

EFFECT OF THE AMINONUCLEOSIDE OF STYLOMYCIN AND PRIMAQUINE ON THE SYNTHESIS OF ANTI EGGS ALBUMIN BY RABBITS

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SUMMARY

The injection of primaquine and of the aminonucleoside of stylomycin leads to a decrease in the levels of serum antibodies in rabbits immunized with crystalline egg albumin.

INTRODUCTION

Although Chagas' disease remains an incurable infection, there are a few drugs that affect its cause and have a marked (pronounced) suppressive effect. Among these drugs, the aminonucleoside of stylomycin was found to be effective against *Trypanosoma cruzi*, the causal agent of Chagas' disease. The drug is active against the free flagellate *in vitro*², remarkably effective against the intracellular forms of the protozoa in tissue culture³, and on mice experimentally infected with a virulent strain of *T. cruzi*³. The association of primaquine enhances its effect in experimental Chagas' disease in mice⁶.

The course of the disease in mice, nearly always fatal with the *T. cruzi* strains used, is accompanied by high levels of parasitemia, and these can be used as criteria to judge the effect of drugs. The same, however, does not occur in the human disease. Very low levels of parasitemia may occur even at the beginning of the acute phase of the disease. Often the parasite cannot be found in the blood by direct examination on the second month of the disease. The more sensitive test for blood parasitism, the xenodiagnosis, is negative in the majority of patients during the chronic phase, and

may be negative even during the acute phase of the disease⁵. Under these circumstances, the sole guide to test the efficacy of a drug, is the complement fixation reaction of Guerreiro and Machado⁴.

The study of the possible effects of these drugs on the levels of circulating antibodies seems to be consequently important before their curative action on human patients can be eventually judged. As a matter of fact, the aminonucleoside of stylomycin is an adenosine analog that could disturb the antibody synthesis through the inhibition of nucleic acid synthesis.

The effects of injection of primaquine and the aminonucleoside of stylomycin on the levels of circulating antibodies in rabbits in the course of immunization with crystalline egg albumin are presented in this paper.

EXPERIMENTAL

Twice recrystallized egg-albumin from Mann Research Laboratories was used as antigen. To the antigen solution, containing 3 mg/ml of egg albumin, 10 to 15 per cent solution of albumin was added. The pH was adjusted with NaOH 0.1 to a point where maximum turbidity was obtained.

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The aminonucleoside of stylomycin was a product of Lederle Laboratories (American Cyanamid Company). The daily dose injected was 10 mg per kg of the animal's body weight.

Primaquine was from Parke Davis Co. The drug (diphosphate form) was dissolved in saline, and a daily dose of 13 mg of the free base per kg of body weight was administered.

Immunization schedule: 10 adult rabbits weighing approximately 2,500 g, were immunized during a three week period.

Each animal received four endovenous injection of equal amounts of antigen each week. In the first week the total antigen dose per animal was 10 mg. In the second and third week, the dose was increased to 20 and 40 mg respectively.

On the 26th day (5 days after the last injection), 20 ml of blood were obtained from each animal by cardiac puncture. After determination of antibody levels, they were divided into three groups: controls (3 rabbits), aminonucleoside of stylomycin (4 rabbits) and primaquine (3 rabbits).

The drugs were administered by intraperitoneal injections beginning on the 33rd day. A daily injection of the drugs was given until the 48th day. Booster doses of antigen were administered to each animal on four consecutive days after the 40th day. On the 40th and 41st days, 4 and 6 mg of egg albumin were administered endovenously to each animal. The other two doses of 10 mg of antigen per animal were injected intraperitoneally.

On the 49th day, a second sample of blood from each rabbit was obtained for determination of antibody levels. The dosage of antibody levels was performed through the quantitative precipitation test¹.

RESULTS

The results obtained are shown in Table I.

It can be seen that while very little if any differences exist between the first and second determinations of antibody among the

control animals, a decrease of about 50% in the levels of serum antibodies was observed after drug administration.

TABLE I

The effect of primaquine and of aminonucleoside of stylomycin on the serum antibody levels of rabbits immunized with egg albumin

Rabbit	Drug administered and dose	Antibody levels (mg/ml serum)	
		Before drug administration	After drug administration
1	Controls	619	796
2		934	728
3		831	780
4	Aminonucleoside of Stylomycin 10 mg/kg/day	2,280	1,045
5		1,020	676
6*		3,434	—
7*		—	—
8	Primaquine 13 mg/kg/day	2,620	1,283
9		5,060	1,265
10		796	275

* Rabbits number 7 and 6 died before the first and second cardiac puncture, respectively.

DISCUSSION

It appears very probable that both primaquine and the aminonucleoside markedly affect the levels of circulating antibodies in immunized rabbits. This effect might be due to the inhibition of antibody synthesis and/or the increased destruction or excretion antibody. The aminonucleoside of stylomycin as an adenosine analogue could inhibit the biosynthesis of antibody through the inhibition of nucleic acid metabolism. Although administration of this drug causes a massive proteinuria, we have (unpublished

observations) results showing that the excretion is mainly of the plasma albumin fraction.

The effects of primaquine on the antibody levels is not clear. The drug is known to remarkably decrease levels of glucose-6-phosphate dehydrogenase⁷. Since this enzyme is indispensable for the principal pathway for pentose synthesis, the primaquine effect could be due to an inhibition of nucleic acid synthesis necessary for antibody synthesis.

These results show the importance of developing good diagnostic tests for Chagas' disease that can be used to evaluate the effect of new, available, therapeutic agents.

RESUMO

Efeito do aminonucleosídeo da estilomicina e da primaquina na síntese da anti-ovalbumina, por coelhos.

A injeção de primaquina e do aminonucleosídeo da estilomicina acarreta uma diminuição do teor de anticorpos sanguíneos em coelhos imunizados com albumina cristalina de ôvo.

REFERENCES

1. COHN, M. — III. Techniques and analysis of the quantitative precipitin reaction. A reaction in liquid media. *Methods in Med. Research* Chicago 5:301-308, 1952.
2. FERNANDES, J. F. & CASTELLANI, O. — Nucleotide and polynucleotide synthesis in *Trypanosoma cruzi*. II. In vitro effect of tioguanine and of the aminonucleoside of stylomycin. *Exptl. Parasitol.* 8:480-485, 1959.
3. FERNANDES, J. F.; PEREIRA, J. P. M. & SILVA, L. H. P. — Nucleotide and polynucleotide synthesis in *Trypanosoma cruzi*. IV. Effect of the aminonucleoside of stylomycin on mouse infections. *Exptl. Parasitol.* 8:496-501, 1959.
4. GUERREIRO, G. & MACHADO, A. — *Brasil Med.* 23:225, 1913.
5. LARANJA, F. S.; DIAS, E.; NOBREGA, G. & MIRANDA, A. — Chagas disease: a clinical, epidemiologic and pathologic study. *Circulation* 14:1035-1060, 1956.
6. MORAES, G. E. S.; FARIA, J. L. & FERNANDES, J. F. — Nucleotide and polynucleotide synthesis in *Trypanosoma cruzi*. V. Effects of primaquine, stylomycin derivatives and analogs, on experimentally infected mice. *Rev. Inst. Med. trop. São Paulo* 2(3): 147-154, 1960.
7. SCHRIER, S. L.; KELLESMEYER, R. W. & ALVING, A. S. — Coenzyme studies in primaquine sensitive erythrocytes. *Proc. Soc. Exp. Biol. Med.* 99:354-356, 1958.
8. SILVA, L. H. P.; YONEDA, S. & FERNANDES, J. F. — Nucleotide and polynucleotide synthesis in *Trypanosoma cruzi*. III. Effect of the aminonucleoside of stylomycin on the parasite in tissue culture. *Exptl. Parasitol.* 8:486-495, 1959.

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