Inhibitory effect of cytokinins on PHA-induced human lymphocyte stimulation

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Summary. The inhibitory effect of various purine derivatives on PHA-induced human lymphocyte blast formation was studied. Two nucleoside cytokinins, N^6-benzyladenosine and N^6-isopentenyladenosine, inhibited blast formation at concentrations as low as 10^{-6} M. However, the other cytokinins, which lacked the ribosyl residue at N^9 position, had to be at the higher molar concentration of 10^{-4} before they could induce the same inhibitory effect.

A potent immunosuppressive factor extracted from the fruitbody of *Lentinus edodes,* one of the most popular edible mushrooms in Japan, has been shown to have a similar structure to lentinacin (2,3-dihydroxy-4-[9-adenyl]-butyric acid), although its final structure has not yet been determined. This new finding prompted us to test the immunosuppressive activities of various cytokinins, which are characterized by being N^6-substituted adenine and adenosine derivatives responsible for the promotion of cell division and expansion in plants. The work reported here was based on the facts that the cytokinins have a remarkably similar structure to our mushroom factor, and also because they are known to act as competitive inhibitors of cyclic-AMP phosphodiesterase[1]. Adenine, adenosine, guanine and guanosine, together with their derivatives and c-AMP, c-UMP, c-CMP and c-GMP, have already been reported[2-4] to be suppressive on lymphocyte responses to PHA, but no detailed studies on the inhibitory activity of cytokinins have been carried out.

Materials and methods. 7 cytokinins were tested. 2 of them (N^6-benzyladenosine and N^6-isopentenyladenosine) were nucleosides and the remaining were sugar-free (kinetin, N^6-benzyladenine, 6-n-amyloxypurine, 6-n-hexyloxypurine and N^6-benzyl-9-n-propyladenine). In addition, adenine, adenosine, 5′-AMP, 3′,5′-c-AMP, dibutyryl 3′,5′-c-AMP, guanine, guanosine, 5′-GMP, 2′,5′-c-GMP and 3′,5′-c-GMP were also examined for their inhibitory activity. The inhibitory effect of these cytokinins and purine analogues was tested in vitro by means of measuring the degree of inhibition of phytohaemagglutinin(PHA)-induced human lymphocyte blast formation as has been described elsewhere[5]. Briefly, each triplicate tube containing human lymphocytes (1.0×10^6) in 0.9 ml of RPMI 1640 medium fortified with 20% fetal calf serum received 1 concentration of the test drug dissolved in 0.1 ml RPMI 1640 medium. After the addition of 15 µg/ml of PHA-P (Difco), these test-tubes, together with control cultures, were incubated for 66 h at 37 °C in humidified air supplemented with 5% (v/v) CO_2. 18 h before the end of the culture period, 0.1 µCi of ^{3}H-thymidine (sp. act. 14.5 Ci/mmole, obtained from Daiichi Pure Chemicals, Tokyo) in 0.1 ml of RPMI 1640 medium was added. The radioactivity incorporated into the DNA was measured in each culture by liquid scintillation spectrometry (Aloka LSC 601). The lymphocytes were obtained from 3 healthy donors and used for determination of the inhibitory activity of each compound.

Results and discussion. The results illustrated in figure 1 indicate that the 2 nucleoside cytokinins, N^6-benzyladenosine and N^6-isopentenyladenosine at 10^{-6} M inhibited PHA-induced lymphocyte blast formation by 50%, as judged by ^{3}H-thymidine incorporation into DNA. The remaining 5 cytokinins showed the same inhibitory effect, but at around 10^{-4} M.

Although adenine (figure 2) and guanine (figure 3) analogues showed some inhibitory activity, as has been reported by others[3,4], 10^{-3}–10^{-4} M were required to induce 50% inhibition. Of these analogues, dibutyryl 3′,5′-c-AMP revealed the highest activity, as was also reported by Smith et al.[4]. Although the effects of extracellular nucleotides and agents including aminophylline, isoproterenol and prostaglandins, which elevate the intracellular cyclic AMP concentrations during human lymphocyte blast forma-

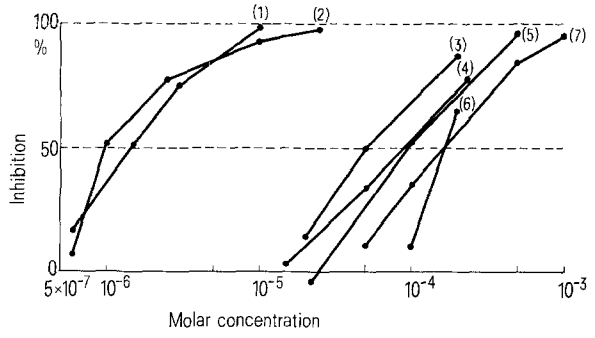

Fig. 1. Inhibitory effect of cytokinins on DNA synthesis in PHA-stimulated lymphocytes. Human peripheral lymphocytes were incubated for 66 h with PHA. ^{3}H-thymidine was present during the final 18 h of culture. The cytokinins tested (final concentrations indicated on the abscissa) were added at 0 time just before the addition of PHA. (1) N^6-Isopentenyladenosine; (2) N^6-benzyladenosine; (3) N^6-benzyl-9-n-propyladenine; (4) 6-n-hexyloxypurine; (5) 6-n-amyloxypurine; (6) kinetin; (7) N^6-benzyladenine.

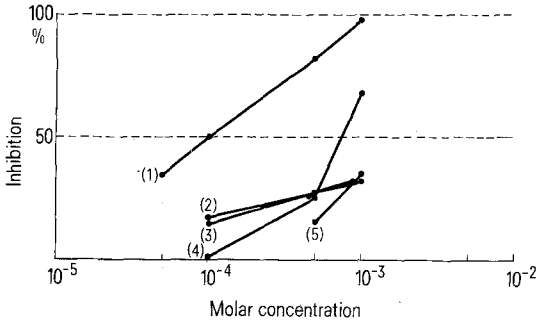

Fig. 2. Inhibitory effect of adenine analogues. (1) Dibutyryl 3′,5′-c-AMP; (2) 5′-AMP; (3) 3′,5′-c-AMP; (4) adenine; (5) adenosine.

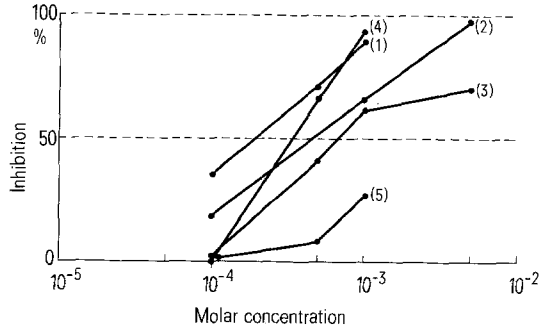

Fig. 3. Inhibitory effect of guanine analogues. (1) Guanosine; (2) 2′,3′-c-GMP; (3) 3′,5′-c-GMP; (4) 5′-GMP; (5) guanine.

tion[3,4], have been examined for their inhibitory activity, cytokinins so far have been omitted from such systemic studies. Only the work reported by Gallo et al. in early 1969 disclosed such an effect in N^6-isopentenyladenosine[6]. A recent work by Hecht et al. suggested the possibility of inhibitory activity in various cytokinins from a biochemical basis[1], but an actual comparison of their inhibitory activity has not yet appeared.

This paper disclosed 1 important finding, i.e., cytokinins with a nucleoside structure, including N^6-benzyladenosine and N^6-isopentenyladenosine, substantially inhibited human lymphocyte metabolism stimulated with PHA at concentrations as low as 10^{-6} M. When compared with the effect of dibutyryl cyclic-AMP, almost $^1/_{100}$ of the concentration was sufficient to inhibit blast formation. Bona et al. reported[7] that 5′-deoxy-5′-S-isobutyl-adenosine, a synthetic analogue of S-adenosyl-homocysteine inhibited the mitogen-induced blast formation of human and rabbit lymphocytes. This compound also has the ability to prevent oncogenic transformation of chicken fibroblasts by Rous Sarcoma virus. The effect of time of addition of 5′-deoxy-5′-S-isobutyl-adenosine was identical to that of N^6-benzyladenosine and N^6-isopentenyl adenosine (data not shown). It would be interesting to compare the activities of N^6-benzyladenosine and N^6-isopentenyladenosine with that of 5′-deoxy-5′-S-isobutyladenosine in preventing oncogenic transformations. Biochemical analyses, such as the effect on cyclic-AMP phosphodiesterase on the compounds tested here, is under way.

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On the effects of thiamphenicol and chloramphenicol on nucleic acid and protein synthesis in rabbit bone marrow cells in vivo and in vitro

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Summary. Besides the in vivo effects of thiamphenicol (TAP), this study shows the in vitro effects of TAP and D- and L-threo-chloramphenicol (CAP) on the synthesis of DNA, RNA, protein and hemoglobin in marrow cells and reticulocytes. The experiments make it likely that TAP exerts its action on a stem cell in a proliferating phase.

TAP and CAP are potent inducers of a reversible marrow depression. Furthermore CAP can cause an aplastic anemia. It is generally accepted that the mechanism of the reversible depression is related to the inhibition of protein synthesis in mitochondria[2-5]. Previous results led us to the conclusion that the toxic effects of TAP have their origin at the start of the differentiation of the erythroid cell line[6]. In this report we describe the relationship between the onset of the decrease of cytochrome c oxidase activity (reflecting the mitochondrial protein synthesis) and the activity of marrow cells with respect to the synthesis of DNA, RNA and protein under a TAP regime in vivo. Moreover we included our in vitro studies because of conflicting conclusions about the action of CAP on mammalian protein synthesis as reported in the literature. The inhibitory effects of CAP on reticulocyte protein synthesis, as found by Weisberger[7], could not be reproduced by Zelkowitz[8]. Recently, Agam[9] demonstrated that CAP inhibited platelet protein synthesis in vivo at low doses and in vitro at high doses. A further aim of our study was to shed more light on the meaning of differences between the effects of TAP and CAP on synthetic processes in vitro in relation to the fact that the aplastic anemia has not been reported for TAP. At very high concentrations of the antibiotics, Yunis[10] did indeed observe differences on the synthesis of DNA.

Materials and methods. Chinchilla rabbits were bled on 4

Table 1. Effects of high concentrations of TAP, D-threo-CAP and L-threo-CAP on the in vitro synthesis of DNA, RNA, protein and haemoglobin in marrow cells and on the synthesis of haemoglobin in reticulocytes

Addition	(mg/ml)	DNA	RNA	Protein	Hb (marrow)	Hb (reticulocytes)
TAP	0.5	91±6	102±14	95±6	80±4	100±4
	1.0	91±5	112± 4	85±4	79±6	100±2
D-CAP	0.5	52±7	60± 4	42±7	41±2	96±8
	1.0	27±4	42± 7	21±5	21±9	81±6
L-CAP	0.5	83±2	76± 4	75±2	80±4	88±2
	1.0	47±2	66± 8	44±3	55±8	85±4

Marrow cells and reticulocytes were obtained from phenylhydrazine-treated rabbits. The in vitro synthesis of the different compounds was measured as described in 'materials and methods'. The incorporation values are expressed as percentages of the results from a 2-h incubation without addition of antibiotics (± SEM; n = 3).