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Comparison of anti-edema effects of iloprost and diclofenac sodium on traumatic rat paw edema

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Objectives: The aim of this study was to compare the anti-edema effects of a stable prostacyclin analogue, iloprost, with parenteral and local forms of a non-steroidal anti-inflammatory drug, diclofenac sodium, on traumatic soft tissue edema.

Methods: Thirty-two adult male rats were randomly divided into 4 equal groups. Traumatic edema in one paw of each rat was produced by established protocol. Different drugs were then administered to each group: intraperitoneal (i.p.) saline (group 1, control group), topical diclofenac gel (group 2), i.p. diclofenac sodium (group 3), and i.p. iloprost (group 4). The volume of the paws was measured at baseline (before trauma) and at 1 hour, 2 hours, 4 hours, 8 hours, 24 hours, 48 hours, and 72 hours after trauma. The anti-edema effects of these 3 drugs (diclofenac gel, diclofenac sodium i.p., iloprost i.p.) were compared to each other and to the control group.

Results: The greatest increase in paw edema in the first, second and fourth hours was seen in the control and iloprost groups. At the 4-hour measurement, edema levels were all equal except control group. Following 4- and 8-hour measurements, edema began to decrease in all groups. After 8 and 24 hours, the fastest decrease in edema was in iloprost group, with complete resolution of edema by 72 hours. The next fastest decrease in paw volume was seen with i.p. diclofenac sodium, followed by diclofenac gel.

Conclusion: Iloprost has experimentally higher anti-edema effect than diclofenac sodium for the conservative treatment of the traumatic soft tissue edema.

Key words: Anti-edema effect; diclofenac sodium; iloprost; rat paw volume; traumatic soft tissue edema.

Foot trauma is among the common problems in orthopedics and traumatology. It is caused by automobile accidents, work accidents, falls, sports injuries, gunshot wounds, and bonesetter manipulations. We can only see soft tissue edema and inflammation; however, tendon, vessel, and nerve injuries may accompany depending on the severity of trauma. Closed or open fractures and compartment syndrome can also be seen together. Soft tissue edema and inflammation are often treated conservatively by such methods as elevation, cold compresses, non-steroidal anti-inflammatory drugs (NSAID), antithrombotic and vasodilator drugs, antioxidants, and stabilization of the foot by cast.

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The main symptoms of foot trauma are pain, inflammation, soft tissue edema, movement restriction, and ecchymoses. Iloprost, a stable long-acting analogue of prostacyclin, has been used for different forms of treatment in recent years. It is known to be a potent vasodilator. In addition, iloprost inhibits platelet aggregation, is an antioxidant, and decreases vessel permeability. Thus, it shows an anti-inflammatory effect by increasing intravascular volume. It is used to treat diabetic foot, pulmonary hypertension, peripheral ischemic vessel disease, early stages of aseptic necrosis of the bone, bone marrow edema, and ischemia of the distal parts of the fingers after microvascular surgery or freezing.

The effect of iloprost on traumatic soft tissue edema is not fully understood. Hitherto, there has been documentation of its effects on ischemia, inflammation and suppression of free oxygen radicals. But, its use in the treatment of traumatic soft tissue edema of the foot has not been examined in the literature.

In this study, we aimed to compare the anti-edema effect of iloprost with the anti-edema effects of the parenteral and gel forms of diclofenac sodium, NSAID.

Materials and methods

Thirty-two Sprague-Dawley young or adult male rats [mean weight 187 g (range 122-260 g)] were included in this study. The rats were randomly divided into 4 equal groups. In each group, the rats were signed from their tails and numbered from one to eight. The ankle of each paw was also marked to ensure correct measurement of volume. After ketamine (100 mg/kg, Ketalar, Eczacıbaşı, İstanbul, Turkey) anesthesia, rats were weighed with a weighing machine (CAS Corp., Korea). The initial volume of the paws was measured by plethysmograph (Ugo Basile 7140, Cornerio VA, Italy) (Fig. 1). Edema of the paws was created by a special mechanism (Fig. 2). From a height of 40 cm, a cylindrical mass weighing 150 g was dropped onto the right paws. After creating traumatic soft tissue edema, drugs were given to each group for 3 days: intraperitoneal (i.p.) saline 0.4 mL/day, half in the morning and half in the evening for group 1 (control group); topical diclofenac gel (Voltaren-Emulgel, Novartis Pharma Stein AG, Basel, Switzerland) 1 mL per day, half in the morning and half in the evening for group 2; i.p. diclofenac sodium (Voltaren amp, Novartis Pharma

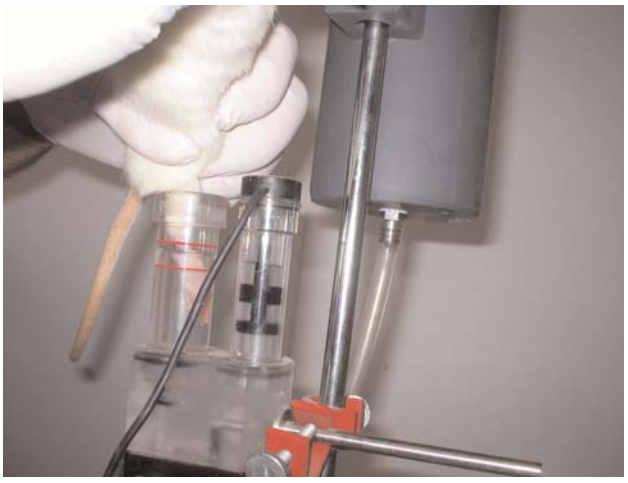

Fig. 1. Measuring the volume of the rat paws by plethysmograph.

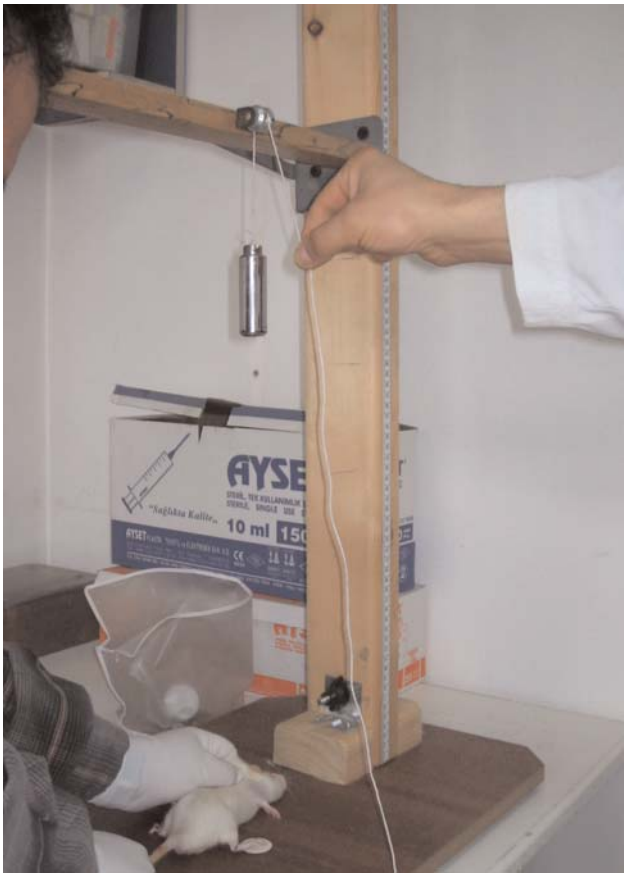

Fig. 2. Producing edema of the rat paws by dropping a mass.

Stein AG, Basel, Switzerland) 25 mg/kg/day, half in the morning and half in the evening for group 3; and i.p. iloprost (Ilomedin, Schering AG, Berlin, Germany) 20 µg/kg/day, half in the morning and half in the evening. The volume of the paws was measured by plethysmograph at 1 hour, 2 hours, 4 hours, 8 hours, 24 hours, 48 hours, and 72 hours after trauma. At the end of the third day, the rats were sacrificed, and their non-traumatized and traumatized paws were evaluated radiologically to rule out any fractures.

Results

In the control group, mean volume of the rat paws was 0.80 mL before trauma, 1.15 mL at 1 hour, 1.19 mL at 2 hours, 1.27 mL at 4 hours, 1.26 mL at 8 hours, 1.13 mL between 24 hours and 48 hours, and 0.96 mL at 72 hours after trauma. Diclofenac gel group was significantly different from control group in terms of paw volume after 24 hours (Fig. 3). Percent increase in volume after trauma was 43.75% at 1 hour, 48.75% at 2 hours, 58.75% at 4 hours, 57.50% at 8 hours, 41.25% at 24 hours, 31.25% at 48 hours, and 20% at 72 hours (Fig. 4).

In diclofenac sodium gel group, mean volume of the paws was 1.04 mL before trauma, 1.25 mL at 1 hour, 1.40 mL at 2 hours, 1.48 mL at 4 hours, 1.51 mL at 8 hours, 1.43 mL at 24 hours, 1.25 mL at 48 hours, and 1.18 mL at 72 hours after trauma (Fig. 3). Diclofenac gel group was statistically different from diclofenac i.p. group starting from 4 hours; from iloprost group at 72 hours; and from control group starting from 24 hours ($p<0.05$). Percent increase in volume after trauma was 20% at 1 hour, 34.6% at 2 hours, 42.3% at 4 hours, 45.2% at 8 hours, 37.5% at 24 hours, 20.2% at 48 hours, and 13.5% at 72 hours (Fig. 4).

In diclofenac sodium i.p. group, mean volume of the paws was 0.83 mL before trauma, 1.05 mL at 1 hour, 1.12 mL at 2 hours, 1.18 mL at 4 hours, 1.13 mL at 8 hours, 1.01 mL at 24 hours, 0.95 mL at 48 hours, and 0.87 mL at 72 hours after trauma (Fig. 3). There was statistically significant difference between diclofenac i.p. and iloprost groups except 4 and 8 hours ($p<0.05$). No statistically significant difference was detected between diclofenac i.p. group and control group. Percent of increase in volume after trauma was 26.5% at 1 hour, 35% at 2 hours, 42% at 4 hours,

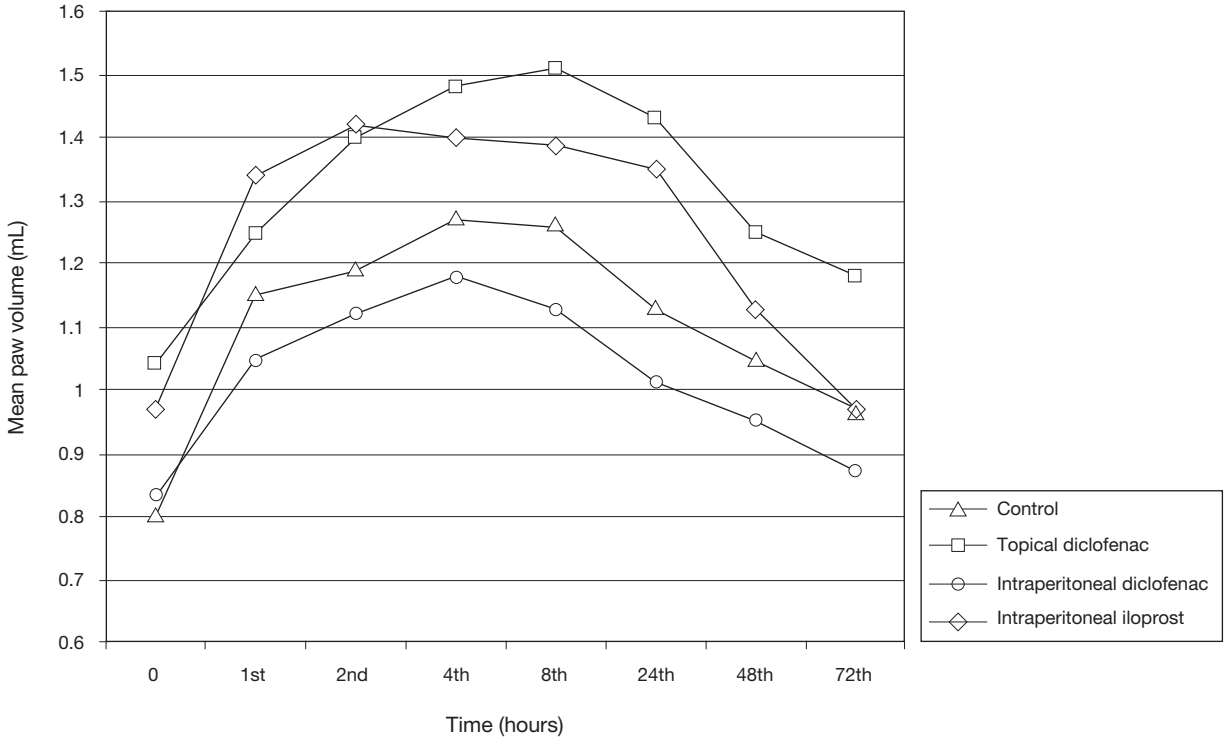

Fig. 3. The change of the mean volume of the rat paws with time.

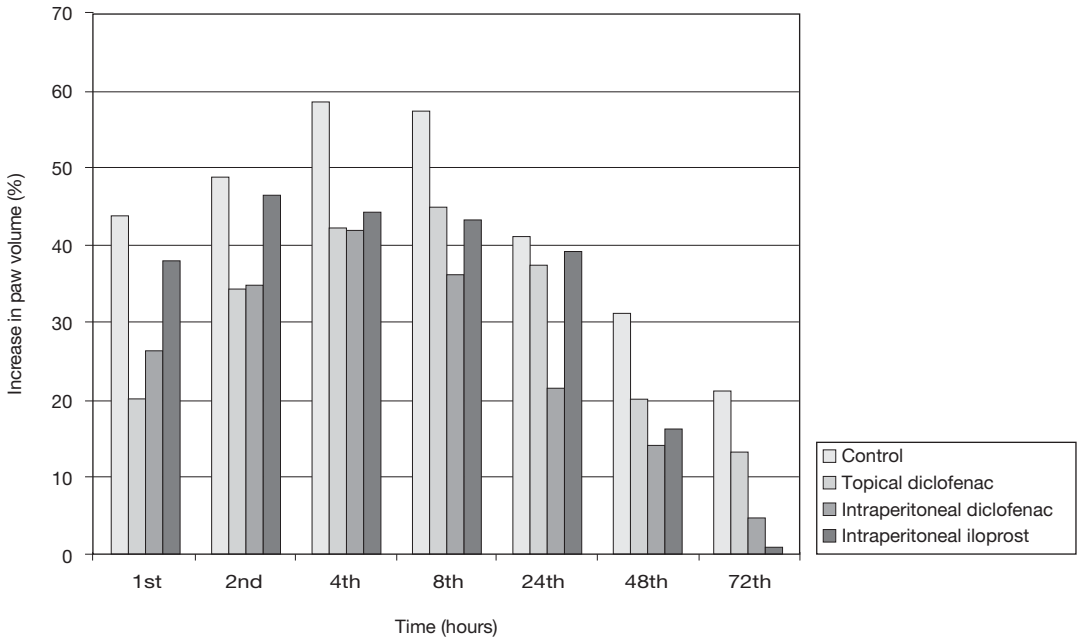

Fig. 4. The percent of increase in the volume of the rat paws with time.

36% at 8 hours, 21% at 24 hours, 14% at 48 hours, and 0.03% at 72 hours (Fig. 4).

In iloprost group, mean volume of the paws was 0.97 mL before trauma, 1.34 mL at 1 hour, 1.42 mL at 2 hours, 1.40 mL at 4 hours, 1.39 mL at 8 hours, 1.35 mL at 24 hours, 1.13 mL at 48 hours, and 0.97 ml at 72 hours after trauma (Fig. 3). There was no statistically significant difference between iloprost and control groups. Percent increase in volume after trauma was 38% at 1 hour, 46% at 2 hours, 44% at 4 hours, 43% at 8 hours, 39% at 24 hours, 16.5% at 48 hours, and 0% at 72 hours (Fig. 4).

For the first hour of the experiment, the greatest increase in edema was seen in the control and iloprost groups. This was also seen at 2 and 4 hours. After 4 hours, edema in all groups started to decrease. The quickest decrease in rat paw edema was noted in iloprost group at 8 and 24 hours. The iloprost group also showed the same paw volume at 72 hours as that seen before trauma, indicating total resolution of edema. After iloprost, the order of effectiveness in decreasing the rat paw edema was diclofenac sodium i.p. and diclofenac sodium gel. At the end of the study, the amount of rat paw edema in the diclofenac sodium i.p. group decreased to 0.003% and it decreased to 13% in diclofenac sodium gel group, and to 20% in the saline control group.

Discussion

Wound healing is a complicated processes including inflammation and soft tissue edema that develops as a response to trauma, involving metabolic, biologic, and physiologic events. The inflammation begins at the time trauma begins. The conditions as a trauma that tether inflammation are microorganisms, cold, hot, radiant energy, chemicals, trauma by electricity, and mechanics, and so on. Damaged cells and foreign bodies are eliminated and tissue repairing process exists.[1]

We attempted to create traumatic soft tissue edema and inflammation on rat paws experimentally, similar to original trauma. There are many ways of creating sterile edema cited in the literature: Freund's adjuvant, carrageenan, and zymosan are chemicals that cause soft tissue edema by activating immune systems components.[2] Another way to create sterile edema is U.V. radiation.[2] The trauma model in the present study was formed resembling the trauma model used to create injuries on rats' heads in the literature.[3,4]

In response to mechanical trauma, we observe changes in the microvessel circulation in soft tissue. Focal capillary density decreases, there is leakage of macromolecules from the vessel walls and an

increase in the number of leukocytes that stimulate local inflammation.[5] As the tissue is damaged, local tissue mediators include histamines, kinins, prostaglandins, and leukotrienes. Some of them increase vessel diameter, some increase vessel permeability, and some regulate edema and pain. Blood clotting in traumatized tissue vessel isolates mediators that cause tissue edema.[1] Inflammation starts with migration of leukocytes and soft tissue cells to the damaged tissues. Cytokines help repair damaged tissues. Primary repair ends with formation of granulation and scar tissue. Early diagnosis and treatment improves functional results.[6,7] Lymphocytes, monocytes, and macrophages take part at the end of the inflammation.[8] Increase in blood pressure and capillary permeability causes liquid transfer from the blood. Increased capillary permeability is a result of inflammation. Low osmotic colloidal pressure is a result of hypoproteinemia and another cause of edema.[9] In clinical studies, mechanical trauma to the extremities causes edema by formation of thrombosis in venules, by decreasing lymphatic drainage, and by activating local inflammation mediators.[10]

The anti-edema and anti-inflammation effects of parenteral and gel forms of the NSAID diclofenac sodium and of iloprost, a stable prostacyclin analogue, were compared with the control saline group in this study. The comparison was made by measurement of edema in the paws of rats for 72 hours following trauma.

Cyclooxygenase (COX) inhibitors are given after trauma to suppress inflammation and edema.[11] Diclofenac sodium is an NSAID for the treatment of inflammation and edema. It also relieves pain related to the mediators. Its target enzyme is COX. In many studies, it has been shown to be effective in suppressing inflammation by inhibiting COX.[11-17] There are two isoenzymes of COX, COX-1 and COX-2, which take part in prostaglandin synthesis. As gastrointestinal side effects are seen by suppression of COX-1, COX-2 is preferred for suppressing inflammation.[18] COX-1 can be found in every tissue, but COX-2 is seen especially in brain, liver, and ovaries. During the inflammation process, COX-2 is produced by interleukin-1 (IL-1), tumor necrosis factors-α (TNF-α), and lipopolysaccharide (LPS).[19] A 50 mg dose of diclofenac taken orally before arthroscopy relieves postoperative pain more than intra-articular ropivacaine injection.[20] Long-term uses of diclofenac include treatment of rheumatoid arthritis, osteoarthritis, and spondyloarthritis; short-term uses include treatment of musculoskeletal trauma, postoperative pain relief, and treatment of dysmenorrhea.

In the present study, diclofenac sodium is effective in decreasing inflammation and tissue edema when used in traumatic tissue edema, and its intraperitoneal administration led to greater and more rapid systemic effects than did local administration.

Gastrointestinal side effects, renal function suppression, and increased hepatic aminotransferase are the major side effects of diclofenac sodium. Gastrointestinal side effects related to NSAID usage cause high mortality rates as 16,000 death per year in USA.[21] The drug goes under biotransformation in the liver, and only 1% gets out of the body from the kidneys unchanged.[12] Twenty-four hours after trauma, diarrhea was observed in all rats taking parenteral diclofenac sodium. Only one rat in this group died before 72 hour; this death was not thought to be caused by the gastrointestinal side effect. Unfortunately one rat in saline group and one rat in diclofenac sodium gel group also died before the study ended.

To reduce the risk of side effects associated with oral administration, NSAIDs that can be locally administered have been developed.[13,22] There are some advantages of local forms compared to the other forms: locally administered forms do not undergo first-pass elimination in the liver, they are easy for the patient to use, and side effects are limited. The only disadvantage is that it is less absorbable from the skin because of the thick stratum corneum layer. When this layer thins, it is more easily absorbed from the skin.[23] The epidermis can absorb 3% diclofenac in hyaluronan gel easily. In this case, the systemic absorption is less, but side effects are less, too.[24] Producing high-dose skin penetration forms will improve the ease of treatment of musculoskeletal inflammation without side effects. Oleic acids and d-limonene can promote greater and more rapid penetration of diclofenac from the skin.[25] Diclofenac gel form can be applied on the skin easily and can suppress inflammation and pain successfully.[26]

In the present study, diclofenac gel form was less effective in the treatment of soft tissue edema than parenteral diclofenac form. Because of the damaged rat paw, the skin gel form was not absorbed enough to show its real anti-inflammatory effect.

Prostacyclins are potent vasodilators that inhibit platelet aggregation and inflammation process.[27] Endothelial mediators including prostacyclins inhibit platelets and leukocytes and protect the tissue from thrombotic events, supply blood to the tissues, and protect the vascular wall from acute damage and chronic remodeling.[28,29] Prostacyclins inhibit the fibrotic response to TGF-α2 by suppressing the Ras/MEK/ERK pathway.[30] Propentofylline and iloprost suppress production of TNF-α from the macrophages.[31-36] Iloprost is a long-acting prostacyclin analogue, a product of endothelial cells. It inhibits platelet aggregation, leukocyte activation, chemotaxis, and superoxide anion production. It is a potent vasodilator.[37] After local application, it has a proinflammatory activity by affecting the cell infiltration detected by leukotriene B4 (LTB_4) on rat skin.[38] Iloprost protects against vessel stenosis by vasodilatation and takes more blood to the tissues. Iloprost decreases platelet aggregation by changing the viscoelasticity of the blood and resolves existing aggregation; it also inhibits the aggregation of waste of inflammation precursors on the damaged blood vessel walls.[39,40] It affects the blood circulation to tissue formed by direct or indirect mechanical trauma. The blood supply and oxygenation of tissues is reduced by ischemia. The vasodilating and anti-aggregation effects of iloprost may be the reason that it is more potent than other drugs in treatment of soft tissue edema. Prostaglandins increase skeletal muscle blood supply during ischemia-revascularization process.[41] In placebo-controlled studies, iloprost has shown treatment value on diabetic foot ulcers.[42-44] Beraprost, a prostacyclin analogue, is used in the treatment of diabetic retinopathy and neuropathy.[45] In treatment of the diabetic foot, iloprost has led to improved wound healing, decreased risk of amputation, and improved pain relief for 3-6 months.[46] Iloprost regulates the genetic role in cell growth and inflammation; it suppresses producing of cytokines in vitro. By its well-known vasodilatation and anti-aggregation effects, it also suppresses inflammation.[47] The positive effects of iloprost on microvessels shows that it can be used effectively in the treatment of ischemic conditions.[48-55] In a study based on the patients with thromboangiitis obliterans, iloprost was more effective than aspirin in pain relief and healing of ulcerated wounds.[51] Some of the common adverse effects are headache, hypotension, syncope, vomiting, pallor of the face and body, nausea, and abdominal cramps.[39] In this study, no side effects of iloprost were observed in rats.

As understood from information in the available literature, drugs we used in this study are the most powerful drugs that can be used in the treatment of soft tissue edema and inflammation. Some forms of diclofenac sodium (parenteral, oral, and local) are used in the clinical treatment at the moment. Iloprost has been discovered in recent years and is given to patients by i.v. infusion and inhalation.[39] Iloprost by i.v. infusion causes patient immobility, and patients must stay in the hospital during treatment.[39,56] Iloprost is usually given by inhalation to patients with pulmonary hypertension and acute pulmonary edema.[27,37,57-59] In the present study, iloprost was given to rats via i.p. injection because of the technical difficulty of intravenous infusion in rats. In the literature, it has been given by intravenous infusion by jugular catheterization under general anaesthesia.

We concluded that parenteral forms of diclofenac sodium and iloprost are more effective than saline or diclofenac gel on traumatic soft tissue edema. Iloprost has particular advantages for treatment of traumatic soft tissue edema because of its effect of vessel vasodilation. Studies are now underway to develop formulations, which are effective and available to be used clinically.

References

1. Rod RS, Trent DS, Philip T. Essentials of anatomy and physiology. 5th ed. New York: McGraw Hill Higher Education; 2004.
2. Davies SL, Siau C, Gary J. Bennett GJ. Characterization of a model of cutaneous inflammatory pain produced by an ultroviolet irradiation–evoked sterile injury in the rat. J Neurosci Methods 2005;148:161-6.
3. Tavazzi B, Signoretti S, Lazzarino G, Amorini AM, Delfini R, Cimatti M, et al. Cerebral oxidative stress and depression of energy metabolism correlate with severity of diffuse brain injury in rats. Neurosurgery 2005;56:582-9.
4. Piper IR, Gorrioch MA, Souter MJ, Andrews PJ, Thomson D. Effects of diaspirin cross-linked haemoglobin on post-traumatic cerebral perfusion pressure and blood flow in a rodent model of diffuse brain injury. Br J Anaesth 1998;80:639-43.

5. Szczesny G, Veihelmann A, Nolte D, Messmer K. Changes in the local blood and lymph microcirculation in response to direct mechanical trauma applied to leg: in vivo study in an animal model. J Trauma 2001;51:508-17.
6. Barlow Y, Willoughby J. Pathophysiology of soft tissue repair. Br Med Bull 1992;48:698-711.
7. Noble KA. Pathophysiology corner: inflammation I. J Perianesth Nurs 2005;20:56-8.
8. Bertani S, Lussignoli S, Andrioli G, Bellavite P, Conforti A. Dual effects of a homeopatic mineral complex on carrageenan-induced oedema in rats. Br Homeopath J 1999;88:101-5.
9. Marrieb EN. Hoehn K. Human anatomy and physiology. 7th ed. Pearson-Benjamin Cumings, 2006.
10. Szczesny G, Olszewski WL, Deszczyński J. Post-traumatic lymphatic and venous drainage changes in persistent edema of lower extremities. [Article in Polish] Chir Narzadow Ruchu Ortop Pol 2000;65:315-25.
11. Gradl G, Gaida S, Finke B, Lindenblatt N, Gierer P, Menger MD, et al. Supernatant of traumatized muscle induces inflammation and pain, but not microcirculatory perfusion failure and apoptotic cell death. Shock 2005;24:219-25.
12. Kirchheiner J, Meineke I, Steinbach N, Meisel C, Roots I, Brockmöller J. Pharmacokinetics of diclofenac and inhibition of cyclooxygenases 1 and 2: no relationship to the CYP2C9 genetic polymorphism in humans. Br J Clin Pharmacol 2003;55:51-61.
13. Konstandinidou M, Kourounakis A, Yiangou M, Hadjipetrou L, Kovala-Demertzi D, Hadjikakou S, et al. Anti-inflammatory properties of diclofenac transition metalloelement complexes. J Inorg Biochem 1998;70:63-90.
14. Mahgoub AA. Grapefruit juice potentiates the anti-inflammatory effects of diclofenac on the carrageenan-induced rat's paw edema. Pharmacol Res 2002;45:1-4.
15. Sacco S, Agnello D, Sottocorno M, Lozza G, Monopoli A, Villa P, et al. Nonsteroidal anti-inflammatory drugs increase tumor necrosis factor production in the periphery but not in the central nervous system in mice and rats. J Neurochem 1998;71:2063-70.
16. Takahashi M, Umehara N, Suzuki S, Tezuka M. Analgesic action of sustained release preparation of diclofenac sodium in a canine urate-induced gonarthritis. J Health Sci 2001; 47:464-7.
17. Marsolais D, Côté CH, Frenette J. Nonsteroidal anti-inflammatory drug reduces neutrophil and macrophage accumulation but does not improve tendon regeneration. Lab Invest 2003;83:991-9.
18. Li MH, Yin LL, Cai MJ, Zhang WY, Huang Y, Wang X, et al. Design, synthesis, and anti-inflammatory evaluation of a series of novel amino acid–binding 1,5 diarylpyrazole derivates. Acta Pharmacologica Sin 2005;26:865-72.
19. Selvam C, Jachak SM, Thilagavathi R, Chakraborti AK. Design, synthesis, biological evaluation and molecular docking of curcumin analogues as antioxidant, cyclooxygenase inhibitory and anti-inflammatory agents. Bioorg Med Chem Lett 2005;15:1793-7.
20. Rautoma P, Santanen U, Avela R, Luurila H, Perhoniemi V, Erkola O. Diclofenac premedication but not intra-articular ropivacaine alleviates pain following day-case knee arthroscopy. Can J Anaesth 2000;47:220-4.
21. Khan AA, Dionne RA. COX-2 inhibitors for endodontic pain. Endodontic Topics 2002;3:31-40.
22. Brunner M, Dehghanyar P, Seigfried B, Martin W, Menke G, Müller M. Favourable dermal penetration of diclofenac after administration to the skin using a novel spray gel formulation. Br J Clin Pharmacol 2005;60:573-7.
23. Choi MJ, Maibach HI. Elastic vesicles as topical/transdermal drug delivery systems. Int J Cosmet Sci 2005;27:211-21.
24. Rivers JK. Topical %3 diclofenac in %2.5 hyaluronan gel for the treatment of actinic keratoses. Skin Therapy Letter 2004;9:1-3.
25. Escribano E, Calpena AC, Queralt J, Obach R, Doménech J. Assessment of diclofenac permeation with different formulations: anti-inflammatory study of a selected formula. Eur J Pharmacol Sci 2003;19:203-10.
26. Bhaskar K, Ramachandran S, Sridhar SK, Rajarajan AT, Ramkumar A, Sanathkumar K, et al. Biopharmaceutical and pharmacodynamic studies on topically applied diclofenac gel available in Indian market. Boll Chim Form 2004;143:208-10.
27. Siobal M. Aerosolized prostacyclins. Respir Care 2004; 49:640-52.
28. Gryglewski RJ, Chłopicki S, Uracz W, Marcinkiewicz E. Significance of endothelial prostacyclin and nitric oxide in peripheral and pulmonary circulation. Med Sci Monit 2001;7:1-16.
29. Nasrallah R, Landry A, Scholey JW, Hébert RL. Characterization of the PGI2/IP system in cultured rat mesengial cells. Prostaglandins Leukot Essent Fatty Acids 2004;70:455-64.
30. Stratton R, Rajkumar V, Ponticos M, Nichols B, Shiwen X, Black CM, et al. Prostacyclin derivatives prevent the fibrotic response to TGF-beta by inhibiting the Ras/MEK/ERK pathway. FASEB J 2002;16:1949-51.
31. Jung S, Donhauser T, Toyka KV, Hartung HP. Propentofylline and iloprost suppress the production of TNF-alpha by macrophages but fail to ameliorate experimental autoimmune encephalomyelitis in Lewis rats. J Autoimmun 1997;10:519-29.
32. Czeslick EG, Simm A, Grond S, Silber RE, Sablotzki A. Inhibition of intracellular tumour necrosis factor (TNF)-alpha and interleukin (IL)-6 production in human monocytes by iloprost. Eur J Clin Invest 2003;33:1013-7.
33. Lehmann C, König JP, Dettmann J, Birnbaum J, Kox WJ. Effects of iloprost, a stable prostacyclin analog, on intestinal leukocyte adherence and microvascular blood flow in rat experimental endotoxemia. Crit Care Med 2001;29: 1412-6.

34. Swartbol P, Truedsson L, Parsson H, Norgren L. TNF-α and IL-6 release from white blood cells induced by different graft materials in vitro are affected by pentoxifylline and iloprost. J Biomed Mater Res 1997;36:400-6.
35. Jörres A, Dinter H, Topley N, Gahl GM, Frei U, Scholz P. Inhibition of tumour necrosis factor production in endotoxin-stimulated human mononuclear leukocytes by the prostacyclin analogue iloprost: cellular mechanisms. Cytokine 1997;9:119-25.
36. Gao IK, Scholz P, Boehme MW, Norden C, Lemmel EM. A 7-day oral treatment of patients with active rheumatoid arthritis using the prostacyclin analog iloprost: cytokine modulation, safety, and clinical effects. Rheumatol Int 2002;22:45-51.
37. Baltalarlı A, Özcan V, Bir F, Aybek H, Saçar M, Önem G, et al. Ascorbic acid (vitamin C) and iloprost attenuate the lung injury caused by ischemia/reperfusion of the lower extremities of rats. Ann Vasc Surg 2006;20:49-55.
38. Ekerdt R, Müller B. Role of prostanoids in the inflammatory reaction and their therapeutic potential in the skin. Arch Dermatol Res 1992;284:S18-21.
39. Bettoni L, Geri A, Danieli E, Cavazzana I, Antanioli C, Chiesa L, et al. Systemic sclerosis therapy with iloprost: a prospective observational study of 30 patients treated for a median of 3 years. Clin Rheumatol 2002;21:244-50.
40. Mazzone A, Cusa C, Bucci L, Vezzoli M, Ghio S, Buggia I, et al. The effects of iloprost infusion on microcirculation is independent of nitric oxide metabolites and endothelin-1 in chronic peripheral ischaemia. Eur J Clin Invest 1999;29:1-5.
41. Rowlands TE, Gough MJ, Homer-Vanniasinkam S. Do prostaglandins have salutary role in skeletal muscle ischemia-reperfusion injury? Eur J Vasc Endovasc Surg 1999;18:439-44.
42. Brock FE, Abri O, Baitsch G, Bechara G, Beck K, Corovic D, et al. Iloprost in the treatment of ischemic tissue lesions in diabetics. Results of a placebo-controlled multicenter study with a stable prostacyclin derivative. [Article in German] Schweiz Med Wochenschr 1990;120:1477-82.
43. Eldor R, Raz I, Ben Yehuda A, Boulton AJ. New and experimental approaches to the treatment of diabetic foot ulcers: a comprehensive review of emerging treatment strategies. Diabet Med 2004;21:1161-73.
44. Duthois S, Cailleux N, Benosman B, Lévesque H. Tolerance of iloprost and results of treatment of chronic severe lower-limb ischaemia in diabetic patients. A retrospective study of 64 consecutive cases. Diabetes Metab 2003;29:36-43.
45. Hotta N, Koh N, Sakakibara F, Nakamura J, Hamada Y, Hara T, et al. Effects of beraprost sodium and insulin on the electroretinogram, nerve conduction, and nerve blood flow in rats with streptozotocin-induced diabetes. Diabetes 1996;45:361-6.
46. Veroux P, Veroux M, Macarone M, Bonanno MG, Tumminelli MG. Efficacy of a novel method of intravenous infusion of the prostaglandin analogue iloprost for the treatment of lower-limb critical ischemia: an open-label, nonrandomized study in two chorts. Curr Ther Res 2004;65:255-65.
47. Di Renzo M, Pieragalli D, Meini S, De Franco V, Pompella G, Auteri A, et al. Iloprost treatment reduces TNF-alpha production and TNF-RII expression in critical limb ischemia patients without affecting IL6. Prostaglandins Leukot Essent Fatty Acids 2005;73:405-10.
48. Müller B, Schmidtke M, Witt W. Action of the stable prostacyclin analogue iloprost on microvascular tone and permeability in the hamster cheek pouch. Prostaglandins Leukot Med 1987;29:187-98.
49. Aigner N, Petje G, Schneider W, Krasny C, Grill F, Landsiedl F. Juvenile bone-marrow oedema of the acetabulum treated by iloprost. J Bone Joint Surg Br 2002;84: 1050-2.
50. Zulian F, Corona F, Gerloni V, Falcini F, Buoncompagni A, Scarazatti M, et al. Safety and efficacy of iloprost for the treatment of ischeamic digits in paediatric connective tissue diseases. Rheumatology (Oxford) 2004;43:229-33.
51. Fiessinger JN, Schäfer M. Trial of iloprost versus aspirin treatment for critical limb ischaemia of thromboangiitis obliterans. The TAO Study. Lancet 1990;335:555-7.
52. Pusey CD. Commentary: iloprost for cholesterol emboli syndrome. BMJ 2002;324:609.
53. Zulian F, Costantini C, Montesco MC, Schiavon F, Zacchello F. Successful treatment of gangrene in systemic necrotizing vasculitis with iloprost. Br J Rheumatol 1998;37:228-30.
54. Disch AC, Matziolis G, Perka C. The management of necrosis-associated and idiopathic bone-marrow oedema of the proximal femur by intravenous iloprost. J Bone Joint Surg Br 2005;87:560-4.
55. Petje G, Radler C, Aigner N, Manner H, Kriegs-Au G, Grill F. Pharmacological management of aseptic osteonecrosis in children. Expert Opin Pharmacother 2004;5:1455-62.
56. Two randomised and placebo-controlled studies of an oral prostacyclin analogue (Iloprost) in severe leg ischaemia. The Oral Iloprost in Severe Leg Ischaemia Study Group. Eur J Vasc Endovasc Surg 2000;20:358-62.
57. Olschewski H, Walmrath D, Schermuly R, Ghofrani A, Grimminger F, Seeger W. Aerosolized prostacyclin and iloprost in severe pulmonary hypertension. Ann Intern Med 1996;124:820-4.
58. Dembinski R, Brackhahn W, Henzler D, Rott A, Bensberg R, Kuhlen R, et al. Cardiopulmonary effects of iloprost in experimental acute lung injury. Eur Respir J 2005;25:81-7.
59. Köksel O, Özdülger A, Aytaçoğlu B, Tamer L, Polat A, Sucu N, et al. The influence of iloprost on acute lung injury induced by hind limb ischemia-reperfusion in rats. Pulm Pharmacol Ther 2005;18:235-41.