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No effect of ketoprofen and meloxicam on bone graft ingrowth

A bone chamber study in goats

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Background and purpose There is increasing awareness that non-steroidal anti-inflammatory drugs (NSAIDs), and especially the cyclooxygenase-2 (COX-2) selective ones, may retard bone healing. We have used NSAIDs (indomethacin for at least 7 days) to prevent heterotopic ossification after acetabular reconstructions using impacted bone grafts. The long-term clinical results have been satisfying, making it difficult to believe that there is an important negative effect of NSAIDs on graft incorporation. We studied the effect of two different NSAIDs on bone and tissue ingrowth in a bone chamber model in goats, using autograft, rinsed allograft, and allograft that had been rinsed and subsequently irradiated.

Methods 9 goats received no NSAIDs, 9 received ketoprofen, and 9 received meloxicam—all for 6 weeks. In each goat 6 bone chambers were implanted: 2 filled with autograft, 2 with rinsed allograft, and 2 with allograft that had been rinsed and irradiated. The amount of bone ingrowth and total tissue ingrowth was compared between the groups.

Results There were no statistically significant differences in bone ingrowth between the different groups. Also, no differences in bone ingrowth were found with respect to the type of graft used. Furthermore, there was no statistically significant difference in the total amount of ingrowth of fibrous tissue between the treatment groups.

Interpretation No differences in bone ingrowth in titanium bone chambers could be detected with both ketoprofen and meloxicam compared to untreated control animals. This confirms our hypothesis that the effect of NSAIDs on the incorporation and ingrowth of bone graft is limited. ■

There is both experimental and clinical evidence of inhibition of new bone formation by NSAIDs (both selective and non-selective) (Goodman et al. 2002, Harder and An 2003, Simon and O'Connor 2007). NSAIDs inhibit bone ingrowth in porous implants (Trancik et al. 1989, Goodman et al. 2002), delay fracture healing (Simon and O'Connor 2007), inhibit spinal fusion in rats (Dimar et al. 1996), and inhibit experimental bone induction (Persson et al. 2005b). Clinically, NSAIDs have been found to retard healing of diaphyseal fractures (Burd et al. 2003) and spinal fusions (Glassman et al. 1998).

NSAIDs are also effective in preventing heterotopic ossification (HO) after total hip arthroplasty (Neal et al. 2000), strongly suggesting that these drugs also have effects on human bone formation on ectopic places. Theoretically, these NSAIDs inhibit bone restoration, which may result in a less stable or unstable implant (Persson et al. 2005a). The effect of NSAIDs could be even more devastating if they are combined with bone grafts to reconstruct bone defects. An inhibitory effect of NSAIDs on bone repair could also jeopardize the ingrowth into these grafts.

We have used NSAIDs (indomethacin for at least 7 days) to prevent heterotopic ossification with acetabular reconstructions in both primary and revision hip surgery. The long-term clinical results are satisfying, making it difficult to believe that NSAIDs have an important negative effect on graft incorporation (Welten et al. 2000, Schreurs et al. 2004). Also, in a study on the outcome of femoral revision with bone impaction grafting at a

minimum follow-up of 10 years, the administration of indomethacin did not appear to influence long-term results (Schreurs et al. 2005).

We studied the effect of 2 different NSAIDs on bone and tissue ingrowth in a bone chamber model in goats using autograft, rinsed allograft, and rinsed and subsequently irradiated allograft. We hypothesized that the effect of NSAIDs on the incorporation of bone grafts is limited.

Materials and methods

Experimental design

30 mature Dutch milk goats (*Capra hircus sana*) (48–61 kg) were obtained from the Central Animal Laboratory, Nijmegen, the Netherlands.

Allograft bone was obtained from the sternum of 3 donor goats; the other 27 goats were divided into 3 groups. The first group did not receive any NSAID, the second group received ketoprofen (a non-selective NSAID) (2.2 mg/kg) once daily subcutaneously, and the third group received meloxicam (COX-2 preferential) (0.5 mg/kg) once daily subcutaneously. The treatment period was 6 weeks for all goats. The treatment regimen for both ketoprofen and meloxicam in goats was based on the literature (Moses and Bertone 2002, Arifah et al. 2003). All procedures were approved by the Animal Ethics Committee of Radboud University, Nijmegen, the Netherlands.

Preparation of grafts

3 donor goats were used to obtain allograft from the sternum. These goats were killed by an overdose of pentobarbital and the sternum was excised under aseptic conditions. All spongy bone from the sternum was retrieved and rinsed with sterile saline using a high-pressure pulsatile lavage system (SurgiLav Plus; Stryker Nederland BV, Waardenburg, the Netherlands). The bone was subsequently pooled and divided in 2 parts. The first part was used without further processing, and the second part was irradiated with 25 kGy using a ⁶⁰Co gamma-ray source (Isotron BV, Ede, the Netherlands) at a temperature of –78.5°C (dry ice). Both allograft and irradiated allograft were stored at –80°C and thawed just before implantation.

Implants

We used the bone conduction chamber (BCC), which is a model for membranous ossification (Aspenberg and Wang 1993, Wang and Aspenberg 1994). The BCC consists of a titanium screw with a cylindrical interior space. It is made up of two threaded half-cylinders held together by a hexagonal closed screw cap. The interior of the chamber has a diameter of 2 mm, and a length of 7.5 mm. There are two ingrowth openings for bone ingrowth located at the bone end of the chamber. Originally developed as a rat model, the BCC was adapted by us for use in goats. The threaded end of the implant is screwed into the bone, allowing direct contact of the ingrowth openings with the endosteal transition from marrow into bone. To accomplish this in goats, a 1-mm-thick plate was inserted into the cap to lower the ingrowth openings through the cortex (van der Donk et al. 2001, Hannink et al. 2007).

Surgical procedure

Anesthesia was accomplished by intravenous administration of pentobarbital (CEVA Santé Animale, Maasluis, the Netherlands) (0.5 mL/kg) and maintained after intubation with nitrous oxide, oxygen, and isoflurane (1.5–2%). In all 27 goats, autograft was obtained from the right femoral condyle under aseptic conditions. A longitudinal incision was made along the lateral epicondyle, and just ventral to the iliotibial tract a hole was made with a hollow drill of 7.3 mm and a plug of cortico-spongy bone was retrieved. The spongy part of this plug was used as autograft.

The goats received 3 chambers at each side in the cortical bone of the proximal medial tibia. 1 chamber at each side was filled with autologous graft, 1 with rinsed allograft, and the third with irradiated, rinsed allograft. A longitudinal incision was made in the skin and fascia over the medial side of the proximal tibia. After raising the periosteum, a hole was drilled through the medial cortex approximately 4 cm from the joint cleft using a 3.1-mm drill. The hole was tapped and bone debris from drilling was removed. The bone chamber was screwed in manually. The second bone chamber was placed at a distance of 10 mm from the first one, and the third was placed 10 mm lower. This was repeated for the other side. The positions of the different grafts were changed to different posi-

tions in different cases, to avoid linking of the different variables (for example, all autografts in the proximal position). The subcutaneous layer and the skin were sutured. All animals were allowed unrestricted movement in their cages and had free access to water and food after the operation. After the implantation procedure, the animals received subcutaneous ampicillin (15 mg/kg/48 h) 3 times.

Evaluation

After 6 weeks, all goats were killed with an overdose of pentobarbital. Tibiae were removed, and the bone chambers with surrounding cortex were fixed in 4% buffered formalin. After 1 day, the content was removed from the chambers and fixed additionally. The specimens were dehydrated using ethanol and embedded in poly(methyl methacrylate) (PMMA). The specimens were cut with a microtome parallel to the longitudinal axis of the chamber. Sections were taken at 0, 300, and 600 μm from the center of the specimens, each section being 5 μm thick. The sections were stained with hematoxylin and eosin, and with Goldner-Masson trichrome for routine histology. All sections in each experiment were investigated in random order.

Histomorphometric analysis was performed using interactive computer-controlled image analysis (analysIS; Soft Imaging System GmbH, Münster, Germany). The bone ingrowth distance in each slide was calculated by dividing the area of new bone by the width of the specimen. In all specimens, marrow cavities surrounded by bone were included in the bone area. The mean of the 3 sections at 0, 300, and 600 μm from the center yielded a value for the specimen. The total tissue ingrowth distance, which is the distance from the end of the ingrowth to the fibrous ingrowth frontier, was measured in the same way as bone ingrowth (Tägil 2000).

Statistics

We applied two outcome measures: fibrous tissue ingrowth and bone ingrowth. Each was possibly related to medication (none, ketoprofen, or meloxicam), graft type (autograft, rinsed allograft, or irradiated rinsed allograft), side (left or right leg), and position of the bone chamber (proximal, distal, or middle).

For each of the two outcome measures, a general linear model for repeated measurements (in one animal) was postulated. We assumed that there was a correlation between all 6 variables in each goat. These models were subsequently analyzed with PROC MIXED software (SAS International, Heidelberg, Germany) and p-values less than 0.05 were considered to be significant. The smallest relevant difference in ingrowth was set at 0.8 mm. The study was designed to have 80% power in detecting this difference of 0.8 mm between the means of all groups, and a standard deviation of this mean of 0.5 mm.

Results

Clinical evaluation

1 goat (not on NSAIDs) died after 2 weeks, due to an unknown pregnancy and complicated delivery. All other goats performed well, with full loading of their legs on the first day after surgery. At preparation of the grafts, 1 bone chamber was lost (autograft from the ketoprofen group), resulting in a total of 155 samples.

Histological analysis

17 of the bone chambers showed no ingrowth or only fatty tissue and no fibrous tissue or bone: 5 in the group without medication (2 autografts and 3 irradiated autografts), 7 in the ketoprofen group (5 autografts and 2 irradiated allografts), and 5 in the meloxicam group (1 autograft, 2 allografts, and 2 irradiated allografts). The remaining 138 specimens showed a typical ingrowth pattern. Nonvascularized remnants of the graft were present in the top of the chamber. A fibrous ingrowth zone was present between the graft and the newly formed bone, and there was a zone of newly formed bone located at the bottom of the chamber (Figure 1). In all treatment groups, the newly formed bone consisted of immature woven bone in a fibrovascular stroma (Figures 2 and 3).

Histomorphometry

No differences in bone growth ($p = 0.5$) or fibrous tissue ingrowth ($p = 0.6$) were found between the different medication groups (Table 1), including the group that did not receive any NSAIDs (Tables 1 and 2).



Figure 1. Example of typical bone ingrowth in a bone chamber (hematoxylin and eosin). This goat received meloxicam and a rinsed allograft was used. Nonvascularized remnants of the graft (G) can be seen at the top of the chamber. A fibrous ingrowth zone (F) can be seen between the graft and the newly formed bone, and a zone of newly formed bone (B) can be seen at the bottom of the chamber. Magnification 12.5 \times .

No differences in bone ingrowth were found with respect to graft type ($p = 0.4$), position of the bone chamber ($p = 0.6$), or operated leg ($p = 0.6$) (Table 3).

In contrast, we found a higher amount of total tissue ingrowth in the irradiated, rinsed bone graft than in the other 2 graft types/treatments ($p = 0.01$), no left-to-right difference ($p = 0.05$), and a higher total amount of tissue in the proximal bone chamber as compared to the middle and distal ones ($p = 0.001$). No differences in total tissue ingrowth were found when comparing the different medication groups ($p = 0.6$).

Discussion

Although there is sufficient evidence that NSAIDs cause reduced fracture healing and bone ingrowth

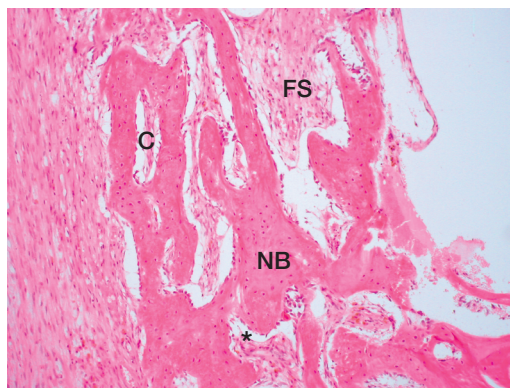


Figure 2. Typical histological section from a sample of a graft from a goat that received ketoprofen (hematoxylin and eosin). Newly formed woven bone (NB) in a fibrovascular stroma (FS) can be seen, with vital osteoblasts (*) and osteoclasts (C) surrounding the newly formed bone. Magnification 50 \times .

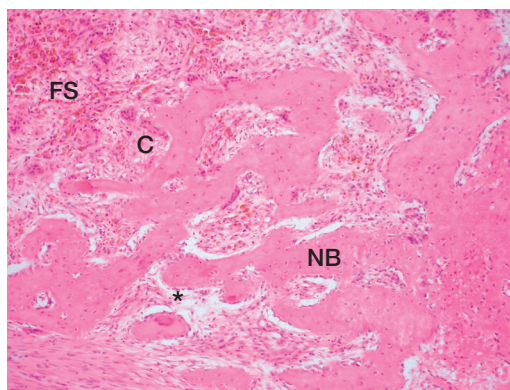


Figure 3. Typical histological section from a sample of a graft from a goat that received meloxicam (hematoxylin and eosin). New woven bone (NB) in a fibrovascular stroma (FS), with osteoblasts (*) and osteoclasts (C) comparable to the bone formation in goats that received ketoprofen. Magnification 50 \times .

in smaller animals, the effects of these drugs on bone graft incorporation in humans and larger animals appears to be limited. The good results of THA despite the use of NSAIDs as prophylaxis for HO makes it difficult to believe that the effect of NSAIDs on bone ingrowth is as large as it is in smaller animals.

We found no difference in bone ingrowth in titanium bone chambers in the goat, irrespective of whether the animals were treated with ketoprofen or meloxicam or no medication at all. Furthermore, there was comparable ingrowth of bone irrespec-

Table 1. Bone ingrowth and fibrous ingrowth, mm (SD)

	No medication		Ketoprofen		Meloxicam	
	Bone	Tissue	Bone	Tissue	Bone	Tissue
Autograft	0.5 (0.6)	3.9 (1.1)	1.0 (1.1)	4.5 (1.6)	0.9 (1.0)	4.1 (1.2)
Allograft	0.8 (0.8)	4.1 (1.2)	1.0 (1.8)	4.2 (1.3)	1.0 (0.8)	4.1 (1.1)
Irradiated allograft	1.0 (1.0)	4.1 (1.5)	0.8 (0.7)	4.5 (1.4)	1.1 (0.9)	4.5 (1.3)

Table 2. Bone ingrowth in the different medication groups

Medication	Difference observed, in mm	95% CI	P-value
No medication vs. ketoprofen	-0.14 (no medication lower than ketoprofen)	-0.66–0.38	0.6
No medication vs. meloxicam	-0.3 (no medication lower than meloxicam)	-0.82–0.22	0.2
Ketoprofen vs. meloxicam	-0.16 (ketoprofen lower than meloxicam)	-0.69–0.34	0.5

Table 3. Tests of fixed effects; relationship between bone ingrowth and graft type, medication, side, and position of the bone chamber

	Degrees of freedom	F-value	P-value
Graft type	2	0.92	0.4
Medication	2	0.73	0.5
Side	1	0.28	0.6
Position	2	0.60	0.6

tive of whether an autograft, rinsed allograft, or irradiated rinsed allograft was used. The findings of the same amount of ingrowth in rinsed allograft and autograft are similar to the findings of van der Donk et al. (2003), who found that with rinsing, total tissue ingrowth increased in the allograft group to approach that of autografts. The effect of lipid extraction from allografts, which enhances bone ingrowth, has been described before by Thorén et al. (1995). The similar amounts of ingrowth in rinsed allografts and irradiated, rinsed allografts are in line with the findings of Hannink et al. (2007).

The bone chamber model we used was developed by Aspenberg and Wang (1993). In this animal model, a single factor can be changed to evaluate

its effect on bone ingrowth. Although the effect of physical load cannot be studied in this model, it is valid for detecting the effects of bone substitutes and signaling molecules involved in bone metabolism that arise under unloaded conditions (Tägil 2000). Most bone chamber studies have been performed in smaller animals, especially rats and mice (Goodman et al. 2002, Gerstenfeld et al. 2003, Simon and O'Connor 2007). It is possible that the pharmacological and pathophysiological regulation of COX or the properties of the isoenzymes in different animal species differ; thus, when anti-inflammatory effects of NSAIDs are determined in animal experiments based on COX inhibition, species differences should be taken in to account (Cheng et al. 1998). The ingrowth itself in this animal model is comparable in rats and goats (van der Donk et al. 2001). We used goats, because we believe that the bone metabolism in goats is similar to the bone metabolism of humans, relative to the metabolism in mice or rats. Furthermore, the pharmacokinetics and pharmacodynamics of the drugs we used are already known (Moses and Bertone 2002, Arifah et al. 2003).

Although we wanted to use a COX-2 selective NSAID, we used the COX-2 preferential drug meloxicam because this is the only one for which the pharmacokinetics and pharmacodynamics have

been studied in the goat. For the same reason, we chose ketoprofen as a non-selective NSAID (Moses and Bertone 2002, Arifah et al. 2003). We administered the drugs for 6 weeks, based on the study by van der Donk et al. (2001) who found representative ingrowth in goats after 6 weeks.

Our data from this animal study should certainly be interpreted with caution. The data on the effects of NSAIDs on fixation of total hip prostheses in humans are conflicting. Ince et al. (2007) found no difference in loosening of uncemented acetabular components after 5 years when comparing groups that received indomethacin, postoperative irradiation, or no prophylaxis, for heterotopic ossification. Similarly, Kjaersgaard-Andersen et al. (1991) could not detect a difference in loosening or lucencies between patients who received indomethacin and the ones who did not. On the other hand, Persson et al. (2005a) found a trend of higher revision rates in an indomethacin treatment group than in patients who did not receive any NSAIDs after 10 years of follow-up.

Based on our study, we conclude that there was no detectable difference when using either ketoprofen and meloxicam in titanium bone chambers in goats loaded with fresh autograft, allograft, or irradiated allograft. Extrapolation of these results to the human situation must be done with caution. On the basis of these data and clinical data, however, the effect of NSAIDs on bone graft ingrowth may be limited.

Contributions of authors

HvdH and GH: study design, surgery, interpretation of data, and manuscript preparation. PB and BS: study design, interpretation of data, and manuscript preparation.

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No competing interests declared.

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