Effect of simvastatin on fracture healing—An experimental study*

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Left femur was osteotomized and fixed with K wire in 21 rabbits. One group was fed simvastatin (120 mg/kg body wt/day) orally, whereas another group without medication served as control. Both groups were assessed radiologically, morphologically, histologically and biomechanically at 4, 8 and 12 weeks. An analysis of various parameters of study showed that simvastatin treated group had improved bone healing at 4 and 8 weeks of follow up, however, the difference was not significant statistically at 12 weeks. So it is concluded that Simvastatin favourably hastened the process of fracture healing in the rabbits at earlier phases.

**Keywords:** Anti-resorptive factor, Fracture healing, Growth factors, Statin

Fracture healing has always been a challenging problem. Since the fracture takes its own course to heal, it is essential to discover factors which affect fracture healing favorably. Much work has been done in the past to evaluate the substances which may accelerate the rate of osteogenesis or have inhibitory action over fracture repair\textsuperscript{1,2}. Past attempts to hasten the fracture healing by various vitamins, hormones and other substances have not shown much promise\textsuperscript{3-5}.

In the recent past, a multitude of drugs or components with either anti-resorptive, anabolic or even combined activities have been identified and their effect is still under investigation\textsuperscript{6-8}. One such new experimental drug is simvastatin—a statin which has shown promising results in fracture healing in rodents\textsuperscript{9}. Recent studies have shown that statins may exert both antiresorptive as well as bone anabolic effects and thus, may enhance fracture healing\textsuperscript{10,11}.

In view of the recent developments, the present study was undertaken to observe the fracture healing process in experimental animals with simvastatin and to understand the role of this agent in the fracture repair process.

**Materials and Methods**
Healthy mature rabbits (n=21) obtained from animal house, Banaras Hindu University, irrespective of sex weighing 1.5 kg or more were selected. The animals were kept in standard steel cages one per cage. Animals had free access to water and food (Pallets; Raman Industries, Ramnagar, India). Simvastatin obtained from Stancare India, was given orally to the animals with surgically created transverse femoral midshaft fractures. Status of healing was observed clinically, radiologically, morphologically, histologically and biomechanically. The present experiments are covered under section of Prevention of Cruelty to Animals Act, 1960. The experiment was approved by the Institute Ethical Committee.

**Experimental design**—A total of 21 rabbits was anaesthetized separately by giving intra muscular 20 mg/kg of ketamine +0.4mg/kg of midazolam. Left lower limb was shaved and cleaned with chlorhexidine, spirit and betadine solution. Animal was placed in right lateral position on the operating table. Length of femur was measured from tip of greater trochanter to knee joint line. Mid shaft of femur was exposed through a lateral incision. A transverse osteotomy of mid shaft of femur was created using a giggly saw. Fractures were fixed by using a K-wire. Wound was closed by self dissolving vicryl sutures and sealed.
with healex spray. Post-operative intramuscular amikacin at dose of 20 mg/kg body wt. and ceftriaxone at dose of 40 mg/kg body wt. was given for 5 days. Each rabbit was given 25 mg of tramadol (im) for post operative pain relief. All animals were observed daily for any signs of wound infection.

Fifteen rabbits for consideration under simvastatin treated group were given the drug orally with the help of NG tube and disposable syringe (10 ml DISPO VAN) at daily dosage of (120 mg/kg body weight) so as to achieve a reasonable activity at fracture site (as determined by pioneer work of Skoglund et al\textsuperscript{12}) since it has a very high first pass metabolism in liver. The drug was started on post-operative day 1 and continued till sacrifice of animal.

**Radiological examination**—Rabbits were examined radiologically at 0, 4, 8 and 12 weeks by using a standard AP skiagram of both limbs. Fracture gap, bridging bone formation, degree of callus formation and fracture end sclerosis, if any, was observed.

**Gross examination at autopsy**—Rabbits were sacrificed by giving an overdose (i m) of ketamine and thiopentone. Intra-medullary wire was removed. Mobility at fracture site was tested. Specimen of bilateral femur was harvested. Soft tissue attachment was cleared as much as possible. The gross findings observed were type of callus, amount of callus, status of bony union and any abnormal mobility.

**Mechanical three-point bending test**—This was done at the Department of Metallurgical Engineering, IT, Banaras Hindu University, Varanasi using an INSTRON machine and a load cell of 2000 g. in compression mode. Three point bending test was performed under displacement control and fracture load was recorded with a load cell. The loading profile consisted of a uniform crosshead speed of 0.05 cm/sec. The diameter and cross sectional area of each femur and callus was measured prior to testing. Load and displacement were recorded. Bending stress, fracture load, displacement and bending stiffness were determined for healing left femur and for intact contra-lateral right femur in same animal. Values were expressed as percentage of mechanical strength of the fractured femur compared with that of intact, contra-lateral control femur.

**Histological examination**—Five and two rabbits each from simvastatin treated and control groups respectively were sacrificed at 4, 8, and 12 weeks intervals. The specimens for histological examination were first decalcified by keeping them in the decalcifying solution, and then they were dehydrated by passing them in increasing concentration of ethanol. The specimen subsequently was treated by xylol, mounted in liquid paraffin, cut into thin sections and were passed through the decreasing grades of alcohol before finally staining with haematoxylin and eosin. Thereafter the fracture callus was observed for degree of cellularity, vascularity, amount of callus, cartilage and bone matrix formation, woven bone and mature bone formation, medullary repair and any remodelling and cortical repair etc. The degree of fracture repair and healing was scored on a five degree scoring system (Rankit score) based on the observation of Allens et al\textsuperscript{1} As per this scoring system the Grade 4: Complete bony union (Fracture site bridged by well formed bony trabeculae); Grade 3: Less than complete bony union as evidenced by presence of small amount of cartilage in fracture callus; Grade 2: Complete cartilaginous union (Well formed plate of hyaline cartilage uniting the fragments); Grade 1: Incomplete cartilaginous union (as evidence by retention of fibrous elements in plate); and Grade 0: Pseudarthrosis formation or nonunion seen as incontrovertible cavity within cartilage plate between fracture fragments containing blood or other fluid and/or lined by low cuboidal mesothelia.

**Statistical analysis**—Data obtained for each set of reading were expressed by their mean and statistical significance was seen by unpaired Student’s \( t \) test. Level of significance was set at \( P = 0.05 \).

**Results**

The results were assessed by radiological examination, gross examination at autopsy (morphological examination), mechanical testing and histological examination. In all cases wound healed in 6-10 days and there was no need for suture removal as absorbable sutures were used for wound closure.

**Radiological examination**—In control group, at four weeks there was minimal to moderate amount of bridging callus seen with fracture line clearly visible in all the rabbits. In simvastatin treated group, there was mild to moderate amount of bridging callus present with fracture line visible in all the rabbits after four weeks of treatment. No fracture gap was observed. The amount of bridging bone callus appeared more than control group radiologically, however, no quantitative assessment was done. At eight weeks in control group, the amount of bridging callus formation increased in all the rabbits. There
was no fracture gap and fracture line was faintly or incompletely visible. Minimal fracture end sclerosis in two rabbits was seen. In simvastatin treated group, after eight weeks of treatment fair amount of well defined bridging callus was seen. Fracture line was disappearing in majority. Healing seemed to be better than control group. After twelve weeks, in control group fracture union and variable amount of remodeling was seen (Fig. 1), whereas in simvastatin treated group fracture united radiologically in all the rabbits. Remodelling and repair of medullary canal was observed better in this group. (Fig. 2). The bridging bone formation increased with time in both the groups, though the remodelling started earlier in simvastatin treated group.

*Morphological examination*—All the rabbits at the

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**Fig. 1**—X-ray photograph of the limb (control group) at 4, 8 and 12 weeks showing complete union.

**Fig. 2**—X-ray photograph of the limb (simvastatin treated group) at 4, 8 and 12 weeks showing complete union and remodeling starting at 8 weeks.
end of experiment were sacrificed as planned by giving lethal dose of anesthesia and bilateral femur were harvested and cleared free from soft tissue. Gross morphological changes at fracture site were observed (Fig. 3). At four weeks in control group, fracture callus was sufficient in amount and assumed colour of surrounding bone but was immature and soft. Abnormal mobility was present in one animal. In simvastatin treated group, minimal to sufficient amount of bridging immature callus was seen in all the rabbits. It was possible to insert a pin into the bridging tissue. Union was incomplete and mainly cartilaginous.

At eight weeks, amount of callus and maturity of callus increased in both the groups. Both the rabbits of control group showed fair amount of osseo-cartilaginous callus and union was nearly complete. All the rabbits of simvastatin treated group showed large amount of mature callus and union was mostly osseous. No abnormal mobility was observed in any of the samples. At twelve weeks, there was decrease in amount of callus in both groups compared with the amount of callus seen at eight weeks – probably due to maturation of callus and remodelling process.

Mechanical analysis—Bilateral femurs were harvested from each rabbit after sacrificing them at four, eight and twelve weeks, respectively. It was observed that at four weeks, simvastatin treated group had a significantly increased strength as compared to control group, but stiffness was not altered in comparison to control group. At eight weeks the stiffness and strength increased more in simvastatin group as compared to control group, whereas at twelve weeks, there was no significant difference in strength and stiffness among the two groups. At this time the specimens had approximately 70% of the strength and 90% of the stiffness of contralateral femora (Figs 4, 5).

Histological analysis—At four weeks, simvastatin treated group showed much higher stages of healing and lesser amount of fibrous tissue. Woven bone formation was more as compared to control. Similar observations were made at eight weeks (Figs 6 a, b). At twelve weeks no statistically significant difference among simvastatin treated and control groups with respect to histological evidence of healing was observed (Fig. 7).

Discussion

In 1967, Urist et al13 have isolated a substrate, bone morphogenetic protein (BMP), which is a transforming growth factor and has been shown to cause osteoinduction and, thus, enhances fracture healing. Role of many other growth factors and hormones

Fig. 3—Gross Specimens of control group (a); and simvastatin treated group (b) at 12 weeks showing callus formation & complete union
were evaluated in the process of fracture healing, however, the past attempts to hasten fracture healing by various hormones and other such substances have not shown much promise.

Many drugs with supposed anabolic effects over bone healing are now under evaluation. One such class of drugs is statins—which were traditionally used as lipid lowering agents. Simvastatin is a statin. It is a reversible competitive 3-hydroxy-3 methyl glutaryl coenzymeA (HMGCoA) reductase inhibitor, commonly used as a lipid lowering agent. It has been found that statins in addition to their lipid lowering action also have many pleotrophic properties. Statins have been shown to possess both bone anabolic and antiresorptive effects and also increases the expression of BMP-2 gene in osteoblasts and thus stimulate new bone formation. A study by Skoglund et al. has demonstrated improved fracture healing in mice treated with simvastatin. Consequently, in experimental studies, statins have shown promising results.

The objective of the present study was to further...
elucidate the role of simvastatin—which has been claimed to enhance bone healing in fracture healing process, with a view to understand the role of these agents in the fracture repair process. Our results showed that simvastatin treated animals had significant radiographic, morphological, biomechanical and histological differences in fracture healing at four and eight weeks as compared to control group, however, these difference were not observed at twelve weeks. Our results are in agreement with the earlier observation of Skoglund et al.12. The results also confirmed the role of simvastatin in fracture and bone healing as an anabolic agent. On similar line increase in bone formation and strength in rats treated with simvastatin has been reported9,16. It has been opined that the intracellular effects of statin may be similar to the mechanism of action of aminobisphosphonates i.e. by blocking the mevalonate pathway, statins may exert antiresorptive effect. In addition, statin may exert bone anabolic effects by increasing the synthesis of BMP-2. Statins have also been shown to stimulate osteoblast proliferation in organ culture of neonatal murine calvaria10. This opens a new avenue in treatment of fracture healing, but further studies are needed to consolidate their anabolic effect. Also studies are needed to find out the exact mechanism and effect of simvastatin in fracture healing. Similarly, other statins could be evaluated to establish the role of various statins and their place in bone healing process.

The dose of simvastatin used in our study was high as statins have a very high first pass metabolism in liver. The present study has opened a new possibility of using statins at doses which improve fracture healing without other side effects, probably by developing statins specific to bone and not liver, so that effective treatment could be achieved at substantially low doses of simvastatin.

Though the number of animals in each group in our study was small, on the basis of findings, it can be concluded that simvastatin (a statin) favourably hastens the process of bone healing, at least in early and intermediate stages of healing though the final outcome may not be altered. Caution must be exercised while extrapolating these data on human beings as biological response of these drugs in humans may vary. Also, further investigations are required to establish the anabolic role of statins in fracture healing and using them in human beings to enhance fracture healing.

References
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