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Indomethacin and celecoxib improve tendon healing in rats

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ABSTRACT Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the formation of bone. However, they have been shown to increase tensile strength in healing tendons. Most NSAIDs inhibit two isoforms of cyclooxygenases called Cox-1 and Cox-2. Thanks to fewer side-effects, the recently introduced selective cyclooxygenase-2 (Cox-2) inhibitors will probably promote more widespread use of this kind of drug. To clarify the effects on tendon healing of a general Cox-inhibitor (indomethacin) as well as a selective Cox-2 inhibitor (celecoxib), we resected 3 mm of the Achilles tendon in rats and measured the strength of the tendon regenerate.

Indomethacin given as daily injections in doses of 1.5, 3.0 and 5.0 mg/kg reduced the thickness (cross-sectional area) of the tendon regenerate at 14 days, as compared to controls, but there was no difference in the failure load or stiffness. In another series of measurements, indomethacin in a dose of 3.0 mg/kg reduced the cross-sectional area at 10, 14 and 18 days after transsection. Failure load was not affected, but tensile stress at failure was increased by indomethacin at 14 and 18 days. Indomethacin (3 mg/kg) was then compared to celecoxib (4.5 mg/kg) and controls 14 days after tendon transsection. No difference between the drugs was seen. Again, the transverse area was smaller in the treated tendons than in the controls. Failure load was unchanged and the tensile stress was higher in the treated tendons than in the controls. Because of the reduction in cross-sectional area without an effect on failure load, the use of Cox-inhibitors may be beneficial in clinical situations where thickening of a healing tendon is a problem—e.g., in the hand or shoulder.

The effect of NSAIDs (Cox-inhibitors) on bone and connective tissue healing remains unclear. The recently introduced selective Cox-2 inhibitors should contribute to even more general use of these drugs because of fewer side-effects. Comparative data about their effects on bone formation and connective tissue (tendon) healing have not been available. Many studies have reported that they inhibit orthopic and heterotopic bone formation (Allen et al. 1980, Altman et al. 1995, Martin et al. 1999).

On the other hand, an increase in tensile strength after treatment with indomethacin has been shown in the healing rat-tail tendon (Vogel 1977) and in the injured as well as the uninjured plantaris longus tendon in rabbits (Carlstedt et al. 1986, Carlstedt 1987). An increase in the mechanical strength of the periodontium in rats has also been found after treatment with indomethacin (Ohkawa, 1982).

In the present paper, we compared the effect of selective (celecoxib) and non-selective (indomethacin) Cox-inhibitors on tendon healing in rats. We performed 3 experiments, using an established method (Forslund and Aspenberg 1998) to study the dose-response of indomethacin, to compare the effects of an optimal dose of indomethacin at some different time points, and to compare the effects of indomethacin to those of celecoxib.

Animals and methods

126 female Sprague Dawley rats (200 g) were used (Table 1). The study was approved by the regional Board of Ethics. The rats were anesthetized with

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chloral hydrate intraperitoneally (4 mg/kg). The right hind limb was shaved and washed with alcohol. The operations were performed under aseptic conditions. A 2 mm transverse skin incision was made on the lateral side of the Achilles tendon. The fascia was cut longitudinally and the Achilles tendon complex exposed. The plantaris tendon was removed to simplify measurements of force at the end of the experiments. The Achilles tendon was cut transversely 1.5 mm proximal to the calcaneal insertion and a 3 mm long segment was removed to enlarge the defect. The tendon was left unsutured with a gap between the tendon ends when the skin was sutured. The animals were not immobilized postoperatively. After the operation, the rats received daily injections of Cox-inhibitors until they were killed with an overdose of pentobarbital sodium (60 mg/mL). The tendon was dissected free from other tissues while attached to the calcaneal bone. The sagittal and transverse diameters of the tendon were measured with a digital calipers. The tensile strength was tested in a testing machine for materials and the tendon was pulled with a constant speed of 1 mm/s until failure. For clamping, the muscle was scraped off the tendon by blunt dissection, to produce a fan of tendon fibers, which was sandwiched between fine sandpaper in a metal clamp. The calcaneus was fixated in a custom-made clamp in 30º dorsiflexion, in relation to the direction of traction. In the first two studies, we used a home-made testing machine for materials, made from a servo-engine and a threaded pin connected to a transducer from a brass ring and strain gauges in a 4-bridge. It was connected to a computer to measure the momentum force. The maximum force was recorded and used for statistical analysis. In the last study, a new materials testing machine was used (100R, DDL Inc., Eden Praire, MN, USA) which enabled us to measure stiffness and energy as well. The diameters were used to calculate the transverse area of the tendons. This calculation has an error of measurement ($2^{-0.5}$ SD (measurement 1–measurement 2)) of 3% of the mean value, based on double measurements of 10 controls in a similar unpublished material. The transverse area was used to calculate tensile stress at failure.

The treatment of the rats was selected at random after the operation had been performed, and the operator of the materials testing machine was blinded to the treatment given.

Table 1. Number of animals operated on

	Operated	Died
Dose-response study		
Control 1.5 and 3 mg/kg	10	1
Indomethacin 1.5 mg/kg	9	0
Indomethacin 3 mg/kg	9	0
Control 5 mg/kg	10	0
Indomethacin 5 mg/kg	10	2
Time-sequence study		
Indomethacin 10 days	8	0
Control 10 days	8	0
Indomethacin 14 days	8	0
Control 14 days	8	0
Indomethacin 18 days	8	0
Control 18 days	8	0
Indomethacin vs. celecoxib study		
Indomethacin 3 mg/kg	10	0
Celecoxib 4.5 mg/kg	10	0
Control	10	0

Dose-response study

The indomethacin, in powder form, was dissolved in water to make a solution (5 mg/mL) for intravenous use. This solution was then further diluted with saline to obtain various concentrations for the subcutaneous injections. The rats in the dose-response study were given subcutaneous injections of indomethacin. First, one group of rats was injected with 0 or 5 mg indomethacin/kg bodyweight (Table 1). This group was then supplemented with more rats which were given injections of 0, 1.5 or 3.0 mg/kg. Each rat received a total volume of 200 µL. The controls were injected with saline. The rats were killed after 14 days. The statistical analysis was done using one-way ANOVA.

Time-sequence study

In this part of the rats received indomethacin in doses of 0 or 3.0 mg/kg, as above (Table 1). They were killed after 10, 14 and 18 days. The statistical analysis was done using two-way ANOVA, followed by post hoc testing with Fisher's PLSD connected to a one-way ANOVA.

Indomethacin vs. celecoxib study

Owing to the low solubility of celecoxib, both celecoxib (tablet) and indomethacin (powder) were

Table 2. Weight-gain (g) in rats injected with indomethacin (im) in a dose of 1.5, 3 and 5 mg/kg and controls. Data from 2 rats are missing

Group	n	Mean	SD
NaCl	9	12	5.6
im 1.5	9	18	13
im 3	9	16	17
NaCl (5)	9	23	6.7
im 5	7	5.4	16

NaCl = controls given 1.5 and 3 mg/kg im
NaCl (5) = controls given 5 mg/kg im

dissolved in 95% alcohol. Before each injection, the solutions were further diluted or dispersed in saline to an alcohol concentration of 10%. The rats were injected intraperitoneally with 250 µL/injection. The dose of indomethacin was 3.0 mg/kg bodyweight and of celecoxib 4.5 mg/kg (Table 1). The controls were injected with 10% alcohol. The rats were killed after 14 days. The statistical analysis was done using one-way ANOVA and Fisher's PLSD.

Results

Dose-response study

1 rat in the control group died after surgery, and 2 rats injected with 5 mg/kg of indomethacin died, probably because of the injections. The weight-gain during the experiment was reduced in rats injected with 5 mg/kg, but not with the other doses (Table 2).

The transverse area of the tendon regenerate was significantly reduced in all indomethacin groups, as compared to their respective control group. The failure load was similar in the indomethacin-treated tendons and their respective controls. Failure stress differed significantly only between tendons from animals treated with the highest (5.0 mg/kg) indomethacin dose and their controls (Figure 1). There was no statistically significant difference between the 2 different control groups.

Time-sequence study

1 control rat in the 14-day group died after the operation. Using two-way ANOVA, we found that the transverse area of the tendon regenerates was reduced by indomethacin ($p = 0.0001$). This effect was statistically significant at all times (post

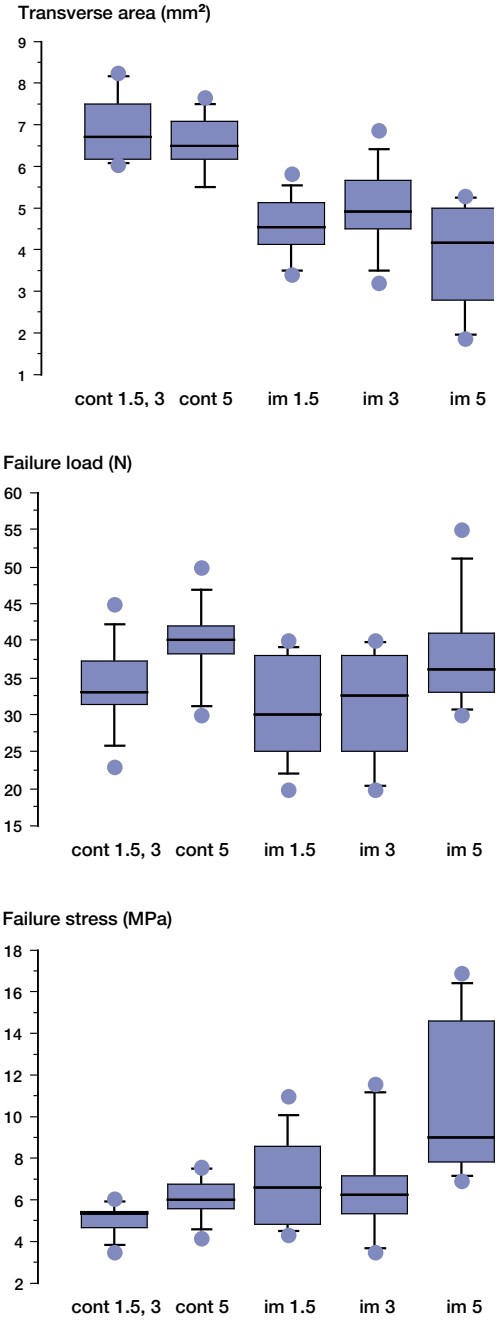

Figure 1. Dose-response study. Results at 14 days. Since indomethacin 5.0 mg/kg was injected on a different occasion, it had its own control group (called cont 5). Indomethacin 1.5 and 3.0 mg/kg had the same control group (called cont 1.5, 3).

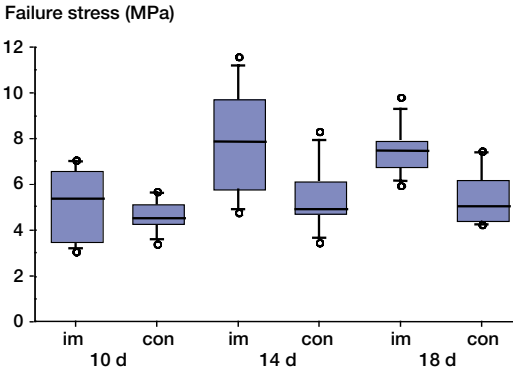

Figure 2. Time-sequence study. Failure stress for tendons treated with indomethacin (im) or controls (con) at 10, 14 and 18 days after surgery.

hoc testing with Fisher's PLSD). The failure load showed a difference only with time, but not with treatment. Failure stress increased significantly with time and treatment (post hoc testing with Fisher's PLSD showed a significant effect of treatment after 14 and 18 days; Figure 2.).

Indomethacin vs. celecoxib study

The failure load, stiffness and energy did not differ between the 3 groups, but the transverse area was smaller in the indomethacin- and celecoxib-treated tendons than in the controls ($p = 0.0003$; Figure 3). Failure stress was higher in both treated groups than in the controls ($p = 0.003$). We found no difference in any parameter between the tendons from rats treated with indomethacin or celecoxib.

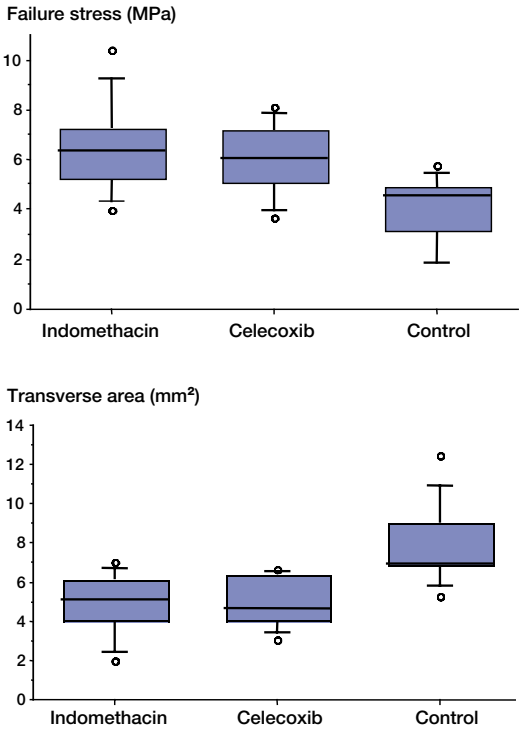

Figure 3. Indomethacin vs. celecoxib study. Failure stress and transverse area.

Discussion

Our data suggest that Cox-inhibitors have a beneficial effect on tendon healing. The material properties of the tendon callus were improved. At the highest dose (5.0 mg/kg), the rats lost weight, which was probably due to the side-effects of indomethacin. None of these effects were noted with the 3.0 mg/kg dose, and the weight-gain was normal.

In rabbits, 16 weeks of systemic treatment with indomethacin (10 mg/kg) increased the failure load of the uninjured plantaris longus tendon (Carlstedt et al. 1987). However, we found no difference in size or failure load of the uninjured tendon after 14 days of daily injections (data not shown).

It has been speculated that one mechanism for the effect of Cox-inhibitors on connective tissue regeneration could be acceleration of collagen maturation. The physical properties of collagen are very dependent on cross-links within and between the collagen molecules. During maturation, the number and quality of the cross-links increase, which increases the tensile strength and reduces collagen solubility (Piez 1968, Vogel 1978, Viidik et al. 1982). The effect of NSAID may be due to interference with collagen metabolism and cross-linking in the process of healing. This view is supported by an increase in the percentage of insoluble collagen and the collagen content in rat-tail tendons after treatment with indomethacin (Vogel 1977). In vivo, bone fractures in rats treated with indomethacin show increases in hydroxyproline incorporation and fibrogenesis, respectively (Ro et al. 1978, Elves et al. 1982). Therefore, the impairment in fracture repair may be due to changes in cell differentiation pathways, rather than a general inhibition of matrix production.

Although indomethacin is a well-known inhibitor of prostaglandin synthesis via cyclooxygenases, it is not clear whether the effect of indomethacin on healing tendons is mediated by this mechanism. Another mechanism, based on changes in collagen maturation, would be through interference with lysol-oxidase synthesis or activity (Carlstedt 1987).

The reduced cross-sectional area was a consistent finding in the indomethacin- and celecoxib-treated groups. This in combination with the unaffected failure load suggests that the expected rise in the use of Cox-inhibitors (especially selective Cox-2 inhibitors) in orthopaedic practice would not have the same drawbacks for tendon repair as they might have for bone repair. On the contrary, the effects of indomethacin and celecoxib, and even other Cox-inhibitors, might be beneficial in clinical situations where swelling of the healing tendon could present a problem—e.g., in digital flexor tendon surgery, or in the subachromial space of the shoulder.

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