Effect of Hypocholesteremic Compounds on Bacterial Multiplication

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SUMMARY

Hypocholesteremic compounds, triparanol, SKF 525-A, SKF 3301-A, SKF 16467-A, inhibited the multiplication of some bacteria and ascomycetes; phycomycetes were not inhibited. Gram-positive bacteria were most sensitive to these compounds. Dimethyl or diethyl aminoethanol and SKF 2314, compounds resembling the major moieties of the SKF compounds, were not inhibitory alone or together; the whole molecule was necessary for inhibition. Inhibition by triparanol of the multiplication of several Gram-positive bacteria was annulled by albumin and ergosterol; oleic acid and squalene were mostly inactive.

INTRODUCTION

Compounds which inhibit steroid metabolism in mammals ('hypocholesteremics') inhibit the multiplication of a wide variety of micro-organisms, especially Gram-positive bacteria (Aaronson, 1965; Martin-Smith & Sugrue, 1964; Smith, Shay & Doorenbas, 1963). We have examined the effect on bacterial multiplication of several related hypocholesteremic compounds to determine (*a*) whether the Gram-positive bacteria are most sensitive; (*b*) the active moiety of the inhibitory compounds; (*c*) compounds which annul the inhibition; (*d*) the potential of these compounds as antibacterial compounds; (*e*) whether the hypocholesteremic compounds can be used to study bacterial metabolism, especially the role of steroids in bacterial metabolism.

METHODS

Micro-organisms from the collection of the Biology Department, Queens College, New York, were used. For disc assay, Whatman no. 50 filter-pad discs (0.6 cm. diam.) were soaked in solutions of the test compound at the concentration indicated, dried, and autoclaved. Bacteria were grown on nutrient agar; fungi on Sabouraud's glucose agar. Cultures in triplicate were incubated at 25° for 2 days or until they showed good growth over the surface of the Petri dish. Triparanol (1-[*p*-(4-diethylaminoethoxy)-phenyl]-1-(*p*-tolyl)-2-*p*-chlorophenylethanol) was supplied by Dr F. J. Murray (W. S. Merrell, Cincinnati, Ohio); the SKF compounds by Dr W. L. Holmes (Smith Kline and French Laboratories, Philadelphia, Pa.). All other chemicals were bought from commercial sources. Triparanol, SKF-2314, oleic acid, squalene, and ergosterol were dissolved in 95 % ethanol in water. Other compounds were dissolved in water.

Table 1. *Inhibition of bacterial multiplication by hypocholesteremic compounds*

Bacteria	Triparanol	SKF compounds*				Amino-ethanols†	
		525-A	3301-A	16467-A	2314		
		Size of zone of inhibition (cm)					
Gram-negative							
<i>Aerobacter aerogenes</i>	0	1.2 ± 0.2	2.1 ± 0.2	1.7 ± 0.2	0	0	
<i>Escherichia coli</i>	0	0	0	0	0	0	
<i>Proteus mirabilis</i>	0	1.1 ± 0.2	1.5 ± 0.1	0	0	0	
<i>Pseudomonas putida</i>	0	0	1.1 ± 0.1	0	0	0	
<i>P. reptilia</i>	0	1.0 ± 0.1	1.8 ± 0.1	0.8 ± 0.1	0	0	
<i>P. vulgaris</i>	0	0	1.8 ± 0.2	1.4 ± 0.1	0	0	
Gram-positive							
<i>Bacillus cereus</i>	0.8 ± 0.1	1.0 ± 0.2	1.8 ± 0	0.8 ± 0.1	0	0	
<i>B. circulans</i>	1.4 ± 0.2	1.8 ± 0.1	3.1 ± 0.1	2.1 ± 0.6	0	0	
<i>B. fusiformis</i>	1.0 ± 0.3	1.0 ± 0.1	1.9 ± 0.4	0.8 ± 0	0	0	
<i>B. mycoides</i>	1.1 ± 0.3	1.9 ± 0.4	1.9 ± 0.5	1.4 ± 0.6	0	0	
<i>B. subtilis</i>	1.1 ± 0.6	2.0 ± 0.1	2.7 ± 0.2	0.9 ± 0.1	0	0	
<i>Micrococcus luteus</i>	1.8 ± 0.2	1.8 ± 0.1	2.6 ± 0.4	1.5 ± 0.3	0	0	
<i>M. lysodeikticus</i>	0.8 ± 0.2	1.9 ± 0.1	2.2 ± 0.2	0.7 ± 0.1	0	0	
<i>M. roseus</i>	2.0 ± 0.3	1.6 ± 0.1	2.1 ± 0.3	0.9 ± 0.1	0	0	
<i>M. sodonensis</i>	1.5 ± 0.3	1.9 ± 0.5	2.2 ± 0.7	0.8 ± 0.1	0	0	
<i>Mycobacterium phlei</i>	2.0 ± 0.4	2.9 ± 0.6	3.3 ± 0.5	1.6 ± 0.6	0	0	
<i>My. smegmatis</i>	1.1 ± 0.2	2.0 ± 0.5	2.4 ± 0.3	1.2 ± 0.2	0	0	
<i>Sarcina flava</i>	1.1 ± 0.3	1.7 ± 0.4	2.1 ± 0.1	1.1 ± 0.2	0	0	
<i>Staphylococcus albus</i>	0.9 ± 0.2	0.8 ± 0.1	1.5 ± 0.1	1.1 ± 0	0	0	
<i>S. aureus</i>	1.2 ± 0.2	1.3 ± 0.1	1.6 ± 0.1	0	0	0	
<i>S. citreus</i>	1.5 ± 0.5	1.3 ± 0.2	2.0 ± 1.0	0	0	0	

* Discs soaked in solution (0.1 M) of compound.

† Dimethylaminoethanol, diethylaminoethanol, dimethyl-3-aminopropanol, diethyl-3-aminopropanol, dimethyl-2-aminopropanol, diethyl-1-aminopropanol.

Table 2. *Effect of concentration on inhibition*

Compound	Discs soaked in conc. (M)	<i>Bacillus subtilis</i>	<i>Micrococcus sodonensis</i>	<i>Mycobacterium smegmatis</i>
		Size of zone of inhibition (cm)		
Triparanol	0.1	1.2 ± 0.2	1.5 ± 0.2	2.3 ± 0.2
	0.05	0.9 ± 0.1	1.4 ± 0.6	2.0 ± 0.2
	0.01	1.2 ± 0.1	1.7 ± 0.3	2.2 ± 0.2
	0.005	1.1 ± 0.2	1.5 ± 0.3	2.4 ± 0.2
	0.001	0	0.9 ± 0.2	1.4 ± 0.4
SKF 525-A	0.1	1.8 ± 0.2	1.9 ± 0.2	2.3 ± 0.5
	0.05	1.4 ± 0.3	1.2 ± 0.3	2.1 ± 0.1
	0.01	1.2 ± 0.5	0	1.3 ± 0.2
	0.005	0	0	0.9 ± 0.1
	0.001	0	0	0
SKF 3301-A	0.1	2.4 ± 0.8	2.0 ± 0.1	3.6 ± 0.4
	0.05	2.1 ± 0.6	1.5 ± 0.4	2.8 ± 0.3
	0.01	0	0.7 ± 0.1	1.0 ± 0.2
	0.005	0	0.7 ± 0	0
	0.001	0	0.7 ± 0	0

RESULTS

Earlier work (Aaronson, 1965) with two hypocholesteremic compounds (triparanol, benzmalecene) showed that Gram-positive bacteria were more sensitive than Gram-negative bacteria. Triparanol again inhibited only Gram-positive bacteria (present work) while the SKF compounds (525-A, 3301-A, 16467-A) inhibited Gram-positive and Gram-negative bacteria; the Gram-positive bacteria were more sensitive to the SKF compounds (Table 1). The bacteria sensitive to the hypocholesteremic compounds varied in sensitivity to the several compounds (Table 2). As with triparanol (Aaronson, 1965) several of the SKF compounds inhibited ascomycetes but not phycomycetes (Table 3). The inhibitory SKF compounds have aromatic and aliphatic parts (Fig. 1). Neither the aromatic moiety (SKF 2314) nor the aliphatic moiety (dimethyl- or diethyl-aminoethanols or their analogues) inhibited bacterial multiplica-

Table 3. *Inhibition of fungal multiplication by hypocholesteremic compounds*

Fungi	Triparanol	SKF compounds*				Amino-ethanols
		525-A	3301-A	16467-A	2314	
		Size of zone of inhibition (cm)				
Ascomycetes						
<i>Hansenula saturans</i>	1.3 ± 0.1	1.0 ± 0.1	0.9 ± 0.3	0	0	0
<i>Neurospora crassa</i>	1.5 ± 0.1	0	0	0	0	0
<i>Penicillium roqueforti</i>	1.6 ± 0.3	0	0	0	0	0
<i>Rhodotorula rubra</i>	1.6 ± 0.1	1.0 ± 0.1	0.9 ± 0.2	0	0	0
Phycomycetes						
<i>Aspergillus niger</i>	0	0	0	0	0	0
<i>Syncephalotium nigricans</i>	0	0	0	0	0	0

* Discs soaked in 0.1 M solution of compound.

Table 4. *Annulment of inhibition by triparanol*

Gram-positive	Triparanol	Triparanol + albumin	Triparanol + oleic acid	Triparanol + squalene	Triparanol + ergosterol
		Size of zone of inhibition (cm.)			
<i>Bacillus cereus</i>	0.8 ± 0.1	0	0.9 ± 0	.	.
<i>B. circulans</i>	1.4 ± 0.2	0.8 ± 0.1	1.8 ± 0.1	1.6 ± 0.2	.
<i>B. fusiformis</i>	1.0 ± 0.3	0	1.3 ± 0.2	.	0
<i>B. mycoides</i>	1.1 ± 0.3	0	0.9 ± 0.2	.	0.7 ± 0
<i>B. subtilis</i>	1.1 ± 0.6	0	0.9 ± 0.2	1.2 ± 0.4	0
<i>Micrococcus luteus</i>	1.8 ± 0.2	0	2.0 ± 0.8	1.4 ± 0.1	0.7 ± 0.1
<i>M. lysodeikticus</i>	0.8 ± 0.2	0	1.1 ± 0.2	0.7 ± 0	0
<i>M. roseus</i>	2.0 ± 0.3	0.8 ± 0	.	1.7 ± 0.2	0
<i>M. sodonensis</i>	1.5 ± 0.3	0	.	1.3 ± 0.1	0.7 ± 0.1
<i>Mycobacterium phlei</i>	2.0 ± 0.4	0.8 ± 0.2	1.8 ± 0.2	2.7 ± 0.3	0.9 ± 0
<i>My. smegmatis</i>	1.1 ± 0.2	0	1.0 ± 0.1	1.8 ± 0.4	1.0 ± 0.1
<i>Sarcina flava</i>	1.1 ± 0.3	0	1.1 ± 0.1	1.1 ± 0.2	0
<i>Staphylococcus albus</i>	0.9 ± 0.2	0	0	1.0 ± 0.1	.
<i>S. aureus</i>	1.2 ± 0.2	0.7 ± 0	1.1 ± 0.1	.	0.8 ± 0
<i>S. citreus</i>	1.5 ± 0.5	0	1.9 ± 0.7	2.0 ± 0.2	.

Concentrations used: triparanol, 0.1 M; oleic acid, 0.1 M; squalene, 0.1 M; ergosterol, 0.01 M; bovine serum albumin, 0.2 %.

β -Diethylaminoethyl-diphenylpropyl acetate.HCl (SKF 525-A)

Diphenylpropylacetic acid (SKF 2314)

2,2-Diphenyl-1-(β -dimethylaminoethoxy)pentane.HCl (SKF 3301-A)

β -Dimethylaminoethylamino-diphenylpropylacetate.HCl (SKF 16467-A)

1-[4-Diethylaminoethoxy]phenyl]-1-(*p*-tolyl)-2-(*p*-chlorophenyl) ethanol
(Triparanol)

Fig. 1. The structure of several hypocholesteremic compounds.

tion at 0.1 M, a concentration at which the other SKF compounds were inhibitory. Discs containing SKF 2314 or diethylaminoethanol added on top of each other or discs soaked in SKF 2314 + diethylaminoethanol were inactive.

In previous work with a photosynthetic bacterium oleic acid, ergosterol and squalene annulled the inhibition by triparanol (Aaronson, 1964). Oleic acid was not as effective

in preventing the inhibition of multiplication by triparanol as were albumin or ergosterol, while squalene seemed to enhance the inhibition by triparanol (Table 4). Alone, none of these lipids or albumin was inhibitory at the concentrations used.

DISCUSSION

Gram-positive bacteria are more inhibited by hypocholesteremic compounds than are Gram-negative bacteria (Aaronson, 1965; Martin-Smith & Sugrue, 1964; Smith *et al.* 1963). The results here with the SKF compounds (Table 1) bear this out. Of the several microbial groups exposed to the SKF compounds, bacteria, ascomycetes, protozoa (Aaronson, 1966) but not phycomycetes were sensitive. These results are like those obtained with triparanol and benzmalecene (Aaronson, 1965). While the combined aliphatic and aromatic moieties, i.e. SKF 525-A, SKF 3301-A, seem necessary for bacterial inhibition (see Table 1), these compounds acted strikingly differently with the eucaryotic phytoflagellate *Ochromonas danica* (Aaronson, 1966) where the aromatic moiety, i.e. SKF-2314, but not the aliphatic moiety, i.e. diethylaminoethanol, was as inhibitory as SKF-525A. The sensitivity of bacteria to a disc soaked in as little as 1 mm of the compound and to a variety of hypocholesteremic compounds, i.e. azasteroids (Martin-Smith & Sugrue, 1964; Smith *et al.* 1963), triparanol and benzmalecene (Aaronson, 1965) and SKF 525-A, SKF 3301-A, and SKF 16467-A, implies that these compounds may offer leads to compounds which inhibit pathogenic bacteria, especially the Gram-positive ones, as well as other pathogens.

Several workers have reported that unsaturated fatty acids (e.g. oleic and linoleic acids) inhibit multiplication of Gram-positive but not Gram-negative bacteria (Kodicek, 1956; Kodicek & Worden, 1945; McQuillen, 1958). McQuillen (1958) found that this inhibition was prevented by lecithin, several steroids, and α -tocopherol for *Lactobacillus helveticus*, while vitamin D₂ annulled the inhibition of *L. casei* by linoleic acid. McQuillen (1958) observed that linoleic acid inhibited the uptake in *L. casei* of ¹⁴C-labelled metabolites such as glutamic acid, lysine, glucose, phenylalanine, adenine and uracil in fixed cellular form (proteins of membranes, etc.) but had far less effect on their uptake into the cellular pools. Vitamin D₂ prevented the inhibition by linoleic acid of the uptake of the compounds into fixed form. Like the inhibition by unsaturated fatty acids, the inhibition of Gram-positive bacteria by triparanol was annulled by ergosterol and also by albumin. While earlier workers did not report on albumin (a well-known annuller of the inhibition of mitochondrial activity by unsaturated fatty acids) it may well be that triparanol (and perhaps other hypocholesteremics) and unsaturated fatty acids inhibit Gram-positive bacteria at the same site, and that these inhibitions are annulled by albumin or steroids. The nature of this site and the reason for the lack of sensitivity of Gram-negative bacteria remain to be determined. It is provocative that steroids annul the inhibition of multiplication by hypocholesteremics in procaryota, which are not supposed to synthesize sterol, while only unsaturated fatty acids annul the inhibition by hypocholesteremics in the eucaryotic *Ochromonas*, which does synthesize steroids (Aaronson, 1965, 1966).

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