The Degradation of Mature Collagen: A Laboratory Study

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The use of triamcinolone (9-a-fluorohydrocortisone acetonide) has been advocated in the treatment of hypertrophic scars and keloids. A controlled clinical series of the intralesional use of this steroid revealed that approximately 90 per cent of all hypertrophic scars and keloids respond.\(^1\) Two burn patients in the reported clinical series were biopsied at weekly intervals following intralesional triamcinolone, and the analysis of the soluble collagen fraction of the mature scar revealed a time dependent increase in solubility. To try and determine the biologic effect of the steroid therapy, a series of laboratory experiments were undertaken to determine both the \textit{in vivo} and \textit{in vitro} effect of this steroid upon mature collagen.

**Method**

For the \textit{in vivo} experiments 90 Sprague-Dawley rats were used, weighing about 200 gm. each. The first 10 served as controls. Their tails were removed, skinned, minced, and the soluble collagen fractions were extracted\(^2\) and hydroxyproline content analyzed.\(^3\) The remaining 80 animals were divided into two groups. In group A each rat tail was injected with 2.5 mg. of triamcinolone acetonide and in group B each tail was injected with 12.5 mg. of hydrocortisone, and equivalent dose. Then animals were sacrificed at weekly intervals over a 4-week period of time. The tails were removed and the soluble fractions of collagen determined.

An \textit{in vitro} study was then designed to determine whether collagenolytic activity per se was exerted by either triamcinolone or hydrocortisone. Twenty-four Sprague-Dawley rats were divided into six groups. The tails were removed, skinned, weighed, minced and placed in beakers. They were then incubated at 5°C for 48 hr. with the following agents: group 1, 2.5 mg. of triamcinolone was added; group 2, 7.5 mg. of hydrocortisone was added; group 3 was the absolute control and nothing was added; group 4, 0.1 mg. of purified bacterial collagenase was added; group 5, both triamcinolone acetonide and collagenase were added; group 6, both collagenase and hydrocortisone were added. Following incubation, the soluble collagen fractions were determined utilizing lactated Ringer's solution as a physiologic extractant.

Lactated Ringer's solution was chosen as the extractant instead of the molar salt and citrate buffer solutions used in the \textit{in vivo} experiment because lactated Ringer's solution, when adjusted to a pH of 7.4, closely approximated extracellular fluid.

**Results**

The \textit{in vivo} studies reveal a consistent time dependent increase in the soluble collagen fractions contained in the mature collagen, and the peak of the solubility occurs between the 2nd and 3rd week, which corresponds to the softening seen clinically when triamcinolone is injected intralesionally (fig. 1). There are some interesting differences in triamcinolone and hydrocortisone treated animals. The yield of salt soluble collagen in the triamcinolone group is twice that of the hydrocortisone group, but the greatest yield of all is in the citrate soluble fraction of the hydrocortisone treated group. In the \textit{in vivo} study, triamcinolone and hydrocortisone alone do not produce a significant increase in the yield of soluble collagen over that of the control when a physiologic extractant was used. Bacterial collagenase did markedly increase the amount of soluble collagen and the combined addition of triamcinolone and collagenase produce a signifi-
cantly greater amount of physiologically soluble collagen than did collagenase alone. The combined use of hydrocortisone and collagenase did increase the yield but was less effective than triamcinolone at the physiologic level (fig. 2).

Discussion

The data from these studies show that triamcinolone, and to a lesser extent hydrocortisone, affect the solubility of collagen. The salt and citrate soluble fractions are increased in vivo to a greater or lesser extent, depending on the agent. Although the in vivo results might suggest that these agents are exerting a collagenase-like activity, the in vitro study showed that they are not collagenases per se, but catalyze or enhance the activity of collagenase to a significant extent, and triamcinolone is the more effective of the steroids tested at the physiologic level.

Summary

Triamcinolone acetonide has proved to be clinically effective in the treatment of hypertrophic scar: keloid and scar contracture. In vitro and in vivo laboratory studies were undertaken to try and determine the method of action of triamcinolone acetonide upon mature collagen. The in vivo use of steroids injected in the rat tail reveals a time dependent increase in the solubility of mature collagen. In vitro studies show that regardless of chemical configuration the steroids tested did not affect the solubility of mature collagen. When combined in vitro, triamcinolone and bacterial collagenase together produced significantly more Ringer's soluble collagen than did collagenase alone.

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FIG. 1. The differential increase in the soluble collagen fractions following the in vivo use of hydrocortisone and triamcinolone.

FIG. 2. The in vitro use of steroids demonstrating enhancement of the action of collagenase but no collagenolytic activity with either triamcinolone or hydrocortisone.
REFERENCES

