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THE INFLUENCE OF THE FATTY ACID COMPOSITION OF *ACHOLEPLASMA LAIDLAWII* MEMBRANES ON THE GROWTH INHIBITORY ACTIVITY OF NARASIN, A POLYETHER IONOPHOROUS ANTIBIOTIC

C.K. SMITH II* and R.G. STOUT

Department of Animal Sciences, University of New Hampshire, Durham, NH (U.S.A.)

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Summary

Narasin, a polyether ionophorous antibiotic capable of acting as a transmembrane carrier of cations, has a growth inhibitory effect on *Acholeplasma laidlawii*, permitting only 20% survival when present at 0.1 μg/ml in an undefined growth nutrient or fatty acid-deficient nutrient supplemented only with palmitic acid. When *A. laidlawii* is propagated in fatty acid-deficient nutrient supplemented with linoleic acid, however, the organisms become 40 times more sensitive to the growth inhibitory effect of this antibiotic. The actual fatty acid compositions of the membranes would indicate that a higher degree of unsaturation enhances ionophore activity.

Narasin (Fig. 1), a fermentation product of *Streptomyces aureofaciens*, is a member of a class of compounds known as the polyether ionophorous antibiotics [1]. A general characteristic of these compounds is that they possess unique physical properties making them capable of acting as carriers of cations across biological membranes [2, 3]. This transmembrane carrier capacity has been demonstrated for narasin in preparations of isolated rat liver mitochondria [4]. Because the kinetics of ionophore-mediated cation transport may be influenced by membrane structure in artificial lipid bilayers [5], it is important to determine whether the membrane composition of intact biological organisms may influence the growth-inhibitory or lethal activity of these compounds.

*Present address: Greenfield Research Laboratories, Eli Lilly and Company, Greenfield, IN 46140, U.S.A.

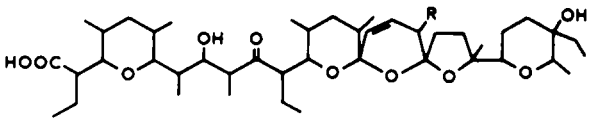

Fig. 1. Narasin, R = H, OH.

The carboxylic ionophore, narasin, is active in vitro against a number of micro-organisms, including the mycoplasma [6]. These organisms, in turn, provide a unique system to study the influence of membrane fatty acid composition on ionophore activity since the lipid composition of the plasma membrane (the only membrane) can be dictated to a large degree by regulating the lipid components of the growth medium [7, 8]. The membranes can easily be isolated by osmotic lysis [9].

This paper describes the lethal activity of narasin on cultures of *Acholeplasma laidlawii* in nutrients with different lipid compositions, and demonstrates that membrane composition influences the activity of these compounds in intact biological systems.

Cultures of *A. laidlawii*, strain A, were grown in a lipid-deficient nutrient [10] supplemented with 100 μM of either linoleic acid or palmitic acid, as well as in an undefined nutrient consisting of 3.7% brain-heart infusion, 2.5% yeast extract and 20% horse serum. Each of these was incubated in the presence of 0.1 μg/ml of narasin (based upon the results of a preliminary dose-toxicity study) for 24 h at 37°C, and the number of surviving organisms was determined by the preparation of spread plates on Barile, Yaguchi and Eveland agar. Identically prepared cultures were harvested, the cells lysed and the plasma membranes collected as described by Razin et al. [7]. These membrane preparations were transesterified [11], dried under N_2, extracted with CH_2Cl_2 and a sample subjected to gas-liquid chromatographic (GLC) analysis for fatty acid composition.

The results of this study show that the presence of proportionately large amounts of the diunsaturated fatty acid, linoleate, in the cell membranes of *A. laidlawii* increases their sensitivity to the growth-inhibitory effect of the carboxylic ionophore, narasin. *A. laidlawii* grown in lipid-deficient nutrient supplemented only with linoleic acid (18:2) incorporates this polyunsaturated fatty acid as a large proportion (60.5%) of the total cell membrane fatty acid composition (Table II). When challenged with 0.1 μg/ml of narasin at 37°C for 24 h these organisms are 40 times more sensitive to the lethal effects of this drug, as determined by percent survival, than those grown in a lipid-rich undefined nutrient supplemented with horse serum, or those organisms propagated in a lipid-deficient nutrient supplemented with palmitic acid (Table I). The membranes of these cells have virtually no unsaturated fatty acids but a high proportion of palmitate (16:0) (Table II). The lipid composition of the membranes of the organisms grown in the undefined nutrient is similar to that reported elsewhere [12].

The fatty acyl composition of a biological membrane will largely dictate the viscosity of that membrane at physiological temperatures (in the liquid-crystalline state). In general, shorter chain length and/or increased unsaturation lowers the viscosity at a given temperature [13—16]. Therefore, the cell

TABLE I

INFLUENCE OF LIPID COMPOSITION OF GROWTH NUTRIENT ON THE LETHAL EFFECT OF 0.1 μg/ml NARASIN AGAINST *ACHOLEPLASMA LAIDLAWII* A

Organisms/ml as determined by plate counts; all counts are the mean of two plates.

	Undefined nutrient			Lipid-poor nutrient + 100 μM palmitate			Lipid-poor nutrient + 100 μM linoleate		
	Untreated	Treated	% survival	Untreated	Treated	% survival	Untreated	Treated	% survival
Trial 1	$69 \cdot 10^6$	$12 \cdot 10^6$	18.0	$21 \cdot 10^4$	$41 \cdot 10^3$	19.5	$57 \cdot 10^5$	$14 \cdot 10^3$	0.2
	$82 \cdot 10^6$	$16 \cdot 10^6$	19.5	$35 \cdot 10^4$	$19 \cdot 10^3$	5.5	$90 \cdot 10^5$	$27 \cdot 10^3$	0.3
Trial 2	$10 \cdot 10^8$	$22 \cdot 10^7$	22.0	$10 \cdot 10^6$	$24 \cdot 10^5$	24.0	$66 \cdot 10^6$	$14 \cdot 10^4$	0.2
	$12 \cdot 10^8$	$23 \cdot 10^7$	19.2	$10 \cdot 10^6$	$25 \cdot 10^5$	25.0	$66 \cdot 10^6$	$22 \cdot 10^4$	0.3
Trial 3	$33 \cdot 10^6$	$82 \cdot 10^5$	25.0	$50 \cdot 10^4$	$80 \cdot 10^3$	16.0	$22 \cdot 10^6$	$24 \cdot 10^4$	1.1
	$31 \cdot 10^6$	$74 \cdot 10^5$	24.0	$31 \cdot 10^4$	$75 \cdot 10^3$	24.2	$22 \cdot 10^6$	$24 \cdot 10^4$	1.1
Mean % survival		21.3			19.1			0.5	
Range		18.0—25.0			5.5—25.0			0.2—1.1	

TABLE II

FATTY ACID COMPOSITION OF THE CELL MEMBRANES OF *ACHOLEPLASMA LAIDLAWII* GROWN IN NUTRIENTS WITH DIFFERENT LIPID COMPOSITIONS

Carbon number	Undefined nutrient*	Lipid-poor nutrient + 100 μM palmitate	Lipid-poor nutrient + 100 μM linoleate
12:0	2.2 ± 0.1	—	—
14:0	21.0 ± 0	2.0 ± 2.0	0.7 ± 0.7
16:0	51.5 ± 2.5	89.7 ± 2.4	18.0 ± 0.6
18:0	2.7 ± 0.2	8.3 ± 0.2	19.5 ± 4.5
18:1	trace	trace	1.7 ± 0.6
18:2	—	—	60.5 ± 2.4
Unidentified	22.6 ± 2.2	—	—

*Percent of total fatty acids.

membranes of the *A. laidlawii* grown in the linoleate-supplemented, lipid-deficient nutrient should be more fluid, due to the high percentage of the diunsaturated fatty acid, than the membranes of cells grown in either palmitate-supplemented, lipid-poor medium or in the undefined nutrient, since the membranes of these cells contain essentially no long-chain unsaturated fatty acids (Table II).

The carboxylic ionophores are capable of acting as mobile carriers of cations. Since the diffusion rate of a molecule is inversely proportional to the viscosity of its environment [17], the rate at which these carrier molecules could move back and forth through a biological membrane would be largely determined by the physical state of that membrane. If the lethal activity of these compounds against *A. laidlawii* is a result of this transmembrane motion, as seems likely, then the severity of the effect over time would be dependent upon the lipid composition of the membrane, as this dictates viscosity. The results of this study can be explained by this reasoning. The greater the degree of unsaturation (i.e., fluidity) of the cell membranes of *A. laidlawii* the greater the susceptibility to the growth inhibitory effect of the ionophore (Table I).

However, the increase in unsaturation of membrane fatty acids (i.e., fluidity) is advantageous to the survival of the organism. The presence of unsaturated fatty acids in the membranes of *A. laidlawii* has been shown to increase the resistance of the organisms to osmotic lysis and to improve growth [7]. An increased amount of palmitate in the cell membranes, on the other hand, retarded the growth of these organisms.

Based upon these facts, increasing the degree of unsaturation of the cell membrane fatty acids of *A. laidlawii* increases the fluidity, enhancing both growth and the sensitivity to the ionophores. The converse is also true.

It is at first perplexing that the organisms grown in the undefined nutrient should show an almost equal response to the ionophore as those grown in palmitate-supplemented nutrient. The membranes of the former group have a greater percentage of short-chain saturated fatty acids that normally indicate a lower viscosity (Table II). Therefore, based upon the reasoning presented, these cells should be more susceptible to the effects of the drug. However, the presence of serum in the growth medium of these organisms provides a supply of cholesterol that is unavailable to the organisms grown in the lipid-deficient nutrient. *A. laidlawii* will incorporate cholesterol into its cell membranes [7, 17], although it is not a requirement for growth. The presence of cholesterol in biological membranes serves to stabilize the fluid nature of the membrane, generally producing a condensing effect at physiological temperature [18—21]. Cholesterol present in biological membranes and in lecithin liposomes lowered the permeability of the membrane for various substances [22—24]. In the case of *A. laidlawii*, cholesterol in the cell membranes results in a reduction in permeability to glycerol and erythritol [17], thus explaining the similar response to narasin by the cells grown in undefined nutrient plus serum and those grown in lipid-deficient medium supplemented with palmitate in the absence of serum providing still further evidence that membrane composition can influence the activity of the ionophorous antibiotics.

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