SOME BIOLOGICAL PROPERTIES OF MYCOPHENOLIC ACID

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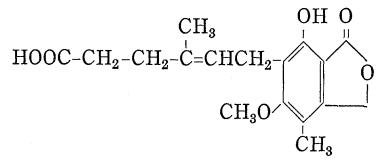
Mycophenolic acid (MA) inhibits the growth of some pathogenic fungi such as *Candida albicans*, *Cryptococcus neoformans* and several species of *Trichophyton* at low concentrations *in vitro*. The antibiotic is more effectvie against fungi at slightly acidic pH than neutral pH. When administered to mice through the intraperitoneal, intramuscular and oral routes MA excreted to a few per cent in the urine. However, at no time was active MA detectable in the blood of mice. MA is effective against experimental trichophytosis of the guinea pigs, without noticeable skin irritation or inflammation.

Mycophenolic acid (MA) is an antifungal antibiotic found by GOSIO[1] in 1896. Later, CLUTTERBUCK and OXFORD[2] reported that MA is produced by *Penicillium brevi-compactum* series to which GOSIO's strain belonged. The structure of MA was elucidated by BIRKINSHAW[3] in 1952, as shown in Fig. 1. Although ALSBERG[4] and FLOREY[5] reported some biological properties of MA, detailed studies of the biological properties have not been made.

Fig. 1. Mycophenolic acid



Recent findings that MA exerts significant antiviral and antitumor activities[6,7] prompted us to study some biological properties of the antibiotic. In this communication we describe *in vitro* antimicrobial properties, absorption and excretion in mice and the effect against experimental trichophytosis in the guinea pig.

Materials and Methods

Nystatin (4,180 u/mg) and griseofulvin were used as positive controls*.

The microorganisms used in this study are type cultures of our laboratory, including 54 strains of 25 species of fungi and 27 strains of 7 species of bacteria. The fungi were grown on malt extract agar (Difco, pH 5.2) supplemented with 2 % glucose. The medium for bacteria was MÜLLER-HINTON agar medium (Eiken, pH 7.2). For determination of minimum inhibitory concentration (MIC) a 2-fold serial dilution method was used. The test organisms were inoculated by streaking to the assay media with a platinum loop from the cell suspensions of the test organisms (bacteria $10^{7.5}$ cells/ml, fungi $10^{6.5}$ cells/ml). The cultures were incubated 2~5 days at 27°C for fungi and 18 hours at 37°C for bacteria.

Quantitative determination of MA ; The thin-agar cylinder plate method was utilized for the quantitative determination of MA using *Candida albicans* as a test organism. The

* Nystatin and griseofulvin samples were kindly supplied by Sankyo Co., Ltd. and Nihon Kayaku Co., Ltd., respectively.

cell suspension of the test organism was mixed with malt-dextrose agar medium. The cultures were incubated at 27°C for 24 hours. The zones of growth inhibitory formed by MA slowly disappeared from the margin with time, so it is necessary to incubate precisely for 24 hours. It is possible to determine MA in the range of 6~100 mcg/ml by this method.

Anti-trichophytosis activity of MA; MA was used as 5% solution in 50% ethanol and griseofulvin, the positive control, was used as 3% phenethyl alcohol solution. The hair was removed from back and both sides of guinea pigs, strain Hartley, weighing 300 ±20 g. After exfoliation with sand-paper on naked skin, the guinea pigs were infected with a spore suspension (10^6 spores/ml) of *Trichophyton asteroides*. The treatment was initiated 40 hours after infection, MA being given on the infected loci twice a day for 7 consecutive days. Anti-trichophytosis activity was determined by observing the symptoms caused by the infection on treated and untreated skin.

Results and Discussions

Antimicrobial Activity of MA

The antimicrobial spectra of MA are demonstrated in Tables 1 and 2. MA shows minimum inhibitory concentration (MIC) values of 3.9~31.2 mcg/ml against some pathogenic fungi such as *Candida albicans*, *C. stellatoidea*, *C. tropicalis*, *C. parakrusei* and *Cryptococcus neoformans*, whereas MA shows no activity against *Candida guilliermondii*, *C. krusei*, *C. pseudotropicalis*, *Hausenula anomala* and *Saccharomyces cerevisiae*. It is noteworthy that several species of *Trichophyton* were inhibited by relatively low

Table 1. Antifungal activity of mycophenolic acid and nystatin

Test organisms		MIC (mcg/ml)		Test organisms		MIC (mcg/ml)	
		Mycophenolic acid	Nystatin			Mycophenolic acid	Nystatin
Candida albicans	Ca-1	7.81	0.97	*Willia anomala*	W-1	500	0.24
〃	Ca-2	3.90	0.48	*Cryptococcus neoformans*	Cr-n-1	15.62	0.24
〃	Ca-3	15.62	0.97	〃	Cr-n-2	15.62	0.97
〃	Ca-4	7.81	0.48	〃	Cr-n-3	15.62	0.97
〃	Ca-5	7.81	0.97	〃	Cr-n-4	15.62	0.97
〃	Ca-6	7.81	0.48	〃	Cr-n-5	31.25	0.97
〃	Ca-7	7.81	0.48	*Saccharomyces carlsbergensis*	S-c-1	62.5	0.97
〃	Ca-8	7.81	0.97	〃	S-c-2	62.5	0.97
〃	Ca-9	7.81	0.48	*S. cerevisiae*	S-ce-1	500	—
〃	Ca-10	7.81	0.97	〃	S-ce-2	500	0.48
〃	Ca-15	15.6	0.97	*Microsporum canis*	Mi-c-1	31.25	1.95
〃	Ca-17	15.6	0.48	*M. gypseum*	Mi-g-1	31.25	3.90
C. guilliermondii	Ca-g-1	500	0.97	*Aspergillus flavus*	As-f-1	500	1.95
〃	Ca-g-2	500	0.24	*A. melleus*	As-m-1	125	1.95
〃	Ca-g-3	500	0.24	*A. amstelodami*	As-a-1	7.81	—
C. krusei	Ca-k-1	500	1.95	*A. chevalieri*	As-c-1	250	3.90
〃	Ca-k-2	500	1.95	*A. fumigatus*	As-f-1	500	7.81
C. parakrusei	Ca-pk-1	31.25	0.48	*Trichophyton mentagrophytes*	T-m-1	15.62	1.95
〃	Ca-pk-2	62.5	0.97	*T. rubrum*	T-r-1	15.6	—
C. tropicalis	Ca-t-1	7.81	0.97	*T. schoenleinii*	T-s-1	500	7.81
〃	Ca-t-2	15.6	0.48	*T. ferrugineum*	T-f-1	15.6	1.95
〃	Ca-t-3	7.81	1.95	*T. asteroides*	T-a-1	15.6	1.95
C. pseudotropicalis	Ca-pt-1	125	—				
〃	Ca-pt-2	500	0.48				
〃	Ca-pt-3	500	1.95				
C. stellatoidea	Ca-s-1	3.90	0.24				

Table 2. Antibacterial activity of mycophenolic acid

Test organisms		MIC (mcg/ml)	Test organisms		MIC (mcg/ml)
Staphylococcus aureus	209P	31.25	*Staphylococcus epidermidis*	S-5	125
〃	209P(R)	125	*Shigella flexneri*	2a	>500
〃	Terajima	62.5	〃	2b	>500
〃	188	62.5	*Proteus vulgaris*	OX-K	250
〃	Kusama	125	〃	OX-2	500
〃	B-5	31.25	*Escherichia coli*	B-19	>500
〃	B-8	31.25	〃	C-6	>500
〃	B-9	125	*Pseudomonas aeruginosa*	Ps-1	>500
〃	B-15	31.25	〃	1905	>500
〃	B-19	31.25	*Salmonella enteritidis*	S-1	>500
〃	B-43	125	〃	S-2	>500
〃	B-46	125			

Table 3. Effect of pH of medium on the activity of mycophenolic acid against various test organisms

Test organisms		MIC (mg/ml)		Test organisms		MIC (mg/ml)	
		pH 5.2	pH 7.3			pH 5.2	pH 7.3
Candida albicans	Ca-3	6.25	200	*Saccharomyces calsbergensis*	S-c-1	200	>200
〃	Ca-6	6.25	200	*Microsporum canis*	Mi-c-1	12.5	100
C. parakrusei	Ca-pk-1	12.5	>200	*Aspergillus amstelodami*	As-a-1	12.5	50
C. tropicalis	Ca-t-1	12.5	>200	*Trichophyton mentagrophytes*	T-m-1	6.25	25
C. stellatoidea	Ca-s-1	12.5	100	*T. asteroides*	T-a-1	6.25	50
Cryptococcus neoformans	Cr-n-1	12.5	200				

Table 4. Effect of inoculum size on the activity of mycophenolic acid against various test organisms

Test organisms	Inoculum size (cells/ml)	MIC (mcg/ml)	Test organisms	Inoculum size (cells/ml)	MIC (mcg/ml)
Candida albicans Ca-4	10^8	125	*Candida krusei* Ca-k-2	10^8	>125
	10^7	15.6		10^7	>125
	10^6	7.8		10^6	>125
	10^5	3.9		10^5	>125
Candida parakrusei Ca-pk-1	10^8	>125	*Candida guilliermondii* Ca-g-1	10^8	>125
	10^7	62.5		10^7	>125
	10^6	31.2		10^6	>125
	10^5	31.2		10^5	125
Saccharomyces carlsbergensis S-c-2	10^8	>125	*Candida pseudotropicalis* Ca-pt-3	10^8	>125
	10^7	125		10^7	>125
	10^6	31.2		10^6	>125
Cryptococcus neoformans Cr-n-3	10^8	125	*Willia anomala* W-1	10^8	>125
	10^7	7.8		10^7	>125
	10^6	7.8		10^6	>125
	10^5	—		10^5	>125

concentration (15.6 mcg/ml). The antifungal spectrum of MA is specific and the activity is not as strong as nystatin which inhibited the growth of all fungi tested at concentrations of 0.2~7.8 mcg/ml.

MA shows moderate inhibition of *Staphylococcus aureus* but the other bacteria tested were insensitive. Most antifungal agents now used are highly toxic to mammals. It is interesting that the acute toxicity of MA is so low that mice could well-tolerate a single intraperitoneal injection of 1,000 mg/kg.[7]

The MIC values of MA against sensitive fungi depends on pH of the assay media and the inoculum size of the test organisms (Tables 3 and 4). For example, several *Candida albicans* strains were inhibited at 6.3 mcg/ml at pH 5.2 but grew normally at pH 7.3 even in the presence of 200 mcg/ml of MA. MIC of MA for *Cryptococcus neoformans* was raised over 16 fold when the inoculum size increased from 10^7 to 10^8 cells/ml.

Absorption and Excretion

Table 5 shows that MA activity was recovered from urine after administration either intraperitoneally, intramuscularly or orally. Upon intramuscular administration of 250 mg/kg of MA urine levels of 42.5 mcg/ml were found after 30 minutes and 27.5 mcg/ml was still excreted in urine after 6 hours (Teble 5). However, no MA was detectable in the blood at any time post administration. This contradictory fact suggests that MA might be present in the blood bound with serum protein, showing no growth inhibitory activity. Although some MA was excreted in the urine, the total sum of recovered presented only a few per cent of the initial dose.

Table 5. Bio-assay values in urine after administration of mycophenolic acid to mice

Dose (mg/kg)	Route	Urine concentration (mcg/ml)			
		30 minutes	1 hour	3 hours	6 hours
250	I M	42.5	11.5	37.5	27.5
250	I P	10.0	17.5	6.0	—
500	P O	37.5	6.0	13.5	15.5

The result is given by the mean of 5 animals.
Test organism : *Candida albicans* Ca-4

Effect against Experimental Trichophytosis

MA suppressed the symptoms caused by infection of *Trichophyton asteroides* without

Table 6. Effect of mycophenolic acid on experimental tricophytosis in guinea pigs

Treatment	Dose	Animal No.	Curative rate*	Redness	Scale	Stimulus
None (Control)		1	0	## ## ##	## ## ##	
		2		## ## ##	## ## ##	
		3		## ## ##	## ## ##	
Griseofulvin (3 % soln.)	0.2 ml/part b. i. d.	4	100 %	— — —	— — —	— — —
		5		— — —	— — —	— — —
		6		— — —	— — —	— — —
Mycophenolic acid (5 % soln.)	0.2 ml/part b. i. d.	7	27.7 %	⊥ + —	— — —	— — —
		8	16.6 %	⊥ ⊥ ⊥	— — —	— — —
		9	23.5 % (22.6 % in average)	— — —	— — —	— — —

* Guinea pigs were sacrificed after treatment to remove small pieces of infected skin (three pieces from back and both abdominal sides of each animal). Each small piece was divided equally into 6 portions to inoculate them onto SABOURAUD agar plates added with penicillin and streptomycin. The fungal growth was examined, after 1-week incubation at 27°C, and the curative rate was calculated as follows :

$$\text{Curative rate } (\%) = \frac{\left(\begin{matrix}\text{Total number}\\ \text{of skin pieces}\end{matrix}\right) - \left(\begin{matrix}\text{Number of skin pieces}\\ \text{indicated fungal growth}\end{matrix}\right)}{\text{Total number of skin pieces}} \times 100\ (\%)$$

irritation and inflammation to the skin (Table 6). When small pieces of treated skin were incubated at 27°C for 7 days on a suitable medium, 77.4 % of the pieces possessed viable *T. asteroides*. This fact indicated that MA is a fungistatic agent rather than a fungicidal one. Since the symptoms caused by the proliferation of the fungus were suppressed, the growth of the organism was inhibited as long as MA was present, but once MA was removed from the treated skin, the fungus began to grow again. Thus, MA shows moderate inhibitory activity against experimental trichophytosis of guinea pigs without adverse side-effects. However, usefulness will be limited because of the fungistatic nature of the compound.

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