COMBINED EFFECT OF HYDROCORTISONE AND SOME WATER-SOLUBLE VITAMINS ON THE BARRIER FUNCTION OF THE INFLAMMATORY FOCUS

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No reference could be found in the literature in which an accurate quantitative experimental or clinical analysis was made of the combined treatment of inflammation with hormones and vitamins. Yet such an analysis is essential before a rational choice can be made of optimal combinations of hormones and vitamins in rational doses.

The object of the present investigation was to study the anti-inflammatory effect of various doses of hydrocortisone, ascorbic acid, and vitamins B_1, B_2, B_6, B_{12}, and Bc, when administered separately, and then to determine the anti-inflammatory activity of combinations of hydrocortisone with each of these vitamins in turn, in doses of equal efficacy.

EXPERIMENTAL METHOD

Experiments were carried out by the method of Islamov and Mednik [1]. Albino mice weighing 22-25 g received a subcutaneous injection of 0.05 ml of purified turpentine, and 24 h later a 1% solution of strychnine nitrate was injected into the focus of inflammation by means of a specially constructed microinjection syringe. The dose of strychnine was 2 μg/g body weight (the total volume of the injected solution was about 0.005 ml). The dose of strychnine used corresponded to LD_{100} for intact animals by the subcutaneous route.

In the untreated mice the focus of inflammation played the role of a reliable barrier obstructing the absorption of the injected strychnine into the blood stream, thus preventing death of about 95% of the animals.

The administration of anti-inflammatory substances after the injection of turpentine prevented the development of an inflammatory focus, weakened its barrier properties, and increased the intensity of the toxic effects parallel to the development of the anti-inflammatory effects.

This technique was used to test the effect on the development of the inflammatory focus of hydrocortisone in doses of between 0.005 and 150 μg/g and the following vitamins: C in doses of 0.05-150 μg/g, B_1 in doses of 0.1-50 μg/g, B_2 in doses of 0.01-40 μg/g, B_6 in doses of 0.05-80 μg/g, Bc in doses of 0.05-60 μg/g, and B_{12} in doses of 0.005-0.5 μg/g.

The hydrocortisone and vitamin were injected subcutaneously 30 min before injection of the turpentine and 6 and 12 h thereafter. Riboflavine and folic acid were given as a suspension in 2% starch mucilage and the remaining vitamins in 0.85% NaCl solution. Each dose was tested on not less than 20 mice.

For studying the combined anti-inflammatory effect of hydrocortisone and vitamin, the half-sums of their equi-effective doses were used, i.e., doses which, when administered after injection of strychnine, resulted in the death of equal numbers of animals. In this way the character of the combined action could be judged, i.e., the presence of additative synergism, potentiation, or antagonism between the hydrocortisone and the corresponding vitamins could be detected.

EXPERIMENTAL RESULTS

The relationship between the anti-inflammatory action of the tested substances and their doses is shown in Figs. 1 and 2. The curves showing the relationship between the anti-inflammatory effect of hydrocortisone and vitamins B, B_1, and B_6 and their doses within the limits of the investigated range (see Fig. 1) approximately followed the normal distribution curves. The highest peaks were shown by the curves of vitamin B_6 and hydrocortisone. The anti-inflammatory activity of vitamins C and B_1 was less marked.

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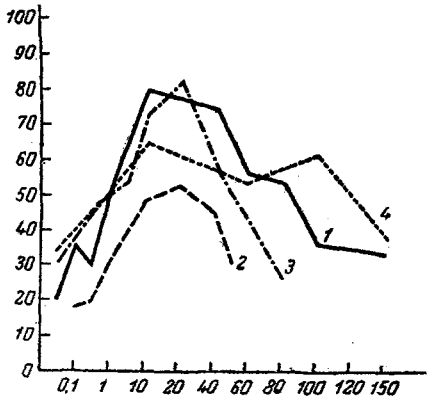

Fig. 1. Anti-inflammatory action of various doses of hydrocortisone and vitamins B_1, B_6, and C (I). Here and in Fig. 2: along the axis of abscissas—doses (in μg per g); along the axis of ordinates—mortality of mice (in %). 1) Hydrocortisone; 2) Vitamin B_1; 3) Vitamin B_6; 4) Vitamin C.

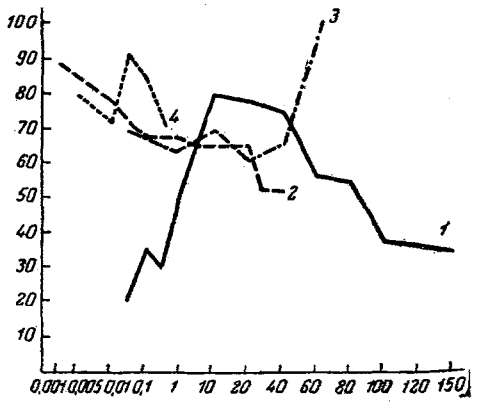

Fig. 2. Anti-inflammatory action of various doses of hydrocortisone and vitamins B_2, Bc, and B_{12}. 1) Hydrocortisone; 2) Vitamin B_2; 3) Vitamin Bc; 4) Vitamin B_{12}.

With vitamins B_2, Bc, and B_{12} (Fig. 2) no such relationship was observed within the dose range tested.

In the case of vitamin B_2 the anti-inflammatory activity, which was strong when minimal doses were used, gradually diminished as the dose was increased. The folic acid curve was a mirror image of the hydrocortisone curves and, finally, the vitamin B_{12} curve remained within the comparatively narrow limits of maximal anti-inflammatory activity.

The results of the experiments to study the effect of hormones and vitamins in combination on the focus of turpentine inflammation are given in the table.

The table shows that if comparatively small doses of hydrocortisone and vitamin B, giving a moderate anti-inflammatory effect and resulting in death of 20-30% of the animals from strychnine poisoning, were combined, marked potentiation of their effect was observed. The half-sums of these doses led to the death of 80% of the animals from strychnine.

With approximation to doses of hydrocortisone and vitamin B_1 giving a more marked anti-inflammatory effect (50% mortality from strychnine injected into the inflammatory focus), their potentiating interaction disappeared and a tendency toward antagonism was observed (the difference was not statistically significant). For instance, the half-sum of the doses of these substances which separately led to a mortality of 50 and 48% respectively from strychnine poisoning, now gave a mortality of only 40%. There was an apparent limiting inhibition of the biological mechanism concerned in the development of the anti-inflammatory effect of the components of this combination.

During the combined action of hydrocortisone and ascorbic acid, the pattern revealed in the experiments with hydrocortisone and vitamin B_1 was repeated.

As a result of the combination of half-doses of hydrocortisone and vitamin B_6, giving an anti-inflammatory effect associated with a mortality of up to 50% inclusive, a statistically significant potentiation of their effect was observed. With a combination of half-doses of these substances giving an anti-inflammatory effect close to the upper limit (80-84% mortality from strychnine), additive synergism was observed.

Obvious antagonism was observed in the experiments with the combined administration of a half-sum of doses of hydrocrotisone and vitamin B_2, which when given separately led to a mortality of 70-80% of strychnine; with an effect corresponding to a mortality of 50%, additive synergism was observed with a tendency toward a decrease in the anti-inflammatory activity of the combination, toward antagonism between the components. In these experiments potentiating synergism was not observed (within the limits of the doses tested). In the experiments with vitamins Bc and B_{12}, signs of additive synergism were found (within the limits of the tested doses).

The results of these experiments thus showed that when hydrocortisone was given along with vitamin C and vitamins of the B group in the simplest conditions of combination (in pairs) their anti-inflammatory action was found to be clearly dependent on the doses of the components of the combination.

Effect of Hydrocortisone (HC), Certain Water-Soluble Vitamins, and Their Combination on the Barrier Properties of a Focus of Turpentine Inflammation 24 h in Duration in Mice Receiving an Injection of Strychnine (2 μg/g)

Treatment	Dose of HC (in μg/g)	Dose of vitamin (in μg/g)	No. of mice	No. dying (%)	χ^2	P
Control, strychnine subcutaneously, 2 μg/g strychnine into focus of inflammation	—	—	25	100		
	—	—	20	5		
HC	0,05	—	25	20	4,48	
B_1	—	0,5	25	20	4,48	
HC+B_1	0,025	0,25	25	80	—	<0,05
HC	0,5	—	20	30	9,0	<0,01
B_1	—	1,0	25	32	9,82	<0,01
HC+B_1	0,25	0,5	25	80	—	
HC	1,0	—	20	50	0,09	
B_1	—	30,0	25	48	0,08	>0,1
HC+B_1	0,5	15,0	25	40	—	
HC	0,5	—	20	30	8,54	<0,01
C	—	0,05	30	33	9,68	<0,01
HC+C	0,25	0,025	30	76	—	—
HC	1,0	—	20	50	0,091	
C	—	1,0	20	50	0,091	>0,1
HC+C	0,5	0,5	25	40	—	
HC	60,0	—	20	55	0,4	
C	—	40,0	20	60	0,9	>0,1
HC+C	30,0	20,0	20	40	—	
HC	0,5	—	20	30	4,08	
B_6	—	0,05	20	30	4,08	<0,05
HC+B_6	0,25	0,025	30	60	—	
HC	1,0	—	20	50	9,41	<0,01
B_6	—	0,05	20	50	9,41	
HC+B_6	0,5	0,025	25	92	—	
HC	10,0	—	25	80	0	—
B_6	—	20,0	25	84	0	—
HC+B_6	5,0	10,0	25	80	—	
HC	10,0	—	20	50		
B_2	—	30,0	20	50	0,1	>0,1
HC+B_2	0,5	15,0	20	40		
HC	10,0	—	25	80	8,96	<0,01
B_2	—	1,0	25	68	5,94	<0,05
HC+B_2	5,0	0,5	30	33	—	
HC	10,0	—	25	80		
B_{12}	—	0,1	25	80	0,37	>0,1
HC+B_{12}	5,0	0,05	30	70		
HC	4,0	—	25	64		
Bc	—	20	25	60	0	
HC+Bc	2,0	10,0	25	60		—

Note. The difference is significant (contingency 2 · 2) when $\chi^2 > 3.84$ ($P < 0.05$).

These results show that ascorbic acid and the vitamins of the B group may have a significant effect on the anti-inflammatory action of hydrocortisone (and possibly of its analogues).

The combined influence of hydrocortisone (and its analogues) and vitamins on inflammation is a problem which should receive further detailed study in both experimental and clinical conditions.

SUMMARY

The object of study was the influence of various separately taken doses of hydrocortisone and vitamins C, B_1, B_2, B_6, B_{12}, and Bc, and of paired combinations of hydrocortisone with each of the vitamins on the barrier properties of a focus of turpentine inflammation. It has been found that in case of a paired combination of small doses of hydrocortisone with vitamins C, B_1, and B_6 there is potentiation of the anti-inflammatory effect. Combination of large doses of cortisone and vitamins gives rise to antagonistic relationships.

LITERATURE CITED

1. I. I. Islamov and G. L. Mednik, Trudy Dushanbinsk, Med. Inst., Vol. 21, No. 3 (1956), p. 257.