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The repair of long bones has not been optimized by Mother Nature. One would think that such a complicated process, leading to replacement of damaged tissue and restoration of function, would be the final result of millions of years of evolution. However, if our hairy and four-legged ancestors were to survive a fracture, they would have needed restoration of long bone function within days. Otherwise, without being able to run, jump or climb perfectly, they would have starved to death or been eaten up by carnivores. Because long bone fracture led to almost certain death, even a mutation that could shorten healing time by half would still not save the animal. Thus, there can hardly have been any evolutionary pressure towards optimized long bone repair. Still, long bones do repair remarkably well: if the animal is helped to survive, the fracture gap will be replaced by newly developed bone with a complex architecture. This is a kind of regeneration rather than repair—quite different from scar formation, which is seen at most other locations. It seems paradoxical that we have a truly regenerative process in the absence of any evolutionary pressure. The explanation is probably that long bone repair uses the same genes as repair of trabecular microfractures. Such fractures often occur with minimal symptoms, and require a repair system for the animal to survive.

Because the repair of long bones is not optimized, it should be quite possible to enhance it by pharmacological or other means. Considerable shortening of the time to radiographic healing in humans has already been achieved with physical treatments such as electromagnetism (Wahlstrom 1984) and ultrasound (Kristiansen et al. 1997).

Fracture repair involves proliferation and differentiation of several tissue types in a sequence, followed by remodeling. All of these processes may be influenced by drugs. Some drugs can have effects on the proliferation of early callus tissue, others on the differentiation of chondrocytes or osteoblasts, capillary formation, sensitivity to mechanical input, etc. The subject of drugs and fracture repair thus involves not only pharmacology and orthopedics but also a wide range of biology. I cannot cover all of these subjects, and will only try to give a brief overview of some studies that I have come across. This is also an emerging field: several drug companies are currently working on new concepts for stimulation of fracture repair, and we can certainly expect publication of new approaches and possibilities in the near future.

Drugs that impair

Cytostatics

Cytostatics work by killing cells that proliferate quickly. Because callus growth involves very fast proliferation, it can be expected that cytostatics should have a strong and negative effect (fracture callus is even hard for pathologists to differentiate from malignant bone tumor). This is all well

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known to orthopedic oncologists and lies partly outside the scope of this article.

Antibiotics

Fluoroquinolones are known to have adverse effects on growing cartilage. Two recent rat studies have shown impaired fracture repair by radiographic, mechanical and histomorphometric criteria when exposed to normal therapeutic serum concentrations of different fluoroquinolones (Huddleston et al. 2000, Perry et al. 2003). This effect appears mostly, but not entirely, to be caused by a disturbance of the cartilaginous callus.

Because tetracyclines are known inhibitors of matrix metalloproteinases (MMPs) and also have other effects on tissue turnover (Lamparter et al. 2002), they could be expected to have an effect on several processes in callus formation. We have recent, unpublished data showing a clear detrimental effect on tendon repair when using relevant serum concentrations, but to my knowledge this has not been shown in fracture models (Alkan et al. 2002).

Corticosteroids

There have been surprisingly few experiments in recent decades studying the effects of corticosteroids on fracture repair. However, as the initiation of fracture repair involves inflammation (as we shall see when discussing NSAIDs), one would expect corticosteroids to have a negative effect. Most experimental studies have used supratherapeutic doses of corticosteroids. Physiologically, the most commonly used animal—the rat—has a different active corticosteroid to that in humans and rabbits, for example, which further compromises the results of many studies. In a recent study in rabbits, however, therapeutic doses of corticosteroids led to a clear inhibition of repair in non-critical size bone defects (defects small enough to heal spontaneously) (Waters et al. 2000).

Thromboprophylactic agents

The coagulation cascade eventually leads to thrombin activation, which then causes a clot to form. However, thrombin also has many other effects. Its proteolytic activity can release and activate growth factors and cell surface receptors. It also has a growth factor-like activity which is independent of its proteolytic activity (Norfleet et al. 2000).

A thrombin-related peptide (TP 508) stimulates fracture repair in several animal models, (Sheller et al. 2004), through complex mechanisms (Wang et al. 2005). Heparin, heparin fragments and direct thrombin inhibitors reduce clot formation by inhibiting thrombin. The question that immediately arises from this information would be whether they also inhibit fracture repair. Surprisingly, there has been little interest in this question. In fact, a PubMed search for "heparin and fracture repair" yields only one single study in the English literature about the effects of low molecular weight heparin on fracture repair (Street et al. 2000). This effect is dramatically negative! In the fractured rabbit rib, a clinically relevant dose of enoxaparin caused a clear reduction in early cellular proliferation and in histological callus maturity at all time points studied. Moreover, there was a decrease in strength, stiffness and energy absorption at 3 weeks. In a German paper, heparin reduced the filling out of bore holes in cancellous rabbit bone, but this could not be shown for heparin fragments (Kock et al. 2002). It turns out that the inhibition of fracture repair by heparin was shown in rabbits and dogs as early as 50 years ago (Stinchfield et al. 1956). Preliminary data from my own laboratory suggest that clinically relevant doses of low molecular weight heparin inhibit both bone implant fixation and soft tissue repair in rats.

Cox inhibitors (NSAIDs)

The term non-steroidal anti-inflammatory drugs (NSAIDs) implies anything except cortisone that has an effect on inflammation. It has come to mean unspecific Cox 1 and Cox 2 inhibitors, in contrast to specific Cox 2 inhibitors. This is quite unclear terminology, and I think that from now on we should talk about Cox inhibitors that can be either unspecific or specific. The Cox 1 and Cox 2 enzymes both catalyze the same rate-limiting enzymatic step in the production of prostaglandins. By and large, Cox 1 is responsible for the small production of prostaglandins that is necessary for cell survival, and Cox 2 is induced whenever excess prostaglandin is needed, e.g. during inflammation or repair. Relatively speaking, Cox 2 produces huge amounts of prostaglandins, so when Cox 2 is turned on, the

production from Cox 1 becomes neglible. Thus, in fracture repair, both unspecific and specific Cox 2 inhibitors dramatically reduce prostaglandin production and should therefore be expected to have similar effects. There is a substantial amount of literature on the effects of Cox inhibitors on bone repair in experimental animals. Some show inhibition of repair and others do not. In those studies where Cox inhibitors appear harmless (Gerstenfeld et al. 2003), the dosing of the Cox inhibitor may be inadequate. In male rats, the selective Cox 2 inhibitors celecoxib and rofecoxib are metabolized so quickly by the liver that also a high dose given once daily will yield too low a serum concentration to be relevant (Halpin et al. 2000, Paulson et al. 2000, Simon et al. 2002). This has not been known, or acknowledged, by all authors. Experiments in mice where the Cox 2 gene has been knocked out demonstrate a dramatic decrease in repair of long bone fractures (Simon et al. 2002, Zhang et al. 2002) and there are also several articles in which Cox inhibitors have decreased fracture repair in experimental animals with adequate dosing (Simon et al. 2002). Some of the discrepancies between different studies and models may also relate to the observation that Cox inhibitors have a less dramatic effect on the periosteal reaction than on the cartilaginous part of the repair process (O'Connor and Simon 2003).

Whereas the inhibition of fracture repair in animals is undisputed, the clinical relevance of these findings is still being debated (Aspenberg 2002, Einhorn 2002). There have, however, been several clinical observations suggesting that Cox inhibitors are harmful. First of all, Cox inhibitors are highly efficient for inhibition of heterotopic ossification in hip surgery (Fransen and Neal 2004). In a controlled but retrospective study of femoral shaft fractures, Cox inhibitors had a strong and highly significant association with delayed union and pseudarthrosis (Giannoudis et al. 2000).

The inhibition of ectopic bone formation in humans only requires treatment with Cox inhibitors during the first 5–7 days after the operation (Neal et al. 2000). This suggests that the initial inflammation is crucial for starting the bone formation response. Also, in fracture repair, some data from animal experiments have suggested that Cox inhibitors are most harmful during the early phase of healing, and that remodeling is little affected (Simon et al. 2002). There seems to be some connection between prostaglandins and BMPs, because the impaired ability of cells from Cox 2 knockout mice to form bone nodules in vitro can be restored by both PGE2 and BMP2 (Zhang et al. 2002). Furthermore, PGE2 can induce expression of BMP2 and BMP7, both in vitro and in vivo (Paralkar et al. 2002, Arikawa et al. 2004).

In conclusion there is no doubt that Cox inhibitors can have clear inhibitory effects on bone formation in humans. It remains to be determined in which clinical situations this is important, and in which situations normal healing will occur anyway. There have been very recent and relevant clinical data on implant fixation. An old randomized study on the prevention of ectopic ossification around hip prostheses was re-evaluated after 10 years (Persson et al. 2005). The patients that had received Cox inhibitors had a higher incidence of revision on account of loosening. However, if radiographic loosening was included, there was no difference. It has been reported in an abstract from the Danish Arthroplasty Registry that uncemented femoral hip components have a threefold increased risk of loosening if Cox inhibitors are given postoperatively.

Coming back to fractures, one would think—from the available literature—that under stable conditions metaphyseal fractures should not be affected much, whereas Cox inhibitors may well be detrimental in long bone shaft fractures, in cases requiring bone grafting, or when healing is impaired for some reason.

Drugs that improve

Growth factors

Several growth factors are expressed during fracture repair, such as fibroblast growth factors (FGFs), transforming growth factor βs (TGFβs), platelet-derived growth factor (PDGFs), insulin-like growth factor 1 (IGF-1), vascular endothelial-derived growth factor (VEGF), and others. Most of these growth factors have been applied using various carriers in animal models. In such models in the rat, FGF-2 has been shown to increase callus size and strength (Kawaguchi et al. 1994).

It also improves metaphyseal repair (Chen et al. 2004). IGF-1 has shown similar effects, especially in combination with TGF (Schmidmaier et al. 2004). Growth hormone (GH) has also had a positive effect on fracture repair in some studies. This effect may be mediated by local synthesis of IGF-1. Many studies have been performed with systemic GH treatment and the effects have mostly been weak. However, a considerable effect was noted in one study on minipigs (Raschke et al. 2001). Andreassen and Oxlund (2003) have shown that local injections of small amounts of GH have a marked positive effect on rat fracture repair, without causing any systemic effects.

To my knowledge, and in contrast to BMPs, these growth factors have not been used in clinical studies.

Bone morphogenetic proteins (BMPs)

BMP research began as early as the 1930s (Levander 1938) and the term BMP was coined by Marshall Urist shortly after he discovered the use of demineralized bone matrix (Urist 1965). Demineralized bone matrix is still widely used in the USA, but there is hardly any evidence that it has a beneficial effect in adult patients. In the past, several studies have been designed to demonstrate bone induction by demineralized bone matrix in humans (Kakiuchi et al. 1985, Aspenberg et al. 1988), but there has been only one study on six patients in which there seems to have been bone induction in a skeletal defect (Geesink et al. 1999). BMP was a hypothetical substance until the genes for BMPs were cloned in 1988. By far the most interest has been paid to BMP2 and BMP7 (also called OP-1). These proteins have similar effects and have been shown to induce bone locally and to speed up skeletal defect repair in a plethora of animal models, including monkeys. Individual BMPs are parts of a complex signaling system consisting of several BMP molecules, and also different receptors and a number of soluble antagonists functioning as decoy receptors. BMP signaling leads to activation of genes for proliferation and differentiation along the chondrogenic and osteogenic pathways. However, in a few early studies in animal models, BMPs were shown to have a negative effect on bone formation (Jeppsson and Aspenberg 1996, Jeppsson et al. 1999). These early findings remained unexplained for a long time, until they were connected to the observation that BMPs also induce osteoclastic bone resorption and that this response often comes before the bone formative response (Kaneko et al. 2000). Thus, application of BMPs locally can lead to a transient bone resorption before the site is filled with newly formed bone (Laursen et al. 1999). It has therefore been suggested that in some situations BMP treatment could be complemented with a bisphosphonate, as protection from this early resorptive phase (Jeppsson et al. 2003). BMP signaling and the response to BMPs are both dependent on the mechanical loading situation (Aspenberg et al. 2000). Thus, the above-mentioned negative response can be changed into increased bone formation by application of mechanical load (Bostrom et al. 1998). On the whole, BMPs have a strong and robust effect in stimulating fracture repair in most animal models.

There have been few clinical studies on the efficacy of BMPs. However, in a large randomized multicenter study on open tibial fractures, BMP-treated patients had a relative risk of 0.56 (confidence interval 0.40–0.78) of requiring secondary intervention because of the delayed union, as compared to placebo controls (Govender et al. 2002). There was also significantly faster healing, and fewer hardware failures and infections. This was a high-quality study and good evidence for the clinical efficacy of BMP2. However, for other indications, BMPs may be problematic. In other studies, BMPs have been compared with bone grafting, and the results have shown no difference between the two groups (Friedlaender et al. 2001, Johnsson et al. 2002). Although this suggests that the two treatments are equally efficacious, a somewhat far-fetched but possible conclusion might also be that neither BMPs nor bone grafting were necessary for successful treatment in these studies. There have also been clinical reports of failures that can be attributed to the bone resorptive effect of BMPs in the case of spinal fractures (Laursen et al. 1999).

The pharmaceutical industry appears to be working with injectable BMP formulations and with smaller and cheaper molecules that can activate BMP receptors.

Parathyroid hormone (PTH)

Although constantly increased levels of PTH

lead to bone resorption, it has been known since the 1930s that parathyroid hormone given intermittently has a strong positive effect on skeletal metabolism (Selye 1932). This had not been explored in any depth until recently. It is now clear that PTH treatment not only prevents osteoporosis in humans, but even leads to its reversion (Lindsay et al. 1997, Neer et al. 2001). Also, several rat studies of long bone fractures have shown a positive effect on repair (Andreassen et al. 1999, 2001). However, the most dramatic effects come relatively late during the repair process, where there is more than a doubling of mechanical strength. In models of implant fixation (stainless steel screws), or bone chambers, there is a more direct bone formation response (Skripitz et al. 2000a, b, Skripitz and Aspenberg 2001a, b). In these models, only three injections during one week are enough to double the proportion of bone-implant contact, and at 2 weeks the pull-out strength is increased 2- to 3-fold. This suggests that the latency of the effect in long bone shaft experiments can be explained by the fact that parathyroid hormone has no effect on the chondroid phase of fracture repair. A positive effect of PTH can be expected only after bridging bone has been developed (Seebach et al. 2004). As previously mentioned, this effect may then be quite dramatic. Consequently, it is uncertain whether PTH will prove useful in nonunions and pseudarthroses.

The combination of these few but clear-cut results from rat studies and the dramatic improvement in bone density in human osteoporosis patients leads to the conclusion that a positive effect on fracture repair can be expected in humans. Currently, randomized studies are being started in order to evaluate this possibility. If these studies become successful, it might be possible for the first time to speed up fracture repair in our patients by using systemic treatment. Although this treatment has few side effects, it involves daily injections.

Selective prostaglandin agonists

As we know, inhibitors of prostaglandin production (Cox inhibitors) have a detrimental effect on fracture repair. Thus, prostaglandins would be expected to stimulate repair. This effect has been shown in a few animal models, and increased bone formation is a known side effect of prostaglandin treatment in infants with an open ductus arteriosus. Even so, this field of research has not been followed because of the possibility of severe side effects. Recently, however, it has been established that there are four different receptors for PGE2, with separate distribution and separate second messenger systems. This has raised the possibility of using selective prostaglandin receptor agonists for stimulation of bone repair. Selective agonists for receptor E2 (EP2) have been shown to stimulate fracture repair in rodents and dogs (Paralkar et al. 2003). In dogs, the agonist was implanted on a PLGA carrier in radial defects that were too large to heal spontaneously, and in plated tibial osteotomies. There is also evidence for a similar effect of EP4 agonists (Tanaka et al. 2004).

Statins and beta-blockers

In a search of over 30,000 small molecules, screening for their ability to turn on the promotor of the BMP2 gene, one of the few molecules that emerged from the study was simvastatin (Mundy et al. 1999). As a result, this common lipid-lowering drug has since been suggested for both osteoporosis treatment and fracture repair. The clinical results derived from re-analysis of clinical studies on cardiovascular disease have, however, been contradictory. There is clear evidence for improved bone formation in rats (Mundy et al. 1999), and there has been only one single study in which simvastatin treatment was found to significantly enhance fracture repair, as measured mechanically in mice (Skoglund et al. 2002). However, there appear to be several problems associated with the reproducibility of these effects, which are for some reason highly dependent on the choice of experimental model. Several issues regarding dose dependence timing and model application also require to be resolved.

It has recently been shown that bone mass is regulated from the hypothalamus via the nervous system, mainly by adrenergic nerves with antiosteogenic effects (Takeda et al. 2002). Thus, propranolol, a common beta-blocker, increases bone mass in wild-type mice (Takeda et al. 2002). A recent, large and well-controlled case-control study has suggested that beta-blockers reduce the risk of osteoporosis fractures (Schlienger et al. 2004). When we read this, my group immediately started

giving propranolol to osteotomized mice, in order to show an effect on healing. Our first attempts failed, and then we found out that a positive effect of propranolol on the repair of bone defects in rats had already been shown 15 years ago (Minkowitz et al. 1991).

Conclusion

Several drugs have had dramatic effects on fracture repair in animal models. Their effects in humans have usually not been studied, and it is possible that in our daily practice, we unknowingly prescribe drugs that affect the healing of fractures. Cox inhibitors have a negative effect, but it is still being debated to what extent and in which situations this is of clinical importance.

Thromboprofylaxis appears to have a negative effect on repair. The clinical relevance of this has not been studied.

As for stimulation of fracture repair, we can expect competition between BMPs, parathyroid hormone and selective prostaglandin agonists in the future, all of which have a dramatic ability to stimulate and improve fracture repair. To date, there is clinical evidence only for the BMPs, which must be implanted on a carrier substance during surgery. Injectable formulations are under development. So far, selective prostaglandin agonists have been applied in a way similar to the BMPs. They are small molecules, are probably much easier to produce, and it might be possible to use them systemically.

If proven to be efficacious, parathyroid hormone variants have the advantage of systemic treatment with few side effects, making the need for surgical implants obsolete. They have not been studied in critical size defects, and in theory one might expect them to be less efficacious in inducing bone formation in such defects. On the other hand, they may possibly prove superior in their ability to accelerate normal repair.

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