SOME OBSERVATIONS ON THE NATURE OF REACTIVE GROUPS INVOLVED IN REVERSAL OF MYCOBACILLIN INHIBITION BY STEROLS AND LIPIDS

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Antifungal action of di- and triacetyl derivatives of mycobacillin is not antagonised by cholesterol or lecithin which antagonise the action of its heptamethyl derivative. Considering the reactive groups of mycobacillin, the antagonism may involve the tyrosine hydroxyl groups of the antibiotic and the 3-hydroxyl group of cholesterol or the oleic acid component of lecithin having unsaturation at the 9: 10 position with *cis*-configuration.

It has been reported that the action of polyene antibiotics as also that of the cyclic polypeptide antibiotic mycobacillin is antagonised by sterols[1,2,11] and phospholipids[3,4,5,11]. Experiments by WEISSMANN and SESSA[6] with artificial phospholipid spherules with and without cholesterol support the "sterol action" hypothesis of KINSKY[7] and LAMPEN[2] for polyene antibiotics. Antagonism of the antifungal action of pyrrolnitrin by lipid has also been reported to depend on physicochemical interaction[8]. In case of mycobacillin, antagonism has been observed not only by commercial cholesterol and lecithin but also by samples isolated from the sensitive strains of *Aspergillus niger*[10]. This reaction involves the 3-hydroxyl group of cholesterol and the oleic acid moiety of lecithin from the side of the antagonist[11]. Now mycobacillin (Fig. 1) contains one serine hydroxyl, two tyrosine hydroxyl and seven carboxyl groups.

In this communication we describe some experiments on the antagonism between mycobacillin and some of its derivatives on the one hand and cholesterol or lecithin or some isomers of oleic acid on the other, so as to throw some light on the nature of reacting groups.

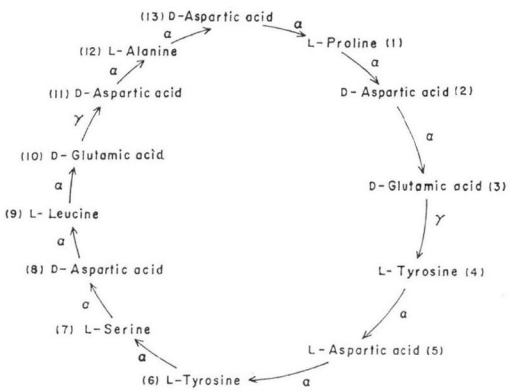

Fig. 1. Structure of mycobacillin

Methods and Materials

Aspergillus niger used as the test organism was maintained on CZAPECK agar medium. Growth inhibition was measured by standard cup-plate method using a 5 days old spore suspension as inoculum. In case of mycobacillin derivatives experiments were carried out in a liquid medium because of low diffusibility of these substances. Derivatives used are diacetyl, triacetyl and heptamethyl mycobacillin. They were prepared according to the method of BANERJEE and BOSE[12,13].

Results

Effect of Cholesterol and Lecithin on Growth Inhibition by Mycobacillin Derivatives

Both diacetyl (acetylated at the two tyrosine hydroxyl groups) and triacetyl (acetylated at two tyrosine and one serine hydroxyl groups) mycobacillin possess antifungal property but their activity is somewhat reduced[12]. Lecithin or cholesterol, when added to the liquid medium along with these mycobacillin derivatives, did not prevent their inhibition of growth. The heptamethyl ester of mycobacillin is also antifungal, although not as active as the parent compound[13], but its action is antagonised by cholesterol or lecithin in liquid medium.

Table 1. Antagonisation of growth inhibitory property of mycobacillin derivatives by cholesterol and lecithin

Mycobacillin and its derivatives	Antagonist			
	Cholesterol		Lecithin	
	Concentration in μg/ml	Growth	Concentration in μg/ml	Growth
Mycobacillin	0	−	0	−
	62.5	−	62.5	−
	125	+	125	+
	250	+	250	+
	500	+	500	+
Diacetyl mycobacillin	0	−	0	−
	100	−	100	−
	200	−	200	−
	400	−	400	−
	800	−	800	−
Triacetyl mycobacillin	0	−	0	−
	125	−	125	−
	250	−	250	−
	500	−	500	−
	1000	−	1000	−
Heptamethyl ester of mycobacillin	0	−	0	−
	100	−	100	−
	150	+	150	+
	300	+	300	+
	600	+	600	+

Antagonistic effect was studied in liquid broth tubes. Each tube contains 0.1 ml of diacetyl (400 μg/ml) or triacetyl (500 μg/ml) or heptamethyl ester derivative (300 μg/ml) of mycobacillin or mycobacillin (250 μg/ml) alone or a mixture of 0.1 ml of mycobacillin or its derivatives and 0.1 ml of antagonists of varying concentration and the total volume of the experimental solution was made upto 1 ml with CZAPECK medium. (−) and (+) indicate inhibition and growth respectively.

Table 2. Effect of oleic acid and its isomers on growth inhibitory property of mycobacillin

Oleic acid		Petroselinic acid		Elaidic acid		*trans*-Vaccenic acid	
Conc. in μg/ml	Diameter of zone of inhibition in mm	Conc. in μg/ml	Diameter of zone of inhibition in mm	Conc. in μg/ml	Diameter of zone of inhibition in mm	Conc. in μg/ml	Diameter of zone of inhibition in mm
62.5	22	62.5	24	62.5	21	62.5	24
125	0	125	23	125	21	125	24
250	0	250	24	250	21	250	23
500	0	500	24	500	21	500	23

Antagonisation of growth inhibition was studied by cup plate method. Each cup contains 0.05 ml of mycobacillin (250 μg/ml) alone or the mixture of 0.05 ml mycobacillin (250 μg/ml) and 0.05 ml oleic acid or its isomers in varying concentration. Diameter of the cup is 12 mm.

'0' indicates absence of zone of inhibition.

Effect of Isomers of Oleic Acid on Antifungal Action of Mycobacillin

The effect of oleic acid and its three isomers namely petroselinic, elaidic and *trans*-vaccenic acids, on growth inhibition by mycobacillin was studied. Antagonism was shown not by the isomers but only by oleic acid which possesses (9: 10) unsaturation with *cis*-configuration.

Discussion

This work was undertaken to determine the nature of the reacting groups involved in antagonism of the growth inhibitory action of mycobacillin on filamentous fungi by cholesterol and lecithin. Experiments were done to see if the antifungal action of di- or triacetyl derivatives of mycobacillin or of its heptamethyl ester is antagonised by cholesterol or lecithin. In course of these studies it has been observed that tyrosine hydroxyl group must be kept free for the antagonisation to occur and that it is immaterial if serine hydroxyl group is free or acetylated. It was also observed that antagonisation may occur whether all the carboxyl groups of mycobacillin are free or esterified.

Remembering the reactive groups present in mycobacillin it appears most likely that its tyrosine hydroxyl groups must be free to take part in the antagonising reaction. The fact that mycobacillin is antagonised by oleic acid but not by its isomers indicates that not only unsaturation but the 9: 10 position of the double bond and its *cis*-configuration are important from the side of the antagonist. Thus the reactive groups involved in the antagonism process include the tyrosine hydroxyl groups of mycobacillin and the 3-hydroxyl group of cholesterol or the given configuration of oleic acid component of lecithin. It has however not been possible to speculate on the nature of the chemical bonding that might occur between the two reacting groups.

References

1) Gottlieb, D.; H. E. Carter, J. H. Sloneker & A. Amman: Protection of fungi against polyene antibiotics by sterols. Science 128: 361, 1958
2) Lampen, J. O.: Interference by polyene antifungal antibiotics (especially nystatin and filipin) with specific membrane functions. Sym. Soc. Gen. Microbiol. 16: 111, 1966
3) Ghosh, B. K. & A. N. Chatterjee: Action of an antifungal antibiotic, nystatin, on the protozoa, *Leishmania donovani*. V. Studies on the absorption of nystatin by *L. donovani*. Ann. Biochem. Exp. Med. 23: 309~317, 1963
4) Ghosh, A. & J. J. Ghosh: Effect of nystatin and amphotericin B on the growth of *Candida albicans*. Ann. Biochem. Exp. Med. 23: 29~44, 1963
5) Ghosh, A. & J. J. Ghosh: Factors affecting the absorption of nystatin by *Candida albicans*. Ann. Biochem. Exp. Med. 23: 101~112, 1963
6) Weissmann, G. & G. Sessa: The action of polyene antibiotics on phospholipid cholesterol structures. J. Biol. Chem. 242: 616~625, 1967
7) Kinsky, S. C.: Comparative responses of mammalian erythrocytes and microbial protoplasts to polyene antibiotics and vitamin A. Arch. Biochem. Biophys. 102: 180~188, 1963
8) Nose, M. & K. Arima: On the mode of action of a new antifungal antibiotic, pyrrolnitrin. J. Antibiotics 22: 135~143, 1959
9) Haider, A.; P. Bhattacharya, N. D. Banerjee & S. K. Bose: Antagonistic action of cholesterol on mycobacillin. J. Bact. 93: 2026~2038, 1967
10) Halder, A. & S. K. Bose: Reversal of mycobacillin inhibition reaction by sterols and phospholipids. J. Antibiotics 24: 779~782, 1971
11) Halder, A. & S. K. Bose: Mechanism of sterol and lipid antagonism of a polypeptide antibiotic, mycobacillin. J. Antibiotics 26: 358~361, 1973
12) Banerjee, P. C. & S. K. Bose: Studies on mycobacillin derivatives, acetyl derivatives of mycobacillin. J. Antibiotics 26: 257~260, 1973
13) Banerjee, P. C. & S. K. Bose: Studies on mycobacillin derivatives, ester derivatives of mycobacillin. Indian J. Biochem. Biophys. 10: 302~304, 1973