Biochemical Pharmacology. Vol. 16, pp. 575–578. Pergamon Press Ltd. 1967. Printed in Great Britain

EFFECT OF VITAMIN K ON CONNECTIVE TISSUE METABOLISM

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(*Received* 15 *September* 1966; *accepted* 3 *November* 1966)

Abstract—Radiosulphate incorporation into calf cartilage slices has been significantly inhibited by vitamins K_1 and K_3 respectively. In rats the uptake of intraperitoneal radiosulphate into costal cartilage has been strongly reduced by vitamin K treatment. The total hydroxyproline, hexosamine, and nitrogen content of inflamed granuloma tissue has been considerably reduced by treatment with vitamins K, while the cutaneous concentration of these components remained unchanged.

AS SHOWN earlier, the development of granuloma tissue could be strongly inhibited by vitamins K_1 (phylloquinone) and K_3 (menadione) in chronic inflammatory tests.[1, 2] Vitamins K have exerted no influence on acute experimental inflammation (various paw oedemas). Their anti-inflammatory action could be demonstrated only in chronic tests, in the late stage of inflammation associated with granuloma tissue development.

Anti-inflammatory glucocorticoids are known to inhibit hydroxyproline and hexosamine synthesis in inflamed skin and granuloma tissue,[3, 4] as well as the incorporation of inorganic sulphate into sulphated mucopolysaccarides.[5, 6]

In order to analyse the anti-inflammatory action of vitamins K, their effect on the synthesis of connective tissue components has been studied under normal and inflammed conditions. For the basis of comparison prednisolon and phenylbutazon were used.

MATERIALS AND METHODS

Vitamin K_1, was used for *in vivo* experiments as an emulsion, for *in vitro* studies dissolved in dimethylformamide. Vitamin K_3 was used as menadione sodiumbisulphite *in vitro*, and as a fine suspension of menadione prepared with tween-80 and carboxymethylcellulose *in vivo*.

The incorporation of inorganic sulphur into cartilage tissue was investigated by the method of Whitehouse and Boström.[6] In case of *in vitro* studies, after preincubation for 3 hr, calf cartilage slices corresponding to 100 mg wet weight were incubated at 37° in Krebs–Ringer's phosphate medium containing $Na_2{}^{35}SO_4$, in oxygen atmosphere. After washing and digestion with pepsine, radioactivity of the cartilage slices was measured by liquid scintillation. *In vivo* male Wistar rats of 150–180 g were given intraperitoneally 3 mc/kg carrier-free $Na_2{}^{35}SO_4$ simultaneously with the substance to be tested. Four animals were used for each dose. Twenty-four hours later the animals were killed with ether, the costal cartilage was cleaned, dried at room

temperature and measured. Fifty to eighty milligrammes of cartilage tissue was dissolved in concentrated nitric acid in a sealed tube at 96°; activity of the obtained aliquot was measured by liquid scintillation.

In studies concerned with the influence of the drug on the chemical composition of the connective tissue, adult male Wistar rats were used. Cotton-wool pellets of 18–20 mg were implanted subcutaneously on both sides of each animal under Nembutal anaesthesia. Five days after the operation one group of the animals was killed, a piece of standard size was cut out of the depilated skin and the developed granuloma tissue was weighed after cleaning and removal of the cotton-wool pellet (wet weight). The respective hexosamine, hydroxyproline and total nitrogen contents of the skin and the granuloma tissue were determined. The remaining animals were treated subcutaneously with vitamins K_1 and K_3 respectively, as well as with prednisolone once daily, while the control group was given the solvent. After five days of treatment the animals were killed, the piece of skin cut out as described above and the developed granuloma tissue were weighed, and hydrolysed for 8 hr at 100° in 4 N HCl/100 mg/ml/ in a sealed tube. After neutralization the respective hydroxyproline,[7] hexosamine,[8] and total nitrogen contents were measured.[9]

RESULTS

In vitro, both vitamin K_1 and K_3 similarly to the well-known effect of prednisolone, inhibited significantly the incorporation of inorganic sulphate into the calf cartilage slices (Table 1).

TABLE 1. INHIBITION OF RADIOSULPHATE (^{35}S) INCORPORATION INTO CARTILAGE SLICES

Compound	Conc. (M)	No. of exp.	counts/min/100mg ± SEM	% of controls
Controls	—	16	40740 ± 3120	100
Vitamin K_1	7×10^{-4}	4	26260 ± 1840	64
Vitamin K_3	1×10^{-3}	12	20560 ± 820	51
Prednisolone	1×10^{-3}	16	25730 ± 1630	63

TABLE 2. INHIBITION OF RADIOSULPHATE (^{35}S) INCORPORATION INTO COSTAL CARTILAGE OF RAT *in vivo*

Compound	No. of Rat	Dose mg/kg i.p.	counts/min/100mg ± SEM	% of control
Controls	5	—	61712 ± 11725	100
Vitamin K_1	4	3·5	12710 ± 254	21
Vitamin K_3	4	3·5	7595 ± 152	12
Prednisolone	4	3·5	13510 ± 811	22
Phenylbutazone	4	20·0	24930 ± 499	40

In vivo, too, both vitamins K have strongly inhibited the incorporation of inorganic sulphate into costal cartilage. With regards to potency there was no significant difference between the two vitamins K, their effect was similar to that of prednisolone. Even in much greater doses, butazolidine has brought about slighter inhibition

(Table 2). *In vivo* both prednisolone and vitamins K have produced stronger inhibition in smaller doses than might have been expected from the *in vitro* results.

Effect on granuloma tissue weight, furthermore on components of cutaneous and granulomatous connective tissue is illustrated in Table 3. Granuloma development and granuloma nitrogen content have been inhibited strongly by vitamin K_3 and

TABLE 3. EFFECT ON THE CHEMICAL COMPONENTS OF CONNECTIVE TISSUE

Treatment	Dose mg/ animal day	Nitrogen (mg)	Nitrogen* increase (%)	Hydroxy-proline (μg)	R_{HI}†	Hexos-amine (μg)	R_{He}†
			Granuloma Tissue				
Controls	—	4·06	137	300 ± 26‡	74	152 ± 19	38
Vitamin K_1	2	2·80	63	250 ± 24	89	101 ± 8	36
Vitamin K_3	2	1·98	15	244 ± 50	123	82 ± 6	42
Prednisolone	1	2·01	16	232 ± 16	116	71 ± 5	35
			Skin				
Controls	—	5·83		926 ± 67	159	214 ± 25	37
Vitamin K_1	2	5·70		1076 ± 62	188	207 ± 8	36
Vitamin K_3	2	6·42		1001 ± 71	156	249 ± 25	39
Prednisolone	1	5·83		1147 ± 84	197	232 ± 19	40

* = Compared to granuloma nitrogen (1·76 mg) prior to treatment.
† = μg hydroxyprolone (hexosamine) per mg nitrogen.
‡ = Average of four animals.

prednisolone, in a lesser degree by vitamin K_1. Granuloma hydroxyproline content has been reduced by all the three drugs. Hydroxyproline calculated for nitrogen has increased significantly under the effect of vitamin K_3 and of prednisolone respectively. In the course of treatment the hexosamine content of granuloma has been reduced in the same proportion as the nitrogen content. The applied treatments have not changed cutaneous nitrogen, hexosamine or hydroxyproline concentrations.

DISCUSSION

On the evidence of Whitehouse's studies, anti-inflammatory agents inhibit the sulphatation of mucopolysaccharides in the connective tissues. The biochemical basis of this inhibition lies in the uncoupling effect of anti-inflammatory agents on oxydative phosphorylation. In their investigations with naphthoquinones, Chen and Dallam[10] found that unlike phylloquinone, menadione exhibited a strong uncoupling action. In the present experiments both quinones exerted on equal inhibition on the incorporation of inorganic sulphate into cartilage tissue, *in vitro* and *in vivo* alike. Our results suggest that the anti-inflammatory effect of vitamins K and their inhibitory action on the uptake of inorganic sulphate are related, while this inhibitory action cannot be explained by the uncoupling effect.

Upon the use of vitamins K and prednisolone, the total nitrogen, hydroxyproline, and hexosamine content of the granuloma tissue was strongly reduced. The increased ratio of hydroxyproline to nitrogen may be explained by non-callogenous nitrogen having been also strongly reduced as a result of treatment. The synthesis of cutaneous

connective tissue components has not been influenced by the tested compounds. This observation confirms the findings of Kowalewski[11] who did not find any change of cutaneous hydroxyproline and hexosamine levels upon cortisone treatment. The inhibitory action of both prednisolone and vitamins K on connective tissue development may be noted in inflamed tissue in the first place.

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