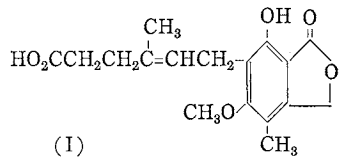
MYCOPHENOLIC ACID: ANTIVIRAL AND ANTITUMOR PROPERTIES

Sir:

The isolation of mycophenolic acid (I) from a penicillium culture was first reported by GOSIO in 1896[1] and again by ALSBERG and BLACK in 1913[2]. Its antibacterial and antifungal properties have been studied by several workers[3,4]. In addition to these biological activities, we now report that mycophenolic acid exhibits *in vitro* and *in vivo* antiviral activity, and significantly inhibits the growth of a broad spectrum of transplantable solid tumors in mice.



(I)

During the screening of fermentation broths from a series of molds, the broth of a strain of *Penicillium stoloniferum* was found to possess reproducible antiviral activity *in vitro*. The active component was isolated by chloroform extraction of filtered broth at pH 3.0, followed by column chromatography on silica gel and crystallization. Comparison of the physical properties of the active crystalline substance with those of mycophenolic acid (I)[5] led to its identification.

Table 1. The effect of mycophenolic acid on virus induced Rous sarcoma (RSV-BRYAN strain)

Mycophenolic acid in diet (%)	Mortality	Av. wt. gain (g)	Mean tumor wt. (g)	Percent inhibition
0.125	2/15	25	.01	94
0.0625	0/15	47	.09	69
0.03125	0/15	90	.33	0
0.00	0/10	125	.32	0

Table 2. The effect of mycophenolic acid on virus induced Friend leukemia

	Weight gain (g)	Av. spleen weight (mg)	Percent inhibition
Uninfected control	+11.2	133	
Infected control	+ 8.9	1,200	
Mycophenolic acid treated (150 mg/kg×4)**	+ 4.0	700	46.8*

* % Inhibition >25 % considered significant.
** Dose schedule: 24 and 4 hour preinfection, 24 and 48 hour post infection.

Table 3. Antitumor spectrum of mycophenolic acid

Tumor system	Host	Dose (I.P.) mg/kg×freq. ×no. of doses	Mean tumor diameter (mm) Treated/Control	Percent activity	Av. survival time (days) Treated/Control	Percent prolongation
Walker carcinosarcoma (solid)	Sprague-Dawley	45×1×10	0/14.3	100		
Walker carcinosarcoma (ascites)		45×1×10			22.7/11.6	96
Mecca lymphosarcoma	AKR	80×1×10	0/8.9	100		
High malignancy clone	C_3H	300×1×10	0/7.1	100		
Plasma cell myeloma	C_3H	100×1×9	1.5/14.1	89		
C_3H mammary	C_3H	300×1×10	11.8/30.1	61		
Adenocarcinoma 755	C57Bl/6	300×1×10	11.5/21.3	46		
Ridgeway osteogenic sarcoma	AKR	300×1×9	3.3/10.0	67		
Gardner lymphosarcoma	C_3H	300×1×10	12.7/31.1	59		
Shionogi carcinoma	dds	300×1×9	7.1/10.9	35		
Sarcoma 180	Swiss	150×1×10	11.0/15.0	27		
Melanoma S91	DBA_1	300×1×9	8.4/9.0	0		
S-180 ascites	Cox std.	75×1×10			15.6/11.4	37
Ehrlich ascites	Cox std.	75×1×10			18.9/16	0
Freunds ascites	Cox std.	150×1×10			18.3/12.0	52
Taper hepatoma ascites	C_3H	75×1×10			24.1/18.2	32
P-1534 leukemia	DBA_2	300×1×10			21.1/17.2	23
L-1210 leukemia	C57Bl/6	300×1×10			18.7/14.6	28
C-1498 leukemia	C57Bl/6	300×1×10			19.5/14.3	36
L5178Y leukemia	DBA_2	300×1×10			15.6/11.4	37

Antiviral activity was demonstrated by the agar diffusion technique in tissue culture against vaccinia, measles, Herpes simplex and Newcastle disease viruses. Activity was seen at concentrations as low as 0.6 μg/pad (1 μg/7 mm pad~50 μg/ml). No gross evidence of cytotoxicity to the mammalian cell line (BS-C-I monkey kidney cell) in the agar system was observed at the highest concentration tested, 40 μg/pad.

Although no significant *in vivo* activity was established against vaccinia, Herpes simplex and influenza virus infections in mice, efficacy was shown in two oncogenic virus systems. Table 1 shows the inhibition of tumor development due to Rous sarcoma virus in chickens by oral administration of mycophenolic acid. The Rous sarcoma assay was performed according to the method of JOHNSON and BAKER[6]. Results are presented in Table 2 showing that splenomegaly in Friend virus infected DBA_2 mice is reduced by treatment with mycophenolic acid. It has been previously reported that splenomegaly is a measure of virus multiplication in Friend virus infections[7]. Activity in this system was detected using the technique previously described by DELONG and coworkers[8].

Subsequent testing has shown that in addition to the activity seen in the oncogenic virus systems, mycophenolic acid effectively inhibited the growth of a large number of transplantable murine solid tumors, transplanted by trocar. Marginal activity was also found against several leukemias and ascites tumors. The spectrum of antitumor activity of mycophenolic acid is given in Table 3. The marked inhibition of growth of the Mecca-lymphosarcoma tumor is noteworthy, since this tumor grows and metastasizes very rapidly. The procedures used for antitumor testing have been described elsewhere by JOHNSON *et al.*[9] The compound was equally effective when administered at the same dose level by either the oral or intraperitoneal route. The LD_{50} in mice of mycophenolic acid was found to be>1,000 mg/kg (oral and intraperitoneal administrations).

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References

1) GOSIO, B.: Ricerche batteriologiche e chimiche sulle alterazioni del mais. Rivista Igiene Sanita Pubblica Ann. 7 : 825~868, 1896
2) ALSBERG, C. L. & O. F. BLACK: Contribution to the study of maize deterioration. U. S. Dept. Agriculture, Bureau of Plant Industry, Bull. 270 : 7~48, 1913
3) FLOREY, H.W.; K. GILLIVER, M.A. JENNINGS & A. G. SANDERS: Mycophenolic acid. An antibiotic from *Penicillium brevicompactum* DIERCKX. Lancet 1946-I : 46~49, 1946
4) GILLIVER, K.: The inhibitory action of antibiotics on plant pathogenic bacteria and fungi. Ann. Botany 10 : 271~282, 1946
5) LOGAN, W. R. & G. T. NEWBOLD: Lactones. V. Experiments relating to mycophenolic acid. J. Chem. Soc. 1957 : 1946~1951, 1957
6) JOHNSON, I. S. & L. A. BAKER: The utility of the Rous sarcoma virus for detection of antiviral activity. Antibiot. & Chemoth. 8 : 113~121, 1958
7) CHIRIGOS, M. A. & R. W. MARCH: Reversal by syngeneic spleen cells of inhibitory effects of drugs and irradiation on Friend virus. Antimicr. Agents & Chemoth. 1966 : 489~496, 1967
8) DELONG, D. C.; L. A. BAKER, N. R. EASTON & R. D. DILLARD: Inhibition of Friend leukemia virus splenomegaly by an acetylenic carbamate. Proc. Soc. Exptl. Biol. & Med. 127 : 845~849, 1968
9) JOHNSON, I.S.; H.F. WRIGHT, G.H. SVOBODA & J. VLANTIS: Antitumor principles derived from *Vinca rosea* LINN. I. Vincaleukoblastine and leurosine. Cancer Res. 20 : 1016~1022, 1960