Mouse Bone Collagenase

The Effect of Heparin on the Amount of Enzyme Released in Tissue Culture and on the Activity of the Enzyme

Seizaburo Sakamoto, Paul Goldhaber, and Melvin J. Glimcher

Harvard School of Dental Medicine and Department of Orthopedic Surgery
Harvard Medical School, Children's Hospital Medical Center,
Boston, Massachusetts

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The amount of mouse bone collagenase recovered in the tissue culture medium of bone cultured in vitro was increased by the addition of heparin at an optimal concentration of approximately 50 units/ml of tissue culture medium. Dextran sulfate and Treburon (a synthetic polysaccharide-sulfuric ester) which are structurally and chemically related to heparin were as effective as heparin in increasing the amount of mouse bone collagenase recovered in the tissue culture medium. In addition to stimulating the synthesis and/or release of mouse bone collagenase, heparin was also found to increase the specific activity of both crude and purified preparations of the enzyme when assayed using collagen as the substrate, but showed no enhancement of enzyme activity when assayed using collagen in solution as the substrate. Dextran sulfate was as effective as heparin in increasing the activity of the enzyme using collagen in the solid state as a substrate. Neither heparin or dextran sulfate enhanced the activity of Clostridium histolyticum collagenase. For the first time, a purified tissue collagenase has been shown to both degrade and solubilize undenatured, insoluble tissue collagen at 37°C. Moreover, since this action was markedly enhanced by the addition of heparin, it suggests that heparin and similar substances may play an important role in the regulation of collagen degradation during the remodeling of collagenous tissues in vivo.

Key words: Bone — Collagenase — Heparin — Collagen.

For reprints: Melvin J. Glimcher, M.D., Department of Orthopedic Surgery, Children's Hospital Medical Center, 300 Longwood Avenue, Boston, Massachusetts 02115, U.S.A.

Introduction

It has been previously found that the addition of small amounts of heparin to the tissue culture medium of bone explants enhanced the bone resorption produced by suboptimal concentrations of parathyroid hormone extract and other substances [2], and that this was accompanied by an increase in the collagenolytic activity released from the explants [6]. The role of heparin as a "cofactor" enhancing bone resorption was also suggested by the studies of Griffith et al. (1965) and Jaffe and Wilson (1965) who showed that osteoporosis developed in patients given large amounts of heparin in the treatment of clotting disorders. More recently, Jowsey et al. (1970) demonstrated that both normal and thyroparathyroidectomized animals showed a significant rise in their serum calcium levels after heparin administration, suggesting that the mobilization of calcium from the skeleton by heparin was a direct effect and was not secondary to increased thyroid or parathyroid gland activity.

Unpublished observations of M. Shimizu in our laboratories which showed that the addition of small amounts of heparin to collagen gels on which mouse bone or skin were cultured in vitro caused an increase of gel lysis, led to the finding that increased amounts of mouse bone collagenase could be recovered from the tissue culture media of bone explants when heparin was added to the medium during culture [15, 14].

In the present study the effects of heparin and structurally and chemically related compounds in enhancing the synthesis and/or release of mouse bone collagenase by bone explants in tissue culture and its direct effect on the enzyme activity of crude and purified preparations of mouse bone collagenase are presented.

Materials and Methods

Tissue Culture of Bone

The tibiae of 5-day-old Swiss albino mice of the Webster strain were cultured in mammalian Tyrode solution containing amino acids, vitamins, L-glutamine, penicillin and streptomycin in a roller tube as previously described. Commercial sodium heparin solution (Eli Lilly & Co., Indianapolis, Indiana) was diluted with Tyrode solution and added to the culture. Heparin