

Preemptive oral rofecoxib plus postoperative intraarticular bupivacaine for pain relief after arthroscopic knee surgery

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ÖZET

Artroskopik diz cerrahisi sonrasında ağrı kontrolünde postoperatif intraartiküler bupivakaine preemtif oral rofekoksib eklenmesi

Bu çalışmada, preemtif oral rofekoksibin, artroskopik diz cerrahisi sonrası intraartiküler bupivakain analjezisine etkisi araştırıldı. Altmışiki olgu üç gruba ayrıldı. Grup 1'e (n=21) ameliyattan 1 saat önce oral 50 mg rofekoksib ve ameliyattan sonra intraartiküler 20 ml % 0.5 bupivakain, Grup 2'ye (n=21) aynı dozda bupivakain ve Grup 3'e (n=20) ameliyat sonrası intraartiküler 20 ml salin uygulandı. Ağrı skorları postoperatif 30. dak., 1, 2, 4, 6, 12 ve 24. saatlerde VAS ile değerlendirildi. Analjezi süresi, analjezik (tramadol, tenoksikam) gereksinimleri ve yan etkiler kaydedildi. Ağrı skorları cerrahiden sonra Grup 3'le karşılaştırıldığında Grup 1'de tüm zaman noktalarında ($p<0.05$, $p<0.001$), Grup 2'de 30. dak ($p<0.001$), 1. ve 4. saatlerde ($p<0.05$) daha düşüktü. Grup 1'de ise Grup 2'den ilk 4 saatte daha düşüktü ($p<0.05$, $p<0.001$). Analjezi süresi Grup 1'de Grup 2 ve 3'den (743.0 ± 480.5 dk vs. 262.4 ± 292.2 dk ve 17.0 ± 12.1 dk; $p<0.05$, $p<0.001$); Grup 2'de Grup 3'ten belirgin ($p<0.05$) olarak daha uzundu. Tramadol gereksinimleri Grup 1'de Grup 2 ve 3'den (4.8 ± 15.0 mg vs. 40.5 ± 43.6 mg ve 67.5 ± 24.5 mg; $p<0.05$, $p<0.001$); Grup 2'de Grup 3'ten anlamlı ($p<0.05$) olarak daha azdı. Tenoksikam gereksinimleri ve yan etkiler gruplar arasında farksızdı. Sonuç olarak, preemtif oral rofekoksib ve postoperatif intraartiküler bupivakainin kombine kullanımının artroskopik diz cerrahisi sonrası belirgin analjezik fayda ve opioid gereksiniminde azalma sağladığı kanısına varıldı.

Anahtar kelimeler: Preemtif, rofekoksib, bupivakain, postoperatif analjezi, artroskopik diz cerrahisi

SUMMARY

This study was designed to test the hypothesis whether preemptive administration of rofecoxib, a novel selective COX-2 inhibitor, can prolong intraarticular bupivacaine analgesia after arthroscopic knee surgery. Sixty-two patients were randomly assigned to one of the three groups. Group 1 (n=21) was administered oral rofecoxib 50 mg 1 h before surgery plus intraarticular 0.5 % bupivacaine 20 ml postoperatively. Group 2 (n=21) was administered the same dose of bupivacaine. Group 3 (n=20) was administered saline 20 ml intraarticularly after surgery. Pain scores (VAS) were assessed at 30 min, 1, 2, 4, 6, 12 and 24 h postoperatively. Analgesia duration, analgesic (tramadol and tenoxicam) requirements, and adverse effects were recorded postoperatively for 24 h. Pain scores were significantly lower in the Group 1 at all time points ($p<0.05$, $p<0.001$) and were significantly lower in the Group 2 at 30 min ($p<0.001$), 1 and 4 h ($p<0.05$) compared to the Group 3. Pain scores were significantly lower in the Group 1 compared to the Group 2 during the first 4 h after surgery ($p<0.05$, $p<0.001$). Analgesia duration was longer in Group 1 than Group 2 or 3 (743.0 ± 480.5 min versus 262.4 ± 292.2 min and 17.0 ± 12.1 min; $p<0.05$, $p<0.001$ respectively), and in Group 2 than Group 3 ($p<0.05$). Tramadol requirements were significantly less in Group 1 than Group 2 and 3 (4.8 ± 15.0 mg versus 40.5 ± 43.6 mg and 67.5 ± 24.5 mg; $p<0.05$, $p<0.001$ respectively), and in Group 2 than Group 3 ($p<0.05$). There were no significant differences among the groups regarding the tenoxicam requirements and adverse effects. In conclusion, the combination of oral rofecoxib administered preemptively and intraarticular bupivacaine administered postoperatively provided a significant analgesic benefit and decreased the opioid requirements after arthroscopic knee surgery, when compared to bupivacaine alone or saline.

Key words: Preemptive, rofecoxib, bupivacaine, postoperative analgesia, arthroscopic knee surgery

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Introduction

A variety of analgesic techniques have been used to relieve pain after arthroscopic knee surgery. Intraarticular local anesthetics have found to be effective to provide postoperative analgesia after such operations (Cagney et al. 1999, Dahl et al. 1990, Chirwa et al. 1989, Eriksson et al. 1986). Addition of either opioids (Allen et al. 1993, Khoury et al. 1992) or α -2 receptor agonists (Reuben and Connelly 1999) to intraarticular bupivacaine improves postoperative analgesia when compared with local anesthetic alone.

Preemptive analgesia may prevent nociceptive inputs generated during surgery from sensitized central neurons and therefore may reduce pain after surgery (Richmond et al. 1993). Opioids or non-steroidal anti-inflammatory drugs (NSAIDs) had been administered systemically for preemptive analgesia (Wadhwa et al. 2001, Dahl and Kehlet 1991). NSAIDs inhibit prostaglandin biosynthesis by inhibition of cyclooxygenase (COX) which has two isoforms: COX-1 and COX-2. Analgesic and anti-inflammatory efficacy occur mainly by inhibition of COX-2, while adverse effects occur by inhibition of COX-1 (Kam and Power 2000, Hawkey 1999). Recently, highly selective COX-2 inhibitor, rofecoxib has become available. Rofecoxib has a relative COX-2/COX-1 selectivity ratio $>800/1$. Since this drug do not inhibit platelet function, its use in the perioperative period may be safe. In some studies, rofecoxib has found to be successful for postoperative pain management (Reuben and Connelly 2000, Ehrich et al. 1999, Morrison et al. 1999b).

This study was undertaken to investigate the effects of preoperative oral rofecoxib administration on postoperative analgesia provided by intraarticular bupivacaine injection in patients underwent arthroscopic knee surgery. This study was designed in a prospective, randomized, double-blinded, and placebo controlled manner.

Material and Method

After obtaining approval from the local ethics committee and written informed consent, we studied 62 patients, ASA physical status I-II undergoing elective arthroscopic knee surgery. Exclusion criteria included a known allergy, sensitivity, or contraindications to opioids, local anesthetics or any NSAIDs, renal insufficiency, a histo-

ry of peptic ulcer, a history of a bleeding diathesis.

The patients were randomly divided into 3 groups: Group 1 (n=21); Group rofecoxib plus bupivacaine, Group 2 (n=21); Group bupivacaine, and Group 3 (n=20); Group saline. The study design was randomized and double-blinded. Patients were randomly allocated according to computer-generated randomization list. For premedication, midazolam 0.07 mg/kg and atropine 0.015 mg/kg i.m. were administered 1 h before surgery. Group 1 was administered oral rofecoxib 50 mg (Vioxx, 25 mg tablets, Merck Sharp & Dohme PTY Limited, South Granville, NSW, Australia) before surgery.

After the patients had been taken to the surgery room, 4 ml/kg 5 % dextrose-lactated Ringer's infusion was started through an i.v. 20-gauge cannula inserted in an appropriate antecubital vein. The heart rate, systolic and diastolic arterial pressure, and peripheral oxygen saturation (Dräger Cato PM 8040, Lübeck, Germany) were monitored. Anesthesia was induced with i.v. propofol (2 mg/kg) and fentanyl (2 mg/kg). Endotracheal intubation was facilitated with i.v. atracurium 0.6 mg/kg. Anesthesia was maintained by 2-3 % sevoflurane in nitrous oxide and oxygen (ratio 2:1). When the surgical procedure was completed, patients' knee joint were injected through the arthroscope with bupivacaine 0.5 % 20 ml in Group 1 and 2, and with the same volume of saline in Group 3.

After tracheal extubation, patients were transferred to the post-anesthesia care unit (PACU). Postoperative pain was assessed using a visual analog scale (VAS; 0: "no pain" and 10: "worse pain imaginable"). Patients were asked in the PACU at 30th min and 1 h, and in the orthopedics clinic at 2, 4, 6, 12 and 24th h after surgery for pain assessment according to the VAS. VAS monitoring was explained to the patients during the preoperative visit. If it was >4 , or on the patients' request, i.m. tramadol 50 mg in the first 4 h and tenoxicam 20 mg at the period of 4-24 h after surgery were administered. Analgesia duration (min) was considered as the time from intraarticular injection to first analgesic requirement. Analgesia duration, total tramadol and tenoxicam requirements, and adverse effects were recorded during postoperative 24 h. Patients were administered i.m. ondansetron (4 mg) when repeated nausea and/or vomiting occurred. All measurements were recorded by the same anesthesia res-

ident who was blinded to the study drugs administered.

Statistical analysis

Age, weight, surgery duration, anesthesia duration, analgesia duration, tramadol and tenoxicam requirements and VAS (cm) were analyzed with one way ANOVA. When a significant result obtained, analgesia duration and tramadol requirements were analysed with Dunnett T3 test, a post-hoc test, because of non-homogen variances. Gender was analyzed with Chi-square test. Adverse effects were analyzed with Kolmogorov-Smirnov test. A value of $p < 0.05$ was considered statistically significant. Data were analyzed using MINITAB for Windows 13.32 (MINITAB, Inc, State College, PA).

Results

There were no significant differences among the three groups with respect to age, weight, gender, surgery and anesthesia duration (Table 1).

Pain (VAS) scores are presented in Table 2. There was a significant difference with respect to VAS scores among the three groups ($p < 0.001$). Pain scores were significantly lower in Group 1 at all

time points ($p < 0.05$, $p < 0.001$), and were significantly lower in Group 2 at 30 min ($p < 0.001$), 1 and 4 h ($p < 0.05$) compared to Group 3. Pain scores were significantly lower in the Group 1 compared to Group 2 during the first 4 h after surgery ($p < 0.05$, $p < 0.001$).

Analgesia duration and analgesic requirements are presented in Table 3. There were significant differences with respect to mean analgesia duration and tramadol requirements among the three groups ($p < 0.001$, $p < 0.001$, respectively). Analgesia duration was longer in Group 1 than Group 2 or 3 (743.0 ± 480.5 min versus 262.4 ± 292.2 min and 17.0 ± 12.1 min; $p < 0.05$, $p < 0.001$ respectively) and in Group 2 than in Group 3 ($p < 0.05$). Tramadol requirements were significantly less in Group 1 than Group 2 and 3 (4.8 ± 15.0 mg versus 40.5 ± 43.6 mg and 67.5 ± 24.5 mg; $p < 0.05$, $p < 0.001$ respectively), and in Group 2 than Group 3 ($p < 0.05$). There were no significant differences among the groups regarding the tenoxicam requirements.

The difference in the incidence of nausea (3 patients in Group 1, 2 patients in Group 2, and 2 patients in Group 3), vomiting (one patient in each group), vertigo and epigastric pain (one

Table 1: Demographic characteristics and surgery data.

Variables	Group1 (n=21)	Group 2 (n=21)	Group 3 (n=20)
Age (yr)	40.7 ± 13.0	40.2 ± 12.8	39.8 ± 12.3
Weight (kg)	73.5 ± 12.7	73.1 ± 10.5	74.2 ± 10.0
Gender(m/f)	11/10	10/11	10/10
Surgery duration (min)	85.2 ± 28.1	85.2 ± 27.3	88.5 ± 26.6
Anesthesia duration (min)	112.1 ± 30.8	116.2 ± 29.1	112.5 ± 25.7

Data are presented as mean ± SD.

No statistically significant difference was found between groups.

Table 2: Pain (VAS) scores in the three groups.

Time after surgery	Group 1 (n=21)	Group 2 (n=21)	Group 3 (n=20)
30 min	1.2 ± 1.4 *††	2.6 ± 1.7 ††	6.1 ± 1.3
1 h	1.1 ± 1.1 **††	3.0 ± 2.0 †	4.3 ± 1.0
2 h	1.0 ± 0.9 **††	1.8 ± 0.7	2.7 ± 1.0
4 h	1.2 ± 1.1 *†	2.2 ± 1.2 †	2.5 ± 1.5
6 h	1.2 ± 1.3 †	1.8 ± 1.0	2.4 ± 0.8
12 h	1.0 ± 1.2 †	2.0 ± 1.4	2.4 ± 1.5
24 h	0.8 ± 1.1 †	1.8 ± 1.6	2.0 ± 1.6

(VAS:Visual Analogue Scale)

VAS scores are presented as mean + SD.

* $p < 0.05$, ** $p < 0.001$, when compared to Group 2

† $p < 0.05$, †† $p < 0.001$, when compared to Group 3

Table 3: Analgesia duration and analgesic requirements in the three groups.

Variables	Group 1 (n=21)	Group 2 (n=21)	Group 3 (n=20)
Analgesia duration (min)	743.0 ± 480.5 *††	262.4 ± 292.2 †	17.0 ± 12.1
Tramadol requirement (mg)	4.8 ± 15.0 *††	40.5 ± 43.6 †	67.5 ± 24.5
Tenoxicam requirement (mg)	53.6 ± 67.6	78.6 ± 60.4	97.5 ± 49.3

Analgesia duration and analgesic requirement is presented as mean ± SD

* $p < 0.05$, when compared to Group 2

† $p < 0.05$, †† $p < 0.001$, when compared to Group 3

patient in Group 1) were not statistically significant between the groups. Number of patients and doses in patients receiving antiemetic drug during the first 24 h after surgery were similar among the groups. None of the patients in the three groups suffered hypotension, respiratory depression, bronchospasm, or allergic reaction.

Discussion

A multimodal approach for postoperative pain management is recommended (Kehlet and Dahl 1993). The pharmacological management includes systemically administered NSAIDs as well as opioids (Power and Barratt 1999, Richmond et al. 1993, Dahl and Kehlet 1991). Since selective COX-2 inhibitors demonstrated analgesic efficacy when administered orally after dental (Ehrich et al. 1999, Malmstrom et al. 1999, Morrison et al. 1999b, Mehlisch et al. 1997, Hubbard et al. 1996) and orthopedic surgery (Reicin et al. 2001), and preemptively used in spinal surgery (Reuben and Connelly 2000), we were interested in determining whether preemptive oral rofecoxib might provide additional postoperative analgesia when combined with intraarticular bupivacaine. Our results confirm the analgesic benefit of preemptive administration of rofecoxib for postoperative analgesia; we observed the lowest VAS scores and analgesic requirements as well as longest analgesia duration in patients who received rofecoxib in the preoperative period. Furthermore, opioid need was also reduced.

Previous studies demonstrated that single oral dose of 50 mg rofecoxib was effective in the treatment of acute pain (Chang et al. 2001, Morrison et al. 1999a). Rofecoxib at the dose of 50 mg was found similar to 550 mg naproxen sodium for post-orthopedic pain. At this dose, rofecoxib had opioid sparing effect when compared to placebo (Reicin et al. 2001). The same dose was recommended for either preoperative (Huang

et al. 2001, Reuben and Connelly 2000) or postoperative (Reicin et al. 2001) administration. We used rofecoxib 50 mg at the preoperative period. Reuben and Connelly (2000) designed a placebo-controlled study to determine the postoperative analgesic efficacy of rofecoxib or celecoxib after spinal surgery. Both groups were administered the drug at preoperative period. Authors found significantly less morphine consumption with i.v. patient-controlled analgesia (PCA) and lower pain scores in rofecoxib group at 8, 12 and 16th h, and in celecoxib group at 8th h than placebo group, postoperatively. At 12 and 16th h, pain scores were found to be lower in rofecoxib group than celecoxib group. Authors concluded that rofecoxib provided for a more sustained analgesic effect compared to celecoxib. In another study, a single oral dose of rofecoxib 50 mg, given 1 h before induction of anesthesia for radical prostatectomy, was similar to placebo for VAS scores at 1, 2, 4, 6, 8 and 24 h, and there was no difference in the postoperative morphine consumption (Huang et al. 2001).

The main benefits of selective COX-2 inhibitors are the reduced incidence of gastric ulceration, perforation and bleeding and reduced effect on platelets (Clemett and Goa 2000, Kam and Power 2000, Van Hecken et al. 2000, Hawkey 1999). These adverse effects occur by concurrent COX-1 inhibition (Kam and Power 2000). Previous studies demonstrated that the most common adverse effects of rofecoxib are headache, fatigue, nausea, dizziness, dyspepsia and dry mouth (Ehrich et al. 1999, Malmstrom et al. 1999, Morrison et al. 1999a, b). In the present study, nausea, vomiting, vertigo and dyspepsia in the combination group, and nausea and vomiting in both bupivacaine and placebo groups were seen. There was no significant difference with respect to adverse effects among the groups.

We conclude that the combination of oral rofecoxib administered preemptively and intraarticular

lar bupivacaine administered postoperatively provided a significant analgesic benefit and decreased the opioid requirements after arthroscopic knee surgery, when compared to bupivacaine alone or saline.

References

- Allen G. C., St Amand M. A., Lui A. C., Johnson D. H., Lindsay M. P.: Postarthroscopy analgesia with intraarticular bupivacaine / morphine. *Anesthesiology* 1993; 79: 475-480.
- Cagney B., Williams O., Jennings L., Buggy D.: Tramadol or fentanyl analgesia for ambulatory knee arthroscopy. *Eur J Anaesth* 1999; 16: 182-185.
- Chang D. J., Bird S. R., Bohidar N. R., Dobbins T. W., Geba G. P.: Superior analgesic efficacy of rofecoxib compared with codeine/acetaminophen in two acute pain studies utilizing the dental-impaction model. *Arthritis Rheumatism* 2001; 44: S124-S124.
- Chirwa S. S., MacLeod B. A., Day B.: Intraarticular bupivacaine after arthroscopic meniscectomy: a randomized double-blind controlled study. *Arthroscopy* 1989; 5: 33-35.
- Clemett D., Goa K. L.: Celecoxib: a review of its use in osteoarthritis, rheumatoid arthritis and acute pain. *Drugs* 2000; 59: 957-980.
- Dahl J. B., Kehlet H.: Non-steroidal anti-inflammatory drugs: rationale for use in severe postoperative pain. *Br J Anaesth* 1991; 66: 703-712.
- Dahl M. R., Dasta J. F., Zuelzer W., McSweeney T. D.: Lidocaine local anesthesia for arthroscopic knee surgery. *Anesth Analg* 1990; 71: 670-674.
- Ehrich E. W., Dallob A., De Lepeleire I., Van Hecken A., Riendeau D., Yuan W., Porras A., Wittreich J., Seibold J. R., De Schepper P., Mehlich D. R., Gertz B. J.: Characterization of rofecoxib as a cyclooxygenase-2 isoform inhibitor and demonstration of analgesia in the dental pain model. *Clin Pharmacol Ther* 1999; 65: 336-347.
- Eriksson E., Haggmark T., Saartok T., Sebik A., Ortengren B.: Knee arthroscopy with local anesthesia in ambulatory patients. Methods, results and patient compliance. *Orthopedics* 1986; 9: 186-188.
- Hawkey C. J.: COX-2 inhibitors. *Lancet* 1999; 353: 307-313.
- Huang J. J., Taguchi A., Hsu H., Andriole G. L. Jr., Kurz A.: Preoperative oral rofecoxib does not decrease postoperative pain or morphine consumption in patients after radical prostatectomy: a prospective, randomized, double-blinded, placebo-controlled trial. *J Clin Anesth* 2001; 13: 94-97.
- Hubbard R. C., et al.: SC-58635, a highly selective inhibitor of COX-2, is an effective analgesic in acute pain model (abstract). *J Invest Med* 44 (1996) 293 A.
- Kam P. C. A., Power I.: New selective COX-2 inhibitors. *Pain Reviews* 2000; 7: 3-13.
- Kehlet H., Dahl J. B.: The value of "multimodal" or "balanced analgesia" in postoperative pain treatment. *Anesth Analg* 1993; 77: 1048-1056.
- Khoury G. F., Chen A. C., Garland D. E., Stein C.: Intraarticular morphine, bupivacaine and morphine / bupivacaine for pain control after knee videoarthroscopy. *Anesthesiology* 1992; 77: 263-266.
- Malmstrom K., Daniels S., Kotev P., Seidenberg B. C., Desjardins P. J.: Comparison of rofecoxib and celecoxib, two cyclooxygenase-2 inhibitors, in postoperative dental pain: a randomized, placebo- and active-comparator- controlled clinical trial. *Clin Ther* 1999; 21: 1653-1663.
- Mehlich D. R., Hubbard R. C., Isakson P., Karim A., Weaver M., Mills S.: Analgesic efficacy and plasma levels of a highly selective inhibitor of COX-2 (SC-58635; SC) in patients with post-surgical dental pain. *Clin Pharmacol Ther* 1997; 61: PIII2-PIII2.
- Morrison B. W., Christensen S., Yuan W., Brown J., Amlani S., Seidenberg B.: Analgesic efficacy of the cyclooxygenase-2 specific inhibitor rofecoxib in post-dental surgery pain: a randomized, controlled trial. *Clin Ther* 1999; 21: 943-953.
19. Morrison B. W., Daniels S. E., Kotev P., Cantu N., Seidenberg B.: Rofecoxib, a specific cyclooxygenase-2 inhibitor, in primary dysmenorrhea. A randomized clinical trial. *Obstet Gynecol* 1999; 94: 504-508.
- Power I., Barratt S.: Analgesic agents for the postoperative period. Nonopioids. *Surg Clin North Am* 1999; 79: 275-295.
- Reicin A., Brown J., Jove M., deAndrade J. R., Bourne M., Krupa D., Walters D., Seidenberg B.: Efficacy of single-dose and multidose rofecoxib in the treatment of post-orthopedic surgery pain. *Am J Orthop* 2001; 30: 40-48.
- Reuben S. S., Connelly N. R.: Postoperative analgesia for outpatient arthroscopic knee surgery with intraarticular clonidine. *Anesth Analg* 1999; 88: 729-733.
- Reuben S. S., Connelly N. R.: Postoperative analgesic effects of celecoxib or rofecoxib after spinal fusion surgery. *Anesth Analg* 2000; 91: 1221-1225.
- Richmond C. E., Bromley L. M., Woolf C. J.: Preoperative morphine pre-empts postoperative pain. *Lancet* 1993; 342: 73-75.
- Van Hecken A., Schwartz J. I., Depre M., De Lepeleire I., Dallob A., Tanaka W., Wynants K., Buntinx A., Arnout J., Wong P. H., Ebel D. L., Gertz B. J., De Schepper P. J.: Comparative inhibitory activity of rofecoxib, meloxicam, diclofenac, ibuprofen, and naproxen on COX-2 versus COX-1 in healthy volunteers. *J Clin Pharmacol* 2000; 40: 1109-1120.
- Wadhwa A., Clarke D., Goodchild C. S., Young D.: Large-dose oral dextromethorphan as an adjunct to patient-controlled analgesia with morphine after knee surgery. *Anesth Analg* 2001; 92: 448-454.