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Effects of ketorolac tromethamine (Toradol[R]) on a functional model of microvascular thrombosis

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SUMMARY. **Ketorolac tromethamine (Toradol[R]), a potent nonsteroidal anti-inflammatory drug used for postoperative pain, also strongly inhibits platelet agregation. The anti-thrombotic effects of intramuscular ketorolac were assessed with a described rat model of microarterial thrombosis. After a single dose of ketorolac mean bleeding times were significantly prolonged ($p < 0.01$) and platelet aggregation was markedly reduced. Patency rates at 20 min were significantly higher in ketorolac groups compared to controls ($p < 0.005$). However, all vessels were thrombosed at 24 h. Scanning electron microscopy demonstrated decreased platelet aggregation and decreased thrombus formation in ketorolac treated animals at 20 min. The prolonged bleeding time and reduction in platelet aggregation add support to concerns of bleeding complications reported in patients treated with ketorolac perioperatively. Thus, ketorolac should probably not be used for pain relief in patients in whom postoperative haematoma formation is a particular concern. In addition, in this model, ketorolac as a single agent was ineffective for long-term prevention of microarterial thrombosis.**

Despite technical advances in microvascular surgery, anastomotic thrombosis continues to be an Achilles' heel for the microvascular surgeon. Thrombosis rates as high as 35% in injured tissues[1] and 10% in uninjured tissues[2] have been reported. In the past, a wide variety of pharmacological regimens have been used in attempts to improve patency rates (particularly in traumatised tissues) but no uniform regimen has been adopted.[3] Many investigators have reviewed the effects of various pharmacologic agents such as heparin and aspirin in numerous microvascular thrombosis models.[4–7] Using a functional model of microvascular thrombosis developed in our laboratory,[8] we have demonstrated improved patency rates with a single perioperative bolus of heparin or Dextran,[9] with fewer complications associated with Dextran. Cox *et al.*[10] also demonstrated improved patency rates with 100 U/mL of heparinised saline irrigating solution using this model. However, possible complications such as excessive bleeding or adverse drug reactions may preclude the use of these anticoagulants in certain cases.[11] Thus, the search for alternative therapies or combined regimens remains an area of interest.

Ketorolac tromethamine (Toradol[R], Syntex Laboratories, Inc., Palo Alto, CA), a potent nonsteroidal anti-inflammatory drug (NSAID) used for postoperative pain, has been shown to inhibit strongly arachidonic acid-induced platelet aggregation.[12] This finding suggests that ketorolac, like aspirin, could be used as anti-platelet therapy in microvascular procedures. In this study, we used our rat model of microvascular thrombosis to assess the role of ketorolac as a single agent in the prevention of microvascular thrombosis. In addition, blood clotting parameters were obtained to assess the anti-thrombotic effects of ketorolac.

Table 1 Mean bleeding times (s)

Group (n = 7)	*30 min*	*24 h*
Normal saline IM	219±7	240±6
1 mg/kg ketorolac IM	291±5*	264±8
3 mg/kg ketorolac IM	370±11*	270±12

* Indicates a statistically significant difference when compared to controls (Student's t-test, all values $p < 0.01$).

Materials and methods

Forty-two Sprague-Dawley rats (275 g body weight) were kept under National Institutes of Health (NIH) guidelines and allowed rat food and water *ad libitum*. Of the 30 animals used for the thrombosis model, 21 underwent bleeding times 1 week prior to operation. An additional 12 animals were killed for the platelet aggregation profiles (6) and scanning electron microscopy (6). Any animals undergoing operation were anaesthetised with sodium pentobarbital (Nembutal, 50 mg/kg) intraperitoneally.

Part 1: Dose determination

Bleeding time. One week prior to operation, bleeding times were obtained on three groups of animals (n = 7 for each group) to determine an appropriate dose of ketorolac for the final study. Group 1 animals (controls) received normal saline intramuscularly. Group 2 animals received 1 mg/kg of ketorolac intramuscularly and Group 3 animals received 3 mg/kg of

ketorolac intramuscularly. Each dose of ketorolac was administered approximately 30 min prior to the bleeding time determination because the time to obtain maximum plasma concentration of ketorolac in rats ranges from 0.25 h to 0.75 h after intramuscular injection.[13] A standardised method of bleeding time determination[14] was performed on all rats 30 min and 24 h after injection.

Aggregation profile. Platelet aggregation profiles were obtained on six animals. Group 1 animals (n = 3) were injected intramuscularly with normal saline and Group 2 animals (n = 3) were injected intramuscularly with 3 mg/kg ketorolac. The animals were anaesthetised 30 min later and a median sternotomy was performed, after which the left ventricle was cannulated. A blood sample (4.5 ml) was removed from each animal and each sample underwent two centrifugations to obtain a platelet-rich and a platelet-poor plasma. These were placed in a standard aggregometer (Platelet Aggregation Profiler, Model PAP-4, BIO-DATA Corp., Hatboro, PA) and 0.10 mg of arachidonic acid was added after 2 min. The percentage of platelet aggregation was measured over a 5-min period.[15]

Part 2: Thrombosis model

Experimental design. Thirty Sprague–Dawley rats were used in the final series. The animals were randomly assigned to one of three groups (n = 10). Initially, 20 of the animals were numbered in sequence (1 to 20). Prior to operation, syringes numbered 1 through 20 were randomly filled with either normal saline (0.6 ml) or ketorolac and normal saline (3 mg/kg ketorolac; 0.6 ml). The contents of each was recorded and the syringes were placed in a box. The operator was blinded to the contents of the syringes and randomly selected the syringes at the time of the operation. The animals received the contents of the syringe intramuscularly prior to dissection of the leg.

In the remaining 10 animals, ketorolac (3 mg/kg) was injected intramuscularly prior to dissection of the leg as above. Each animal then received further intramuscular injections of ketorolac (3 mg/kg) at 6 h intervals for 24 h.

Surgical technique. The femoral and inguinal areas were shaved and a linear incision was made over the femoral vessels. The superficial femoral arteries, approximately 1 mm in outside diameter, were identified and dissected free under a Zeiss operating microscope. A background sheet was placed behind the vessel and extra adventitia was sharply dissected away. A bulldog clamp exerting approximately 175 g of force was placed on the vessel for 2 min to simulate a crush injury. After removal of the bulldog clamp, two microvascular clamps were applied proximally and distally to the crush site. An arteriotomy was made through one half the circumference of the vessel at the location of the bulldog crush site. The intima was abraded for 40 s using a BV 75–3 microsurgical needle (Ethicon Inc., Somerville, NJ). The arteriotomy was closed with three 10–0 nylon sutures and patency was assessed by the distal "milking test". To produce stasis, the microvascular clamps were replaced proximally and distally for 20 min and then removed. Patency was determined at 20 min and at 24 h by both direct visualisation and the distal "milking test". The same operator performed all operations and detailed operative notes were recorded, with particular regard to operative complications and haematoma formation.

Part 3: Scanning electron microscopy

Six animals were divided into two groups (n = 3) and the thrombosis model was carried out in a blind fashion. One group received normal saline (0.6 ml) intramuscularly and the other received an equal volume of ketorolac and saline (3 mg/kg ketorolac) intramuscularly prior to dissection of the leg. In each animal, the superficial femoral artery was transected after completion of the model, immediately irrigated of nonthrombosed blood products, and fixed in sodium cacodylate buffered 4% glutaraldehyde at 4°C for 3 h. The specimens were removed from this solution and placed in sodium cacodylate buffer for 3 days. This was followed by placement of the specimens in 1% osmium tetroxide for 45 min, dehydration in a graduated series of alcohol solutions, critical point drying in liquid CO_2 and coating with gold-palladium. Scanning electron microscopy examination was accomplished with a JEOL 100 CX CHEMSCAN (JEOL USA, Inc., Peabody, MA, magnification 1500 ×).

Results

Part 1

Bleeding times in those animals injected with ketorolac were compared to those in controls using the Student's

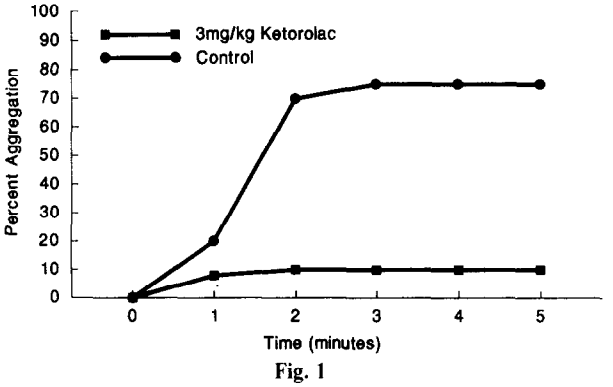

Fig. 1

Figure 1—Platelet aggregation profile demonstrates a marked inhibition of platelet aggregation in those animals injected with 3 mg/kg ketorolac (note only 10% aggregation).

Table 2 Patency rates

Group (n = 10)	*20 min* (%)	*24 h* (%)
Normal saline IM	10	0
3 mg/kg ketorolac IM	90	0
3 mg/kg ketorolac IM q 6 hours	90	0

* Indicates a statistically significant difference when compared to controls (Fisher's exact test, all values $p < 0.005$).

Fig. 2

Figure 2—Scanning electron micrograph of superficial femoral artery exhibiting platelet aggregates (small arrow) and red blood cells (large arrow) forming a thrombus in a control animal 20 min after performing the model (magnification × 1500).

t-test. A highly significant prolongation of the mean bleeding time was noted at 30 min in those animals injected with both 1 mg/kg ketorolac (291 ± 5 s) and 3 mg/kg of ketorolac (370 ± 11 s) compared to control animals (219 ± 7 s; $p < 0.01$). At 24 h, however, there was no clinically significant difference in the mean bleeding times of the ketorolac groups when compared to controls (Table 1).

Aggregation profiles revealed a marked inhibition of platelet aggregation in those animals injected with 3 mg/kg ketorolac. The ketorolac group exhibited only 10% aggregation as compared to approximately 75% aggregation in the controls (Fig. 1).

Examination of the results of the bleeding times and aggregation profiles suggested that a dose of 3 mg/kg of ketorolac was appropriate for the remainder of the study.

Part 2

Patency rates in the ketorolac groups (single and multiple dose) were compared to those in the control group using the Fisher exact test. Although both ketorolac groups exhibited 90% patency at 20 min (compared to 10% patency in controls; $p < 0.005$), all vessels were thrombosed at 24 h. Thus patency rates in both ketorolac groups were significantly different at 20 min, but not at 24 h, when compared to controls (Table 2).

Part 3

Scanning electron microscopy of the vessels from the ketorolac group revealed minimal platelet aggregation and decreased thrombus formation 20 minutes after performing the model. Vessels in the control group, however, exhibited numerous platelet aggregates and red blood cells forming a sizeable thrombus (Figs 2, 3).

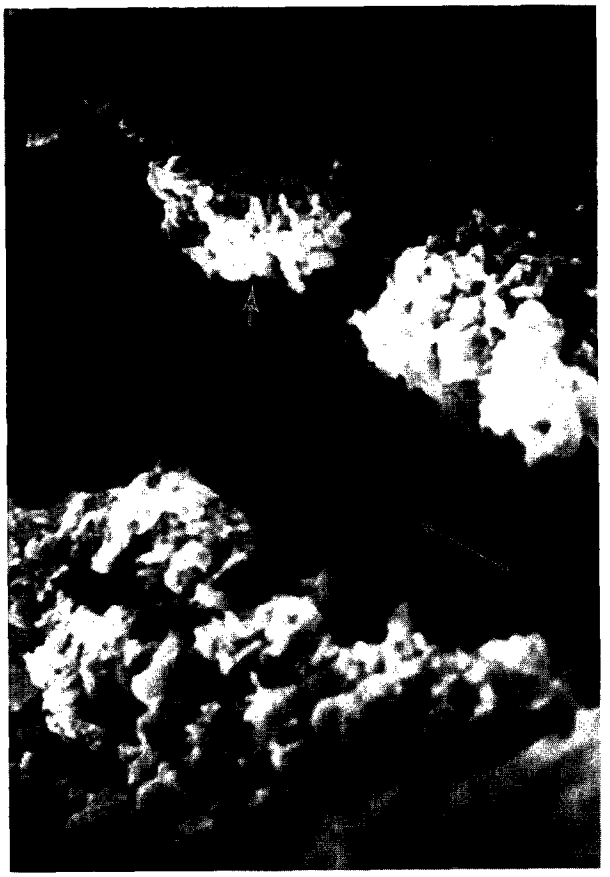

Fig. 3

Figure 3—Scanning electron micrograph of superficial femoral artery exhibiting minimal platelet aggregates and decreased thrombus (single arrow) in a 3 mg/kg ketorolac-injected animal 20 min after performing the model. The patent lumen of the vessel is denoted by the double arrow (magnification × 1500).

Discussion

Ketorolac tromethamine has been shown to be more potent than aspirin in inhibiting arachidonic acid-induced platelet aggregation.[12] Greer[16] demonstrated that ketorolac not only significantly inhibited platelet aggregation in response to arachidonic acid and collagen, but also inhibited Thromboxane A_2 production. Clinical trials[12,16] have demonstrated that ketorolac produces a modest prolongation of bleeding times, no change in prothrombin times or partial thromboplastin times, and a clinically insignificant reduction of platelet counts. In contrast to aspirin, however, ketorolac's inhibition of platelet aggregation is reversible within 24–48 h after the drug is discontinued.[12,17]

Despite ketorolac's potent effects on platelet aggregation, its use in microvascular surgery has been limited. Ideally, ketorolac could be used as a postoperative pain reliever, thus precluding the use of morphine or meperidine, as well as a reversible antithrombotic agent. However, unwanted complications such as difficult haemostasis or postoperative haematoma formation are a concern. Garsha and Bostwick[18] described postoperative haematomas in 5 patients who received postoperative injections of ketorolac for pain after breast surgery, though Concannon *et al.*[19] used ketorolac in two aspirin-allergic patients who required microvascular surgery, with successful suppression of platelet function and no reported complications.

Our bleeding times and aggregation profiles at a dose of 3 mg/kg in the rat were consistent with ketorolac's potent, reversible platelet inhibition properties. Scanning electron microscopy further demonstrated minimal platelet aggregation and decreased thrombus formation at 20 min in ketorolac-treated animals. In addition, patency rates were significantly different from controls at 20 min. These findings indicate that ketorolac has a significant early effect on clot formation, and this raises further questions about the use of ketorolac in certain plastic surgery procedures in which haematoma formation is of major concern.

Despite ketorolac's significant early effect on clot formation, all vessels in this study were thrombosed at 24 h. The formation of a thrombus is considered to be the result of two haemostatic processes: the formation of the haemostatic plug through platelet adherence and aggregation, and the formation of fibrin through the coagulation cascade. Our results indicate that ketorolac provided a short-term inhibition of platelet aggregation but did not alter the coagulation cascade sufficiently to prevent eventual thrombus formation. Theoretically, the addition of an agent that alters fibrin formation, such as heparin or dextran, may provide better protection against thrombus formation, but possible bleeding complications associated with these combinations merit further investigation.

References

1. Weiland AJ, Villarreal-Rios A, Klein HE, Kutz J, Atasoy E, Lister G. Replantation of digits and hands: analyses of surgical technique and functional results in 71 patients with 86 replantations. *J Hand Surg* 1977; 2: 1–9.
2. Shaw WW, Converse JM. A survey of 2680 free flaps: survival, donor site and applications among experienced microvascular surgeons. Proceedings of the Fiftieth Annual Convention of the American Society of Plastic and Reconstructive Surgery. *Plast Surg Forum* 1987; 4: 93–98.
3. Davies DM. A world survey of anticoagulation practice in clinical microvascular surgery. *Br J Plast Surg* 1982; 35: 96–99.
4. Rosenbaum TJ, Sundt TM, Jr. Thrombus formation and endothelial alterations in microarterial anastomoses. *J Neurosurgery* 1977; 47: 430–441.
5. Cooley BC, Ruas EJ, Shaw-Wilgis EF. Scanning electron microscopy of crush/avulsion arterial trauma: effect of heparin and aspirin administration. *Microsurgery* 1987; 8: 11–16.
6. Greenberg BM, Masem M, May JW, Jr. Therapeutic value of intravenous heparin in microvascular surgery: an experimental vascular thrombosis study. *Plast Reconst Surg* 1988; 82: 463–470.
7. Khouri RK, Cooley BC, Kenna DM, Edstrom LE. Thrombosis of microvascular anastomoses in traumatized vessels: fibrin versus platelets. *Plast Reconst Surg* 1990; 86: 110–117.
8. Davidson SF, Brantley SK, Talbot PJ, Das SK. A functional model of microvascular thrombosis. *Plant Reconst Surg* 1990; 86: 579–581.
9. Davidson SF, Brantley SK, Das SK. Comparison of single dose antithrombotic agents in the prevention of microvascular thrombosis. *J Hand Surg* 1991; 16: 585–589.
10. Cox GW, Runnels S, Hsu HS-H, Das SK. A comparison of heparinised saline irrigation solutions in a model of microvascular thrombosis. *Br J Plast Surg* 1992; 45: 345–348.
11. Goodman LS, Gillman AG. The Pharmacologic Basis of Therapeutics. 7th edition. New York: MacMillan. 1985: 1354, 1366.
12. Conrad KA, Fagan TC, Mackie MJ, Mayshar PV. Effects of ketorolac tromethamine on hemostasis in volunteers. *Clin Pharmacol Ther* 1988; 43: 542–546.
13. Mroszcak EJ, Frank WL, Combs D et al. Ketorolac tromethamine absorption, distribution, metabolism, excretion and pharmacokinetics in animals and humans. *Drug Metab Dispos* 1987; 15: 618–626.
14. Dejana E, Callioni A, Quintana A, deGaetano G. Bleeding time in laboratory animals. II – A comparison of different assay conditions in rats. *Thromb Res* 1979; 15: 191–197.
15. Moore M. UMMC platelet aggregation protocol. Jackson, Ms: University of Mississippi Medical Center Department of Pathology, 1990.
16. Greer IA. Effects of ketorolac tromethamine on hemostasis. *Pharmacotherapy* 1990; 10 (6 part 2): 715–765.
17. Syntex Product Monograph of Toradol (Ketorolac tromethamine). Palo Alto, CA: Syntex Laboratories, Inc, 1990.
18. Garsha IS, Bostwick J. Postoperative hematomas associated with Toradol. *Plast Reconst Surg* 1991; 88: 919–920.
19. Concannon MJ, Welsh CF, Puckett CL. Inhibition of perioperative platelet aggregation using Toradol. *South Med J* 1991; 84: 2S–72.

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