Effects of Beta-Carotene, Vitamin A, and Glucocorticoids on Collagen Synthesis in Wounds[1] (35698)

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Vitamin A antagonizes the inhibitory effects of glucocorticoids upon repair of wounds (1, 2). Large doses of glucocorticoids deplete stores of vitamin A in the liver and impair the conversion of beta-carotene into vitamin A (3). If glucocorticoids inhibit the conversion of beta-carotene into vitamin A, it should then follow that beta-carotene will not reverse the effect of glucocorticoid on inhibition of healing. An experimental trial was set up to investigate whether beta-carotene administered in high systemic doses could mimic the antagonistic effect of vitamin A on the action of glucocorticoids. The degree of healing was assessed by measuring the tensile strength and hydroxyproline content of the wound. Hydroxyproline is only found in collagen.

Materials and Methods. Adult male Sprague-Dawley rats, weighing 350 to 400 g, were divided into four groups: Group A (controls), five rats were given no treatment. (Sham injections have no effect upon healing.) Group B (corticoid alone), five rats received an injection of 8 mg of Depo-Medrol (methylprednisolone acetate, purchased from the Upjohn Company, Kalamazoo, Mich.) subcutaneously and 0.1 ml of sterile peanut oil on days 1, 3, and 5 after wounding. Methylprednisolone acetate is a slowly-released, long-acting glucocorticoid preparation which has a constant anti-inflammatory action for 10 days or more. Group C (corticoids + vitamin A), five rats received 8 mg of prednisolone acetate and three injections of 0.1 ml of Aquasol A (equiv to 5000 IU of vitamin A palmitate, purchased from the U.S. Vitamin and Pharmaceutical Co., New York, N.Y.) intramuscularly on days 1, 3, and 5 after wounding. Group D (corticoids + beta-carotene), five rats received one injection of 8 mg of prednisolone acetate and three injections (2 mg each) of beta-carotene (equiv to 3330 IU of vitamin A) dissolved in 0.1 ml of sterile peanut oil intramuscularly on days 1, 3, and 5.

A standard incision 6 cm long was made on the shaved back of each rat 1.5 cm away from and parallel to the spinal cord. The wound was closed with a running stainless steel suture, penetrating the skin at intervals of 0.5 cm. The wounds were left uncovered.

The abdomen was also shaved and a full thickness skin incision 1 cm long was made. One Ivalon polyvinyl Intermedic (Unipoint Laboratories, High Point, North Carolina) sponge disc was inserted under the subcutaneous muscle on each side of the incision, and the incision was closed with stainless steel sutures.

On day 8 after surgery, the rats were weighed, and the tensile strength of the wound was measured with a Sandblom-Muren tensiometer (2). The sponge discs were removed and their wet and dry weights were recorded. The dried implants were hydrolyzed in 6 *N* HCl at 110° for 24 hr, filtered, dried, and dissolved in water. The hydroxyproline content was determined with a Technicon AutoAnalyzer (4).

Results. Glucocorticoids reduced the mean tensile strength of the wounds (Table I). Vitamin A and beta-carotene were equally effective in reversing this retardation and both restored the wound tensile strength to

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TABLE I. Results of Treatment with Vitamin A, Beta-Carotene and Glucocorticoids.

Group	Treatment	No. of animals	Tensile strength	No. of animals	Total hydroxyproline (μg $\pm$ SE)	Hydroxyproline/sponge (μg/mg)
A	Control	9	342 $\pm$ 29	6	516 $\pm$ 28	10.9
B	Corticoid alone (prednisolone acetate, 8 mg)	8	225 $\pm$ 31[a]	8	296 $\pm$ 8[a]	7.9[a]
C	Corticoid + vitamin A (prednisolone acetate, 8 mg; total vitamin A, 15,000 IU)	14	324 $\pm$ 27[b]	9	358 $\pm$ 22[a/b]	9.1[b]
D	Corticoid + β-carotene (prednisolone acetate, 8 mg; total β-carotene, 6 mg)	15	328 $\pm$ 23[b]	7	366 $\pm$ 24[a/b]	9.6[a]

[a] $p < .05$ for control vs treated.
[b] $p < .05$ for group B and groups C and D.

normal. Glucocorticoids reduced the total amount and the weight of hydroxyproline per unit weight of the sponge in the implants. Vitamin A and beta-carotene were identical in their ability to reverse this retardation, but they were unable to restore the total amount of collagen to normal. The rats in the three groups that received glucocorticoids lost similar amounts of weight (about 60 g) while the control rats gained an average of 15 g during the 7-day period.

Discussion. Amtruda *et al.* (5) reported that after glucocorticoid therapy is withdrawn, the amount of vitamin A in plasma increases significantly. Their explanation for this was that beta-carotene accumulates during glucocorticoid therapy, presumably because corticoids block its conversion to vitamin A. They reasoned that after cessation of therapy, beta-carotene is rapidly converted into vitamin A. Beta-Carotene is assumed to be biologically inactive in mammals, although it serves as a precursor for vitamin A, the biologically active compound. Our results indicate that either the conversion of beta-carotene into vitamin A is not blocked by high exogenous doses of glucocorticoids or that beta-carotene is actually biologically active and can reverse the retarding effects of glucocorticoid upon collagen synthesis and repair.

Both vitamin A and beta-carotene restored steroid-retarded wound strength to normal. Although they did not restore the accumulation of collagen to normal levels, the amount was increased as compared to rats treated with glucocorticoid alone.

There is a direct relationship between collagen accumulation and wound tensile strength (6) but Freihoffer *et al.* (7) pointed out that the degree of extracellular cross-linking is the critical factor in the relationship. A possible explanation for the finding that rats receiving a combination of beta-carotene or vitamin A and glucocorticoids accumulate less collagen than normal, but have the same wound strength, may be that there is more cross-linking in the collagen. Alternatively, it is possible that connective tissue components other than collagen are important in wound strength. Still another possibility is related to the degree of inflammation which has a direct relationship with the quantity of collagen synthesized in a granuloma. Glucocorticoids inhibit inflammation, and the quantity of vitamin A used in this experiment did not restore inflammation to normal in the implants (unpublished re-

sults). The number of inflammatory cells indirectly involved in giving optimum collagen accumulation in a wound may be less than the number needed to provide similar conditions in a polyvinyl sponge. The degree to which vitamin A or beta-carotene restores inflammation may be sufficient to restore the synthesis of connective tissue in the small volume of an incised wound, but may not suffice to restore synthesis in the relatively large volume of a sponge granuloma.

Summary. Beta-Carotene, a precursor of vitamin A, was as effective as vitamin A itself in reversing the retarding effects of glucocorticoids on the tensile strength of incised wounds and on the accumulation of collagen in sponge granulomas.

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