Acta Medica Okayama

Volume 25, *Issue* 4 1971 *Article* 7

AUGUST 1971

Immunosuppressive effect of 6-mercaptopurine-riboside. IV. Studies on antibody biosynthesis

Masana Ogata*

*Okayama University,

Immunosuppressive effect of 6-mercaptopurine-riboside. IV. Studies on antibody biosynthesis*

Masana Ogata

Abstract

The effect of 6.MPR on the antibody formation of rabbits challenged with bovine serum albumin has been studied in comparison with that of 6.MP. Observation revealed that the antibody formation is profoundly suppressed when the animal is treated with 6.MPR in an appropriate dose and period in relation with the introduction of antigen. Discussion was made of the possibility of 6.MPR as a superior therapeutic agent for autoimmune diseases.

*PMID: 4263562 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **25**, 287—293 (1971)

IMMUNOSUPPRESSIVE EFFECT OF 6-MERCAPTOPURINE-RIBOSIDE PART IV. STUDIES ON ANTIBODY BIOSYNTHESIS

Masana OGATA

Department of Public Health, Okayama University Medical School, Okayama, Japan

Received for publication, July 14 1971

Alkylating agents (nitrogen mustard and cyclophosphamide), purine antagonists (6-mercaptopurine (1), azathioprine (Imuran) and 6-thioguanine), and the folic acid analog (amethopterine), are widely used as chemoimmunosuppressive agents for the therapy of "autoimmune diseases", e. g. progressive hepatitis and rheumatoid arthritis.

Since these agents are mostly antimetabolic and quite toxic, it is desirable to get chemicals having less toxic side-effects and keeping a relatively high immunosuppressive activity. In this sense, six-mercaptopurine riboside (6-MPR), which is much less toxic, may be useful as an immunosuppressive agent in place of 6-mercaptopurine (6-MP). REGELSON *et al.* (2) have reported that the survival rate of the patients suffering from acute leukemia treated with 6-MPR was higher than that of those treated with 6-MP. In animal experiment, they have also demonstrated that 6-MPR was less toxic than 6-MP, showing that the maximum tolerant dose of 6-MPR in mice was 5 times that of 6-MP, when the agents were administered through intraperitoneal route (2). Thus it has been elucidated that the toxicity of 6-MPR is extremely low, but it remains uncertain yet whether 6-MPR has any immunosuppressive effect. This communication describes that 6-MPR has a relatively high immunosuppressive effect comparable to 6-MP demonstrating a marked suppression of antibody production in rabbit treated with 6-MPR and challenged with bovine serum albumin.

MATERIALS AND METHODS

Twenty-seven white New-Zealand strain rabbits weighing about 2.5 kg and kept on stock *ad libitum* diet were used. All animals received intramuscular injection of crystalline bovine serum albumin (100 mg/kg) supplemented with equal volume of complete Freund's adjuvant (Difco). The albumin was dissolved in saline and used as 1% solution. After the albumin injection, the animals were

divided into 3 groups, 9 animals each respectively.

In Group 1, three animals received 6-MPR intravenously 5.4 mg/kg/day for 13 days after the injection of serum albumin, the other three animals received one mole equivalent of 6-MP, 3.0 mg/kg/day for the same period, and the remaining 3 animals were left without any treatment. All the nine animals were challenged with the second injection of bovine serum albumin 73 days after the first injection of albumin, 0.5 ml of 1% solution intravenously.

In Group 2, all the animals received the second challenge, 5 mg of crystalline bovine serum albumin solution intravenously 55 days after the first injection. Of them three animals were treated with 6-MPR for two separate periods. In the first period 6-MPR, was given 2.7 mg/kg/day for 31 days starting from the day of the first albumin injection and in the second period a similar treatment for 19 days from 51st day of the first albumin injection. The other 3 animals were treated with 6-MP, 1.5 mg/kg/day, for the same period and at the same intervals as in the former 3 ones. The rest 3 animals were not treated with immunosuppressive agent and served as controls.

In Group 3, all the 9 animals received the second challenge with the albumin 40 days after the first injection of albumin by the same method as in Groups 1 and 2. Of these, three animals were given 6-MPR and the other 3 were given 6-MP in the same dose as in Group 2 respectively but only one period of 19 days from 37th day of the first challange with albumin. The remaining 3 animals received no immunosuppressive agents and served as control.

Antibody was measured by the tannic acid hemoagglutination method of STAVITSKY (3) with the blood from a marginal ear vein, and the results were expressed as the $\log_2$ of the highest serum dilution which showed a+1 pattern.

RESULTS

In Group 1, where the rabbits were given the immunosuppressive agents for 13 days from the first albumin injection, only a slight suppression of antibody producitons was observed on 19th day of experiment, but thereafter actually no immunosuppressive effect of the agents was detected.

According to the results observed on 19th day, the suppressive effect of 6-MPR on antibody production appeared to be a little higher than that of 6-MP. The enhanced antibody production observed after the second injection of antigen was not suppressed (Fig. 1).

In Group 2, 3 control animals gave a relatively low titer of antibody on 8th day, as the primary response which reached the maximum on 29th day. Thereafter, the value of titer decreased gradually, but it increased by the second challenge with albumin. The rabbits given 6-MPR, showed a marked delay in immuno-response and less in intensity of antibody titer. 6-MP also showed a similar effect, but somewhat in-

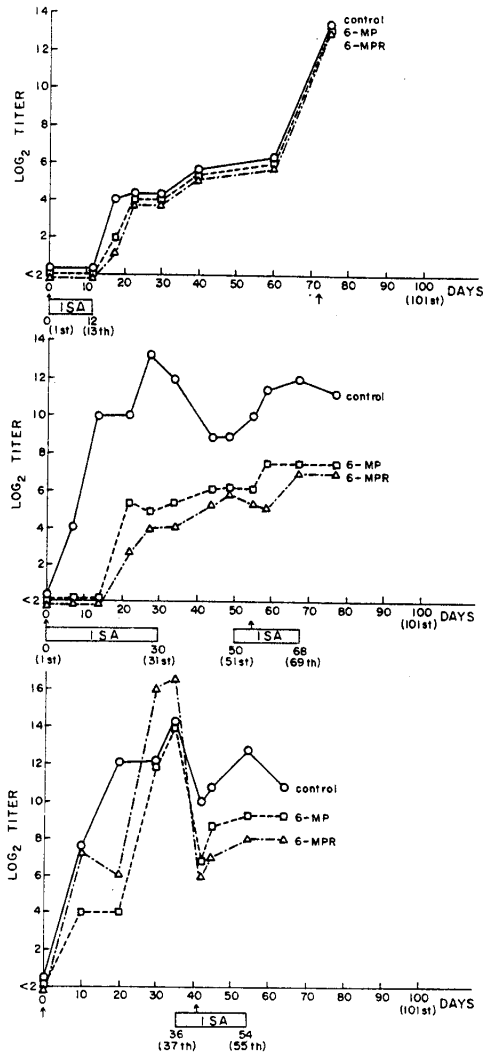

Fig 1. Immune response of rabbits against bovine serum albumin. Some delay and suppression in response were observed by treating the animals with 6-MP (squares) and 6-MPR (triangles). Open circles mean the values of controls receiving no immunosuppressive treatment. Each vertical arrow represents antigen injection. The clear block. I S A, means the period of administration of immunosuppressive agents. Each value depicted on the graph represents the mean of 3 animals. Method: Three tests, Fig. 1-A, 1-B and 1-C show the results of 3 groups differing in treatment with 6-MPR and 6-MP.

ferior to that of 6-MPR. The antibody titer of the control animals was found to have increased on 60th day, 4 days after the second challenge. The rate of the increase in antibody titer again appeared to be lower in the rabbits treated with the immunosuppressive agents compared to that of the control. The effect of 6-MPR seemed to be more intense than that of 6-MP (Fig. 2).

In Group 3, all the animals gave a similar value of the antibody titer after the first injection of albumin as in the control animals in Group 2, and the maximum value appeared on 36th day. In this group, 3 animals were treated with 6-MPR, and other 3 with 6-MP for 19 days, from 37 to 55th experimental day. That is, the treatment of animals with immunosuppressive agents started from 5 days before the second challenge with antigen and terminated 13 days after the second challenge. The remaining 3 animals received the second injection of antigen but were not treated with the immunosuppressive agents and served as control. The animals treated with the immunosuppressive agents gave a marked fall in antibody titer and a minimized response to the second injection of the antigen. In the case with 6-MPR it appeared to be superior to 6-MP in immunosuppressive effect.

TABLE 1. VARIATION OF LEVEL OF ANTIBODY TITER OF THE RABBITS INJECTED WITH BOVINE SERUM ALBUMIN MEASURED BY TANNIC ACID HEMAGGLUTINATION TECHNIC OF STAVITSKY AND EFFECT OF 6-MPR AND 6-MP ON ANTIBODY PRODUCTION.

Group 1.

Group \ Days*		1st 0	13th +12	19th +18	24th +23	31st +30	41st +40	61st +60	75th +74 (+1)**
Control	1	$<2^4$	$<2^4$	2^4	2^4	2^4	2^6	2^6	2^{14}
	2	$<2^4$	$<2^4$	2^4	2^4	2^4	2^6	2^6	2^{14}
	3	$<2^4$	$<2^4$	2^4	2^4	2^4	2^4	2^6	2^{12}
Average		$<2^4$	$<2^4$	2^4	2^4	2^4	$2^{5.3}$	2^6	$2^{13.3}$
6-MPR	1	$<2^4$	$<2^4$	$<2^4$	2^4	2^4	2^4	2^6	2^{14}
	2	$<2^4$	$<2^4$	2^4	2^4	2^4	2^6	2^6	2^{14}
	3	$<2^4$	$<2^4$	$<2^4$	2^4	2^4	2^6	2^6	2^{12}
Average		$<2^4$	$<2^4$	$2^{1.3}$***	2^4	2^4	$2^{5.3}$	2^6	$2^{13.3}$
6-MP	1	$<2^4$	$<2^4$	2^4	2^4	2^4	2^6	2^6	2^{14}
	2	$<2^4$	$<2^4$	$<2^4$	2^4	2^4	2^6	2^6	2^{14}
	3	$<2^4$	$<2^4$	2^2	2^4	2^4	2^4	2^6	2^{12}
Average		$<2^4$	$<2^4$	2^2***	2^4	2^4	$2^{5.3}$	2^6	$2^{13.3}$

Group 2.

Group \ Days*		1st 0	8th +7	15th +14	23rd +22	29th +28	35th +34	45th +44	50th +49	57th +56 (+1)**	60th +59 (+4)**	69th +63 (+13)**	79th +78 (+23)**
Control	1	<2^4	2^4	2^{10}	2^{10}	2^{14}	2^{12}	2^8	2^8	2^{10}	2^{12}	2^{12}	2^{12}
	2	<2^4	2^4	2^8	2^8	2^{12}	2^{10}	2^6	2^6	2^8	2^{10}	2^{10}	2^{10}
	3	<2^4	2^4	2^{12}	2^{12}	2^{14}	2^{14}	2^{12}	2^{12}	2^{12}	2^{12}	2^{14}	2^{12}
Average		<2^4	2^4	2^{10}	2^{10}	$2^{13.3}$	2^{12}	$2^{8.7}$	$2^{8.7}$	2^{10}	$2^{11.3}$	2^{12}	$2^{11.3}$
6-MPR	1	<2^4	<2^4	<2^4	<2^4	2^4	2^4	2^4	2^6	2^4	2^5	2^7	2^7
	2	<2^4	<2^4	<2^4	2^4	2^4	2^4	2^6	2^6	2^4	2^4	2^6	2^6
	3	<2^4	<2^4	<2^4	2^4	2^4	2^4	2^6	2^6	2^8	2^6	2^8	2^8
Average		<2^4	<2^4	<2^4	$2^{2.7}$***	2^4	2^4	$2^{5.3}$	2^6	$2^{5.3}$	2^5	2^7	2^7
6-MP	1	<2^4	<2^4	<2^4	2^4	2^4	2^6	2^6	2^6	2^4	2^8	2^8	2^8
	2	<2^4	<2^4	<2^4	2^6	2^6	2^6	2^6	2^6	2^6	2^8	2^6	2^6
	3	<2^4	<2^4	<2^4	2^6	2^4	2^4	2^6	2^6	2^8	2^6	2^8	2^8
Average		<2^4	<2^4	<2^4	$2^{5.3}$	$2^{4.7}$	$2^{5.3}$	2^6	2^6	2^6	$2^{7.3}$	$2^{7.3}$	$2^{7.3}$

Group 3.

Group \ Days*		1st 0	11th +10	21st +20	31st +30	36th +35	43rd +42 (+1)**	46th +45 (+4)**	55th +54 (+13)**	65th +64 (+23)**
Control	1	<2^4	2^8	2^{12}	2^{12}	2^{14}	2^{12}	2^{12}	2^{14}	2^{12}
	2	<2^4	2^6	2^{12}	2^{12}	2^{12}	2^{10}	2^8	2^{10}	2^{12}
	3	<2^4	2^8	2^{12}	2^{12}	2^{16}	2^8	2^{12}	2^{14}	2^8
Average		<2^4	$2^{7.3}$	2^{12}	2^{12}	2^{14}	2^{10}	$2^{10.7}$	$2^{12.7}$	$2^{10.7}$
6-MPR	1	<2^4	2^6	2^6	2^{16}	2^{18}	2^6	2^6	2^8	2^6
	2	<2^4	2^{10}	2^6	2^{16}	2^{16}	2^6	2^8	2^8	2^{10}
	3	<2^4	2^6	2^6	2^{16}	2^{16}	2^7	2^7	2^8	2^8
Average		<2^4	$2^{7.2}$	2^5	2^{16}	$2^{16.7}$	2^6	2^7	2^8	2^8
6-MP	1	<2^4	2^4	2^4	2^{12}	2^{16}	2^8	2^8	2^{10}	2^{10}
	2	<2^4	2^4	2^4	2^{12}	2^{14}	2^6	2^{10}	2^{10}	2^{10}
	3	<2^4	2^4	2^4	2^{12}	2^{12}	2^6	2^8	2^8	2^8
Average		<2^4	2^4	2^4	2^{12}	2^{14}	$2^{6.7}$	$2^{8.7}$	$2^{9.3}$	$2^{9.3}$

* The days after the first injection of antigen

** Titers inside the parenthesis show the days after the second injection

*** Calculated 2^4 below as 2^0

DISCUSSION

The observations reported here have demonstrated clearly that 6-MPR effectively suppresses the production of antibody against bovine serum albumin in rabbit. Its immunosuppressive effect was comparable to that of 6-MP or superior to the latter, when one mole equivalent dose of the agents was injected intramusculary or intravenously. Such a property of 6-MPR should be common to other animal strains and antigens, and suggests that 6-MPR will be a useful medicament for autoimmune diseases in man. It has been reported that 6-MP competes with hypoxanthine for the binding to inosinic acid pyrophosphorylase and reacts with 5-phosphoribosyl pyrophosphate to be converted into thioinosic acid (4), which interferes with the incorporation of purines in nucleonic acid synthesis.

Although the exact mechanism of the action of 6-MPR with respect to antibody synthesis is not known, its suppressive effect on antibody production will mainly be related to the suppression of general protein formation through the suppression of RNA synthesis, as it is assumed that 6-MPR will be converted to thioinosic acid by the action of nucleoside kinase and ATP (5).

Thus, 6-MPR would be superior to 6-MP as an immunosuppressive agent, but it should be an agent to be used carefully considering its serious side-effects. Therefore, it should be noted that further experiments on toxicity of 6-MPR on various animals for long term administration are required before 6-MPR is applied to patients as immunosuppressive medicament.

SUMMARY

The effect of 6-MPR on the antibody formation of rabbits challenged with bovine serum albumin has been studied in comparison with that of 6-MP. Observation revealed that the antibody formation is profoundly suppressed when the animal is treated with 6-MPR in an appropriate dose and period in relation with the introduction of antigen. Discussion was made of the possibility of 6-MPR as a superior therapeutic agent for autoimmune diseases.

REFERENCES

1. SCHWARTZ, K. STACK, J. and DAMESHEK, W.: Effect of 6-mercaptopurine on antibody production. *Proc. Soc. Exp. Biol. & Med.* **99**, 164, 1958
2. REGELSON, W., HOLLAND JF. FTEI, E. GOLD, G. L., HALL, T., KRANT, M., MILLER, S. O.

and SHNIDER, B. I.: Comparative clinical toxicity of 6-mercaptopurine (NSC-755)[1] and 6-mercaptopurine ribonucleoside (NSC-4911)[2] Administered intravenously to patients with advanced cancer[3,4]. *Cancer Chemother Reports* **36**, 41, 1964

3. STAVITSKY, A. B.: Micromethods for the study of proteins and antibodies. *J. Immunol.* **72**, 360, 1954
4. LUKENS, L. W. and HERRINGTON, K. A.: Enzymic formation of 6-mercaptopurine ribotides. *Biochem. Biophys. Acta* **24**, 432, 1957
5. TŌRU, NAKAMURA.: Report of Second Thioinosin Research Meeting. p18, 1970, Tokyo