ANTITUMOR ACTIVITY OF MYCOPHENOLIC ACID

SEIKICHI SUZUKI, TAKAO KIMURA, KUNIO ANDO,
MIKIO SAWADA and GAKUZO TAMURA*

Research Laboratories, Chugai Pharmaceutical Co., Tokyo, Japan
*Laboratory of Microbiology, Department of Agricultural Chemistry,
The University of Tokyo, Japan

(Received for publication April 5, 1969)

Mycophenolic acid shows significant antitumor activity against several strains of transplantable mouse tumors, some of which were originally established as syngeneic in tumor-host realtionship. Mycophenolic acid was more effective against solid than ascitic tumors. Oral, subcutaneous or intraperitoneal administration was equally effective.

In our screening for antiviral and antitumor antibiotics using the agar diffusion method as a primary assay and fungi as the screening organisms[1,2], it was found that mycophenolic acid was active. In this paper the report of antitumor activity for mycophenolic acid as described by WILLIAMS *et al.* is extended to several additional tumor systems.

Materials and Methods

1. Tumor cells:

(1) EHRLICH ascites carcinoma has been maintained in albino swiss mice strain 4CS in our laboratory since 1963.

(2) Mouse ascites mammary carcinoma, MM2, was previously converted into ascitic form by TAKEUCHI and YAMAMOTO in 1957. It arose from a spontaneous mammary carcinoma in a C3H/He mouse[3], but soon lost its strain specificity. It has been maintained in our laboratory in 4CS mice.

(3) Mouse ascites hepatoma, MH134, was converted into ascitic form by SATO *et al.*[4], from a C3H/HeN mouse hapatoma induced after treatment with carbon tetrachloride by ANDERVONT *et al.*[5] It has been maintained in C3H/He mice with distinct strain specificity in tumor take in our laboratory.

(4) Mouse ascites sarcoma, SR-C3H/He Th62, was originally induced with Schmidt-Rupin strain of Rouse sarcoma virus in C3H/He mouse and converted into ascitic form by YAMAMOTO and TAKEUCHI[6]. This tumor has the viral genom in carrier state and continuously possesses histocompatible antigens in C3H/He mice.

All strains of tumor were kindly supplied by Dr. T. YAMAMOTO and Dr. M. TAKEUCHI of the Institute of Medical Science, University of Tokyo.

2. Mice: Swiss albino mice, strain 4CS, which is a substrain of ddY mice, was used for EHRLICH carcinoma. C3H/He mice for MH134 and SR-C3H/He, and (C3H/He×4CS) F_1 mice for MM2 were employed. Animals were of both sexes and 5 weeks of age at the time of inoculation, being given water and cubed chow *ad libitum*.

3. Agents: Mycophenolic acid was supplied from Department of Fermentation of our laboratory in purified form. It was brought into phosphate buffer solution (PBS) with the minimal amount of 0.1 N NaOH necessary to dissolve the drug at a concentration

of 30 mg/ml (pH 7.4). Mitomycin C was obtained commercially (Kyowa Hakko Co.). Mitomycin C was dissolved in PBS at a concentration of 0.05 mg/ml.

Results

1. EHRLICH Carcinoma

(1) EHRLICH ascites tumor cells (1×10^6 cells) were inoculated intraperitoneally to 4CS mice and the mice were treated with various doses of mycophenolic acid or mitomycin C by the same route once daily for 10 consecutive days, initiating 18 hours after the inoculation. The accumulation of ascites was inhibited or retarded during the period of first 5 days after inoculation at a dose of 240 mg/kg. All treated mice died due to tumor growth and their survival rates were not remarkable when compared with the control group (Fig. 1).

(2) In case of solid form where 2×10^6 cells of EHRLICH ascites tumor cells were inoculated subcutaneously to 4CS mice and treated with various doses of mycophenolic acid or mitomycin C by intraperitoneal route with the same dose schedule as above. Mycophenolic acid inhibited solid tumor growth when the mice were sacrificed at the 11 th day and the tumor was compared with the control group (Table 1). More than 60 % inhibition of tumor growth was observed at dose levels of more than 30 mg/kg per day.

2. Mouse Ascites Mammary Carcinoma MM2

(1) (C3H/He×4CS) F_1 mice were inoculated intraperitoneally with 1×10^6 cells of MM2 ascites tumor cells and treated with the agents by the same route. As shown

Fig. 1. Antitumor effect of mycophenolic acid on EHRLICH carcinoma (ascites form) in 4CS mice.

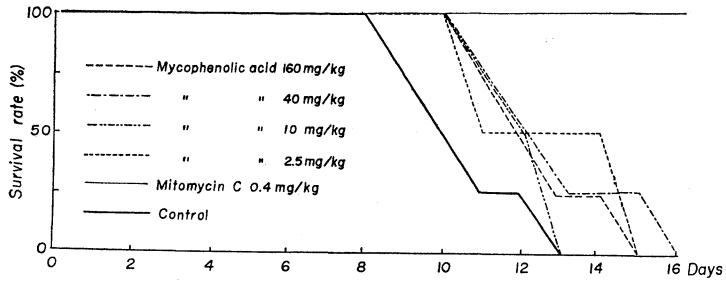


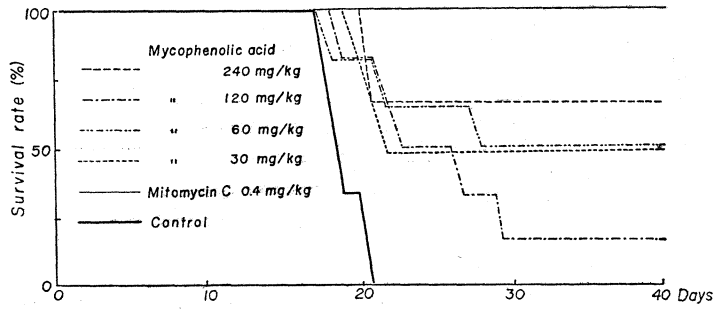
Table 1. Antitumor effect of mycophenolic acid on EHRLICH carcinoma (solid form) in 4CS mice.

	Daily dose* (mg/kg)					
	Mycophenolic acid				Mitomycin C	PBS
	240	120	60	30	0.4	
Av. tumor wt (mg)	trace	67	141	146	237	406
% Inhibition	95	83.7	65.4	64.1	41.6	0
Av. body wt. gain** (g)	1.3	2.4	2.5	2.4	2.3	3.4

* Injected intraperitoneally daily for 10 days.

** Difference in the body weight before and at 11 days after inoculation.

Fig. 2. Antitumor effect of mycophenolic acid on MM2 tumor (ascites form) in (C3H/He×4CS)F_1 mice.



in Fig. 2, mycophenolic acid was moderately effective on the ascitic form of this tumor. Mitomycin C, at a dose of 0.4 mg/kg per day, was remarkably effective on this type of the tumor. All mitomycin C treated mice were alive more than 40 days.

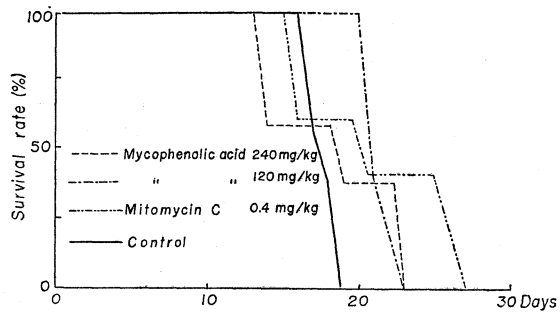
(2) (C3H/He × 4CS) F_1 mice were inoculated subcutaneously with 2×10^6 cells of MM2 ascites tumor cells. The mice were treated by intraperitoneal route for 10 consecutive days, starting at 18 hours after inoculation. When the weight of tumor after 11 days was compared with the control group, more than 50 % inhibition of tumor was observed at dose levels of more than 120 mg/kg per day.

Table 2. Antitumor effect of mycophenolic acid on MM2 tumor (solid form) in (C3H/He× 4CS)F_1 mice.

	Daily dose* (mg/kg)					
	Mycophenolic acid				Mitomycin C	PBS
	240	120	60	30	0.4	0
Av. tumor wt. (mg)	164	116	182	188	248	232
% Inhibition	72.5	50.0	22.1	14.7		0
Av. body wt. gain** (g)	1.4	2.1	2.3	2.5	2.3	3.6

* Injected intraperitoneally daily for 10 days.
** Difference in the body weight before and at 11 days after inoculation.

Fig. 3. Antitumor effect of mycophenolic acid on MH134 tumor (ascites form) in C3H/He mice.

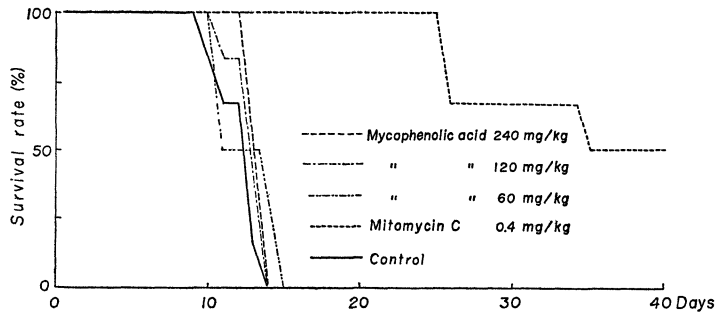


3. Mouse Ascites Hepatoma MH134

(1) C3H/He mice were inoculated intraperitoneally with 1×10^6 cells of MH134 ascites tumor cells and treated with agents by the same route (Fig. 3). Mycophenolic acid or mitomycin C gave almost no effect on the ascitic form of this tumor; that is, mycophenolic acid at the dose of 240 and 120 mg/kg per day showed relative survival times of 103.3 and 120.4 % respectively.

(2) C3H/He mice were inoculated subcutaneously with 2×10^6 cells of MH134 ascites tumor cells and treated by intraperitoneal route for 10 days starting at 18 hours after the tumor inoculation (Table 3). At a dose of 240 mg/kg of mycophenolic acid per day, the tumor growth was inhibited at a rate of 72.6 % when the weight of tumor at 11 days was compared with the control group. At a dose of 120 mg/kg

Fig. 4. Antitumor effect of mycophenolic acid on SR-C3H/He sarcoma (ascites form) in C3H/He mice.



per day mycophenolic acid gave 64.0 % inhibition of tumor growth.

4. SR-C3H/He Th62 Sarcoma

(1) C3H/He mice were inoculated intraperitoneally with 1×10^6 cells of the syngeneic tumor cells and treated by the same dose schedule as in 1-(1). Mycophenolic acid was ineffective on this type of SR-C3H/He Th62 tumor, whereas mitomycin C was effective. A half of mitomycin C treated mice survived more than 40 days (Fig. 4).

Table 3. Antitumor effect of mycophenolic acid on MH134 tumor (solid form) in C3H/He mice.

	Daily dose* (mg/kg)				
	Mycophenolic acid			Mitomycin C	PBS
	240	120	60	0.4	0
Av. tumor wt. (mg)	540	710	1,500	1,340	1,970
% Inhibition	72.6	64.0	23.9	32.0	0
Av. body wt. gain** (g)	1.2	2.1	2.3	2.1	3.1

* Injected intraperitoneally daily for 10 days.
** Difference in the body weight before and at 11 days after inoculation.

Table 4. Antitumor effect of mycophenolic acid on SR-C3H/He sarcoma (solid form) in C3H/He mice.

	Daily dose (mg/kg)				
	Mycophenolic acid			Mitomycin C	PBS
	240	120	60	0.4	0
Av. tumor wt. (mg)	634	1,117	1,555	1,243	1,257
% Inhibition	49.6	21.2	−23.7	1.1	0
Av. body wt. gain** (g)	1.2	2.1	2.3	2.1	3.1

* Injected intraperitoneally daily for 10 days.
** Difference in the body weight before and at 11 days after inoculation.

(2) C3H/He mice were inoculated subcutaneously with 2×10^6 cells of the syngeneic tumor cells. The mice were treated by intraperitoneal route for 10 consecutive days, starting at 18 hours after inoculation. Mycophenolic acid was moderately effective on the solid form of this tumor, showing, at a dose of 240 mg/kg per day, about 50 % inhibition of tumor growth. Although mitomycin C was effective on the ascites form, it was ineffective on the solid form of this tumor.

5. Route of Administration and Antitumor Activity

(1) 4CS mice were inoculated subcutaneously with 2×10^6 cells of EHRLICH carcinoma cells and treated with various doses of mycophenolic acid by intraperitoneal, subcutnaneous and oral routes once daily for 10 consecutive days starting from 18 hours

after inoculation. Tumors were weighed 11 days after inoculation. When the inhibition rates of the tumor growth were compared in the three administration groups, the rates were approximately equal in each other (Table 5), indicating that mycophenolic acid can be effective through all the administration routes tested.

Table 5. Antitumor effect of mycophenolic acid on EHRLICH carcinoma (solid form) in 4CS mice by various treated routes.

	Administration route and daily dose* (mg/kg)						Control
	I. P.		Oral		S. C.		
	240	120	240	120	240	120	
Av. tumor wt. (mg)	80	52	64	64	20	72	396
% Inhibition	79.3	86.9	83.9	83.9	95.0	81.8	0
Av. body wt. gain** (g)	1.3	1.8	1.2	2.0	0.6	1.4	2.8

* Injected daily for 10 days.
** Difference in the body weight before and at 11 days after inoculation.

(2) C3H/He mice were inoculated subcutaneously with 2×10^6 cells of MH134 tumor cells and the mice were treated intraperitoneally and orally with mycophenolic acid once daily for 10 consecutive days starting from 18 hours after inoculation. Tumors were weighed 11 days after inoculation. Oral administration at the dose of 240 and 120 mg/kg per day resulted in 79.5% and 61% inhibition while intraperitoneal administration of the same doses yielded 72% and 50% inhibition, again showing almost equal effectiveness by either route of administration (Table 6).

Table 6. Comparison of the antitumor effect following intraperitoneal and oral administration of mycophenolic acid on MH 134 tumor (solid form) in C3H/He mice.

		Daily dose (mg/kg)			
		240	120	60	0
Intraperitoneal administration*	Av. tumor wt. (mg)	276	490	786	978
	% Inhibition	71.8	49.9	20.0	0
	Av. body wt. gain** (g)	0.9	1.2	1.6	1.8
Oral administration	Av. tumor wt. (mg)	206	462	710	954
	% Inhibition	79.8	61.4	25.6	0
	Av. body wt. gain** (g)	1.1	0.8	1.3	1.9

* Injected daily for 10 days.
** Difference in the body weight before and at 11 days after inoculation.

Discussion

The main purpose of the present study is to search for antitumor activities of mycophenolic acid on our transplantable mouse tumor systems, which are syngeneic in tumor-host relationship.

The data presented in this paper indicate that mycophenolic acid is effective on the syngeneic tumor systems as well as others. The results of the comparative study on four kinds of tumor of ascites and solid form show that mycophenolic acid was more effective on solid tumors than ascites tumors.

The study on the route of administration for the agent show mycophenolic acid is effective by oral and subcutaneous routes as well as intraperitoneal route. These results are in good accordance with the findings of WILLIAM *et al.*[7]

In the previous experiment, the antifungal activity of mycophenolic acid is inactivated by serum. Since it is known that the bound as well as unbound forms of certain antitumor agents are effective, *e. g.*, iyomycin[8], it is likely that the antitumor activity of mycophenolic acid is due in part to the bound form of the acid.

Acknowledgement

The author express their sincere thanks to Dr. T. YAMAMOTO and Dr. M. TAKEUCHI for gift of tumors and advice in directing the work.

References

1) ANDO, K.; S. SUZUKI, G. TAMURA & K. ARIMA: Antiviral activity of mycophenolic acid. J. Antibiotics 21 : 649～652, 1968.
2) NOTO, T.; M. SAWADA, K. ANDO & K. KOYAMA: Some biological properties of mycophenolic acid. J. Antibiotics 22 : 165～169, 1969.
3) TAKEUCHI, M. & T. YAMAMOTO: On assay method of antitumor agents relating to enhanced resistance of living body. J. Antibiotics, Ser. B 16 : 142～145, 1963.
4) SATO, H.; M. BELKIN & E. ESSNER: Experiments on an ascites hepatoma. III. The conversion of mouse hepatomas into ascites form. J. Natl. Cancer Inst. 17 : 1～22, 1956.
5) ANDERVONT, H. B. & T. B. DUNN: Transplantation of hematomas in mice. J. Natl. Cancer Inst. (Suppl.) 15 : 1513～1524, 1955.
6) YAMAMOTO, T. & M. TAKEUCHI: Studies on Rous sarcoma virus in mice. I. Establishment of an ascites sarcoma induced by Schmidt-Ruppin strain of Rous sarcoma virus in C3H/He mouse. Jap. J. Exp. Med. 37 : 37～50, 1967.
7) WILLIAMS, R. H.; D. H. LIVELY, D. C. DELONG, J. C. CLINE, M. J. SWEENEY, G. A. POORE & S. H. LARSEN: Mycophenolic acid. Antiviral and antitumor properties. J. Antibiotics 21 : 463～464, 1968.
8) UMEZAWA, I.; N. KANDA & T. HATA: Iyomycin, a new antitumor antibiotic from *Streptomyces*. V. Experimental treatment of sarcoma 180 with iyomycin-B_1 complex and its derivatives. J. Antibiotics 21 : 30～36, 1968.