Comprehensive Summaries of Uppsala Dissertations
from the Faculty of Medicine 1316

Heterotopic Ossification

Clinical and Experimental Studies on Risk Factors, Etiology and Inhibition by Non-steroidal Anti-inflammatory Drugs

BY

PER-ERIK PERSSON

ACTA UNIVERSITATIS UPSALIENSIS
UPPSALA 2004

Dissertation presented at Uppsala University to be publicly examined in Sal Hanö, Centralsjukhuset, Kristianstad, Friday, January 30, 2004 at 13:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

Abstract

Persson, P-E. 2004. Heterotopic Ossification. Clinical and Experimental Studies on Risk Factors, Etiology and Inhibition by Non-steroidal Anti-inflammatory Drugs. Acta Universitatis Upsaliensis. *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1316. 47 pp. Uppsala. ISBN 91-554-5840-8

In this thesis, occurrence of heterotopic ossification (HO) following total hip arthroplasty (THA) was studied. Preventive effects and complications with non-steroidal anti-inflammatory drugs (NSAIDs) were analyzed. Experimental investigations on bone formation were employed to gain insight to the mechanism of NSAIDs action on bone.

(I). Fifty-six patients with bilateral THAs were analyzed. We found a strong correlation between HO on the two sides. Incidence and grade of HO were higher in men than in women.

(II). Sixty-nine patients with bilateral THAs who had been treated with NSAIDs after one or both THAs were analyzed for HO. Widespread HO occurred in untreated THAs, but in none of the treated THAs.

(III). A consecutive series of THAs were analyzed for HO. No widespread HO occurred in patients treated with NSAIDs for 21 days. In contrast, widespread HO occurred in 23% of patients not treated.

(IV). A randomized, double-blind, prospective study on 144 patients was performed to determine the efficacy and minimum treatment time with Ibuprofen for prophylaxis of HO after THA. Treatment with Ibuprofen was effective for preventing HO and a treatment time of 8 days was sufficient.

(V). A ten-year follow-up examination was performed on the patients from study IV. Thirteen patients had been revised. All but one belonged to groups treated with Ibuprofen. However, the prosthetic survival time was not statistically different for patients treated with NSAIDs compared to the control group. Eighty-four more patients underwent radiographic examination10 years after THA. Nine loose prostheses were found. These were equally distributed between NSAIDs-treated and non-treated THAs. When combining complications (revisions and radiographic loosening) no significant effects could be verified.

(VI). Experimental induction of heterotopic new bone with demineralized allogeneic bone matrix (DABM) and with bone autografts, was used in rats to study effects of NSAIDs on new bone formation. Indomethacin inhibited net bone formation in DABMs and in orthotopic fractured bone. In contrast, a net mineral loss occurred in autografts, but neither mineral content nor ^{45}Ca incorporation was affected by Indomethacin treatment. The amount of bone formed per mg implanted DABM was linearly correlated to implant size.

Keywords: Heterotopic ossification, Hip prosthesis, NSAIDs, Prophylaxis, Prosthesis loosening, Reoperation, Animal model, Bone formation

Per-Erik Persson, Department of Surgical Sciences, Akademiska sjukhuset, Uppsala University, SE-75185 Uppsala, Sweden

ISSN 0282-7476
ISBN 91-554-5840-8
urn:nbn:se:uu:diva-3908 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-3908)

‘Forty-two!’ yelled Loonquawl. ‘Is that all you’ve got to show for seven and a half million years’ work?’

‘I checked it very thoroughly,’ said the computer, ‘and that quite definitely is the answer. I think the problem, to be quite honest with you, is that you’ve never really known what the question is . . . Once you know what the question is, you’ll know what the answer means.’

Quotation from: *The Hitchhiker’s Guide to the Galaxy* by Douglas Adams.

List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I Sodemann B, Persson PE, Nilsson OS. Periarticular heterotopic ossification after total hip arthroplasty for primary coxarthrosis. *Clin Orthop.* Dec 1988(237):150-157.

II Sodemann B, Persson PE, Nilsson OS. Prevention of heterotopic ossification by nonsteroid antiinflammatory drugs after total hip arthroplasty. *Clin Orthop.* Dec 1988(237):158-163.

III Sodemann B, Persson PE, Nilsson OS. Prevention of periarticular heterotopic ossification following total hip arthroplasty. Clinical experience with indomethacin and ibuprofen. *Arch Orthop Trauma Surg.* 1988;107(6):329-333.

IV Persson PE, Sodemann B, Nilsson OS. Preventive effects of ibuprofen on periarticular heterotopic ossification after total hip arthroplasty. A randomized double-blind prospective study of treatment time. *Acta Orthop Scand.* Apr 1998;69(2):111-115.

V Persson PE, Berggren AM, Nilsson OS. Ten-year risk of failure in patients treated with ibuprofen after total hip arthroplasty. (Submitted)

VI Persson PE, Sisask G, Nilsson O. Indomethacin inhibits bone formation in inductive allografts but not in autografts. (Submitted)

Contents

Abbreviations

BMP	Bone morphogenetic protein
^{45}Ca	Radioactive calcium (isotope 45)
COX	Cyclooxygenase
COX-1	Cyclooxygenase type 1
COX-2	Cyclooxygenase type 2
DABM	Demineralized allogeneic bone matrix
HO	Heterotopic ossification
NSAIDs	Non-steroidal anti-inflammatory drugs
PG	Prostaglandin
SD rat	Sprague-Dawley rat
TGF-β	Transforming growth factor-β
THA	Total hip arthroplasty

Introduction

Definition

Heterotopic ossification (HO) means bone that is formed outside the skeleton. HO can occur in a variety of medical conditions. Morphologic, biochemical and immuno-histochemical studies of HO at different locations and in different pathological conditions, have shown that HO contains the same constituents as bone within the skeleton during rapid bone formation, as in repair and growth [1]. The development of HO generally follows a similar sequence of events, but the rate of maturation of the tissue may vary considerably. In most instances trauma seems to be the eliciting factor for HO, but HO may also occur without obvious trauma.

Different types of HO

HO may occur in different conditions, such as after surgical trauma, burns and other trauma to soft tissues, in neoplasms, after neurologic injuries and in hereditary disorders.

Surgical trauma

The most common HO develops in the soft tissues around the hip following total hip arthroplasty (THA). The location may vary and two patterns have been identified, namely, central around the neck of the femoral component and lateral to the acetabular rim and to the greater trochanter of the femur [2]. Surgery for acetabular fractures and/or dislocation of the hip joint, are other events often complicated by HO [3, 4]. Widespread HO often forms and constitutes a significant clinical problem with decreased range of movement and pain, if prophylactic treatment is not given.

Also, HO frequently appears at entrance sites for intra-medullary nails after fracture treatment [5].

HO is rarely observed after arthroplasties of the knee. The overall incidence of HO after total knee arthroplasty is about 5-10%, but severe HO occurs very rarely and interferes with the clinical outcome only in exceptional instances [6-8]. When HO occurs after knee arthroplasty, the most common location is in the quadriceps muscle, a muscle that is prone to form bone in response to traumatic events.

In shoulder arthroplasties, HO occurs more often, with a reported incidence of 25-45%, but only rarely does the HO interfere with range of motion, or cause pain [9, 10]. Similarly to hip arthroplasties, male patients and patients undergoing surgery for degenerative osteoarthritis have an increased risk of developing heterotopic bone. Thus, HO following shoulder arthroplasty is fairly frequent, but seldom a disabling condition.

HO has not been reported to create a significant clinical problem after elbow arthroplasties. In the few existing reports, the incidence is about 1-5 %. However, significant HO may occur in patients operated for anchylosis or post-traumatic arthritis [11]. HO is also known to appear after elbow fracture/fracture-dislocations and after burn injuries, resulting in restrictions in elbow joint movement [12, 13].

Other traumas and burn injuries

Blunt trauma to soft tissues, most often encountered in sports, may cause HO. When this happens, the location of the trauma seems to be an important factor. The vastus intermedius muscle, the gluteal muscles and the region around the elbow are the regions most susceptible to this type of HO [14].

HO may also occur around major joints and in large muscle groups after burn injuries [15].

Neoplasms

Frequently heterotopic bone forms in response to neoplasms of mesenchymal origin. Tumors derived from osteogenic cells can produce bone, and bone can also be produced as a reactive phenomenon adjacent to the tumor. Histochemical studies by Bosse show that the development of HO in some aspects is similar to the development of an osseous autonomous neoplasm [16]. In the early stages, the new bone formed may be difficult to distinguish from neoplastic bone [17].

Cells from the urogenital tract seem especially prone to form bone. HO has been reported in the kidneys, uterus and corpora cavernosa. Interestingly, extracts from transitional epithelium of the urinary tract were the first cells shown to contain a soluble osteoinductive factor [18].

Neurologic injuries

Severe head trauma or paraplegia after spinal injury vastly increases the risk for HO, especially around the hip and knee [19]. The mechanism for this is not known, but the muscles may be susceptible to trauma as a consequence of the neurologic injury - or the nerve system itself might have a regulatory function on mesenchymal cells which have receptors for some of the neuropeptides [20]. Interestingly, fracture healing in patients with a neurologic injury often occurs with an exuberant callus, much like the heterotopic bone in the same patients.

Hereditary disorders.

In the rare genetic disorder Fibrodysplasia (Myositis) Ossificans Progressiva, HO occurs progressively and trauma is only of minor importance. This is the most severe form of HO. In this disease the patient reacts with a rapid and exuberant formation of new bone in the soft tissues even after minor insults. Cells from patients with this disease show an enhanced transcription of bone morphogenetic protein-4 messenger ribonucleic acid, which probably plays a critical role in the pathophysiology of the disease [21].

Hereditary factors are probably of importance in any type of HO, but their relative importance may vary. Different individuals react to surgical or other trauma with the development of more or less excessive HO. The formation of HO following a fairly standardized stimulus such as THA covers a large spectrum, which shows the important role of the individual response of HO development. This response is probably genetically programmed, but can be modified by different treatments.

Heterotopic ossification after total hip arthroplasty

Historical background

Treatment of degenerative hip conditions was a big challenge for surgeons during the first two thirds of the 20th century. Inter-trochanteric osteotomy was often performed with the purpose to give these patients a temporary relief of pain. In 1970, John Charnley presented the “low friction arthroplasty”, a concept which eventually proved to be a long-lasting solution [22]. When his first paper on this method was published, he had already nearly 10 years of experience of the concept, using high-density polyethylene for the acetabular component and acrylic cement for fixation of

the prosthetic components to the bone. The short- and long-term results were excellent [23] and the patients mostly achieved a good joint mobility.

However, Charnley noted that in some instances, patients developed large amounts of bone in the soft tissues around the new artificial joint. A number of these patients did not experience full satisfaction with the operation due to stiffness, and in some cases even pain in the hip. Sir John's concept for hip joint replacement conquered the world, but the problem of how to avoid HO was yet to be solved.

Incidence and prevalence of HO

HO is the most frequent complication in hip replacement [24-28], with a reported incidence of between 9 and 90%.

Larger studies using the same or similar grading systems show considerably smaller variations, with HO ranging between 35-60 %.

Widespread HO is reported to occur in between 5 and 30% of patients, while severe HO with clinical symptoms, such as pain and restricted joint motion leading to functional impairment occurs in fewer than 10% [23, 26, 29, 30].

Natural course of HO

Irrespective of the etiology of HO, the morphological features follow a similar pattern.

Morphological and biochemical analysis of the heterotopic bone has shown an intense turnover and a high content of growth factors, indicating HO to be a metabolically active tissue [16]. In the acute phase, round cell infiltration is seen with edema and degeneration of the muscle. After a few weeks, cartilage and then bone replace the inflammation. Clinically, the development of HO in severe cases may mimic early, low-grade infection with swelling, edema and even elevated temperature [31]. Progression of the HO then continues for between 3 and 6 months, with maturation of the bone. After 6 months HO rarely increases [26, 32, 33]. Interestingly, the HO does not disappear or decrease in mass with time, in spite of the fact that it is not exposed to direct weight-bearing. However, forces created by muscular activity and motion will affect the HO by creating mechanical load.

Correlation of HO to bone metabolism in general

Heterotopic bone formation after a standardized trauma to the soft tissues - such as total hip arthroplasty - is related to the specific bone biology of the individual. This is further supported by the finding that patients with HO have greater spinal bone densities than matched controls [34]. In addition, other

conditions with increased new bone formation, such as anchylosing spondylitis, diffuse idiopathic skeletal hyperostosis and hypertrophic osteoarthritis, are associated with an increased risk of developing HO [26, 35]. This implies that HO is not an isolated or local phenomenon but an expression of a general tendency of the individual to form bone, whether it is formed within the skeleton or in the soft tissues.

Risk-factors, correlation and etiology of HO

The mechanism that causes HO is not fully known, but trauma to the soft tissues and bone is an eliciting factor. The early inflammatory reaction following the surgical trauma appears to be essential for the induction of HO. In addition to trauma, a genetic factor or "predisposition" is necessary in order for HO to occur.

THA, together with surgically treated acetabular fractures, are the surgical procedures most often complicated by HO. In HO after THA, several important relationships to bone metabolism and bone response to trauma in general have been determined. In addition, the reaming often results in remains of bone marrow in the muscle bed. Thus, the surgical procedure creates a direct access for osteogenically competent progenitor cells to a well vascularized muscle site, while osteoinductive factors and growth factors are released from the traumatized tissues, especially the bone, and may act to mobilize these immature cells and induce them to develop into mature osteoblasts.

Furthermore, there are individual, and probably genetic relationships that can be described in terms of risk factors for HO:

- Males are more prone to develop HO than females, and the amount of bone formed is also greater [24, 29, 36].
- Patients with a previous history of HO almost invariably develop HO after hip arthroplasty [26, 27, 35].
- Anchylosing spondylitis, diffuse idiopathic skeletal hyperostosis and hypertrophic OA are conditions associated with increased bone formation and increased risk of HO [26, 34].

Even if the trauma is fairly standardized it can result in a spectrum of responses, ranging from no bone induction at all, to virtual anchylosis of the joint.

Grading of HO

Several radiographic grading methods have been applied to categorize HO. Most of these methods are based on antero-posterior radiographs of the hip.

Some of the grading systems also include the localization of HO in relation to the joint, while others are based on the extent or the "bridging" (in one plane) of the joint [24-26, 29, 33, 37, 38].

The classification according to Brooker is by far the most frequently used classification in current literature.

Clinical significance

The most common symptoms of HO are decreased range of motion and sometimes pain.

HO has been found to correlate to a lower grade of function. In general, HO must be widespread (Brooker grade III or IV) in order to cause symptoms [23, 39]. Patients with severe grades of HO after THA show early clinical symptoms and radiographic signs of calcification in the gluteus medius muscle area, even within 1 month of surgery. The early clinical symptoms in the relatively rare cases (less than 10%) of severe HO may mimic early, low-grade infection, indicated by an inflammatory reaction with swelling, tenderness and a slightly elevated temperature.

Treatment

Three kinds of treatment have been proposed to prevent HO for patients at risk, or as a general prophylactic treatment after THA.

1. Medication with non-steroidal anti-inflammatory drugs (NSAIDs) postoperatively,
2. Pre- or postoperative irradiation of the hip region and
3. Medication with bisphosphonates postoperatively

NSAIDs

A number of prospective and randomized studies have confirmed the efficacy of NSAIDs to prevent the significant grades of HO both in cemented and uncemented THA, and in patients at risk. The shortest effective treatment time is probably between 5 and 10 days, starting the treatment on the day of surgery [40-43]. NSAIDs have also been proved to prevent the recurrence of HO after resection [39, 44, 45].

NSAIDs may affect one or several of the key steps in the bone-forming process and several different NSAIDs have been investigated for efficacy in the prophylaxis for HO. While Indomethacin has been most thoroughly

investigated, it appears that most substances (Indomethacin, Ibuprofen, Diclofenac, Aspirin, Naproxen, Ketorolac, Tenoxicam) in this group are effective [41-43, 46-49]. Therefore, the preventive effect of NSAIDs on HO is probably mediated through their general inhibitory effect on prostaglandin (PG) synthesis.

Prostaglandins and bone

All cells in the human body synthesize prostaglandins. In bone-cells (osteoblasts, osteocytes and osteoclasts), prostaglandin-E_2 (PGE_2) is the most common prostaglandin.

PGs are essential for the response of cells to trauma by initiating the production of various cytokines in order to start a healing process (inflammatory cell response). Basal production of PGs is necessary for the protection of mucosal membranes such as the gastric mucosa. The enzyme cyclooxygenase (COX) is involved in an important step in the synthesis of PGs from the precursor arachidonic acid. NSAIDs act as inhibitors of COX and thereby down-regulate the synthesis of PGs.

In recent years it has been found that (at least) two different COX exist. The same genes do not produce the two enzymes. COX-1 is responsible for the synthesis of constitutional (protecting) levels of PGs, and COX-2 is up-regulated when some extraordinary condition affects the cellular tissue. For example, COX-2 levels, and thereby the synthesis of PGs, rise markedly in inflammatory conditions. The recently introduced COX-2 inhibitors are now widely used as analgesics throughout the world since they produce fewer adverse gastro-intestinal effects. These drugs are probably potent inhibitors of both heterotopic bone formation as well as fracture healing in orthotopic bone. The COX-2 inhibitors have not yet been tested for prevention of HO after THA, but in animal experiments, inability to synthesize COX-2 has been shown to result in the inhibition of fracture healing [50, 51].

Dosage of NSAIDs

Standard dosages of the different NSAIDs (Indomethacin 25 mg three times daily, Ibuprofen 400 mg 3 times daily, Aspirin 1 g 3 times daily, Naproxen 0.5g 2 times daily) during the first postoperative weeks, have been shown to inhibit HO after total hip arthroplasty and to be effective in preventing the recurrence of excised HO after previous hip surgery [39, 44, 45, 52, 53].

Duration of Treatment with NSAIDs

The duration of treatment with NSAIDs has been investigated in several prospective studies and it appears that treatment for more than 3 weeks does not reduce the incidence of HO further [42, 49, 54].

The minimum duration of treatment appears to be 5 to 10 days. The treatment should also be started at the time of surgery in order to be effective [53, 55]. However, no additional effect is achieved if the treatment is initiated prior to surgery, and, furthermore, this might cause increased peroperative bleeding. Thus, the early postoperative onset of medication is sufficient for a full effect, but the start of medication should probably not be delayed beyond the first postoperative days. In one of our own studies, delay of medication for 5 days resulted in increased rates of HO [53].

Side-effects of NSAIDs

In most double-blind studies the tolerance to medication is good and dropouts are not more frequent in the treatment groups than in the placebo-treated groups. Some double-blind studies have shown discontinuation of the medication in as many as 20% of the patients treated with Indomethacin, mainly due to gastrointestinal discomfort or central nervous effects, but similar symptoms and a similar rate of discontinuation were noted in the placebo-treated groups [39, 42, 49, 55].

The most serious concern about medication with NSAIDs is based on experimental findings of inhibition of bone remodelling, and reduced bone ingrowth into porous implants [56]. These findings have prompted questions concerning whether medication with NSAIDs can increase the risk of mechanical loosening of the prosthesis. However, to date, there are no clinical studies that show increased rates of radiographic loosening or increased rates of revision in cemented or uncemented THA [39, 57]. However, the number of patients and the follow-up time in these studies are not sufficient to completely exclude a long-time effect on the fixation of the prosthesis.

Irradiation

New bone formation is dependent on cellular proliferation, and transformation of cells into osteogenic cell lines. Ionising radiation is known to exert an inhibitory effect on rapidly dividing cells by interfering with their production of nuclear DNA. This is the rationale for the prophylactic treatment with local prophylaxis by radiation for patients at risk of developing HO after THA. The first report on this type of treatment was by Coventry and Scanlon in 1981 [58]. Their patients were successfully treated postoperatively with fractionated doses of 2 Gy given 10 times. Subsequently, the doses have been reduced to 5 to 8 Gy given as a single pre- or postoperative dose, and still a good prophylactic effect on HO is maintained. Thus, pre- or postoperative irradiation as a single dose or fractionated in 5 doses gives a good preventive effect in patients at risk of developing HO [58-60]. The finding of a prophylactic effect of preoperative

radiation with a relatively low single dose [61, 62] facilitates this treatment, but cost and a small risk of radiation-induced sarcoma impose a restriction on its use. Radiation also decreases bone ingrowth and the strength of fixation of porous implants [63]. Shielding of the prosthetic implants can possibly minimize this effect.

Bisphosphonates

Bisphosphonates have also been used to prevent HO after THA. The medication is effective only as long as it is continued, but it results only in a delay of the calcification of the osteoid, since the ossification develops when the medication is discontinued. Therefore bisphosphonates have now largely been abandoned as prophylactics for HO [64, 65].

Experimental HO

A laboratory model for inducing bone formation in soft tissues was first described by Marshall Urist in 1965 [66]. Allogeneic bone-segments, which have been demineralized by hydrochloric acid and implanted into muscle pouches, induce bone-formation at the implant site. Implantation of demineralized allogeneic bone matrix (DABM) has since then been widely used to study bone metabolism under various conditions.

This model of new bone formation has many advantages; especially the ease with which the net amount of new bone can be quantified, as all bone is new bone. In addition, the time sequence of events can be monitored. The bone formation follows a sequence resembling orthotopic endochondral bone formation [67]. In respect to HO, bone induction by DABMs has many similarities, e.g. the morphological features and the heterotopic site, while there are also some differences in terms of the inductive stimulus.

Marshall Urist early identified the paramount scientific importance of bone induction. He noted that the sequence of events resulting in new bone were similar to those of the developing skeleton and hypothesized that the inducing agent was a regulator of skeletal development, and that this substance also controlled regeneration and bone healing. Consequently he named the substance Bone Morphogenetic Protein (BMP). Subsequently, a large number of BMPs have been identified and synthesized, the majority of them belonging to the Transforming Growth Factor β (TGF-β) superfamily. Also, in agreement with Urist's original hypothesis, the BMPs have not only proved to be essential in bone development, but also in many other key events in the development of mesenchymal tissue.

Aims

Clinical studies

- To study the incidence and risk factors for heterotopic ossification (HO) after total hip arthroplasty (THA).
- To analyze the effects of postoperative prophylaxis for HO with Indomethacin or Ibuprofen in patients operated with THA.
- To determine the shortest effective treatment time for prophylaxis with Ibuprofen to prevent clinically significant HO after THA.
- To study the tolerance and adverse effects of medication with Indomethacin and Ibuprofen after THA.
- To determine the short- and long-term revision rate and the incidence of aseptic loosening after treatment with Ibuprofen for HO in THA.

Experimental study

- To study the effects of Indomethacin on bone induction by demineralized allogeneic bone matrix (DABM) compared to bone autografts and fracture healing in Sprague-Dawley (SD) rats.
- To analyze the effects of different amounts of inductive DABM on the yield of new bone in young and old SD rats.

Patients and methods–Clinical Studies

All five clinical studies in this thesis are based on radiographic analyses of HO in patients operated with THA for degenerative osteoarthritis. Studies I–III are retrospective analyses of consecutive series of patients, while Study IV has a prospective, randomized and double-blind design. Study V is a long-term follow-up of the patients in study IV.

Patients in studies I–III were recruited from a rural population with a high incidence of HO.

Half of the patients in study IV–V were recruited from the same rural population, while the other half was recruited from an urban population.

In studies I and II, only patients who had been operated on both sides with THA, were included.

Patients in Study I - V

Table 1. Study I & II (Bilateral THA)

	Patients	*THA*	*M/W*	*Mean age*
Untreated both sides	56	112	34/22	66 (51-83)
Treated both sides	31	62	18/13	67 (53-81)
Treated one side only	38	76	20/18	66 (55-80)
All	125	250	72/53	66 (51-83)

Table 2. Study III-V

	Patients	*THA*	*M/W*	*Mean age*
Study III	175	200	98/77	69 (45-88)
Study IV	144	144	72/72	67 (46-85)
Study V	144	144	72/72	

Mean age denotes the age at THA.

Surgery

In all clinical studies the patients were operated on by a posterior approach without trochanteric osteotomy. A Lubinus, an Exeter or a Charnley prosthesis was used. The stem and the acetabular cup were fixed to the bone by a conventional cementing technique. The patients had prophylactic treatment for thrombosis with either Dextrane infusions or low-molecular Heparin injections. A schedule for prophylactic antibiotic treatment was followed.

Medication with NSAIDs

In studies I to V, the medical records of the patients were thoroughly studied.

All peri- and postoperative medication and treatment were noted. None of the patients had received peri- or postoperative irradiation therapy or treatment with bisphosphonates. All postoperative analgesic treatment was recorded, including treatment with NSAIDs.

In study I no NSAIDs were given to the patients.

In studies II and III NSAIDs were given to some of the patients, as prophylaxis for HO. Standard dosages of Indomethacin (50 mg 2 times daily) or Ibuprofen (400 mg 3 times daily), were used. The duration of treatment and the time of the initiation of treatment with NSAIDs varied in these studies.

In studies IV and V, which are based on the same patients, Ibuprofen (400 mg 3 times daily) or placebo was used in a 3-treatment group design. In the groups treated with Ibuprofen the treatment continued for 8 or 21 days. Medication was started on the morning of surgery.

Radiographic examinations, evaluation of HO and prosthetic loosening

In studies I to IV, all patients were investigated with ordinary anterior-posterior (AP) radiographs preoperatively, within a few days after the operation, and at 3, 6 and 12 months postoperatively.

For the evaluation and grading of the amount of HO, we used the classification according to Brooker [25]. This is by far the most commonly used classification in studies of HO after THA. This classification is also easy to apply and is proven to give a good inter- and intra-observer reliability, [68] even though it has also been criticized [69].

In the Brooker classification, HO is given one of five grades on the radiographs.

- Grade 0 means no HO at all.
- Grade I means one or two isolated areas of ossification, each less than 1 cm in diameter.
- Grade II means a more widespread area of HO, covering less than half the distance between the femur and the pelvis.
- Grade III means HO that covers more than half this distance, but not bridging the entire distance between femur and pelvis.
- Grade IV means HO that apparently bridges the entire distance.

It is important to also assess the radiographs taken preoperatively and a short time postoperatively so that pre-existing osteophytes or small parts of radio-opaque bone cement are not mistaken for HO on later radiographs. Examples of different grades of HO are shown in figure 1.

In study V, a questionnaire was sent out by mail to patients still alive 10 years after the primary THA. The responses, in conjunction with the medical records provided information concerning whether the patients had had their THAs re-operated and, if so, the reason for the revision. All patients still alive, who had not been re-operated, were offered a follow-up radiographic examination to assess loosening and HO. The radiographs were centered on the symphysis, so that all prosthetic material appeared. They were examined in a standardized way by an experienced radiologist who had no information of what treatment the patients had received 10 years earlier. Prior to the examination, the following criteria for radiographic loosening were defined.

The acetabular cup was considered loose when one or more of the following criteria were fulfilled;

- A visible zone around the whole circumference of the cement-bone interface on an AP-radiograph, the width of the zone exceeding 2 mm along the entire circumference.
- Migration of more than 3 mm in any direction compared to the postoperative radiographs.

The femoral component was considered loose when one or more of the following criteria were fulfilled;

- Distal migration of the femoral component within the cement mantle > 5 mm.
- Visible fracture of the cement mantle.
- Distal migration of the femoral component between bone and cement > 5mm, and presence of radiolucency between bone and cement > 2 mm wide in more than one Gruen zone.

- Mid-stem pivoting of the prosthesis with a lateral stem-tip shift > 3mm.
- Medial calcar shift of the prosthesis > 3mm.
- Visible metal fatigue fracture of the femoral stem.

The THA was defined as radiographically loose when one or both components fulfilled one or more of the criteria above.

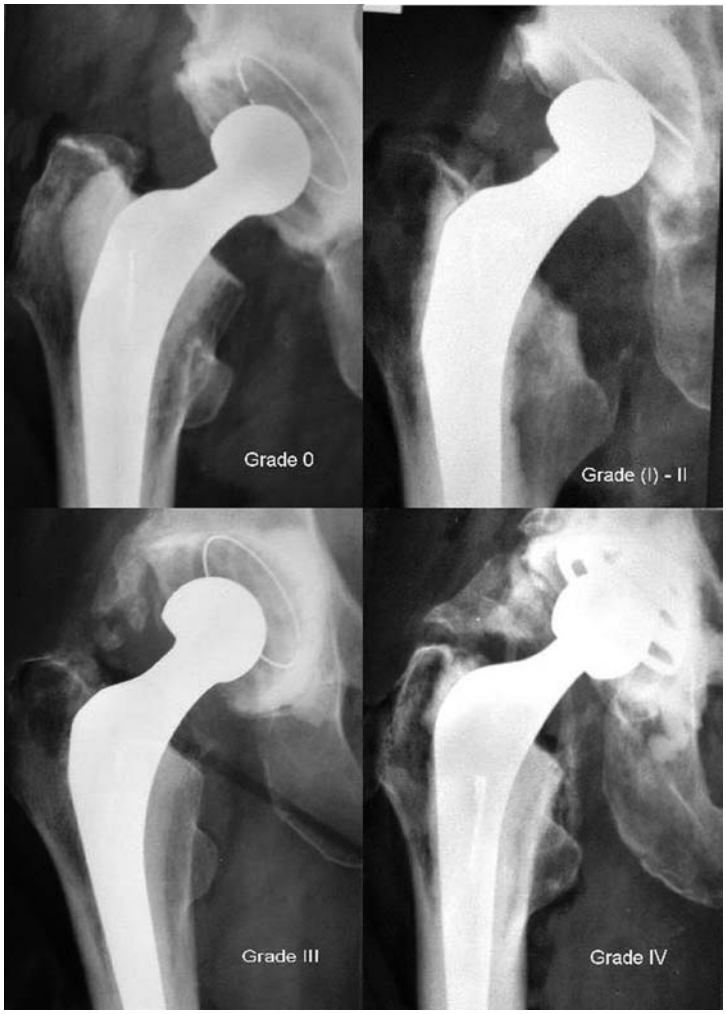

Figure 1 Examples of different grades of HO according to Brooker.

Design of clinical studies

Studies I–II were retrospective analyses of all patients operated on both sides with THA for degenerative osteoarthritis in Kristianstad during 1973–1984. Study III was a retrospective analysis of all patients with degenerative osteoarthritis, consecutively operated with THA in Kristianstad during January 1, 1981–April 1, 1984. Thus, some patients are represented as well in study II as in study III.

In the studies I–III, we focused mainly on analyzing HO. Therefore the THA patients were not evaluated specifically concerning the clinical result of the operation.

Prior to study IV, we decided that 48 patients in each group would be sufficient in order to investigate the effects of Ibuprofen on the development of HO. This was based on the incidence of HO in studies I to III. We hypothesized that the number of patients would also be sufficient to find out whether a shorter period of treatment was sufficient to prevent the development of HO. A randomization plan was made, and the test medication (Ibuprofen and Placebo) was provided by a pharmaceutical industry (A/S Alfred Benson). The medication was provided in numbered cases, each number randomly representing one of the treatment groups. A standardized protocol was set up for each patient.

The clinical parameters for study V were procured as described under the previous heading. All patients still alive and not re-operated were offered a follow-up radiographic examination to assess loosening and HO. These radiographs were examined by an experienced radiologist, who had no information of what treatment the patients had received 10 years earlier.

Statistics

In study I, we investigated the frequency of HO and the question of whether there were differences due to gender. For the statistical analysis we used a Chi^2 test. HO grades 0 and I and HO grades II to IV were grouped in the calculations. We also studied whether there was a correlation in grade of HO between the two sides of the same patient. For comparison of the two sides in the same patient (cross-over design), the Stuart modification of the McNemar test was used.

In study II, the effect of prophylactic treatment with NSAIDs on HO was studied. The intra-individual correlation of development of HO was also studied, and the same statistical methods as in study I were used.

In study III, a series of consecutive THAs were investigated for the effect of Ibuprofen and Indomethacin on HO. HO grade 0 and I and II to IV, were

grouped in the same way as in studies I and II and a Chi^2 test was used for the statistical analysis.

Study IV investigated the effects of Ibuprofen on HO with special regard to the duration of treatment. A Fisher's exact test, together with a log-linear transformation with ordering of the grade of HO (0 to IV), related to the factors gender and treatment (Goodman 1984) was used for the statistical analyses.

In study V, we studied the long-term effects of Ibuprofen on prosthetic fixation after total hip arthroplasty. A Cox Proportional Hazards Regression Model was used to determine whether the average survival time or risk of prosthetic loosening differed significantly between untreated patients and those treated with Ibuprofen. The difference between grades of HO, with respect to prophylactic treatment with Ibuprofen, at 1 and 10 years were also analyzed with a Chi^2 test, combining grades 0 and I, and grades II to IV, as in the previous studies.

Methods–experimental study

Animals

Study VI describes two different experiments. In the first, we studied differences in bone induction initiated by demineralized allogeneic bone matrix (DABM) versus autologous bone grafts.

In the second experiment we studied whether bone induction by DABM was influenced by the size of the implanted allograft.

In both experiments Sprague-Dawley rats (SD rats) were used.

In experiment 1, we used only adult male rats (body weight 500g). Thirty-six rats had DABM and autografts implanted and were then divided into 4 groups, treated for either 3 or 6 weeks with either Indomethacin or placebo.

In experiment 2, we studied the effects of different amounts of the inductive agent and also the difference between young and older rats. Therefore we used 12 young (body weight 90g), and 12 older (body weight 300g) SD rats. Each rat had DABMs of different sizes implanted. The DABMs were harvested after 3 weeks.

Surgery

The implants of DABM or autografts were placed in muscle pouches in the abdominal wall of male SD rats under neurolept analgesia (Hypnorm, Leo, Helsingborg, Sweden, 1.0 ml/kg body weight).

Autografts – DABM

The autografts in Experiment 1 were taken from the distal femur condyles using a small troachar (Craig-Kogler). The autografts contained the two cortices with trabecular bone in between.

DABM was prepared from long bones of SD rats by removing soft tissue, demineralizing in 0.6 N HCl, defatting with chloroform/methanol and

washing in cold water [70]. Diaphyses and metaphyses were used after lyophilization.

Experimental design

Experiment 1 was designed to study the effects of Indomethacin on bone induction in bone autografts and DABM, and also in femurs fractured by the autograft harvesting procedure. Thirty-six adult male SD rats each had 2 autografts and 4 pieces of DABM implanted. The rats were divided into four groups and treated for 3 or 6 weeks with Indomethacin or placebo by daily, subcutaneous injections. The animals were randomized to the different treatments. Twenty-four hours prior to death the rats were each given a single intramuscular injection of carrier-free ^{45}Ca. The animals were killed by carbon dioxide 3 or 6 weeks after surgery. The implants, femurs, tibias and humeri were retrieved. Mineral content and isotope activity were analyzed in the grafts, and in one tibia, femur, and humerus for each rat.

Experiment 2 was designed to study the effects of the size of the DABM on bone induction in young and older rats. Twelve SD rats, 90g body weight and 12 rats, 300g body weight each had 6 implants of DABM of the following weights, 0.5, 1, 2, 3, 4, or 5 mg implanted. Twenty-four hours prior to death the rats were each given a single intramuscular injection of carrier-free ^{45}Ca. The animals were killed by carbon dioxide 3 weeks after implantation and the implants were retrieved. Ash weight and ^{45}Ca activity were determined for each implant.

Analyses and statistics

The implants and the orthotopic bones were ashed and weighed. The ash was dissolved in HCl. The radioactivity of the samples was counted in a liquid scintillation counter. The ash weight, the absolute ^{45}Ca activity, and the ^{45}Ca specific activity (counts per minute/mg ash), were calculated for the different implants and the different orthotopic bones. The mean values of the two types of grafts and the values for the orthotopic bone were then used to calculate the mean and standard deviation of each of the 4 groups. In addition, the ratio of ^{45}Ca specific activity of the DABMs, autografts and femurs to the ^{45}Ca specific activity of the humerus was determined in each rat (Osteoquantum index) as a measure of relative calcium accretion rate.

The Wilcoxon Rank Sum test was used for all statistical analyses.

Results and comments

Clinical studies

HO is the most common complication after THA, and sometimes the clinical outcome is affected. This is especially true in patients who suffer high grade HO around both hips after bilateral THA operations. Joint motion in both hips may be severely restricted, and since these patients also often suffer from lumbar hyperostotic degenerative disease, even sitting in a chair may cause discomfort.

The observations by Dahl in 1975, that Indomethacin inhibits HO, raised considerable interest for a possible treatment for this complication. At the Department of Orthopedics, Kristianstad we started prophylactic treatment for HO with Indomethacin in 1980. The first patient treated had previously been operated with THA and developed significant HO (Brooker grade IV) leading to impairment of the mobility of the joint. At the THA in the other hip, she received prophylactic treatment with Indomethacin 25 mg three times daily for three weeks. At the follow-up after six months she was much more satisfied with her NSAID-treated hip with regard to motion and comfort, and the radiograph showed only moderate HO (Brooker grade II). This encouraged us to go on with prophylactic treatment with Indomethacin for HO. By April 1981 prophylactic treatment with Indomethacin, 50 mg twice daily for the first 21 postoperative days after THA, was established as a routine.

Studies I and II

To evaluate the efficacy of this protocol, and to gather insight into the incidence and etiology of HO, we investigated the effectiveness of prophylaxis for three weeks. We also investigated whether patients who develop HO after one THA, are prone to develop HO on the contralateral side to the same extent, as was suggested by DeLee et al [26]. Further, we wanted to investigate whether there was a difference in incidence and grade of HO in men and women, as suggested by others [1, 24].

For this purpose patient data and radiographs for all patients operated with bilateral THA for degenerative osteoarthritis, from Jan 1 1973 to April 1 1984, were collected.
We found the outcome in the bilaterally operated patients to be of special interest since they had a high intra-individual correlation to the grade of HO if the hips were treated similarly in terms of prophylactic treatment with Indomethacin/Ibuprofen (Study I and II). If only one of the hips was treated this correlation totally disappeared (Study II). These results show that the most important factor for the development of HO is the individual's ability to respond to bone-inductive stimuli, such as surgical trauma. This response can clearly be modified by prophylactic treatment with NSAIDs, as patients treated after one but not the other THA may be regarded as their own control (cross-over design).

When examining the non-treated THA we also found a statistical difference between men and women. There was a difference both in incidence and grade of HO, showing women to be less prone to form HO. The incidence of any grade of HO for hips not treated with NSAIDs was 79% for men and 48% for women (Both sexes together 67%). This implies, in accordance with the results of other investigators, that one of the risk factors for HO is male gender [24, 32, 35]. When both hips were treated with NSAIDs the corresponding figures were 14%, 4% and (9,5%).

Thus, medication and other treatments that might influence the development of HO, the sex ratio of the patient populations and the diagnosis for receiving a THA must be considered when studying the incidence of HO.

Study III

To further evaluate the efficacy of prophylactic treatment with Indomethacin/Ibuprofen on HO after THA, we collected data and radiographs from consecutive patients operated with THA for degenerative osteoarthritis at our clinic from January 1, 1981 to April 1, 1984. We also wanted to assess compliance with the medication and adverse effects (Study III).

In 200 consecutive THAs, we found that the prophylaxis was not given as intended in 34 THAs. In 14 patients (14 THAs) the routine prophylaxis was not initiated at all. In another 5 patients (5 THAs) medication was initiated, but not until 6-12 days after surgery. In 2 of these 5 THAs we found HO grade III. This indicates the importance of initiating NSAID treatment immediately following trauma. In addition, medication with NSAIDs was contra-indicated in 5 patients (6 THAs) due to previous allergic reactions or a recent history of gastric or duodenal ulcer. In 8/128 (6%) of THA patients

treated with Indomethacin and in 1/31 (3%) treated with Ibuprofen, the medication had to be discontinued due to adverse reactions. These 34 THAs, for which the prophylaxis was not given as intended, had a 53% incidence of all grades of HO, and grade III or IV occurred in 24%.

When prophylactic treatment was successfully completed, the incidence for HO was only 5.5%, and there were no instances of grade III or IV. Since we were of the opinion that Ibuprofen gave fewer side effects than Indomethacin, we used Ibuprofen (400 mg three times daily for 21 days) for patients who had previously experienced discomfort from using Indomethacin as an analgesic. This view was later corroborated by others [71, 72].

In this series the medication was carried out according to intention in more than 80%. In these patients we found no instances of high grade HO. In contrast, we found high grade HO in 8 of 34 THAs (24%) when treatment was delayed or not given. Thus, prophylactic treatment for HO with Indomethacin or Ibuprofen for three weeks is both effective and safe.

After evaluation of this study we changed the routine and used Ibuprofen instead of Indomethacin as prophylactic treatment, due to an expected higher compliance. Indeed, this assumption was later supported by the findings of similar rates of adverse effects in the placebo and the Ibuprofen groups, using a double-blind design in study IV.

Study IV

Based on these findings we wanted to analyze the shortest time of treatment with Ibuprofen for effective prophylaxis. The previous retrospective findings gave an indication that the early postoperative period was essential for the development of HO. Thus we decided to compare a considerably shorter postoperative treatment period (8 days), with our standard treatment period (21 days), and with a placebo control group. Using this experimental design, we were also able to analyze compliance and potential side-effects of the treatment, such as increased bleeding. Both 8 days and 21 days of treatment effectively prevented the development of significant HO. However, there was a slight tendency towards lower efficacy in the group treated for 8 days. There were 2 patients with grade II, and 2 patients with grade III HO in this group, compared to 1 patient only with grade II and none with grade III in the group treated for 21 days. In conclusion, we found several indications that the early period following initiation of a bone inductive stimulus is important for the development of HO. In addition to the effectiveness of short-term treatment with NSAIDs for the inhibition of HO, we also found very little progression of HO after 3 months.

In accordance with our previous findings, HO was more frequent in men. Grade III and IV occurred in 5/21 men, compared to 2/24 women in the placebo group, when evaluated 12 months after THA.

Half of the patients in this study came from an urban population, the other half from a rural district. We found a higher incidence of HO in the rural population. This finding correlates with a higher incidence in the rural population of hypertrophic osteoarthritis, which is a well-known risk factor for HO.

A total of 22 patients were replaced due to adverse effects of the medication or due to non-compliance. The most common adverse effect was nausea or other gastro-intestinal discomfort. None of the patients suffered from serious complications. Gastro-intestinal discomfort was as common in the placebo group as in the groups with active treatment. The substitutes were equally distributed among the three groups. This indicates that most adverse reactions are not due to the drug itself, but to surgery and unspecific effects of the medication.

The total amount of bleeding was not affected, in spite of the fact that treatment was initiated on the morning prior to surgery. This is somewhat surprising, since it is known that NSAIDs affect the aggregation of platelets by inhibiting the synthesis of the PG tromboxane. However, the amount of bleeding varied considerably among patients, and the methods to determine blood loss are somewhat inexact, so that limited effects of NSAIDs might not be detected.

Study V

One major concern when using NSAIDs in a newly operated THA, has been their possible effect on late prosthetic loosening. To address this concern we reviewed all hospital records from study IV and used the Swedish population registry to find out if patients were still alive or deceased. In addition, we sent a questionnaire to all patients still alive 10 years after surgery to obtain more treatment data. Two levels of failure were decided upon prior to the 10-year follow-up:

1. Revision surgery performed with exchange or removal of one or both prosthetic components.
2. Radiographic loosening and patients in level 1.

The reasons for revision surgery in the patients are given in table 3.

Table 3. Revisions at long-term follow-up in patients randomized to placebo or Ibuprofen treatment after THA (V)

Treatment group	*A-septic loosening*	*Peri-prosthetic fracture*	*Dis-location*	*Septic loosening*	*All revisions*
Men					
Placebo	1	0	0	0	1
Ibu 8 days	3	1	1	0	5
Ibu 21 days	5	1	0	0	6
Women					
Placebo	0	0	0	0	0
Ibu 8 days	0	0	0	1	1
Ibu 21 days	0	0	0	0	0
All					
Placebo	1	0	0	0	1
Ibu 8 days	3	1	1	1	6
Ibu 21 days	5	1	0	0	6
N	*9*	*2*	*1*	*1*	*13*

Each group comprised 24 patients at the beginning of the study. Follow-up consisted of a review of all medical records and a questionnaire at a minimum of 8 years after surgery.

We found that 13 patients had undergone revision, 9 patients due to prosthetic loosening (stem and/or cup), 2 patients due to fracture of the femur in the prosthetic region after mild trauma, 1 due to recurrent dislocations and one due to septic loosening. It is notable that all 12 revisions due to aseptic complications occurred in men, 11 belonging to the treatment groups. However, the difference in revisions between patients treated and not treated with Ibuprofen, was not significant. Nevertheless, in spite of the relatively small numbers of patients available for evaluation at follow-up, the statistical analysis gave a p-value of 0.076. Thus, a statistical type II error is possible. The revision rate was not unexpectedly high, 9% of the 144 patients after 10 years.

Thirty-seven patients were deceased at the 10-year follow-up. The remaining 94 patients were sent a letter with a questionnaire, and an offer of a radiographic follow-up. Eighty-four patients accepted (33 men and 51 women). There were 33 patients in the placebo group and 51 in the groups treated with Ibuprofen. Radiographic loosening was found in 9 patients. Four

patients belonged to the placebo group and 5 patients had been treated with Ibuprofen. The 84 patients in the radiographic follow-up are described in table 4.

Table 4: Radiographic 10-year follow-up

Treatment group	*N*	*Loose cup only*	*Loose stem*	*All loosenings (cup and/or stem)*	*No complication*
Men					
Placebo	15	1	2	3	12
Ibu 8 days	9	0	0	0	9
Ibu 21 days	9	0	1	1	8
Women					
Placebo	18	0	1	1	17
Ibu 8 days	17	0	0	0	17
Ibu 21 days	16	2	2	4	12
All					
Placebo	33	1	3	4	29
Ibu 8 days	26	0	0	0	26
Ibu 21 days	25	2	3	5	20

N denotes number of patients who could be analyzed for radiographic loosening after 10 years and who had not been excluded due to revision surgery.

When revisions and radiographic loosening were combined, the difference in prosthetic failure for the combined Ibuprofen groups was not statistically significant, compared to the control group.

Experimental and clinical studies show that the early phase of bone formation is the period essential for inhibition with NSAIDs. This is confirmed by the present studies (III and IV), that show reduction of HO in patients treated for the first 8 days, but not in patients for whom the treatment was delayed. These findings are similar to findings in experimental heterotopic bone formation [73].

Is this early phase also important for the long-term fixation of the prosthesis? On one hand, the total rate of revision was not increased in study V, but on the other hand the findings that 8/9 of the revisions for loosening were in the two treated groups is of concern, even if the number of patients is too small to draw far-reaching conclusions. However, as mentioned above, there is ample theoretical (experimental) evidence that NSAIDs have effects on bone biology, which might be of importance for prosthetic fixation. If these findings can be verified in larger studies they are of major importance

since a great number of patients are treated postoperatively with NSAIDs. However, other authors have not found increased rates of loosening of cemented or uncemented implants, or decreased rates of union of trochanteric osteotomies in patients treated with NSAIDs [44, 57, 74].

In spite of the fact that the follow-up study of THAs treated with NSAIDs for HO, did not show an increased incidence of loosening (study V), theoretically there could be a negative effect of prophylactic treatment with NSAIDs for HO on endoprosthetic fixation. In fact, both cemented and uncemented implants are dependent on new bone formation and apposition for their long-term fixation and survival.

When considering the risk of decreased initial prosthetic fixation after THA in patients treated with NSAIDs, relevant experimental data must also be considered. No doubt NSAIDs have an inhibitory effect on heterotopic bone formation, but studies have also shown effects of NSAIDs on orthotopic bone in animal experiments. Delayed fracture healing and a slower regain of torsion strength after weakening of bone by drilling have been reported [75-77]. Also, in-growth of bone into a porous implant is delayed by treatment with NSAIDs [56]. In humans these effects of NSAIDs have not been investigated, but it has been shown that mechanical load on human osteoblast-like cells *in vitro* gives a response of enhanced production of PGE2 [78]. Interestingly, this response was found in cell strains from only some of the individuals.

In conclusion, several large prospective and randomized studies have been performed with different NSAIDs to prevent HO and it is my opinion that these studies should now be evaluated in a similar manner to define possible long-term effects of NSAIDs on implant fixation.

Experimental study (study VI)

One way to address the etiology and treatment of heterotopic ossification is to use a relevant experimental model that allows specific morphological and biochemical analysis of bone formation. However there is no such model that closely resembles HO after THA in man. The fact that the etiology of HO is only partly known adds to the difficulties in the development of a relevant experimental model. However, HO can be induced in rodents in several ways. The most well-defined model is based on the application of an inductive bone-matrix. Marshall Urist first described this model in 1965, and defined the process leading to bone-formation in detail [66]. In brief, this experimental model entails demineralization of cortical bone, followed by its implantation in muscle. Osteoinductive factors, such as bone morphogenetic proteins (BMPs) from within the bone matrix act on the surrounding

immature mesenchymal cells, causing chemotaxis, proliferation and differentiation into cartilage and bone. Within two to three weeks a small ossicle develops. This type of heterotopic bone formation has several similarities to HO after THA: The sequence of events leading to bone formation is similar. The bone formed is metabolically highly active and contains bone marrow. The bone-forming cells appear to be local mesenchymal cells which, by the osteo-inductive stimulus (BMP), are induced to follow a bone-forming pathway. Trauma to the soft tissues also appears to be necessary for bone formation to occur. However, there is at least one major difference in that the inductive stimulus in the experimental model is caused by paracrine factors (BMPs) while the inductive stimulus in HO after THA is not known.

Experimental induction of heterotopic new bone with demineralized allogeneic bone matrix (DABM) has previously been used to study the effects of NSAIDs on the inductive process. A low grade, but statistically significant inhibition of new bone formation occurs in rats treated with Indomethacin or Ibuprofen. Similarly to the clinical situation it was the early phase of the inductive process that was sensitive to inhibition. Once bone formation was established, treatment could not cause inhibition [70, 73]. We found that Indomethacin also inhibited new bone formation and bone induction following fracture of the femurs after harvesting the autografts. In both these instances the rate of new bone formation was high, compared to the activity in orthotopic, non-traumatized bone.

Our clinical findings of a much more pronounced inhibition of HO after THA led us to investigate the effects of Indomethacin on autografts implanted in a heterotopic site. This experimental model of heterotopic autografts mimics the clinical situation, since one possible cause of heterotopic ossification after THA is that particles are released by the reaming of the acetabulum or the femoral canal. These particles contain agents that may act as inducers to the surrounding soft tissues or to immature mesenchymal cells within the tissue. If the inductive effect of an autograft (which is fully mineralized and probably less inductive) initiates a bone-forming process, it might be sensitive to treatment with NSAIDs.

It turned out that the autografts exhibited a low-grade bone formation at their heterotopic sites, and that the mineral accretion was not affected by the treatment.

In addition, resorption was analyzed in the established model of inducing heterotopic bone formation in rats by implanting DABM into abdominal muscle. New bone formation was accompanied by a decrease in isotope activity of pre-labeled collagen, reflecting resorption. Treatment with Indomethacin reduced new bone formation by 20% and also inhibited the release of isotope pre-labeled collagen to a similar extent. This finding

indicates that it is the early phase of bone induction and resorption of the graft that is influenced by Indomethacin [79].

In another experiment, we studied whether the size of the implant giving rise to the inductive stimulus had an effect on bone formation. After 3 weeks there was no difference in the amount of bone formed per mg of implanted DABM. Thus, bone induction does not depend on the size of the implant. We also found that considerably less bone was formed in the older SD rats, emphasizing the importance of the receptor site in the process inducing bone formation.

It can be concluded that Indomethacin reduces formation of new bone early in a reparative process, when the rate of bone formation is elevated, while bone turnover in steady-state conditions is not affected. These findings are in agreement with the findings of Keller et al. who noted an inhibition by Indomethacin of the early metabolic response after fracture of the tibia, resulting in retarded bone healing [80].

General discussion

Heterotopic ossification after THA

Patient-related risk factors

DeLee, Ferrari and Charnley described the development of HO after previous THA surgery, to be a risk factor concerning HO at the next THA [26]. Several other authors have confirmed this to be one of the most important risk factors for developing HO. We wanted to specifically address this by analyzing bilaterally operated patients. By using this approach we could exclude patient-related differences, except age, since the patients were not operated on both hips at the same time. The mean time elapsed between operations was 1.4 years. Most treatment-related differences were also excluded, as all patients were operated at the same clinical center, all the THAs were cemented and a similar operating technique and postoperative regime were used. In study I, in which patients did not receive prophylactic treatment for HO, there was a very strong correlation between HO on the two sides, only 2 of 56 patients differed more than one grade on the Brooker scale. Figure 2 shows the grades of HO after bilateral THA in these 56 men and women, when no treatment for HO was given after either operation.

The very significant importance of the individual disposition to form bone was evidenced by these results. However, we do not know what causes the individual propensity to react with bone formation to trauma, but it is well known that genetic factors determine many aspects of bone metabolism and bone repair. In the patients who develop HO, the immature, soft tissue mesenchymal cells are more prone to become active in the process leading to the development of new bone [1, 24].

The incidence of HO is higher in men than in women. In our material (studies I – IV), there were 229 THAs (124 male, 105 female), not prophylactically treated for HO. Severe (Grade III-IV) HO occurred in 36 of the male (29%) but only in 11 of the female THAs (10%). Thus, men form HO more frequently than women and they also suffer from the clinical

consequences much more often [1, 26, 81]. The explanation for this is unknown but hormonal and genetic factors are probably of importance.

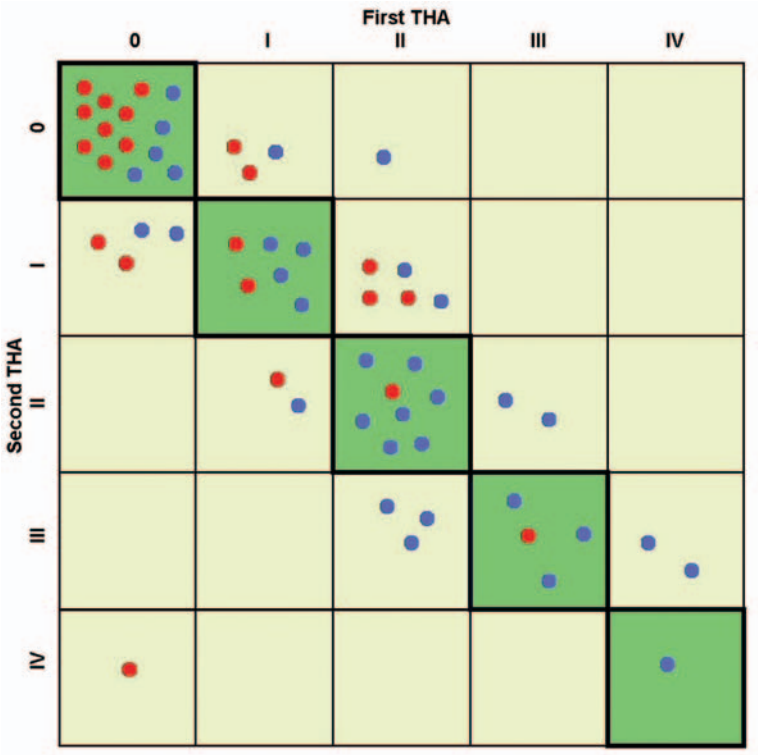

Figure 2 Grades of HO after bilateral THA, where no prophylactic treatment for HO was given after either operation. The figure shows a good correlation of grade of HO between the two THAs. It also shows that women (red dots) are less prone to develop HO than men (blue dots).

The incidence of HO after THA varies within a wide range, as reported in different studies. NSAIDs are potent analgesics and widely used for this purpose. Since treatment with NSAIDs at the appropriate time in connection with the THA is an efficient inhibitor of HO, the use of these drugs must be noted when the incidence of HO is calculated. In our own material (594 THAs), HO of any grade (I-IV), occurred in only 76/365 = 21% of the treated hips, while 140/229 = 61% showed HO in the untreated hips. For HO grades III –IV, the figures become even more striking (2/365 = <1% and 47/229 = 21%, respectively).

Furthermore, we found that increasing age also reduces the risk of HO, in both men and women (Study I). This is more pronounced in women than in

men. Figure 3 show this phenomenon in women with 105 untreated THAs from studies I to V.

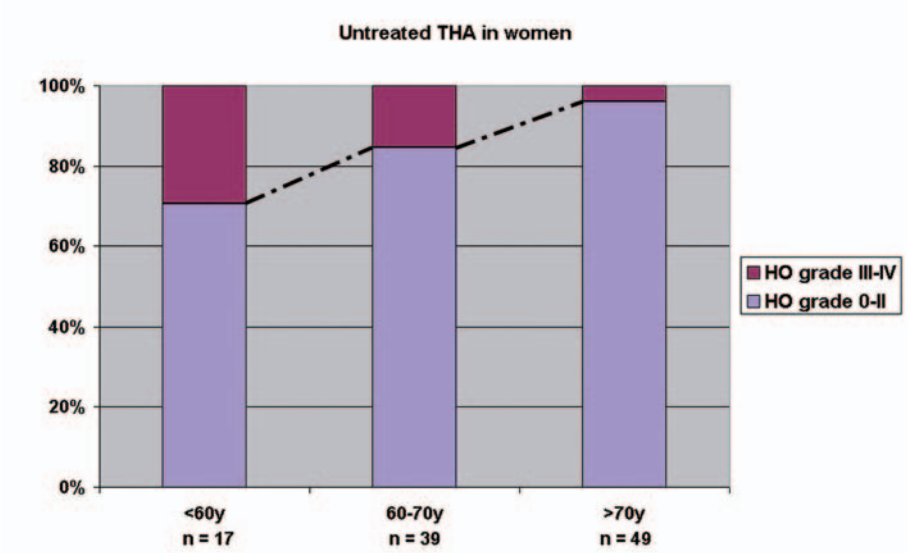

Figure 3 Incidence of HO for 105 THAs in women in relation to age. No prophylactic treatment given.

One possible explanation for this, is less responsiveness in the stem cells in elderly people, or a less reactive/inductive local environment. Another explanation can be that older people have been found to have less capacity to induce bone, probably due to lower amounts of BMP [82]. The finding of less bone induction in older rats is also in agreement with the findings of less HO in the elderly.

Patients that have developed high grade and clinically significant HO after THA are high-risk patients for developing HO. Therefore they are very likely to develop high grade HO once again after a second THA or after an operation to excise the HO, if no prophylactic treatment is given. Figure 4 shows an example where prophylactic treatment with a NSAID after the second THA was successful. Thus, it may be concluded that a genetically determined bone-inductive reactivity is the most important risk factor for HO.

These patient-related factors all indicate that the risk of developing HO after THA is related to bone metabolism in general. This is further supported by the fact that patients with HO have greater bone density of the lumbar spine, and that hypertrophic osteoarthritis, anchylosing spondylitis and

diffuse idiopathic skeletal hyperostosis (DISH) are known to increase the risk of HO [24, 25, 34].

Thus, when the incidence of HO after THA is to be evaluated, several parameters must be considered, e.g. diagnosis, gender, medication, irradiation and probably also surgical technique.

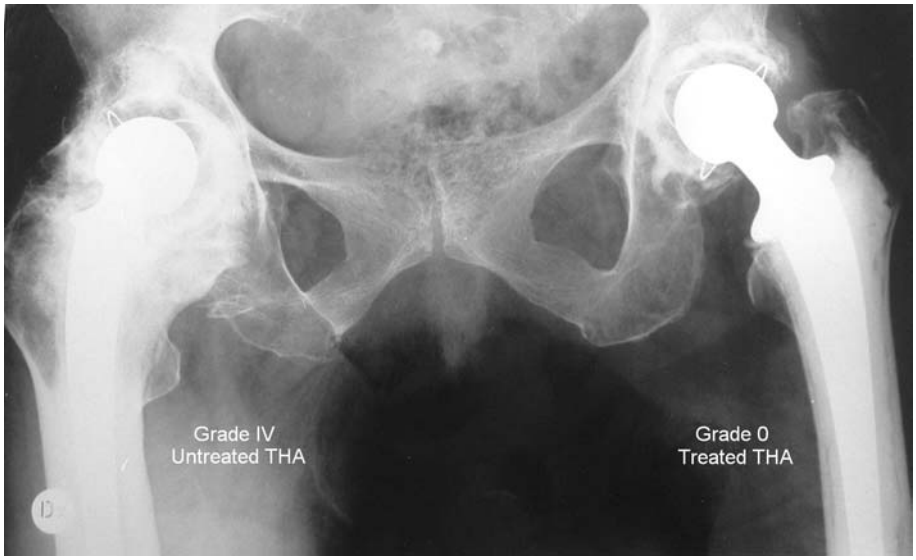

Figure 4 Radiograph of a man who developed very pronounced HO after THA on the right side. No prophylactic treatment with NSAIDs was given. After THA on the left side, the patient was treated with Indomethacin 50 mg twice daily for 3 weeks.

Cellular and biochemical events in the development of HO

The cellular mechanism that causes HO after joint replacement is not known, but trauma to the soft tissues and bone is one eliciting factor. Nevertheless, a fairly standardized trauma such as THA results in a spectrum of responses ranging from no bone induction to anchylosis of the joint. Thus, in addition to trauma, a disposition for bone formation is necessary for HO to occur.

The clinical symptoms in severe HO often develop early with radiographic signs of calcification in the gluteus medius muscle region within 3-4 weeks of surgery. Some progression of HO then occurs, with maturation of the bone between 3 and 6 months. After 6 months HO rarely increases in amount but some further maturation occurs [26, 27]. Morphologic and biochemical analysis of the heterotopic bone has shown an intense turnover and a high content of growth factors indicating a metabolically

active tissue [17, 34]. Interestingly, the HO does not decrease in mass with time, in spite of the fact that the bone is not exposed to weight bearing.

The early inflammatory reaction following the surgical trauma of THA appears to be essential for the induction of HO. In hip arthroplasties, the reaming often results in the spread of trabecular bone and bone marrow into the muscles. Surgery thereby creates direct access for osteogenically competent cells to well-vascularized soft tissue, while at the same time osteoinductive factors and growth factors are released from the traumatized tissues, especially from the bone, and may further induce mesenchymal, immature cells to develop into osteoblasts.

The surgical trauma probably also causes favorable conditions for bone induction by providing an appropriate set of local, paracrine factors such as various cytokines and PGs. Osteoinductive growth factors such as the bone morphogenetic proteins (BMPs), transforming growth factor beta (TGF-β) and insulin-like growth factors (IGF-I, IGF-II) are abundant in bone and they are probably released during surgery[67, 83]. These cytokines probably play different roles in new bone formation. TGF-β seems to stimulate cell proliferation, while BMPs stimulate the differentiation of immature mesenchymal cells into osteoblasts [83]. Thus, pluripotential mesenchymal stem cells are abundant in the soft tissues surrounding the hip joint, and these cells are mobilized and transformed into osteogenic stem cells by the release of inducing agents, caused by the surgical trauma [1].

One way to study the key events in the formation of HO is by analyzing the effects of known treatments that inhibit HO. The efficacy of pre-operative irradiation suggests that osteogenic precursor cells residing in the local tissues are important for the formation of new bone at the heterotopic site [84]. This finding is in agreement with the conclusions concerning the risk factors; i.e. different responsiveness of the mesenchymal cells in different individuals is decisive for the development of HO.

NSAIDs and HO

Prophylaxis with NSAIDs

NSAIDs given during the first postoperative weeks have been shown to be potent inhibitors of HO after total hip arthroplasty. They are also effective in preventing the recurrence of HO after excision of heterotopic bone that developed after previous hip surgery [39, 44, 45, 52, 53, 85, 86]. A number of prospective and randomized studies have confirmed the efficacy of NSAIDs in preventing the significant grades of HO both in cemented and uncemented

THA and in patients at risk [39, 43, 46, 49, 54, 55]. I have found only one study on a relatively small number of patients (47), that could not confirm this effect [87].

Interestingly, in rats, autografts induced a very limited bone-forming response in the heterotopic site, and no significant effect on bone formation or graft resorption by NSAID treatment could be discovered. In contrast, DABMs and femur fractures induced a substantial bone-forming response, where NSAID treatment created an early inhibitory effect.

The mechanism of the action of NSAIDs

NSAIDs may affect one or several of the key steps in the process of bone formation. However, NSAIDs probably act mainly through their well-known inhibitory effects on prostaglandin synthesis.

Cyclooxygenase type 1 and 2 (COX-1, COX-2) are enzymes that are active in the synthesis of Prostaglandin G2 (PGG2) from arachidonic acid in various tissues in the body. COX-1 is responsible for the synthesis of constitutional (protecting) levels of PGs, and COX-2 is up-regulated when some extraordinary condition affects the cellular tissue. COX-1 and COX-2 also enhance the synthesis of PGH2 from PGG2. The prostaglandins abundant in bone (PGE1, PGE2 and PGF) are synthesized from PGH2. PGE1 and PGE2 are known to be potent stimulators of bone formation. Production of PGs is affected both by cytokines which stimulate bone resorption, such as some of the interleukins and tumor necrosis factor alpha (TNF-α), but also by cytokines which stimulate bone formation, such as TGF-β, BMPs, insulin-like growth factors (IGFs), platelet derived growth factors (PDGFs) and vascular endothelial growth factor (VEGF). All these cytokines enhance PGE production by up-regulating COX-2 production [83].

In conclusion, there is strong evidence that prostaglandins have important roles in bone regeneration and repair. Thus, it is not surprising that NSAIDs have effects on these processes. Theoretically, these effects may be mediated through different mechanisms; Prostaglandins may alter the local environment by enhancing the inflammatory response to surgery and thereby increase the release of the different cytokines necessary for the mobilization and differentiation of mesenchymal cells. By inhibiting PG synthesis, NSAIDs affect the local environment and inhibit the inflammatory response to surgery. Prostaglandins (and NSAIDs) may also exert their effects by a direct action on the bone-forming or the bone-resorbing cells, and/or on the differentiation of mesenchymal cells into osteogenic tissue. Furthermore, the effects may also be indirect through action on processes such as angiogenesis.

Duration and timing of treatment with NSAIDs

Since NSAIDs are potent inhibitors of bone formation, their possible influence on prosthetic fixation has been of some concern. For that reason, we, as also other investigators, have tried to shorten the treatment time as much as possible. We found treatment with Ibuprofen for 8 days to be sufficient to prevent HO after THA (study V). In other studies, 5 to 10 days of treatment with Indomethacin was sufficient to prevent HO [43, 54, 88, 89]. Prophylactic treatment with Tenoxicam or Ketorolac for the first 5 postoperative days resulted in reduced rates of HO [48, 49], while 8 days of treatment with Indomethacin was slightly more effective than 4 days of treatment [40]. Thus, the minimum duration of treatment is now established to be 5 – 10 days. There are many indications that the very early postoperative period is of crucial importance, since NSAIDs do not prevent HO if treatment is shorter than 5 days or delayed for more than 5 days, [53, 90]. This also applies in experimental induction of HO [73].

Risks with NSAID treatment

NSAIDs are effective analgesics and are widely used for this purpose. Since they act by inhibiting the synthesis of prostaglandins in various tissues, especially the NSAIDs that act as unselective COX inhibitors have many adverse effects. Tromboxane (PGB2) is a PG that is essential for the aggregation of blood platelets. Therefore bleeding-time is somewhat increased in patients using unselective NSAIDs. PGE2 is another PG, which is important for the protection of the gastric mucosa against acid gastric juice. Indigestion and development of gastric ulcers are common complications in the use of NSAIDs. Allergic reactions to NSAIDs can also occur. Thus, there are many contra-indications to the use of NSAIDs, and these must always be considered.

When NSAIDs are used for the prophylactic treatment for HO, it is important not to treat patients with a history of gastric ulcer, with asthmatic disease or with known allergy to acetylsalicylic acid or NSAIDs. In our prospective and randomized study, we compared per- and postoperative bleeding in patients treated with Ibuprofen to patients treated with placebo. We could not find significant differences. However, in clinical practice NSAIDs are often discontinued 1 week prior to arthroplastic surgery in order to diminish the risk of excessive bleeding.

In the same study medication was discontinued due to adverse effects in 22 patients. In most cases the reason was nausea or gastro-intestinal discomfort as could be expected since this is by far the most common adverse effect of medication with NSAIDs. However, it turned out that there were the same numbers of patients with adverse effects in the groups treated

with NSAIDs as in the placebo group. Lately it has been shown that Ibuprofen in low doses induces less risk of gastric bleeding than, for example, Indomethacin [72]. In one study Indomethacin was not tolerated by 1/3 of the treated patients [91].

NSAIDs to prevent recurrence of HO

During the period 1980-1986, in the Department of Orthopedics in Kristianstad, ten patients, who were reoperated for prosthetic loosening, had also developed high grade HO (3 grade III, 7 grade IV) after the first operation. In the reoperation we also excised the heterotopic bone, and the patients were treated postoperatively with NSAIDs for 2 – 3 weeks. In one of the patients the treatment had to be withdrawn after one week, due to nausea and gastrointestinal discomfort. No other complications were noted. Range of motion (only flexion) was measured, and AP radiographs were taken before surgery and at the follow-up examination 2 – 5 years after the reoperation. Only one patient developed grade II HO, the remaining 9 patients did not develop significant HO. Seven patients had an increased range of motion at the time of follow-up, compared to the preoperative measurement [45].

When high grade HO occurs and gives the patient problems with impaired gait and discomfort when sitting, or pain, operation for the excision of HO may be indicated. Since these patients are at high risk of experiencing recurrence of HO, prophylactic treatment is necessary, as otherwise HO will invariably reoccur. Even for this indication NSAIDs give a prophylactic effect, a fact that has also been shown by others [52, 86].

Influence of NSAIDs on prosthetic fixation

The most serious concern regarding medication with NSAIDs comes from the experimental findings of an inhibitory effect on bone remodeling after trauma [75, 92, 93], reduced bone ingrowth in porous implants [56, 94] and inhibition of formation of new bone in response to bone induction [73, 95]. These results have raised the question as to whether the medication might cause an increase in mechanical loosening of the prosthesis due to a decreased initial prosthetic fixation. However, clinical evidence of increased aseptic loosening, such as increased radiolucencies [57], increased rates of revision [53, 96], and increased rates of non-union of trochanteric osteotomies in cemented or uncemented THAs has not been presented to date [44].

In study V we performed a 10-year follow-up on the patients from study IV. In spite of the high proportion of revised THA in the two Ibuprofen treatment groups (11/12) we could not find statistical evidence for an

increased revision rate or increased incidence of prosthetic loosening. Although long observation periods and large number of patients might be necessary to detect small effects on prosthetic fixation, it is concluded that at present there is no definite evidence that medication with NSAIDs causes aseptic loosening of the prosthesis.

In contrast, NSAIDs may be beneficial in slowing down the process of osteolysis once the prosthesis is loose. Some experimental studies have examined macrophage activity in the pseudomembrane between the prosthetic material (cement) and bone, due to different forms of debris from the process of loosening. They found an enhanced expression of COX, PGE2 and nitric oxide together with certain interleucines produced by the macrophages. This accelerated the osteoclast activity, and some NSAIDs seemed to be able to slow down the process of osteolysis [97, 98].

Based on both relevant experimental data and the pronounced effects on HO, we propose that NSAIDs should be used with caution in all orthopedic procedures requiring bone regeneration, including THAs. Also, it is necessary to perform long-term follow-up investigations on the frequency of revisions and loosening of THAs, especially in the many randomized clinical trials that have been performed during the recent decades.

Conclusions

- The most important risk factor for the development of HO after THA is the individual's propensity to react to an osteoinductive stimulus; patients who develop HO after one THA almost invariably develop the same amount of HO after a subsequent THA, provided no specific treatment is given (I).
- Men are more prone to develop HO after THA than women (I-IV).
- The risk of developing high-grade HO decreases with age in women (II).
- Postoperative medication with the NSAIDs Indomethacin or Ibuprofen is effective as prophylactic treatment for HO after THA (II-IV).
- To be efficient, treatment with Indomethacin and Ibuprofen must commence immediately after the THA operation (III).
- The duration of prophylactic treatment with Ibuprofen for HO can be reduced to 8 days, with the inhibitory effect on clinically relevant HO maintained (IV).
- Treatment with Indomethacin and Ibuprofen results in few adverse reactions (II-IV).
- Prophylactic treatment with NSAIDs has no definite effect on the fixation of the prosthesis, but this matter should be further investigated (V).
- The amount and grade of HO does not change within an observation time of 10 years (V).
- Under experimental conditions, Indomethacin inhibits the early phase of bone induction and fracture healing, but not bone formation or resorption in autografts (VI).
- Bone induction by DABM implants is directly correlated to the size of the implant (VI).

Sammanfattning på svenska

Bakgrund

Vår kropp har förmåga att bilda benvävnad även utanför skelettet. Ben som bildas i mjuk vävnad kallas för heterotopt ben (HO). När HO bildas, är en vävnadsskada (trauma) oftast nödvändig. Traumat startar ett komplext händelseförlopp så att bildning av HO kan initieras. Vissa kroppsregioner har större benägenhet att bilda HO än andra. Höftregionen är en av dessa.

När man opererar patienter med total höftledsplastik (THA) finns risk att HO bildas kring den nya höftleden. I vissa fall blir HO så utbredd att leden blir stel. Höftledsförslitning är ofta dubbelsidig, varför många patienter opereras på båda sidor. Om patienten får utbredd HO efter båda operationerna, kan resultatet bli dåligt.

1974 presenterades rön som visade att HO minskade om man medicinerade patienterna med icke-steroid anti-inflammatorisk medicin (NSAIDs). Eftersom vi hade noterat förekomst av HO och eftersom patienter med utbredd HO ofta inte var helt nöjda med sina nya höftleder, väcktes vårt intresse att studera och försöka förhindra HO.

Cyklooxygenas (COX) är ett enzym som behövs för att celler ska kunna bilda prostaglandiner (PG). PG utgör en familj av substanser som är viktiga i samband med inflammatoriska och vävnadsskyddande processer. PG har stor betydelse när nytt ben ska bildas. NSAIDs hämmar COX och därmed bildningen av PG, varvid det inflammatoriska svaret efter trauma och därmed bildandet av HO minskar.

Studierna I till IV i denna avhandling, undersöker hur ofta HO utvecklas efter THA, vilka patienter som har störst risk att utveckla HO samt när och hur länge man behöver behandla med NSAIDs för att förhindra HO.

Studie V är en 10-års uppföljning av studie IV och undersöker om behandling med NSAIDs påverkar beninfästningen av höftproteserna negativt, dvs. ger upphov till en ökad lossningsfrekvens.

Studie VI är en experimentell studie på råttor, som studerar bildandet av experimentell HO med hjälp av två olika slag av framkallande stimuli (implantat), och hur NSAIDs påverkar bildandet av nytt ben.

Resultat

I studie I och II studerades patienter som hade opererats med THA på båda sidor. Vi kunde visa en stark korrelation mellan de båda sidornas utbredning av HO, vilket talar för en genetisk benägenhet att bilda HO. Risken att bilda HO visade sig vara högre hos män än hos kvinnor. Patientens ålder hade också betydelse, med lägre risk för HO i högre åldrar.

Behandling med Indomethacin eller Ibuprofen, två olika NSAIDs, förhindrade utveckling av HO efter THA. Även patienter som bildat hög grad av HO i den höftled som opererades utan förebyggande medicinering, hade god effekt av behandling med NSAIDs vid nästa THA.

I studie III undersöktes 200 THA som opererats efter varandra och som medicinerats i 3 veckor med Indomethacin eller Ibuprofen. Tre veckors behandling visade sig vara effektiv mot HO utan att ge svåra eller frekventa biverkningar. I de fall (34 av 200) där behandlingen ej kunde genomföras förekom patienter med hög grad av HO. Undersökningen gav indikationer att behandlingen måste påbörjas i anslutning till operationen för att vara effektiv, men att behandlingstiden sannolikt ytterligare kunde förkortas.

Studie IV var en randomiserad studie för att utröna om behandlingstiden kunde kortas ner. Studien var placebokontrollerad, dvs. en patientgrupp fick overksam medicin. Varken patienten eller undersökaren visste huruvida patientens medicin var verksam eller ej (dubbel-blind studie). Studien omfattade 144 patienter. Patienterna behandlades med Ibuprofen i 1 alternativt 3 veckor, eller med placebo. Efter 3 och 12 månader förekom HO i mindre grad och mera sällan i Ibuprofenbehandlade grupper jämfört med i placebogruppen. Det fanns ingen skillnad avseende HO mellan patienter behandlade 1 eller 3 veckor varför behandling med Ibuprofen under 1 vecka visade sig vara tillräcklig för att förebygga HO. Allvarliga korttidsbiverkningar av medicineringen kunde inte observeras.

Eftersom Ibuprofen bl.a. har benbildningshämmande egenskaper, skulle dess användning som profylax mot HO teoretiskt kunna ge sent uppträdande, negativa effekter på höftprotesens fixering till skelettet. Studie V är en uppföljande studie av överlevande patienter från studie IV. Vi undersökte om skillnader förelåg mellan patienter behandlade med Ibuprofen jämfört med de som hade fått placebo, 10 år efter THA. Statistiskt signifikant skillnad avseende proteslossning kunde inte verifieras, men 11 av 13 omoperationer p.g.a. lossning skedde i de två Ibuprofenbehandlade grupperna.

Studie VI omfattar två experiment på råttor, där vi i experiment 1 studerade Indomethacins effekt på benbildning som stimulerats av såväl autologa (från samma individ) som demineraliserade allogena (från annan individ av samma ras) benimplantat. Implantaten opererades in i fickor i

råttornas bukmuskulatur. Råttor har därvid en känd förmåga att utveckla HO. Djuren injicerades med Indomethacin alternativt fysiologisk koksaltlösning dagligen under 3 eller 6 veckor. Ett dygn före avlivning injicerades radioaktivt kalcium (^{45}Ca). Kalkinnehåll och radioaktivitet analyserades.

Demineraliserade alloimplantat uppvisade snabb nybildning av benvävnad. Indomethacin reducerade bennybildningen med 30%, vilket var statistiskt signifikant. De autologa implantaten visade däremot förlust av mineralinnehåll, men vare sig omfattningen av benmineralförlusten eller den ^{45}Ca specifika aktiviteten (bennybildningstakten) påverkades av Indomethacin. Icke traumatiserat skelettben var opåverkat av Indomethacin, medan traumatiserat lårben (tagställe) uppvisade mindre benläkningstakt vid 3 veckor hos de Indomethacinbehandlade råttorna. Vid 6 veckor hade benläkningstakten återhämtat sig trots Indomethacinbehandling.

I experiment 2 studerades huruvida storleken av allogena transplantat eller åldern hos råtta hade betydelse för benbildningstakten. Unga och äldre råttor fick inopererat demineraliserade allogena benimplantat av 6 olika storlekar. Djuren injicerades med ^{45}Ca och avlivades efter 3 veckor.

Mängden nybildat ben korrelerade lineärt till storleken av implantatet. Yngre råttor bildade förhållandevis större mängd ben och benbildningstakten var dubbelt så stor jämfört med de äldre råttorna.

Slutsatser

- Män och individer som tidigare har visat sig bilda HO, har störst risk att ånyo bilda HO efter THA. Äldre kvinnor har minskad risk att bilda HO.
- Medicinering med Indomethacin eller Ibuprofen förhindrar utveckling av HO efter THA. Behandlingen måste starta direkt efter operationen.
- Åtta dagars behandling med Ibuprofen räcker till för att förhindra HO.
- Profylax mot HO med Ibuprofen hade ingen säkerställd negativ effekt på protesfixationen efter THA, men saken bör undersökas ytterligare.
- Under djurexperimentella förhållanden hämmar Indomethacin tidig fas av frakturläkning och demineraliserade alloimplantats förmåga att framkalla ben, men inte resorption eller benbildning förorsakad av autoimplantat.
- Demineraliserade alloimplantats förmåga att framkalla ben är direkt relaterad till implantatens storlek.

Acknowledgements

I would like to thank all those people who have made the completion of this thesis possible. I especially express my gratitude to:

Olle Nilsson, my tutor, supervisor and great friend, for great support and for never, never giving up. Thanks for pushing me all the way.

Bent Sodemann, my mentor and co-author, for teaching me an imaginative and critical way of thinking. Luckily, someone did not have to eat his hat.

Elli Nilsson, for her great hospitality whenever I visited Elli's and Olle's home. I surely did not loose weight in Uppsala.

Ann-Marie Berggren, an experienced radiologist, for skillful investigation of all those radiographs, looking for zones and bones.

Lars Berglund, for his help with the more difficult statistics in paper V.

Gregor Sisask, for support and help with operating and medicating rats.

The staff at the policlinic of Orthopedics in Kristianstad, for helping me to order and to carry radiographs and patients records.

My colleague orthopedists in Uppsala, Kristianstad and Hässleholm, for kindness and for cheering on.

My children, Karin, Jonas and **Erik** for love and support and for just existing.

Anna-Maria, my wife for putting up with me all these years, even if life has been tough at times. I love you.

References

1. Jowsey J, Coventry MB, Robins PR. Heterotopic ossification: Theoretical conciderations, possible etiologic factors and a clinical review of total hip arthroplasty patients exhibiting this phenomenon. *The Proceedings of the Hip Society*: C.V. Mosby, U.S.A.; 1977:210-221.
2. Kjaersgaard-Andersen P, Sletgard J, Gjerloff C, Lund F. Heterotopic bone formation after noncemented total hip arthroplasty. Location of ectopic bone and the influence of postoperative antiinflammatory treatment. *Clin Orthop.* Mar 1990(252):156-162.
3. Kaempffe FA, Bone LB, Border JR. Open reduction and internal fixation of acetabular fractures: heterotopic ossification and other complications of treatment. *J Orthop Trauma.* 1991;5(4):439-445.
4. Ghalambor N, Matta JM, Bernstein L. Heterotopic ossification following operative treatment of acetabular fracture. An analysis of risk factors. *Clin Orthop.* Aug 1994(305):96-105.
5. Marks PH, Paley D, Kellam JF. Heterotopic ossification around the hip with intramedullary nailing of the femur. *J Trauma.* Aug 1988;28(8):1207-1213.
6. Harwin SF, Stein AJ, Stern RE, Kulick RG. Heterotopic ossification following primary total knee arthroplasty. *J Arthroplasty.* Apr 1993;8(2):113-116.
7. Hasegawa M, Ohashi T, Uchida A. Heterotopic ossification around distal femur after total knee arthroplasty. *Arch Orthop Trauma Surg.* Jun 2002;122(5):274-278.
8. Rader CP, Barthel T, Haase M, Scheidler M, Eulert J. Heterotopic ossification after total knee arthroplasty. 54/615 cases after 1-6 years' follow-up. *Acta Orthop Scand.* Feb 1997;68(1):46-50.
9. Sperling JW, Cofield RH, Rowland CM. Heterotopic ossification after total shoulder arthroplasty. *J Arthroplasty.* Feb 2000;15(2):179-182.
10. Kjaersgaard-Andersen P, Frich LH, Sojbjerg JO, Sneppen O. Heterotopic bone formation following total shoulder arthroplasty. *J Arthroplasty.* 1989;4(2):99-104.
11. Kozak TK, Adams RA, Morrey BF. Total elbow arthroplasty in primary osteoarthritis of the elbow. *J Arthroplasty.* Oct 1998;13(7):837-842.
12. Holguin PH, Rico AA, Garcia JP, Del Rio JL. Elbow anchylosis due to postburn heterotopic ossification. *J Burn Care Rehabil.* Mar-Apr 1996;17(2):150-154.
13. Ring D, Jupiter JB. Operative release of complete ankylosis of the elbow due to heterotopic bone in patients without severe injury of the central nervous system. *J Bone Joint Surg Am.* May 2003;85-A(5):849-857.
14. Nalley J, Jay MS, Durant RH. Myositis ossificans in an adolescent following sports injury. *J Adolesc Health Care.* Nov 1985;6(6):460-462.
15. Evans EB. Heterotopic bone formation in thermal burns. *Clin Orthop.* Feb 1991(263):94-101.

16. Bosse A. [Clinical aspects, differential diagnosis and histogenesis of heterotopic ossification]. *Veroff Pathol.* 1997;146:1-168.
17. Bosse A, Wanner KF, Weber A, Muller KM. Morphological and clinical aspects of heterotopic ossification in sports. A case study. *Int J Sports Med.* Aug 1994;15(6):325-329.
18. Huggins CB. The formation of bone under the influence of epithelium of the urinary tract. *Clin Orthop.* Jul-Aug 1968;59:7-19.
19. Mital MA, Garber JE, Stinson JT. Ectopic bone formation in children and adolescents with head injuries: its management. *J Pediatr Orthop.* Jan-Feb 1987;7(1):83-90.
20. Bjurholm A, Kreicbergs A, Terenius L, Goldstein M, Schultzberg M. Neuropeptide Y-, tyrosine hydroxylase- and vasoactive intestinal polypeptide-immunoreactive nerves in bone and surrounding tissues. *J Auton Nerv Syst.* Dec 1988;25(2-3):119-125.
21. Olmsted EA, Kaplan FS, Shore EM. Bone morphogenetic protein-4 regulation in fibrodysplasia ossificans progressiva. *Clin Orthop.* Mar 2003(408):331-343.
22. Charnley J. Total hip replacement by low-friction arthroplasty. *Clin Orthop.* Sep-Oct 1970;72:7-21.
23. Charnley J. The long-term results of low-friction arthroplasty of the hip performed as a primary intervention. *J Bone Joint Surg Br.* Feb 1972;54(1):61-76.
24. Ritter MA, Vaughan RB. Ectopic ossification after total hip arthroplasty. Predisposing factors, frequency, and effect on results. *J Bone Joint Surg Am.* Apr 1977;59(3):345-351.
25. Brooker AF, Bowerman JW, Robinson RA, Riley LH, Jr. Ectopic ossification following total hip replacement. Incidence and a method of classification. *J Bone Joint Surg (Am).* Dec 1973;55:1629-1632.
26. DeLee J, Ferrari A, Charnley J. Ectopic bone formation following low friction arthroplasty of the hip. *Clin Orthop.* Nov-Dec 1976(121):53-59.
27. Sodemann B, Persson PE, Nilsson OS. Periarticular heterotopic ossification after total hip arthroplasty for primary coxarthrosis. *Clin Orthop.* Dec 1988(237):150-157.
28. Morrey BF, Adams RA, Cabanela ME. Comparison of heterotopic bone after anterolateral, transtrochanteric, and posterior approaches for total hip arthroplasty. *Clin Orthop.* Sep 1984(188):160-167.
29. Nollen AJ, Slooff TJ. Para-articular ossifications after total hip replacement. *Acta Orthop Scand.* 1973;44(2):230-241.
30. Ornsholt J, Espersen JO. Para-articular ossifications after primary prosthetic replacement ad modum Austin T. Moore. *Acta Orthop Scand.* Sep 1975;46(4):643-650.
31. Jensen LL, Halar E, Little JW, Brooke MM. Neurogenic heterotopic ossification. *Am J Phys Med.* Dec 1987;66(6):351-363.
32. Ahrengart L, Lindgren U. Heterotopic bone after hip arthroplasty. Defining the patient at risk. *Clin Orthop.* Aug 1993(293):153-159.
33. Holz U, Kraner F, Weller S. [Periarticular ossification following replacement of the hip-joint. Clinical and radiologic examination of 697 hip-joints (author's transl)]. *Z Orthop Ihre Grenzgeb.* Apr 1977;115(2):146-158.
34. Puzas JE, Miller MD, Rosier RN. Pathologic bone formation. *Clin Orthop.* Aug 1989(245):269-281.

35. Nollen JG, van Douveren FQ. Ectopic ossification in hip arthroplasty. A retrospective study of predisposing factors in 637 cases. *Acta Orthop Scand.* Apr 1993;64(2):185-187.
36. Ahrengart L. Periarticular heterotopic ossification after total hip arthroplasty. Risk factors and consequences. *Clin Orthop.* Feb 1991(263):49-58.
37. Arcq M. [Ectopic ossification: a complication after total hip replacement (author's transl)]. *Arch Orthop Unfallchir.* Nov 13 1973;77(2):108-131.
38. Schmidt J, Hackenbroch MH. A new classification for heterotopic ossifications in total hip arthroplasty considering the surgical approach. *Arch Orthop Trauma Surg.* 1996;115(6):339-343.
39. Schmidt SA, Kjaersgaard-Andersen P, Pedersen NW, Kristensen SS, Pedersen P, Nielsen JB. The use of indomethacin to prevent the formation of heterotopic bone after total hip replacement. A randomized, double-blind clinical trial. *J Bone Joint Surg Am.* Jul 1988;70(6):834-838.
40. Dorn U, Grethen C, Effenberger H, Berka H, Ramsauer T, Drekonja T. Indomethacin for prevention of heterotopic ossification after hip arthroplasty. A randomized comparison between 4 and 8 days of treatment. *Acta Orthop Scand.* Apr 1998;69(2):107-110.
41. Gebuhr P, Wilbek H, Soelberg M. Naproxen for 8 days can prevent heterotopic ossification after hip arthroplasty. *Clin Orthop.* May 1995(314):166-169.
42. Persson PE, Sodemann B, Nilsson OS. Preventive effects of ibuprofen on periarticular heterotopic ossification after total hip arthroplasty. A randomized double-blind prospective study of treatment time. *Acta Orthop Scand.* Apr 1998;69(2):111-115.
43. Wurnig C, Auersperg V, Boehler N, et al. Short term prophylaxis against heterotopic bone after cementless hip replacement. *Clin Orthop.* Jan 1997(334):175-183.
44. Ritter MA, Sieber JM. Prophylactic indomethacin for the prevention of heterotopic bone formation following total hip arthroplasty. *Clin Orthop.* Jun 1985(196):217-225.
45. Sodemann B, Persson PE, Nilsson OS. Nonsteroid anti-inflammatory drugs prevent the recurrence of heterotopic ossification after excision. *Arch Orthop Trauma Surg.* 1990;109(2):53-56.
46. Wahlstrom O, Risto O, Djerf K, Hammerby S. Heterotopic bone formation prevented by diclofenac. Prospective study of 100 hip arthroplasties. *Acta Orthop Scand.* Oct 1991;62(5):419-421.
47. Freiberg AA, Cantor R, Freiberg RA. The use of aspirin to prevent heterotopic ossification after total hip arthroplasty. A preliminary report. *Clin Orthop.* Jun 1991(267):93-96.
48. Pritchett JW. Ketorolac prophylaxis against heterotopic ossification after hip replacement. *Clin Orthop.* May 1995(314):162-165.
49. Gebuhr P, Sletgard J, Dalsgard J, et al. Heterotopic ossification after hip arthroplasty: a randomized double-blind multicenter study tenoxicam in 147 hips. *Acta Orthop Scand.* Feb 1996;67(1):29-32.
50. Simon AM, Manigrasso MB, O'Connor JP. Cyclo-oxygenase 2 function is essential for bone fracture healing. *J Bone Miner Res.* Jun 2002;17(6):963-976.
51. Zhang X, Schwarz EM, Young DA, Puzas JE, Rosier RN, O'Keefe RJ. Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. *J Clin Invest.* Jun 2002;109(11):1405-1415.

52. Ritter MA, Gioe TJ. The effect of indomethacin on para-articular ectopic ossification following total hip arthroplasty. *Clin Orthop.* Jul 1982(167):113-117.
53. Sodemann B, Persson PE, Nilsson OS. Prevention of periarticular heterotopic ossification following total hip arthroplasty. Clinical experience with indomethacin and ibuprofen. *Arch Orthop Trauma Surg.* 1988;107(6):329-333.
54. Knelles D, Barthel T, Karrer A, Kraus U, Eulert J, Kolbl O. Prevention of heterotopic ossification after total hip replacement. A prospective, randomised study using acetylsalicylic acid, indomethacin and fractional or single-dose irradiation. *J Bone Joint Surg Br.* Jul 1997;79(4):596-602.
55. Elmstedt E, Lindholm TS, Nilsson OS, Tornkvist H. Effect of ibuprofen on heterotopic ossification after hip replacement. *Acta Orthop Scand.* Feb 1985;56(1):25-27.
56. Keller JC, Trancik TM, Young FA, St Mary E. Effects of indomethacin on bone ingrowth. *J Orthop Res.* 1989;7(1):28-34.
57. Kjaersgaard-Andersen P, Schmidt SA, Pedersen NW, Kristensen SS, Pedersen P. Early radiolucencies following cemented total hip replacement. Influence of postoperative treatment with indomethacin. *Orthopedics.* Mar 1989;12(3):401-406.
58. Coventry MB, Scanlon PW. The use of radiation to discourage ectopic bone. A nine-year study in surgery about the hip. *J Bone Joint Surg Am.* Feb 1981;63(2):201-208.
59. Ayers DC, Evarts CM, Parkinson JR. The prevention of heterotopic ossification in high-risk patients by low-dose radiation therapy after total hip arthroplasty. *J Bone Joint Surg Am.* Dec 1986;68(9):1423-1430.
60. Fingeroth RJ, Ahmed AQ. Single dose 6 Gy prophylaxis for heterotopic ossification after total hip arthroplasty. *Clin Orthop.* Aug 1995(317):131-140.
61. Pellegrini VD, Jr., Konski AA, Gastel JA, Rubin P, Evarts CM. Prevention of heterotopic ossification with irradiation after total hip arthroplasty. Radiation therapy with a single dose of eight hundred centigray administered to a limited field. *J Bone Joint Surg Am.* Feb 1992;74(2):186-200.
62. van Leeuwen WM, Deckers P, de Lange WJ. Preoperative irradiation for prophylaxis of ectopic ossification after hip arthroplasty. A randomized study in 62 hips. *Acta Orthop Scand.* Apr 1998;69(2):116-118.
63. Sumner DR, Turner TM, Pierson RH, et al. Effects of radiation on fixation of non-cemented porous-coated implants in a canine model. *J Bone Joint Surg Am.* Dec 1990;72(10):1527-1533.
64. Thomas BJ, Amstutz HC. Results of the administration of diphosphonate for the prevention of heterotopic ossification after total hip arthroplasty. *J Bone Joint Surg Am.* Mar 1985;67(3):400-403.
65. Nollen AJ. Effects of ethylhydroxydiphosphonate (EHDP) on heterotopic ossification. *Acta Orthop Scand.* Aug 1986;57(4):358-361.
66. Urist MR. Bone: formation by autoinduction. *Science.* Nov 12 1965;150(698):893-899.
67. Urist MR, DeLange RJ, Finerman GA. Bone cell differentiation and growth factors. *Science.* May 13 1983;220(4598):680-686.
68. Wright JG, Moran E, Bogoch E. Reliability and validity of the grading of heterotopic ossification. *J Arthroplasty.* Oct 1994;9(5):549-553.

69. Della Valle AG, Ruzo PS, Pavone V, Tolo E, Mintz DN, Salvati EA. Heterotopic ossification after total hip arthroplasty: a critical analysis of the Brooker classification and proposal of a simplified rating system. *J Arthroplasty.* Oct 2002;17(7):870-875.
70. Tornkvist H, Nilsson OS, Bauer FC, Lindholm TS. Experimentally induced heterotopic ossification in rats influenced by anti-inflammatory drugs. *Scand J Rheumatol.* 1983;12(2):177-180.
71. Carson JL, Strom BL, Morse ML, et al. The relative gastrointestinal toxicity of the nonsteroidal anti-inflammatory drugs. *Arch Intern Med.* Jun 1987;147(6):1054-1059.
72. Henry D, Lim LL, Garcia Rodriguez LA, et al. Variability in risk of gastrointestinal complications with individual non-steroidal anti-inflammatory drugs: results of a collaborative meta-analysis. *Bmj.* Jun 22 1996;312(7046):1563-1566.
73. Tornkvist H, Bauer FC, Nilsson OS. Influence of indomethacin on experimental bone metabolism in rats. *Clin Orthop.* Mar 1985(193):264-270.
74. Nolan DR, Fitzgerald RH, Jr., Beckenbaugh RD, Coventry MB. Complications of total hip arthroplasty treated by reoperation. *J Bone Joint Surg Am.* Oct 1975;57(7):977-981.
75. Bo J, Sudmann E, Marton PF. Effect of indomethacin on fracture healing in rats. *Acta Orthop Scand.* Dec 1976;47(6):588-599.
76. Sudmann E, Dregelid E, Bessesen A, Morland J. Inhibition of fracture healing by indomethacin in rats. *Eur J Clin Invest.* Oct 1979;9(5):333-339.
77. Tornkvist H, Lindholm TS, Netz P, Stromberg L, Lindholm TC. Effect of ibuprofen and indomethacin on bone metabolism reflected in bone strength. *Clin Orthop.* Jul-Aug 1984(187):255-259.
78. Fermor B, Gundle R, Evans M, Emerton M, Pocock A, Murray D. Primary human osteoblast proliferation and prostaglandin E2 release in response to mechanical strain in vitro. *Bone.* Jun 1998;22(6):637-643.
79. Nilsson OS, Persson PE, Ekelund A. Heterotopic new bone formation causes resorption of the inductive bone matrix. *Clin Orthop.* Aug 1990(257):280-285.
80. Keller J, Bunger C, Andreassen TT, Bak B, Lucht U. Bone repair inhibited by indomethacin. Effects on bone metabolism and strength of rabbit osteotomies. *Acta Orthop Scand.* Aug 1987;58(4):379-383.
81. Lazansky MG. Complications revisited. The debit side of total hip replacement. *Clin Orthop.* Sep 1973;95:96-103.
82. Urist MR, Nilsson OS, Hudak R, et al. Immunologic evidence of a bone morphogenetic protein in the milieu interieur. *Ann. Biol. Clin.* 1985;43:755-766.
83. Lind M. Growth factor stimulation of bone healing. Effects on osteoblasts, osteomies, and implants fixation. *Acta Orthop Scand Suppl.* Oct 1998;283:2-37.
84. Pellegrini VD, Jr., Gregoritch SJ. Preoperative irradiation for prevention of heterotopic ossification following total hip arthroplasty. *J Bone Joint Surg Am.* Jun 1996;78(6):870-881.
85. Kjaersgaard-Andersen P, Schmidt SA. Indomethacin for prevention of ectopic ossification after hip arthroplasty. *Acta Orthop Scand.* Feb 1986;57(1):12-14.
86. Schmidt SA, Kjaersgaard-Andersen P. Indomethacin inhibits the recurrence of excised ectopic ossification after hip arthroplasty. A 3-4 year follow-up of 8 cases. *Acta Orthop Scand.* Oct 1988;59(5):593.

87. Ahrengart L, Blomgren G, Tornkvist H. Short-term ibuprofen to prevent ossification after hip arthroplasty. No effects in a prospective randomized study of 47 arthrosis cases. *Acta Orthop Scand.* Apr 1994;65(2):139-141.
88. Amstutz HC, Fowble VA, Schmalzried TP, Dorey FJ. Short-course indomethacin prevents heterotopic ossification in a high-risk population following total hip arthroplasty. *J Arthroplasty.* Feb 1997;12(2):126-132.
89. Vastel L, Kerboull L, Dejean O, Courpied JP, Kerboull M. Prevention of heterotopic ossification in hip arthroplasty. The influence of the duration of treatment. *Int Orthop.* 1999;23(2):107-110.
90. van der Heide HJ, Koorevaar RT, Schreurs BW, van Kampen A, Lemmens A. Indomethacin for 3 days is not effective as prophylaxis for heterotopic ossification after primary total hip arthroplasty. *J Arthroplasty.* Oct 1999;14(7):796-799.
91. Cella JP, Salvati EA, Sculco TP. Indomethacin for the prevention of heterotopic ossification following total hip arthroplasty. Effectiveness, contraindications, and adverse effects. *J Arthroplasty.* 1988;3(3):229-234.
92. Sudmann E, Bang G. Indomethacin-induced inhibition of haversian remodelling in rabbits. *Acta Orthop Scand.* Dec 1979;50(6 Pt 1):621-627.
93. Keller J, Kjaersgaard-Andersen P, Bayer-Kristensen I, Melsen F. Indomethacin and bone trauma. Effects on remodeling of rabbit bone. *Acta Orthop Scand.* Feb 1990;61(1):66-69.
94. Trancik T, Mills W, Vinson N. The effect of indomethacin, aspirin, and ibuprofen on bone ingrowth into a porous-coated implant. *Clin Orthop.* Dec 1989(249):113-121.
95. Nilsson O, Bauer H, Brosjo O, Persson PE. Influence of indomethacin on experimental heterotopic bone formation in rats. *Rev Chir Orthop Reparatrice Appar Mot.* 1988;74 Suppl 2:330-332.
96. Kjaersgaard-Andersen P, Schmidt SA. Total hip arthroplasty. The role of antiinflammatory medications in the prevention of heterotopic ossification. *Clin Orthop.* Feb 1991(263):78-86.
97. Hukkanen M, Corbett SA, Batten J, et al. Aseptic loosening of total hip replacement. Macrophage expression of inducible nitric oxide synthase and cyclo-oxygenase-2, together with peroxynitrite formation, as a possible mechanism for early prosthesis failure. *J Bone Joint Surg Br.* May 1997;79(3):467-474.
98. Lavigne P, Shi Q, Jolicoeur FC, Pelletier JP, Martel-Pelletier J, Fernandes JC. Modulation of IL-1beta, IL-6, TNF-alpha and PGE(2) by pharmacological agents in explants of membranes from failed total hip replacement. *Osteoarthritis Cartilage.* Nov 2002;10(11):898-904.

Acta Universitatis Upsaliensis

Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine.* (Prior to October, 1985, the series was published under the title "Abstracts of Uppsala Dissertations from the Faculty of Medicine".)

Distribution:
Uppsala University Library
Box 510, SE-751 20 Uppsala, Sweden
www.uu.se, acta@ub.uu.se

ISSN 0282-7476
ISBN 91-554-5840-8