Effects of Thromboxane A₂ Inhibition on Osteogenic Sarcoma Cell-induced Platelet Aggregation¹

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ABSTRACT

There is evidence that tumors may stimulate platelet aggregation, causing release of thromboxane A₂. Thromboxane A₂ may potentiate tumor metastasis by stimulating tumor cell growth and proliferation and by enhancing platelet-tumor cell aggregate formation. Despite potential significance of thromboxane A₂ in tumor metastasis, agents which inhibit thromboxane A₂ synthesis have not been uniformly effective in reducing tumor metastasis. We, therefore, evaluated the effects of a thromboxane A₂ receptor antagonist SQ-29,548 compared to those of a thromboxane A₂ synthetase inhibitor OKY-046 on osteogenic sarcoma-induced platelet aggregation and thromboxane A₂ release. Osteogenic sarcoma cells added to platelet-rich plasma caused complete and irreversible platelet aggregation as well as thromboxane A₂ release. Preincubation of platelet-rich plasma with SQ-29,548 (2 to 20 nM) decreased platelet aggregation induced by tumor cells, but it had no effect on thromboxane A₂ release. In contrast, preincubation of platelet-rich plasma with OKY-046 (0.1 to 10 μM) had no effect on platelet aggregation despite a decrease in thromboxane A₂ synthesis. These results suggest that thromboxane A₂ receptor blockers, rather than synthetase inhibitors, may prevent tumor cell-induced platelet aggregation.

INTRODUCTION

Evidence exists that certain tumor cells stimulate platelet aggregation (1-4), which may be an early step in metastasis of these tumors. Tumor cell-induced platelet aggregation was first observed by Gasic et al. (5), who identified 16 different tumors which directly stimulated platelets. Subsequently, others have confirmed the platelet-stimulatory capacity of rat renal sarcoma (6), glioblastoma (4), neuroblastoma (4), and melanoma cells (7, 8). We have recently observed induction of platelet aggregation by cultured MG63 human osteogenic sarcoma cells.

Thromboxane A₂ is released during platelet aggregation. It has been suggested that release of thromboxane A₂ may be pathogenic for tumor metastasis for the following reasons. (a) Some tumors release large amounts of thromboxane A₂ compared to normal tissue (9). (b) Thromboxane A₂ potentiates tumor growth in culture and increases metastasis in animals (8). (c) Thromboxane A₂ is a potent stimulant of platelet aggregation (10), causing vascular injury which may promote implantation of tumor cell-platelet aggregates.

If thromboxane A₂ participates in tumor metastasis, it may be hypothesized that thromboxane A₂ inhibitors should decrease tumor metastasis. However, nonspecific thromboxane A₂ inhibitors, such as aspirin, have not yielded uniformly beneficial results (11-14). The reasons for the lack of consistently beneficial effect may relate to several factors. (a) Many of these agents have actions unrelated to platelet inhibition which may affect tumor metastasis. (b) Nonselective thromboxane A₂ synthetase inhibitors decrease prostacyclin, a potential antimetastatic prostaglandin (8). Selective thromboxane A₂ synthetase inhibitors result in diversion of precursor endoperoxides to other products, such as prostaglandin E₂, prostaglandin D₂, and prostaglandin F₂α, which may promote tumor metastasis. Accumulation of cyclic endoperoxides exerts pro-platelet-aggregatory actions similar to those of thromboxane A₂. In contrast, thromboxane A₂-endoperoxide receptor antagonists have potential advantages over thromboxane A₂ synthetase inhibitors, since these agents do not cause diversion of endoperoxides to other prostaglandins or alteration of prostacyclin synthesis. Since endoperoxides share the same receptors as thromboxane A₂, the prometastatic effects of endoperoxides as well as those of thromboxane A₂ may be suppressed.

The aim of this study was to determine if thromboxane A₂ inhibition would modify osteogenic sarcoma cell-induced platelet aggregation. We evaluated effects of osteogenic sarcoma cells on human platelet aggregation function before and after treatment of platelets with a selective thromboxane A₂ synthetase inhibitor or a receptor antagonist.

MATERIALS AND METHODS

Cell Culture. Human osteogenic sarcoma cells (MG-63) were obtained from the American Type Tissue Collection (Rockville, MD). The cells were grown in Dulbecco's modified Eagle's medium ( Gibco Grand Island, NY) and were supplemented with 15% fetal bovine serum and antibiotics.

Harvesting of Adherent Cells. Adherent cell populations were brought to a single cell suspension by a 5-min incubation in PBS¹ supplemented with 0.5 mM EDTA and glucose (1.0 g/liter). At the end of the incubation period, the cells were diluted with a 5-fold excess of Dulbecco's modified Eagle's medium. Cells were then washed twice with PBS and counted in a hemocytometer. Viability was determined by trypan blue dye exclusion. There was more than 95% viability in every experiment. The suspensions were adjusted to obtain a final cell concentration in platelet aggregation cuvets at 2 x 10⁶ cells/ml. Cells were maintained at 4°C until use for platelet aggregation.

Tumor Cell-induced Platelet Aggregation. Blood was collected from normal volunteers who had not ingested any drugs within the previous 2 wk. Blood was drawn into tubes containing heparin (10 units/ml) and was then centrifuged at 150 x g for 10 min to obtain PPP. Remaining blood was then centrifuged at 1500 x g for 30 min to obtain PPP. Platelet aggregation was calibrated at 100% with PPP and 0% with PRP. Tumor cell-induced platelet aggregation was measured by comparing light transmission through PRP alone and through PRP incubated with tumor cells (2 x 10⁶ cells/ml). In control experiments, tumor cells were pretreated with collagenase or latex particles to distinguish stimulatory effects of tumor cells from those due to collagen or inert particles, respectively. In other experiments PRP was preincubated at 37°C with thromboxane A₂-endoperoxide receptor antagonist SQ-29,548 or with thromboxane A₂ synthetase inhibitor OKY-046 for 1 min prior to addition of tumor cells.

Tumor Cell-induced Platelet Thromboxane A₂ Generation. After incubation of PRP with tumor cells, thromboxane A₂ generation was measured by gas chromatography-mass spectrometry. The results were expressed as nanograms per milliliter of PPP.

Received 3/27/86; revised 6/12/86; accepted 6/13/86. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹Supported in part by grants from the USPHS (CA 09126 and CA 40351), the National Cancer Institute, American Heart Association, Florida Affiliate, St. Petersburg, FL, and the American Cancer Society, Florida Affiliate, Tampa, FL. Presented in part at the annual meeting of the American Association for Cancer Research, Los Angeles, CA, May 1986 (19).

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³The abbreviations used are: PBS, phosphate-buffered saline; PRP, platelet-rich plasma; PPP, platelet-poor plasma.

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stopped by addition of 10 μM indomethacin and 4.5 mM EDTA (final concentration), and the PRP was centrifuged. The supernatant was stored at −70°C. Thromboxane B2 was measured as the stable metabolite of thromboxane A2 by specific radioimmunoassay. Levels of thromboxane B2 in PRP alone were less than 1 ng/ml and were less than 1%.

RESULTS

Tumor Cell-induced Platelet Aggregation. MG-63 tumor cells caused complete and irreversible platelet aggregation of human platelets (Fig. 1; Table 1). Addition of epinephrine to PRP after incubation with tumor cells did not increase the extent of aggregation, indicating that aggregation induced by osteogenic sarcoma cells was maximal. Pretreatment of tumor cells with collagenase did not decrease platelet aggregation, indicating that aggregation induced by osteogenic sarcoma cells was due to tumor cells and not to collagen. In addition, latex particles alone did not induce platelet aggregation.

Tumor Cell-induced Platelet Thromboxane A2 Synthesis. Thromboxane B2 in PRP alone was less than 1 ng/ml and was unchanged when PRP was incubated with diluents for SQ-29,548 or OKY-046. After stimulation of PRP with tumor cells, thromboxane B2 levels increased to 41.5 ± 21.9 ng/ml (P ≤ 0.02 compared to PRP alone) (Table 1).

Effect of Thromboxane A2 Synthetase Inhibitor and Receptor Antagonist on Tumor Cell-induced Platelet Aggregation and Thromboxane A2 Biosynthesis. Preincubation of SQ-29,548 (2 to 20 nm) with PRP decreased platelet aggregation induced by tumor cells (Fig. 1). However, SQ-29,548 did not decrease thromboxane A2 formation. In contrast, OKY-046 in concentrations up to 10 μM had no effect on platelet aggregation induced by tumor cells despite significant reduction in thromboxane A2 biosynthesis (Table 1). It is noteworthy that MG-63 cells alone produced no significant amount of thromboxane A2.

DISCUSSION

Thromboxane A2 is a potent stimulus of platelet aggregation (10) and may be important in causing tumor cell metastasis. Platelet aggregation may result in platelet aggregate formation around tumor cells, such as is seen within minutes after injection of tumor cells into animals (1, 2). The surrounding of tumor cells by platelets may protect tumor cells from immune surveillance (15), may facilitate formation of a “bridge” between the tumor cells and vascular surface (1, 2), and may promote growth and proliferation of tumor cells (16). Synthesis by tumor cells of thromboxane A2 may also stimulate tumor growth and proliferation as well as further platelet aggregation. Injured blood vessels also generate large amounts of thromboxane A2 locally (17) which could facilitate tumor cell-platelet aggregate formation and implantation of the aggregate. It is, therefore, logical to suggest that thromboxane A2 inhibitors may decrease tumor metastasis. Indeed, tumor metastasis is markedly decreased with thromboxane A2 inhibitors in rats given injections of melanoma cells (18).

In the present studies, we have shown that cultured MG-63 tumor cells per se do not generate significant amounts of thromboxane A2 (19). However, these cells stimulate human platelet aggregation and thromboxane A2 biosynthesis. We reasoned that thromboxane A2 inhibition may prevent aggregation of human platelets in response to tumor cells. The effects of a thromboxane A2 synthetase inhibitor OKY-046 were different from those of thromboxane A2 receptor antagonist SQ-29,548. OKY-046 had no significant effects on osteogenic sarcoma cell-induced platelet aggregation, while thromboxane A2 synthesis was markedly reduced. The lack of inhibitory effects of the drug on platelet aggregation is probably related to small amounts of uninhibited thromboxane A2 which may
be sufficient to cause complete platelet aggregation. In addition, thromboxane A₂ synthetase inhibitors cause accumulation of precursor cyclic endoperoxides which alone are capable of stimulating platelet activity and could result in platelet aggregation. We have performed preliminary experiments to distinguish the roles of endoperoxides and uninhibited thromboxane A₂ in MG-63-induced platelet aggregation. In these experiments, we pretreated platelet-rich plasma with acetylsalicylic acid (aspirin). Aspirin in final concentrations of 9 to 90 μg/ml inhibited this phenomenon, which suggests a primary role of thromboxane A₂ in osteogenic sarcoma-induced platelet activation.

In contrast to the effects of OKY-046, the thromboxane A₂ receptor antagonist SQ-29,548 prevented platelet aggregation in response to MG-63 osteogenic sarcoma cells, but it did not affect thromboxane A₂ formation. Since thromboxane A₂ and endoperoxides act at a common receptor site, it can be reasoned that SQ-29,548 abolished platelet-proaggregatory effects of the endoperoxides. It is noteworthy that thromboxane A₂ synthesis was not affected.

The compound SQ-29,548 inhibits platelet aggregation induced by arachidonic acid or the thromboxane A₂ "mimic" U-46,619, but not that induced by ADP, and does not inhibit thromboxane A₂ synthesis. The basic molecule, 7-oxabicyclo[2.2.1]heptane is similar to several other thromboxane A₂ mimics and receptor antagonists. SQ-29,548 antagonizes the activity of endoperoxides on guinea pig trachea and rat aorta (20). This compound also competitively antagonizes tracheal responses to 11,9-epoxymethanoprostaglandin H₂ and thromboxane A₂, but not to prostaglandin E₂, serotonin, norepinephrine, carbachol, histamine, or KCl. These data suggest that SQ-29,548 is a highly specific thromboxane A₂-endoperoxide receptor antagonist (20, 21).

In conclusion, this study shows that osteogenic sarcoma cells stimulate human platelet aggregation and thromboxane A₂ synthesis. The activation of platelets by these tumor cells is abolished by pretreatment of platelets with a specific thromboxane A₂-endoperoxide receptor antagonist but not with a selective thromboxane A₂ synthetase inhibitor. These observations suggest that platelet-tumor cell interaction may occur in the presence of small amounts of uninhibited thromboxane A₂ or cyclic endoperoxides. As such, use of thromboxane A₂ receptor antagonists may possibly be more beneficial than thromboxane A₂ synthetase inhibitors in the prevention of tumor cell-platelet interactions.

ACKNOWLEDGMENTS

The authors wish to thank Kelly Greetham for her assistance in the preparation of this manuscript.

REFERENCES

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