96.27 ± 44.94	98.22 ± 38.83	0.280

ADP, adenosine diphosphate; Amp, amplitude; Dex, dexmedetomidine.
The Friedman repeated measures analysis of variance was used. Data were expressed as mean ± SD. $p < 0.05$ was considered statistically significant.

platelet $ß_2$-adrenoreceptors blunts aggregation by increase of intracellular c AMP, and aggregatory response to epinephrine may be enhanced by non-selective blockage because of unopposed α_2-adrenoreceptor stimulation.[13,14] It was speculated that catecholamine surge might trigger a hypercoagulable state and enhance the odds of overt thrombosis in patients with atherosclerotic disease and hypertension.[15-18] Martins et al. evaluated effects of dexmedetomidine on blood coagulation by thromboelastography and concluded that dexmedetomidine had significantly increased reaction time and decreased coagulation index in final curves as compared to initial values. They reported that, although dexmedetomidine had a mild hypocoagulative effect, coagulation remained within normal ranges.[19] Although it is wise to think that dexmedetomidine may have a decreasing effect on thrombotic events during an operation which stimulates sympathetic nervous system and leads to increased catecholamine levels, it is never used as a sole agent, not in patients undergoing surgery nor in the ICU. Hence, it is important to clarify the effects of dexmedetomidine on platelet functions for other medications, which may have possible additive effects on platelet function. We designed this study because of the fact that we could not find any published investigation related to the effects of dexmedetomidine on platelet functions in English medical literature.

A study that investigated the effects of clonidine and its analogue para-aminoclonidine showed that both agents potentiated platelet aggregation induced by a submaximal concentration of ADP and epinephrine-induced aggregation in a dose-dependent fashion. Stamp et al. concluded that these two agents are partial agonists for the α_2-adrenergic receptors on blood platelets and that this receptor exists predominantly in the low-affinity state in the intact cell.[20] Similarly, Petrusewicz et al. tested sixteen imidazole derivative drugs with regards to their aggregatory and antiaggregarory effects on human blood platelets and found a significant inhibition of the epinephrine induced aggregation in a dose dependent fashion by using clonidine in their study.[21] In another study, it was shown that aggregation induced by ADP was potentiated by clonidine greater than or equal to epinephrine.[22] In the present study, we used the recommended therapeutic dose range of dexmedetomidine in vitro and we could not find any statistically significant difference in platelet aggregation response to ADP (5 and 10 final concentrations), collagen (3 µg.mL^{-1} final concentrations) or epinephrine (10 µM final concentrations). While it is 8 to 10 fold more potent than clonidine for the α_2-adrenoceptors, the lack of the potentialisation of the platelet aggregation by using dexmedetomidine can be explained by the low doses of dexmedetomidine. Additionally, it is notable that the increased platelet aggregation by which obtained clonidine in previous studies was also dose-dependent. Recent reports show that imidazole α-adrenergic agents inhibit epinephrine-induced aggregation in canine platelets more effectively than non-imidazole ones.[23] In a previous report, nonadrenergic imidazole-prefered binding sites were shown in human platelets.[24] Because both clonidine and dexmedetomidine have an imidazole structure, the imidazole structure could not be responsible for this situation. Although the results of platelet aggregation tests were not different with the therapeutic dose range of dexmedetomidine when compared with the control group in this study, these results may show an increased platelet aggregation with the usage of increased doses of the agent like clonidine. We preferred to study only the recommended dosage of this agent because of the fact that dexmedetomidine can cause unwanted cardiovascular effects with the usage of overdoses in clinical administration.

In conclusion, when used in similar concentrations to recommended dose-range, dexmedetomidine had no effect on the platelet functions in healthy individuals in vitro. However, platelet functions can be affected by several additional pathological and physiological mechanisms in clinical administration. Hence, it should be used cautiously especially if there is concomitant administration of the other medications, which can alter the platelet functions.

Conflicts of interest

The authors declare no conflicts of interest.

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