EFFECT OF INTRAARTICULAR INJECTIONS OF EMOXYPINE ON THE COURSE OF IMMUNOGENIC ARTHRITIS IN RABBITS

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There is evidence of intensification of free-radical oxidation (FRO) taking place in many inflammatory and noninflammatory diseases, including rheumatic diseases: rheumatoid arthritis, systemic lupus erythematosus; systemic scleroderma; reactive types of arthritis, gout, etc. It has been shown that products of FRO, including free oxygen radicals, other active forms of oxygen (AFO), and lipid radicals, may be important mediators of inflammation [10]. Recognition of this role has led to the development of a particular trend in the treatment of rheumatic diseases, namely antioxidant therapy [12].

The aim of this investigation was to study the effect of intra-articular injections of the antioxidant emoxypine (2-Et-6-Met-3-oxypyridine) on the development of experimental immunogenic (allergic) arthritis.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male Chinchilla rabbits weighing 1200-1600 g. Bovine serum albumin was used. The animals were sensitized by intradermal injection of 4.5 mg albumin in 0.5 ml of Freund's complete adjuvant in the interscapular region. After 10 days a second injection of albumin (2 mg) was given in the same region, in order to reinforce the effect of sensitization. Allergic arthritis was induced in the rabbits by intraarticular injection of a reacting dose of the antigen (bovine serum albumin, 2 mg) into the knee joint 20 days after primary immunization. Treatment of the rabbits with the antioxidant emoxypine then began. The animals were divided into four groups. Treatment schedules for the animals are given in Table 1.

Blood for biochemical and immunologic tests was taken from the auricular vein of the rabbits before the experiment began (before immunization), 2 days after injection of the reacting dose of albumin (at the peak of inflammation), and on the 8th day after injection of the reacting dose of albumin (before sacrifice). Emoxypine (0.5 ml of a 1% solution) was injected intraarticularly (Table 1). The blood was subjected to general analysis. AFO generation during phagocytosis of particulate material by leukocytes was determined by a chemiluminescence method (Chl) [1]. The content of thiol groups [7], ceruloplasmin (CP) [9], C-reactive protein (CRP) [3], circulating immune complexes (CIC) [8], and activity of beta-glucuronidase (β-GU) [4] in the blood serum, the concentration of malonic dialdehyde (MDA) [5], in the blood plasma, and activity of superoxide dismutase (SOD) [11] and catalase [6] in the erythrocytes were determined. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Through the use of correlation between biochemical analysis and immunological indicators on intact animals (before immunization) a direct correlation between enzyme β-GU lysosome activity and CIC content ($r = 0.55$, $p < 0.05$) as well as between CRP concentration and CP ($r = 0.72$, $p < 0.01$) has been detected; the tendency of the

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TABLE 1. Schedules of Experiments on Treatment of Allergic Arthritis with Emoxypine

Day of experiment	Site of injection of preparation (knee joint)	Group of animals			
		1st (5)	2nd (5)	3rd (5)	4th (5)
1-	Left	PS	PS	PS	PS
	Right	Ar	Ar + Em	Ar	Ar
3-	Left	PS	PS	PS	PS
	Right	PS	Em	Em	Em
4-	Left	—	—	—	—*
	Right				
6-	Left	PS	PS	PS	PS
	Right	PS	Em	Em	Em

Legend. Ag) Antigen (2 mg), PS) physiological saline (0.5 ml), Em) emoxypine (5 mg); number of animals shown between parentheses, asterisk indicates emoxypine injected subcutaneously.

opposite correlation between antioxidation defense indicators (SOD, catalase, thiol groups) with one side and β-GU activity and CIC content in blood serum with the other has been detected.

Table 2 shows that the development of arthritis was accompanied by a rise of the ESR and of levels of CRP and CP. The peak of the Chl-response of the phagocytes of whole blood and the MDA concentration in the plasma also increased, evidence of a tendency for the blood phagocytes to give increased generation of AFO and, under the influence of the latter, enhanced lipid peroxidation. Definite changes took place in the state of antioxidant protection of these bodies: the level of serum thiol groups in all groups of animals except group 2 had a tendency to fall, whereas in the rabbits of group 2, which received an intraarticular injection of emoxypine simultaneously with the reacting dose of antigen, the content of thiol groups at the peak of inflammation was significantly depressed ($p < 0.001$). SOD activity of the erythrocytes, on the other hand, rose significantly ($p < 0.05$) in the animals of group 2, but in the remaining groups it remained within normal limits; the catalase activity of the erythrocytes was virtually unchanged. Differences in the change of activity between group 2 and the other groups were observed at the peak of inflammation with respect to the lysosomal enzyme β-GU also: whereas in groups 1, 3, and 4 serum β-GU activity showed a tendency to rise, in group 2 it showed a tendency to fall. These results are evidence of stabilization of the cell membranes under the influence of emoxypine. This action of emoxypine also has been observed by other workers [2]. Injections of emoxypine into an inflamed joint at the height (3rd day after injection of antigen into the joint) and on the 6th day of the inflammatory process had no significant effect on the biochemical parameters studied (animals of groups 3 and 4), but at the same time they caused a significant increase in SOD activity in the erythrocytes of these animals at the end of treatment (before sacrifice). Additional subcutaneous injections of the preparation (group 4) were active only against SOD activity. In this group of animals the highest activity of this enzyme was observed.

In the rabbits of group 2, which received an intraarticular injection of emoxypine simultaneously with the reacting dose of the antigen, the arthritis did not attain the degree observed in rabbits of the control group (group 1) macroscopically in any single case, unlike the animals of groups 3 and 4. This was reflected in the smaller rise of the CRP and CIC levels and absence of an increase in β-GU activity in the blood serum. The CIC level in this group of animals was virtually indistinguishable from normal, whereas in the remaining groups, at the peak of inflammation, it was significantly raised. In the rabbits of group 2 there was a particularly clear increase in SOD activity of the erythrocytes. Thus, 3 days after injection of the antigen, compared with its initial level it was increased in group 1 by 26%, in group 2 by 99%, group 3 by 23%, and group 4 by 38%. In the rabbits of groups 3 and 4 a comparable level of SOD activity (an increase by 101 and 77% respectively) was reached only after 1 week, whereas in the rabbits of group 2 it was already reduced a little.

Induced Chl of whole blood, reflecting ability to generate the superoxide radical O_2^- in the course of phagocytosis of microcrystals of barium sulfate, was virtually identical in magnitude and in its time course in the animals of groups 1 and 3, whereas in those of groups 2 and 4 a tendency was found for it to decrease after 7 days compared with the magnitude of the Chl response 3 days after injection of the antigen.

TABLE 2. Effect of Emoxypine on Biochemistry and Immunology of Indicators in Rabbit Blood

Biochemical and immunologic parameters	Period of investigation				
	before immunization	at peak of inflammation	significance of difference p	before sacrifice	significance of differences p
ESR, mm in 1 h	3,8±1,3	11,3±1,9	<0,001	5,6±1,9	>0,05
Ceruloplasmin, g/liter	0,248±0,017	0,386±0,008	<0,001	0,332±0,018	<0,001
CRP, mg/ml	0,95±0,04	1,58±0,42	<0,01	1,09±0,04	<0,05
Chl, quanta/sec·4 π	9,98±1,67	27,79±4,67	<0,001	31,81±7,12	<0,001
MDA, c.u./ml	10,8±2,4	27,4±4,7	<0,001	10,8±1,7	>0,05
β-GU, nM 4-MUF/ml/h	414,6±58,8	477,0±80,8	>0,05	300,0±42,4	>0,05
CIC, optical density units	119,2±20,3	173,8±40,2	<0,01	20,0±10,5	<0,05
SHO-group, μM/liter	344,35±18,06	299,0±18,70	>0,05	319,0±23,20	>0,05
SOD, c.u./mg Hb	11,6±1,7	14,7±1,0	>0,05	14,0±1,3	>0,05
Catalase, c.u./mg Hb	0,17±0,01	0,17±0,01	>0,05	0,16±0,02	>0,05
ESR, mm in 1 h	2,4±0,4	12,6±4,2	<0,001	2,0±0,3	>0,05
Ceruloplasmin, g/liter	0,307±0,032	400±0,012	<0,01	0,351±0,019	<0,05
CRP, mg/ml	0,99±0,04	1,17±0,06	<0,05	1,15±0,03	<0,05
Chl, quanta/sec·4 π	16,05±3,36	39,31±10,12	<0,05	28,62±4,69	<0,05
MDA, c.u./ml	9,4±2,6	19,7±2,4	<0,05	9,4±0,8	>0,05
β-GU, nM 4-MUF/ml/h	417,6±34,0	393,0±66,6	>0,05	340,8±23,3	>0,05
CIC, optical density units	75,8±16,6	93,8±25,8	>0,05	42,6±6,2	>0,05
SH-group, μM/liter	388,6±19,8	319,8±7,4	<0,001	320,5±11,3	<0,05
SOD, c.u./mg Hb	10,4±0,8	20,7±4,7	<0,05	18,3±3,9	<0,05
Catalase, c.u./mg Hb	0,02±0,01	0,19±0,03	>0,05	0,15±0,01	<0,001
ESR, mm in 1 h	2,7±0,4	10,2±2,1	<0,001	4,5±1,4	>0,05
Ceruloplasmin, g/liter	0,320±0,023	0,432±0,026	<0,001	0,371±0,021	>0,05
CRP, mg/ml	0,98±0,05	1,63±0,04	<0,001	1,25±0,05	<0,001
Chl, quanta/sec·4 π	8,61±2,18	25,4±6,65	<0,05	27,72±7,95	<0,05
MDA, c.u./ml	12,2±2,1	16,0±1,3	>0,05	11,6±1,6	>0,05
β-GU, nM 4-MUF/ml/h	350,4±63,4	409,2±48,5	>0,05	384,0±44,8	>0,05
CIC, optical density units	39,4±5,0	187,0±20,9	<0,001	30,1±7,4	>0,05
SHO-group, μM/liter	351±10,1	324,7±15,0	<0,05	300,1±15,7	>0,05
SOD, c.u./mg Hb	11,0±0,2	13,6±2,9	>0,05	22,2±4,9	<0,05
Catalase, c.u./mg Hb	0,21±0,02	0,16±0,01	>0,05	0,15±0,01	<0,05
ESR, mm in 1 h	2,0±0,4	12,3±2,3	<0,001	3,2±0,8	>0,05
Ceruloplasmin, g/liter	0,299±0,024	0,390±0,020	<0,01	0,348±0,010	>0,05
CRP, mg/ml	0,90±0,04	1,20±0,09	<0,001	1,42±0,09	<0,001
Chl, quanta/sec·4 π	6,43±2,02	35,24±3,63	<0,001	22,76±3,57	<0,001
MDA, c.u./ml	14,0±2,6	31,2±17,4	<0,05	17,4±1,5	>0,05
β-GU, nM 4-MUF/ml/h	385,2±19,8	448,8±42,9	>0,05	410,4±48,8	>0,05
CIC, optical density units	50,2±9,7	180,0±30,4	<0,001	33,8±8,2	>0,05
SHO-group, μM/liter	372,0±1,6	323,6±18,3	>0,05	313,7±12,6	<0,05
SOD, c.u./mg Hb	14,7±0,6	20,4±4,9	>0,05	26,1±4,3	<0,05
Catalase, c.u./mg Hb	0,15±0,01	0,13±0,02	>0,05	0,13±0,01	>0,05

Note. Reliable differences calculated through relation to norm.

The relationship thus revealed in intact animals between activity of the lysosomal enzyme β-GU and the content of CIC in the blood serum, on the one hand, and of SOD activity of the erythrocytes on the other hand, was preserved both as the peak of inflammation and during treatment of the animals with emoxypine.

Histologic investigation of the adipose synovial membrane of the right knee joints on the 8th day after injection of the reacting doses of antigen still revealed residual signs of synovitis in the animals of groups 1, 3, and 4, in the form of areas of proliferation of the covering cells and foci of lymphoid infiltration of the subendothelial layer, in some cases with an admixture of plasma cells. These features were absent in the rabbits of group 2.

Thus in the case of early intraarticular injection of the antioxidant emoxypine it had a marked effect on the course of experimental immunogenic (allergic) arthritis in rabbits, namely: on the severity of the clinical manifestations of arthritis, on some biochemical, immunologic, and histologic manifestations of inflammation, and on mobilization of an important enzyme of antioxidant protection, namely erythrocytic SOD.

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