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THE EFFECT OF INDOMETHACIN UPON EXPERIMENTAL FRACTURES IN THE RAT

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The effect of Indomethacin upon the process of fracture repair and osteogenesis in bone isografts has been studied in the rat. It was found that the drug had no significant effect upon new bone formation in heterotopic bone grafts. It had no effect upon the osteogenesis in repairing drill holes in 2 month old rats. A significant impairment of osteogenesis was detected, however, in older (6–9 months) animals given continuous treatment with Indomethacin from the week before fracture. This effect was not apparent if treatment was terminated on the day after induction of the lesion. There is histological evidence of increased fibrogenesis and decreased osteogenesis and remodelling in fractures in old rats given high dosages of Indomethacin.

Key words: bone grafts; fracture repair; Indomethacin; osteogenesis

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Although short-term treatment with Indomethacin appears to have no effect upon healing of fractures (Allgower et al. 1963), it has been claimed that longer term treatment with this drug may have an inhibitory effect upon bone repair (Allen et al. 1980, Rø et al. 1976, Sudmann 1975). On this basis, Indomethacin has been used to reduce unwanted callus formation in a patient with fracture-dislocation of the ankle joint (Sudmann & Hagen 1976). Since then, it has been suggested that Indomethacin therapy in patients with osteoarthritis leads to accelerated progression of their disease and development of radiological features suggestive of a failure of bone repair and ossification (Rønningen et al. 1979).

The experiments reported here are part of a study to determine the effects of Indomethacin upon healing of fractures in rats of various ages, and also upon osteogenesis in heterotopic bone isografts.

MATERIALS AND METHODS

Animals: Male rats of the AS inbred strain were used at various ages. All animals were kept in wire-mesh bottomed cages and fed and watered *ad libitum.* For all operative procedures the animals were anaesthetised using ether and aseptic techniques employed. Indomethacin or saline was administered by the oral route using a gastric tube.

Production of fractures: The fractures studied in this investigation were drill hole defects produced in the body of a caudal vertebra. A 1 cm long incision was made about one-third of the way down from the proximal end of the tail, and the tendons and blood vessels were carefully retracted exposing the body of a vertebra. Taking precautions to avoid damage to blood vessels, a 1.5 mm diameter drill hole was made in the bone using a dental burr in an electric dental drill. After haemostasis, the tendons and blood vessels were allowed to regain their normal positions and the skin wounds were closed using silk sutures.

Bone grafts: Bone isografts were obtained from the iliac bones of donor rats of the same age as the recipient.

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These were dissected free of adherent muscle, the periosteum was scraped off and the iliac crest removed. The bones were then cut in half. The upper half provided grafts which had thin cortical surfaces and were mainly cancellous bone; the lower grafts had thicker cortical shells. All grafts were kept on gauze moistened with culture medium TC 199 until they were transplanted. Pockets were created in the skin of the dorsal surface of recipient rats by making a 1 cm long incision through the skin and splitting the dermis from the panniculus carnosus muscle by means of curved scissors. A graft was then placed in each pocket and the wound closed by metal clips.

Indomethacin: Indomethacin was supplied as a powder by Merck, Sharp and Dohme Ltd., and was weighed and dissolved in a minimum quantity of absolute ethanol. This was then diluted with isotonic saline containing 2.2 g sodium bicarbonate/100 ml to give the dose in 1 ml of fluid. Either Indomethacin or diluent alone was administered daily at a dose of 4 mg/kg.

Assessment of osteogenesis: $Strontium^{85}$ retention was used to assess osteogenesis of both fractures and bone grafts. The basic technique has been previously described (Elves 1974). In the case of bone grafts, the whole graft was recovered, weighed, fixed in formal saline and counted intact. The tail vertebra *in situ* were first dissected free of soft tissue and then, taking care not to disturb any external callus, the bone with the defect was identified. This vertebra was next separated, weighed, fixed and counted, as were each of the three normal vertebrae immediately above and below the fracture. In the case of all bones being examined the Sr^{85} content was expressed on a unit weight basis (cpm/mg). After the radioactivity had been counted, all bones were processed for histological examination.

RESULTS

Pattern of bone repair

Two time intervals were chosen to examine the effects of Indomethacin upon the fractures; at 21–28 days for early osteogenesis and at 56 days for late osteogenesis. These times were chosen on the basis of preliminary experiments in which the pattern of osteogenesis in the early phase of fracture repair was examined in young animals (2 months old) and 2 groups of older rats (6–7 and 8–9 months). From the results, shown in Table 1, it will be seen that the level of Sr^{85} retention in the normal vertebrae in the old animals was about 50 per cent of that in the young ones. This is in agreement with earlier observations (Elves 1974). It will also be seen that in both age groups there was an increase in Sr^{85} retention in the fractured bone. The pattern of osteogenesis can be seen more clearly when a ratio is calculated as follows:

$$\frac{\text{Specific activity of fractured vertebra (F)}}{\text{Specific activity of normal vertebra (N)}}$$

Table 1. The pattern of osteogenesis in normal bone and during the early phase of fracture repair in rats of different ages*

Age of rats (months)	Type of bone	Age of fracture 7 days	14 days	21 days	28 days
2	Normal	583.1±33.9	453.6±97.4	457.6±53.0	447.4±80.5
	Fracture	647.7±68.2	577.8±125.3	757.8±124.7	678.2±67.4
	F/N**	1.11±0.07	1.28±0.09	1.66±0.20	1.44±0.15
	No. per group	6	6	11	6
6–7	Normal	–	–	248.9±8.7	194.8±37.7
	Fracture	–	–	487.0±184.4	402.1±58.9
	F/N**	–	–	1.97±0.33	2.08±0.26
	No. per group	–	–	13	11
8–9	Normal	325.9±25.3	242.3±25.1	221.9±39.7	197.9±61.8
	Fracture	392.5±38.0	393.8±57.3	451.5±77.6	410.0±169.3
	F/N**	1.20±0.06	1.62±0.12	2.05±0.22	2.04±0.03
	No. per group	5	5	15	9

* Osteogenesis = Sr^{85} uptake in cpm/mg ± SD.

** $F/N = \frac{\text{Activity in fractured bone}}{\text{Activity in normal bone}} \pm SD.$

Now it can be seen that in young rats the osteogenic activity increased in the fractured bone to reach a peak at 21 days. In older rats, the increase in relative activity was more marked and a plateau was reached between 21 and 28 days. The differences in activity in fractures between young and old animals are significant from day 14 onwards ($P = 0.001$ – Student's t test). When 6–7 month old rats were studied, the results were not significantly different from those obtained using 8–9 month old rats. On the basis of these observations osteogenesis was examined in fractures in 2 month old animals after 23 days, and in 6–7 month animals after 26 days in short-term studies.

Effects of Indomethacin treatment on fractures

The results of both short- and long-term studies are shown in Table 2. In all cases, treatment with either Indomethacin or diluent began 7 days before the bone defects were made. After 4 weeks of continuous treatment, there was little evidence of any effect of Indomethacin upon the normal skeletal elements (i.e. pelvis and tail vertebrae) in either young or old rats.

In the 2 month old rats, there was a drop in specific activity in vertebrae with fractures after Indomethacin treatment, but neither this depression nor the difference in F/N ratio was significant. In the case of 6–7 month old animals, the drug did cause a significant lowering of osteogenesis in the fractured bones ($P = 0.001$). This was also reflected in the F/N ratio. A second group of 6–7 month old rats had their treatment terminated on the day after producing the defect. Under these circumstances, no significant effect upon osteogenesis was observed 3 to 4 weeks later. Thus the inhibitory effect of the Indomethacin on osteogenesis was short-lived, and dependent on the continued presence of the drug. In a further experiment, 8–9 month old rats were used and were examined 14 days after creation of the defect, after 3 weeks treatment. In this case, although the drug had no effect upon osteogenesis in the normal vertebrae, there was a drop in the level of Sr^{85} retention in the pelvic bone ($P = 0.02$). In these animals too, there was significant impairment of osteogenesis at the fracture site (F/N: $P = 0.001$).

Six to 7 month old rats were given 9 weeks continuous treatment, and defects were examined when they were 56 days old. There was little effect upon the amount of osteogenesis in normal bone of the vertebrae and pelvis. Surprisingly, no effect was observed upon the level of Sr^{85} retention in the defect-containing bone compared with the normal bone. It will be noticed that the level of Sr^{85} uptake in these late defects was higher than in the earlier defects.

Histological examination was made of all defects. In control 3–4 week old defects there was

Table 2. Effect of Indomethacin on osteogenesis in the skeleton and in fractures

Age of rats (months)	Treatment*	Time of examination after fracture (days)	Osteogenesis (cpm Sr^{85}/mg) in: Pelvis	Vertebrae	Fracture	No. of rats	F/N ratio
2	Nil	23	816.50±60.92	450.25±56.51	757.86±124.72	12	1.66±0.20
2	Indo	23	817.86±91.75	427.46±60.82	686.18±113.18	12	1.61±0.17
6–7	Nil	26	516.30±93.89	194.78±37.72	402.13±58.90	11	2.08±0.26
6–7	Indo	26	512.46±152.46	200.49±49.34	306.03±44.83	9	1.56±0.18
6–7	Indo**	26	518.04±110.69	196.57±49.66	431.51±108.77	8	2.22±0.34
8–9	Nil	14	521.75±35.51	242.30±25.11	393.78±57.34	5	1.62±0.12
8–9	Indo	14	466.04±27.41	249.14±29.94	319.88±35.61	6	1.29±0.11
6–7	Nil	56	593.48±83.30	283.86±50.27	466.41±117.77	10	1.65±0.30
6–7	Indo	56	540.10±98.62	278.77±35.43	473.44±95.53	10	1.67±0.32

* Indomethacin given continuously p.o. daily starting at day –7 until death or ** until day +1 after fracture.

fibrous tissue in the space with some cartilaginous metaplasia, and a considerable amount of new bone growth into the defect from the endosteum. In all cases there was evidence of reorganisation of woven bone to form a lamellar bone plate. There were signs of periosteal new bone formation. The only difference seen between the defects in young and old animals was the extent of new bone formation, which was usually less in the latter. In the young animals that had been treated with Indomethacin the amount of fibrous tissue in the defect was increased, and there was slightly less new bone formation in the drill holes. In some animals there was evidence of remodelling to form lamellar bone, but not as much as in defects in control animals. In defects in the older, treated, animals there was obviously less new bone formation, and in some cases the new bone was extremely sparse. In these defects there was considerable fibrous tissue, with bone debris still to be seen amongst it.

In the long-term defects (56 days) in untreated rats there was a lot of new bone present, mostly of the lamellar type, and there was usually a complete bridge of new bone across the defect. The new bone surface was, however, usually below the surface of the old bone and had a thin covering of fibrous tissue. The defects in the treated rats were markedly different. In most cases, the defect was still filled with dense fibrous tissue, with areas of immature woven bone around the edges. Very few lesions had a bony plate, and where this was present, it was thin and incomplete.

Effect of Indomethacin upon osteogenesis in bone grafts

The early phase osteogenesis has been measured in 2 month old rats (after 23 days) and 6–7 month old rats (after 26 days), and the late phase new bone formation has been examined in old rats after 56 days. From the data presented in Table 3, it will be seen that the level of osteogenesis in the upper graft was always higher than that in the lower grafts. The results derived from the two types of graft have, therefore, been kept separate. The graft weights were all approximately the same for the age, at the time of grafting, and there was no difference between groups at the end of the experiment. It is clear from the results that the treatment of the recipient rats had little effect upon osteogenesis in grafts in young or old animals. In the long-term experiments using old animals there was no evidence of any resorption defect in the treated grafts and the Sr^{85} retention levels were not significantly lower. Thus, there is no evidence in these experiments that

Table 3. Effect of Indomethacin upon Sr^{85} retention in bone isografts

Treat-ment****	Age of host (months)	Time of examination of graft (days)	Osteogenesis* in:		Weight of:	
			Top**	Bottom***	Top**	Bottom***
Nil	2	23	1427.6±103.9	1075.7±180.2	13.5±3.2	28.1±10.0
Indo Day –7→21	2	23	1472.8±171.8	1146.6±213.7	13.6±4.3	29.2±12.9
Nil	6–7	26	1784.2±159.2	1390.42± 94.8	32.9±5.0	38.3± 3.7
Indo Day –7→28	6–7	26	1873.0±283.0	1389.95±134.9	35.4±5.0	38.7± 4.0
Nil	6–7	56	1352.1±322.4	890.5±115.2	20.1±4.8	32.8± 6.4
Indo Day –7→56	6–7	56	1211.5±211.2	815.8±152.6	18.6±4.4	35.2± 9.1

* Osteogenesis = Sr^{85} cpm/mg.
** Top = grafts from top part of ilium.
*** Bottom = grafts from bottom part of ilium.
**** Treatment as in Table 2.

Indomethacin treatment had any effect upon either resorption of bone from grafts or upon the osteogenesis occurring within them. Histologically there was no discernible difference between grafts from treated and control animals.

DISCUSSION

The experiments presented above have examined the effects of Indomethacin upon osteogenesis in two different circumstances. In the case of the bone isografts, the graft cells surviving transplantation provide most of the osteogenesis in the first 3 weeks, with cells from the host supplementing and eventually supplanting them later (Elves & Pratt 1975, Elves 1976a, b). Indomethacin proved to have no deleterious effects on graft-derived or host-derived osteogenesis, in these experiments.

The second situation we investigated was fracture repair. The model chosen for this study offers some advantages over those used by others. A drill hole through one cortical surface of a bone provides a lesion in the bone which is stable and immobile. It is also produced under visual control and so can be made consistently and reproducibly. Mobile fractures produce a large external callus which can vary in its extent depending upon factors such as degree of mobility, damage to blood vessels, apposition of fragments, etc. (Rhinelander & Baragry 1962, Rhinelander et al. 1968, Lindholm et al. 1969, Sarmiento et al. 1977). A model producing a variable fracture does not allow the degree of precision needed to obtain statistically meaningful results.

The experiments reported above have shown impairment of bone repair in mature rats treated with Indomethacin at a dose level of 4 mg/kg. In a pilot study of the effect of this drug upon drill hole defects in the rat femur, some impairment of healing was observed with a dose level of 2.8 mg/kg. Rø and his colleagues (1976) showed that an oral dose of 2 mg/kg of Indomethacin impaired healing of non-immobilised closed fractures in rat femora: Mineralisation of the callus was impaired and fibrous tissue was a prominent feature in fractures in treated rats. More recently, Allen and his colleagues (1980), using closed complete fractures of the radius and ulna, have shown that impairment of fracture repair occurred in rats receiving Indomethacin at dosages of 2 mg/kg and 4 mg/kg. The present results are in good agreement with these workers. Inhibitory effects upon the healing of fractures in the rat have also been shown for Aspirin (Allen et al. 1980) and Ibuprofen (Tornkrist & Lindholm 1980).

The effect of Indomethacin upon bone repair is at present difficult to understand, mainly due to a lack of clear understanding of the cellular basis of the process of bone healing. Although the histological sequence of events during this process is well-establised, the question which is still unanswered is, where do the cells giving rise to osteoblasts and osteocytes, and ultimately new bone, come from? There is evidence that at least some of the new bone formation seen in fractures is due to the activity of local periosteal cells (Ham 1930). Endosteal cells are also involved and in our model endosteal new bone formation is a constant feature (Melcher & Irving 1962, Kruse & Kelly 1974). The potentially osteogenic cells can also give rise to cartilage cells and the direction of differentiation they take depends upon factors such as blood flow and oxygen tension (Brighton et al. 1969, Brighton & Krebs 1972). High oxygen tension is said to delay healing of fractures (Brooks 1971) and may lead to delayed union (Laurnen & Kelly 1969). It is unlikely therefore that the effect of prostaglandin synthesis inhibitors is due to a change in blood supply to the fracture site as prostaglandins would tend to increase blood flow and oxygen tension.

In addition to the participation of local osteogenic cells in bone healing, there is also involvement of other mesenchymal cells with osteogenic potential which enter the damaged area (Tonna & Cronkite 1961, Tonna & Pentel 1972). These cells also have chondrogenic potential and are probably pluripotential cells (Tonna & Pentel 1972). We suggest that the drug is not affecting the local, established, osteogenic cells, such as will be present in the bone grafts. Tonna (1969) has shown that local osteogenic cells in old animals will, if stimulated by trauma, respond and give rise to cells which are as osteogenically "potent" as those in young animals. The defect probably lies in the development of external callus which does decline with age (Tonna & Cronkite 1962). This may be due to an age-related fall in the number of mesenchymal osteogenic progenitor cells in the stem cell pool. Hence, some impairment of the differentiation of these cells will have a more marked effect in old rats than in young animals. The two histological features of the defects in the Indomethacin-treated rats, which may be relevant in this context, are the reduced quantities of new bone formation and the increased amount of fibrous tissue which remains in the defect. The drug may be affecting the differentiation of the pluripotential cells, causing them to become fibroblastic rather than osteogenic. Further experiments are needed to test this hypothesis.

The role of prostaglandins in resorption of bone is well-established (Klein & Rainz 1970, Powles et al. 1973). There was some suggestion of a resorption defect in these experiments, as there was evidence of lack of remodelling of woven bone in the long-term fractures in treated rats. However, this action of Indomethacin cannot account for the impairment of strontium retention (i.e. osteogenesis) in these animals.

From the clinical point of view, these results, together with those obtained by others (Allen et al. 1980, Sudmann 1975, Rø et al. 1976), suggest that continued administration of Indomethacin to patients with fractures ought to be carefully considered. Our results indicate, however, that the effect of this drug on osteogenesis is short-lived and cessation of treatment soon after a fracture has occurred will allow normal healing to occur. So far there is only one report which suggests that human fracture repair is inhibited by Indomethacin (Sudmann & Hagen 1976). Whether these results and those reported elsewhere have any relevance to drug-induced progression of joint disease, as has been suggested (Rønningen et al. 1979), is not possible to decide. Our experiments and those of Sudmann (1975), and Allen and his co-workers (1980) were made using rats or rabbits. Clearly their relevance to the human situation remains to be clarified. In one study of the effects of non-steroidal anti-inflammatory drugs upon osteoarthritic femoral heads, there was no indication of drug-induced modification of new bone formation nor impaired healing of microfractures (Robinson 1980).

REFERENCES

Allen, H. L., Wase, A. & Bear, W. T. (1980) Indomethacin and Aspirin: Effect of non-steroidal anti-inflammatory agents on the rate of fracture repair in the rat. *Acta Orthop. Scand.* **51**, 595–600.

Allgower, M., Burri, C., Graffenried, P., Guber, U. F., Heim, U., Meng, J., Segmueller, G., Siegrist, J. & Studer, E. (1963) Quantitative analysis of the effect of anti-phlogistic substances in lower leg fractures. *Schweiz. Med. Wochenschr.* **93**, 565–567.

Brighton, C. T. & Krebs, A. G. (1972) Oxygen tension of healing of fractures in the rabbit. *J. Bone Joint Surg.* **54**-A, 323–332.

Brighton, C. T., Ray, K. D., Soble, L. W. & Kuettner, K. E. (1969) In vitro epiphyseal-plate growth in various oxygen tensions. *J. Bone Joint Surg.* **51**-A, 1383–1396.

Brooks, M. (1971) *The blood supply of bone. An approach to bone biology.* Appleton-Century-Crofts, New York.

Elves, M. W. (1974) An evaluation of the use of Strontium^{85} for the assessment of experimental bone grafts. *Acta Orthop. Scand.* **45**, 641–651.

Elves, M. W. (1976a) The effect of X-irradiation upon the fate of cancellous bone allografts in inbred rats. *Transplantation* **22**, 31–36.

Elves, M. W. (1976b) Newer knowledge of the immunology of bone and cartilage. *Clin. Orthop.* **120**, 232–259.

Elves, M. W. & Pratt, L. M. (1975) The pattern of new bone formation in isografts of bone. *Acta Orthop. Scand.* **46**, 549–560.

Ham, A. W. (1930) A histological study of the early phases of bone repair. *J. Bone Joint Surg.* **12**, 827–844.

Klein, D. C. & Raisz, L. G. (1970) Prostaglandins: stimulation of bone resorption in tissue culture. *Endocrinology* **86**, 1436–1440.

Kruse, R. L. & Kelly, P. J. (1974) Acceleration of fracture healing distal to a venous tourniquet. *J. Bone Joint Surg.* **56**-A, 730–739.

Laurnen, E. L. & Kelly, P. J. (1969) Blood flow, oxygen consumption, carbon dioxide production, and blood-calcium and pH changes in tibial fractures in dogs. *J. Bone Joint Surg.* **51**-A, 298–308.

Lindholm, R. V., Lindholm, T. S. & Toikkanen, S. (1969) Effect of forced inter-fragmental movements on the healing of tibial fractures in rats. *Acta Orthop. Scand.* **40**, 721–728.

Melcher, A. H. & Irving, J. T. (1962) The healing mechanism in artificially created circumscribed defects in the femora of albino rats. *J. Bone Joint Surg.* **44**-B, 928–936.

Powles, T. J., Clarke, S. A., Easty, D. M., Easty, G. C. & Neville, A. M. (1973) The inhibition by Aspirin and Indomethacin of osteolytic tumour deposits and hypercalcaemia in rats with Walker tumour and its possible application to human breast cancer. *Brit. J. Cancer* **28**, 316–321.

Rhinelander, F. W. & Baragry, R. A. (1962) Micro-angiography in bone healing: I. Undisplaced closed fractures. *J. Bone Joint Surg.* **44**-A, 1273–1298.

Rhinelander, F. W., Phillips, R. S., Steel, W. M. & Beer, J. C. (1968) Micro-angiography in bone healing: II. Displaced closed fractures. *J. Bone Joint Surg.* **50**-A, 643–662.

Rø, J., Sudmann, E. & Marton, P. F. (1976) Effect of Indomethacin on fracture healing in rats. *Acta Orthop. Scand.* **47**, 588–599.

Robinson, H. J. (1980) The effect of non-steroidal anti-inflammatory drugs on bone structure in osteoarthritis. *Trans. Orthop. Res. Soc.* **5**, 316.

Rønningen, H., Langeland, N. & Foss Hauge, M. (1979) Indometacin ved hofteleddartrose. *Tidsskr. Nor. Laegeforen.* **99**, 432–433.

Sarmiento, A., Schaeffer, J. F., Beckerman, L., Latta, L. L. & Enis, J. E. (1977) Fracture healing in rat femora as affected by functional weight bearing. *J. Bone Joint Surg.* **59**-A, 369–375.

Sudmann, E. (1975) Effect of indomethacin on bone remodelling in rabbit ear chambers. *Acta Orthop. Scand.*, Suppl. 160, 91–115.

Sudmann, E. & Hagen, T. (1976) Indomethacin-induced delayed fracture healing. *Arch. Orthop. Unfallchir.* **85**, 151–154.

Tonna, E. A. (1969) Skeletal aging and its effects on the osteogenetic potential. *Clin. Orthop.* 57–81.

Tonna, E. & Cronkite, E. P. (1961) Autoradiographic studies of cell proliferation in the periosteum of intact and fractured femora of mice utilizing DNA labeling with H^3-Thymidine. *Proc. Soc. Exp. Biol. Med.* **107**, 719–721.

Tonna, E. A. & Cronkite, E. P. (1962) Changes in the skeletal cell proliferative response to trauma concomitant with aging. *J. Bone Joint Surg.* **44**-A, 1557–1568.

Tonna, E. & Pentel, L. (1972) Chondrogenic cell formation via osteogenic cell progeny transformation. *Lab. Invest.* **27**, 418–426.

Tornkrist, H. & Lindholm, T. S. (1980) Effect of Ibuprofen on mass and composition of fracture callus and bone. *Scand. J. Rheumatol.* **9**, 167–171.

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