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EFFECT OF DIPYRIDAMOLE ON IMMUNE RESPONSE IN MICE

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Summary. — Dipyridamole in the dose of 50 mg/kg body weight stimulated the primary humoral response in BALB/c and C57Bl mice. The cell-mediated immune response was not influenced in BALB/c mice.

Key words: dipyridamole; immune response; mouse

Introduction

Dipyridamole [persantin, 2,6-bis(diethanolamino)-4,8-dipiperidino-pyrimido(5,4-d)pyrimidine], a well known coronary vasodilatator and antithrombotic drug, was found to possess broad antiviral activity in vitro against RNA as well as DNA viruses (Tonew and Dzeguze, 1977; Tonew *et al.*, 1977; Oehring and Schmidt, 1978; Bańkowski *et al.*, 1981).

The antiviral activity of dipyridamole could be enhanced by its irradiation with artificial visible light (Tonew, 1980). Effect on the influenza virus A/England infection in mice was described by Tonew *et al.* (1982). Kobus *et al.* (1982) reported on the activity against herpes virus infection in mice enhanced by additional irradiation with microwaves. Günther *et al.* (1977) observed a therapeutic and prophylactic effect in patients with herpes labialis and recidivans, respectively. The therapeutic effect of dipyridamole increased in combination with irradiation by short wave visible light.

Recent investigations of biological activities of dipyridamole have emphasized that the compound acts as an interferon inducer in vitro in different cell cultures as well as in vivo in mice (Galabov and Mastikova, 1982, 1983). This paper describes the effects of dipyridamole on the immune response in mice.

Materials and Methods

The effect of dipyridamole on humoral response was studied by plaque assay for antibody forming cells of mice immunized with sheep red blood cells (SRBC).

Dipyridamole administration. Dipyridamole — 2,6-bis(diethanolamino)-4,8-dipiperidino-pyrimido (5,4-d)pyrimidine), supplied by Dr. E. Tonew, was suspended in sterile 0.9% saline

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and stored at 4 °C for the period of each experiment. The drug was injected subcutaneously in the dose of 1 mg/20 g mouse twice daily for consecutive days. First injection of dipyridamole was applied on the day of SRBC inoculation and next for the three following days. The control groups of mice received 0.9% saline.

Immunization procedure. SRBC were stored at 4 °C in Alsevier' s solution for a period of 7 to 10 days. Before use they were washed three times in PBS. Six BALB/c and six C57/B1 mice of both sexes, weighing 18—20 g formed the dipyridamole-treated and control groups. Animals were immunized intravenously with 5% suspension of SRBC in 0.2 ml volumes. On day 5 mice were sacrificed and spleen cells were obtained. Direct haemolytic plaque assay for antibody forming cells was performed according to the methods described (Cunningham and Szenberg, 1968; Jerne *et al.*, 1974).

The cellular response was studied by oxazolone hypersensitivity test (Asherson and Ptak, 1968, 1970). Briefly, BALB/c mice, six dipyridamole treated and six controls, were sensitized by application of 3% alcohol solution of oxazolone (BDH, England) on the skin of clipped abdomen. Seven days later, the mice were anaesthetized. The thickness of the ears was measured with micrometer before and 24 hr after smearing both sides of the ears with 1% solution of oxazolone in olive oil.

Results and Discussion

The effect of dipyridamcle on the humoral immune response presented in Table 1 is expressed in numbers of pla que forming cells per 10^6 spleen cells. A statistically significant enhancement of the primary humoral response was detected in both dipyridamole-treated mouse strains.

The effect of dipyridamole on the cellular immune response was expressed as the increase in ear thickness (Table 2). In two independent experiments, no differences in cell-mediated immune response between control and di-dipyridamole treated BALB/c mice were found.

Galabov and Mastikova (1982) showed that dipyridamole induced interferon production, especially in mouse peritoneal leukocytes and, to a much lesser extent, in primary cultures of mouse embryo fibroblasts and in human diploid embryo lung fibroblasts.

White mice given low doses of dipyridamole (0.1 mg/kg body weight) responded with a high interferon titre of 128 IU/ml. However, when this compound was administered in a high dose of 50 mg/kg body weight (i.e. in such a dose that used in our study) low interferon response in titre of 8 IU/ml was induced. When dipyridamole was applied intraperitoneally, as in our studies, it stimulated interferon production in much lower titre than after oral administration (Galabov and Mastikova, 1983). It is known that inter-

Table 1. The effect of dipyridamole on the number of cells producing antibodies to SRBC in mice

Mouse strain	PFC/10^6 spleen cells (mean ± SD)*		P
	SRBC control	SRBC + dipyridamole	
BALB/c	762 ± 164	1 296 ± 117	< 0.005
C57B1	1 415 ± 427	2 364 ± 649	< 0.05

* Groups of six mice.

Table 2. The effect of dipyridamole treatment on the response to oxazolone in BALB/c mice

Mouse strain	Increase in ear thickness (mean ± SD)*		P
	Oxazolone control	Oxazolone + dipyridamole	
BALB/c	12.5 ± 3.1*	14.2 ± 5.0	> 0.05**
BALB/c	8.2 ± 2.6	6.5 ± 1.9	> 0.05

Groups of six mice. Two experiments, each one was repeated three times.
* In cm 10^{-3}.
** Not significant.

ferons do have immunomodulating properties (Baron *et al.*, 1982; De Maeyer and De Maeyer-Guignard, 1982). All three types of interferon inhibit various factors of the immune response, although under some conditions, e.g. when applied in low doses interferon might cause an immunity enhancement. Therefore, we suppose that our results could be explained by the fact that large doses of dipyridamole stimulated low titres of interferon in mouse sera which in turn, further increased the number of antibody-forming cells.

The reported data showed a new, so far unknown biological effect of dipyridamole, i.e. the enhancement of humoral immunity. This important fact should be taken into consideration when interpreting the results of antiviral activity in vivo.

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