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Minireview

Antibacterial effects of fatty acids and related compounds from plants

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Fatty acids are important constituents of plants and are commonly known to possess antimicrobial activities. The structure-activity relationship of fatty acids, including the effects of hydrocarbon chain length, unsaturation and presence of functional groups, is reviewed. The biological activity of fatty acids is significant as they are often isolated following bioassay-guided fractionation of plant extracts. The possibility of the therapeutic use of fatty acids as antimicrobial agents is worthy of note.

Introduction

Fatty acids are carboxylic acids with long, unbranched carbon chains, some of which may contain double bonds. Fatty acids with *cis* double bonds occur widely in nature, but those with *trans* double bonds are rare. Triacylglycerols (fatty acids linked to glycerol) of animal origin, which contain a relatively large proportion of saturated fatty acids, are generally solid at room temperature, while those of plant origin, which contain a greater percentage of unsaturated fatty acids, are usually liquid (Lehninger 1982). In general, fatty acids do not exist as free carboxylic acids because of their affinity for many proteins (Gurr and James 1980). One result of this is an inhibitory action on most enzymes. Where free acids have been reported as major constituents they may be artefacts due to cell damage which allows lipases to act on acyl lipids of the tissue (Gurr and James 1980).

During the bioassay-guided fractionation of plant extracts, it has on occasion been discovered that fatty acids are responsible, at least in part, for the observed antibacterial action of the crude extract (Cerdeiras *et al*. 2000, Dilika *et al*. 2000, McGaw *et al*. 2002, Yff *et al*. 2002). Researchers often choose to perform an initial defatting or tannin-removing procedure prior to embarking upon activity-directed fractionation, precisely to avoid being left with compounds known to possess antibacterial activity such as tannins or fatty acids. The non-specific binding of these compounds to proteins is recognised to interfere with enzyme and receptor-based assays.

Fatty acids as constituents of plants

Fatty acids occur mainly in bound form in plants, esterified to glycerol, as fats or lipids (Harborne and Baxter 1993). These lipids comprise up to 7% of the dry weight in leaves in higher plants, and are important membrane constituents in cell membranes, chloroplasts and mitochondria (Harborne and Baxter 1993). Membranes are important sources of signaling molecules, many of which are derived from fatty acids (Weber 2002). These fatty acid-derived molecules can act as intracellular mediators or as extracellular signals, and are important in interspecies communication and plant defence (Weber 2002). The seeds and fruits of many plants also contain lipids in considerable amounts, providing a storage form of energy to use during germination (Harborne and Baxter 1993).

The common fatty acids in plants are either saturated or simple unsaturated compounds of C_{16} or C_{18} chain length (Harborne and Baxter 1993). Palmitic acid (C_{16}) is the major saturated acid in leaf lipids, also occurring in some seed oils, while stearic acid (C_{18}) is less prominent in leaf lipids but is a major saturated acid in seed fats in several plant families (Harborne and Baxter 1993). Unsaturated acids based on C_{16} and C_{18} are widespread in leaf and seed oils (Harborne and Baxter 1993). The tri-unsaturated linolenic acid is common, as are linoleic and oleic acids (Harborne and Baxter 1993). Quantitatively, the major fatty acids are palmitic, linoleic and, in particular, α-linolenic acids (Hitchcock and Nichols 1971, Harwood 1980). The membranes of chloroplasts contain exceptionally high (about 90% in some lamellae) percentages of α-linolenic acid (Hitchcock and Nichols 1971, Harwood 1980).

The significance of fatty acids in chemotaxonomy

Whereas plant seeds contain a wide variety of fatty acids,

those from leaf tissue are remarkably constant from plant to plant (Harwood 1980). Just as the complex lipid composition of higher plant leaves is relatively constant, so the fatty acid content is also quite characteristic.

Variation in the fatty acid and sterol composition of plants has long been of interest to chemotaxonomists. Fatty acids from seeds and leaves, more than sterols, have been used for plant family classification into classes and subclasses (Perdetzoglou *et al*. 1996). Since variation in fatty acid composition is rather small among plant families, quantitative differences of the fatty acid composition have been used as chemotaxonomic tools (Perdetzoglou *et al*. 1996).

Fatty acids as antimicrobial agents

The bactericidal and antifungal properties of fatty acids are well known (Kabara *et al*. 1972).

Fatty acids with antibacterial activity have been isolated from several plants using bioassay-guided fractionation. Cerdeiras *et al*. (2000) identified 11-O-(6'-O-acetyl-β-D-glucopyranosyl)-stearic acid as the main antibacterial component of aerial parts of *Ibicella lutea*. This fatty acid derivative showed an interesting antibacterial activity, being active against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* with the Minimal Inhibitory Concentration (MIC) value of 9μgml^{-1} against *S. aureus* (Cerdeiras *et al*. 2000). Dilika *et al*. (2000) described the antibacterial activity of linoleic and oleic acids isolated from the leaves of *Helichrysum pedunculatum*. Linoleic and oleic acids inhibited the growth of Gram-positive *B. subtilis*, *Micrococcus kristinae* and *S. aureus* and linoleic acid also showed activity against *Bacillus cereus* and *Bacillus pumilis* (Dilika *et al*. 2000). Both acids displayed no activity against Gram-negative *Enterobacter cloacae*, *E. coli*, *Klebsiella pneumoniae*, *P. aeruginosa* and *Serratia marcescens* (Dilika *et al*. 2000).

In 2002, Yff *et al*. isolated palmitic acid using bioassay-guided fractionations from *Pentanisia prunelloides* in an effort to rationalise the use of this plant by indigenous healers in the treatment of inflammation and bacterial and viral infections. Similarly, McGaw *et al*. (2002) reported the isolation of linolenic acid and methyl-5,11,14,17-eicosatetraenoate from *Schotia brachypetala*, a tree used in traditional remedies to relieve diarrhoea and dysentery. The MIC value for linolenic acid against *B. subtilis*, *E. coli* and *K. pneumoniae* was 3.13mgml^{-1}, and against *S. aureus* was 1.56mgml^{-1}. Methyl-5,11,14,17-eicosatetraenoate possessed MIC values of 0.78mgml^{-1} against the Gram-positive *B. subtilis* and *S. aureus*, and 3.13mgml^{-1} against the Gram-negative *E. coli* and *K. pneumoniae*.

Of interest was the synergistic effect observed by Dilika *et al*. (2000) between linoleic and oleic acids against *S. aureus* and *M. kristinae*. The MIC of each of the fatty acids alone against the two bacterial species was 1mgml^{-1} but when administered in combination, the MIC was 0.05mgml^{-1}, indicating a strongly synergistic effect. Lee *et al*. (2002) reported that the MIC of linolenic acid on *B. cereus* and *S. aureus* was 20mgl^{-1} and 50mgl^{-1} respectively, but linolenic acid at 10mgl^{-1} combined with 10mgl^{-1} of monoglyceride (glycerol laurate or glycerol myristate) showed stronger antibacterial activity than using linolenic acid alone. There is a noticeable lack of studies such as these investigating the synergistic effect of antibacterial fatty acids.

Linolenic acid: a possible therapeutic agent?

All-*cis*-9,12,15-octadecatrienoic acid (α-linolenic acid, 18:3) occurs in higher plants and algae, especially as a component of galactosyl diacylglycerol (Gurr and James 1980). Linolenic acid is the most usual form of triethenoid C_{18} acid found in seed fats (Hilditch 1956). It forms 50% or more of the mixed fatty acids in linseed oil, and also occurs in other seed oils (Hilditch 1956). Ohta *et al*. (1995) demonstrated the antibacterial activity of α-linolenic acid against methicillin-resistant *S. aureus* (MRSA). McDonald *et al*. (1981) reported on the susceptibility of several strains of MRSA to linolenic acid and hydrolysed linseed oil (containing 52% linolenic acid in the ester form). McDonald *et al*. (1981) described the potential role of preparations containing hydrolysed linseed oil in the eradication of staphylococcal infection, and in the prophylaxis of infection in debilitated patients.

The possibility of the therapeutic use of linolenic acid as an antibacterial agent should be explored (Lacey and Lord 1981). α-Linolenic acid is generally considered to have low toxicity, so it may potentially be administered to patients infected with MRSA as a dietary treatment (Ohta *et al*. 1995). It is naturally occurring, and hence unfavourable reactions would not be anticipated, it should not destroy the commensal flora, resistance to it would not develop, and there would apparently be no risk of resistance to antibiotics developing during its use (Lacey and Lord 1981). Linolenic acid, although present in small quantities in human skin (Wilkinson 1972, cited by Lacey and Lord 1981) may well be an important naturally occurring antibacterial agent, and its presence could explain why pathogenic staphylococci are rarely found on intact skin (Lacey and Lord 1981). Nieman (1954) stated that some natural defence systems of higher organisms, such as the self-disinfection of the skin, are due at least partly to the presence of antibacterial fatty acids *in situ*. However, the potential antibacterial activity of fatty acids *in vivo* may be neutralised by adsorption on proteins in the bloodstream (Nieman 1954).

Giamarellos-Bourboulis *et al*. (1995) demonstrated the inhibition of the Gram-negative *E. coli* by gamma-linolenic acid (GLA). At 5mgl^{-1} and 300mgl^{-1}, GLA inhibited 9.5% and 33.3% of the 42 *E. coli* strains tested respectively The possible mechanism of GLA action on *E. coli* could be attributed either to an alteration of cell membrane properties induced by GLA or to the generation of free radicals from GLA, which may lead to membrane damage and ultimately bacterial death (Giamarellos-Bourboulis *et al*. 1995). Further studies are necessary to clarify the mechanism of GLA action on *E. coli*, and the clinical importance of these findings.

Cooper *et al*. (1985) described the antibacterial activity of linolenic acid against *B. subtilis* and *Vibrio parahaemolyticus*. The marine bacterium, *V. parahaemolyticus*, is a major cause of gastroenteritis in countries where large amounts of sea fish and its products are consumed (Cooper *et al*. 1985). Linolenic acid had inhibitory activity against spores of *Clostridium botulinum*, *Clostridium sporogenes* and *B.*

cereus (Ababouch *et al*. 1992).

α-Linolenic acid possesses additional interesting biological activities. The fatty acid has strong anti-conidial germination activity against blast fungus (Sekizawa *et al*. 1981). Linoleic and linolenic acid have been identified as antialgal substances in the culture medium of the green alga *Chlamydomonas reinhardtii* (McCracken *et al*. 1980, cited by Ohta *et al*. 1995). α-Linolenic acid has also shown selective inhibitory activity against the inducible cyclooxygenase-2 (COX-2), an enzyme involved in the process of inflammation (Huss *et al*. 2002).

Fatty acid structure and antimicrobial activity

There is an intimate relationship between the structure of fatty acids and their ability to function as antimicrobial agents. A summary of the effect of structure on fatty acid antibacterial action is represented in Table 1. The most effective saturated, mono-unsaturated, and polyunsaturated fatty acids are those with chain lengths of C_{12}, $C_{16:1}$ and $C_{18:2}$, respectively (Kabara 1980). The number and position of double bonds is more important to fatty acids longer than 12 carbons than for fatty acids with fewer carbons (Kabara 1980). Gram-positive bacteria are more susceptible to fatty acids than are Gram-negative bacteria (Knapp and Melly 1986). Yeasts are affected to a greater extent by fatty acids containing 10–12 carbons, while Gram-positive bacteria are more affected by slightly longer chain lengths (Kabara 1980). Gram-negative organisms are affected by very short chain fatty acids, that is C_6 or less (Kabara 1980). Fatty acids with greater than eight carbons are not inhibitory to Gram-negative bacteria (Kabara 1980).

Fatty acid esters of sucrose have been reported to have antimicrobial properties against Gram-negative bacteria, although to a much lower extent than those shown for Gram-positive bacteria (Marshall and Bullerman 1994). Fungi are inhibited to a greater extent by acetylenic derivatives than ethylenic derivatives of fatty acids (Marshall and Bullerman 1994). Fatty acids esterified to monohydric alcohols have no antimicrobial activities, while esterification of fatty acids to polyhydric alcohols, such as glycerol or sucrose, increases the antimicrobial effectiveness of the fatty acids (Kabara 1980). The fatty acid used to esterify the polyol dictates the potency of the ester. Lauric acid (C_{12}) and palmitoleic acid ($C_{16:1}$) form the most active saturated and unsaturated esters, respectively, and monoester forms are more potent than polyester forms (Kabara 1980). Marshall and Bullerman (1994) concluded that the antimicrobial properties of sucrose fatty acid esters, in addition to other functional properties such as emulsification or stabilisation, suggests potential use of these and related compounds in the food processing, cosmetic and pharmaceutical industries.

Ohta *et al*. (1995) tested ten fatty acids and their methyl esters against *S. aureus* and MRSA. They found that palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$) and oleic acid ($C_{18:1}$) had no activity, but fatty acids with two or more double bonds (starting with $C_{18:2}$) were active. Of the polyunsaturated fatty acids tested, the activity of γ-linolenic acid ($C_{18:3}$) was the highest, and α-linolenic acid ($C_{18:3}$), eicosapentaenoic acid ($C_{20:5}$), and docosahexaenoic acid ($C_{22:6}$) also had strong activity. The methyl esters were either inactive or much less active than the other form (Ohta *et al*. 1995). In a similar experiment with *E. coli*, Ohta *et al*. (1995) found that all fatty acids were inactive.

In general, inhibitory properties of fatty acids appear more pronounced with longer and more unsaturated compounds (Nieman 1954). It appears that unsaturation is not essential for inhibition of fungi and mycobacteria by fatty acids (Nieman 1954). The position of unsaturation affects biological activity (Kabara 1986). The stereochemistry of the fatty acids also has an effect, with the (natural) *cis*-forms of unsaturated fatty acids exhibiting a greater antibacterial activity than the corresponding *trans*-isomers (Nieman 1954, Kabara 1986). Unsaturation in contrast to esterification is less effective with low chain fatty acids as compared to higher chain fatty acids (Kabara 1986). Monoesters of fatty acids are more active than the corresponding fatty acid (Kabara 1986).

Mechanism of antimicrobial action of fatty acids

The antimicrobial effects of fatty acids and their derivatives have been known for many years, and there have been various suggestions as to the mechanism of antibacterial action (Knapp and Melly 1986). Fatty acids can inhibit the growth of numerous types of bacteria, as well as protozoans, viruses and fungi (Nieman 1954, Knapp and Melly 1986). As has been mentioned, fatty acid sensitivity is considered to be a characteristic of Gram-positive bacteria, with few Gram-negative species being susceptible. Members of the Gram-negative families Neisseriaceae, Enterobacteriaceae and Parvobacteriaceae are inhibited by small amounts of fatty acids (Nieman 1954).

Chain length and antibacterial activity

Short-chain saturated fatty acids and long-chain polyunsaturated fatty acids appear to exert their antimicrobial effects by different mechanisms. Short-chain fatty acids are generally toxic in high concentrations in a pH-dependent manner

Table 1: Summary of the effect of structure on antibacterial activity of fatty acids

Fatty acid structure	Bacterial toxicity
Short chain	Gram-negative (at high concentration, pH dependent)
Long chain	Gram-positive (lower concentration, pH independent)
Methyl ester	Decreases activity
Sucrose ester	Increases activity
Stereochemistry	*Cis*-isomer more active than *trans*-isomer
Unsaturation	Increases activity against Gram-positive bacteria

and are known to have an adverse effect on the energy metabolism of a wide variety of micro-organisms (Knapp and Melly 1986). On the other hand, polyunsaturated fatty acids affect only certain types of bacteria and are toxic at much lower concentrations in a more pH-independent fashion (Knapp and Melly 1986).

Tsuchido *et al*. (1985) discovered that the addition of saturated C_6, C_8, C_{10}, and C_{12} fatty acids appeared to lyse actively growing cells of *B. subtilis* 168, as judged by a decrease in the optical density of the culture. Tsuchido *et al*. (1985) suggested that fatty acid-induced lysis of *B. subtilis* 168 is due to the induction of autolysis by an autolytic enzyme rather than massive solubilisation of the cell membrane by the detergent-like action of the fatty acids. According to Tsuchido *et al.* (1985), it is unclear how fatty acids induce autolysis. Tsuchido *et al*. (1985) stated that it is unlikely that fatty acids directly activate autolytic enzymes in the cell wall, but rather, the primary target of action of fatty acids may be the cell membrane. One explanation for the induction of autolysis by fatty acids is that the fatty acids solubilise autolysins from the membrane at relatively low concentrations; this is supported by the results of experiments on protoplasts (Tsuchido *et al*. 1985). In addition, the dependency of the lytic action of fatty acids on the carbon chain length of the molecule suggests that the surface-active action of the acid might participate in the dissociation of autolysins from the membrane (Tsuchido *et al*. 1985). To summarise, the short and medium chain fatty acids tested by Tsuchido *et al*. (1985) seem to have a dual effect on cellular lysis, depending on their concentration, with the action on the cell membrane leading to the induction of autolysis being detectable at relatively low concentrations, and the association with the autolytic enzyme itself being strong at relatively high concentrations. The mechanism of lytic action of fatty acids seems to be different from that of their bactericidal action (Tsuchido *et al*. 1985).

Fatty acids inhibit the growth and oxygen consumption of *B. subtilis* in nutrient medium by inhibiting the transport of substances such as amino acids and keto acids through the cellular membrane (Freese *et al*. 1973). The effectiveness of inhibition increased with increasing chain length (Freese *et al*. 1973). In contrast, the inhibitory effect on *E. coli* increased only up to a fatty acid chain length of six carbons, while long-chain fatty acids had no effect (Freese *et al*. 1973).

Esters of fatty acids

Marshall and Bullerman (1994) reported that the mechanism of action of sucrose esters of fatty acids appears to be biostatic rather than biocidal. Bergsson *et al*. (1999) tested several fatty acids and their 1-monoglycerides against *Neisseria gonorrhoeae*, a Gram-negative coccus. Lauric acid ($C_{12:0}$), palmitoleic acid ($C_{16:1}$) and monocaprin ($C_{10:0}$) effectively killed the pathogen. Bergsson *et al*. (1999) hypothesised that the lipids kill the bacteria by disruption of their cell membranes (visible with electron microscopy), and because of this lipid action on biological membranes, the emergence of resistance is unlikely.

Tsuchido *et al*. (1987) examined the lytic action of glycerol and sucrose esters of fatty acids with different carbon chain lengths. It was found that glycerol dodecanoate and sucrose hexadecanoate induced autolysis in cells of *B. subtilis* 168, and during treatment a great loss of viability occurred preceding lysis (Tsuchido *et al*. 1987). The esters caused morphological changes in the cells, but an apparent adaptation of the cells to the esters was noted (Tsuchido *et al*. 1987). As with short- and medium-chain fatty acids (Tsuchido *et al*. 1985), the lysis of *B. subtilis* cells induced with glycerol dodecanoate and sucrose hexadecanoate may be due to the action of autolytic enzymes and not to the direct action of solubilisation of the bacterial membrane (Tsuchido *et al*. 1987). After lysis the surviving cells grew, suggesting that these cells adapted to esters, as indicated by their renewed growth and tolerance to esters if they were added again (Tsuchido *et al*. 1987). The cell death observed by Tsuchido *et al*. (1987) during the treatment with esters was very rapid. This suggests that the mechanism that causes death is different from that which causes lysis and that direct interaction of esters with the cell membrane may cause the cell death, although the possibility that an irreversible triggering of cell lysis induces apparent rapid death cannot be ruled out (Tsuchido *et al*. 1987). The fact that cells treated with esters altered cell morphology suggests the inhibition of some process of synthesis or regulation of the cell envelope which is possibly related to the induction of autolysis (Tsuchido *et al*. 1987). Glycerol and sucrose esters of fatty acids are supposed to be effective non-toxic antimicrobial agents, but the adaptation seen in this study may be a problem if this phenomenon exists in micro-organisms that cause food spoilage or that produce toxins (Tsuchido *et al*. 1987).

The cytoplasmic membrane as the site of fatty acid action

Galbraith and Miller (1973a) discovered that both Gram-positive and Gram-negative bacteria reversibly adsorbed fatty acids, and uptake increased with decreasing pH value and increasing chain length. Upon investigating the interaction between antibacterial fatty acids and the bacterial membrane, Galbraith and Miller (1973b) recorded that fatty acids of chain length greater than 10 carbons induced lysis of protoplasts at pH 7.4. The experiments of Galbraith and Miller (1973b) on protoplasts implicated the cytoplasmic membrane as the site of action of the fatty acids and reflected the relative physicochemical properties of the acids on the whole cells since the order of bactericidal activity coincided with that of lytic activity.

Sheu and Freese (1972) found that fatty acids of different chain length inhibited growth of *B. subtilis*, but the effect was reduced in the presence of glycolytic compounds and reversed by transfer to medium without fatty acids. The concentration required for inhibition of growth, oxygen consumption and adenosine triphosphate (ATP) synthesis increased with decreasing molecular weight of the fatty acids (Sheu and Freese 1972). Sheu and Freese (1972) postulated that the fatty acids reversibly react with the cell membrane or cell proteins in it; they could either alter the membrane structure or uncouple the electron transport

chain from two types of proteins, those used for ATP regeneration and others needed for the transport of certain compounds into the cells. In another study, Sheu *et al*. (1972) reported that acetate and other short-chain fatty acids (C_1–C_6) inhibited strongly the uptake of L-serine or other L-amino acids of *B. subtilis*. It was concluded that the fatty acids 'uncouple' the amino acid carrier proteins from the cytochrome-linked electron transport system, to which they may be coupled via protein interaction or via a cation gradient (Sheu *et al*. 1972). Freese *et al*. (1973) reported that the concentration of fatty acids required to produce a certain amount of growth inhibition of *B. subtilis* (at pH 6.5) decreases with increasing chain length, with saturated and unsaturated fatty acids being about equal. Freese *et al*. (1973) presumed that the more lipophilic long chain carbons are more effectively partitioned into the cell membrane. This membrane attachment was confirmed to be reversible (Freese *et al*. 1973).

In an interesting case study, Saito and Tomioka (1988) compared the susceptibilities of colonial variants of various strains of *Mycobacterium avium* complex to long-chain fatty acids with a strong hydrophobicity. Smooth T variants, which have an outer, regularly structured polysaccharide layer, showed a much higher resistance to various antimicrobial agents than do the smooth D variants, which lack the outer layer (Saito and Tomioka 1988). This polysaccharide layer may be related to an impaired permeability in smooth T variants, resulting in decreased susceptibility (Saito and Tomioka 1988). The smooth D variant was more susceptible to all the test fatty acids, which included capric, lauric, oleic and linolenic acids (Saito and Tomioka 1988). Saito and Tomioka (1988) noted that for *M. avium*, the fatty acid susceptibility of a particular variant correlates well with its virulence, i.e. the smooth T variant is much more virulent than is the smooth D variant. An interesting observation was that activated macrophages with an enhanced mycobactericidal activity secreted a large amount of antimycobacterial fatty acids such as oleic and linolenic acids (Hui *et al*. 1977). Saito and Tomioka (1988) stated that, since fatty acids penetrate through cell surface structures, including the outer membrane and cell wall, before they reach their target sites and act on the target molecules on the cell membrane, the permeability of the surface structures for fatty acids may dominate the susceptibility of a given organism. The antibacterial action of fatty acids has been explained by the insertion of the nonpolar moieties of the fatty acids into the phospholipid layer of the bacterial cell membrane, causing a change in membrane permeability, alteration of the activity of some membrane proteins essential for maintenance of cellular functions, and uncoupling of the oxidative phosphorylation system (Saito and Tomioka 1988). The hydrophilic moiety of the outer membrane of Gram-negative bacteria probably prevents fatty acids from penetrating the interior target sites of *E. coli* (Saito and Tomioka 1988). It is possible that the hydrophilic nature of the polysaccharide outer layer of the *M. avium* smooth T variants, which is lacking in the smooth D variants, might obstruct fatty acid penetration into the cell wall and thereby inhibit their transport to the target sites on the membrane (Saito and Tomioka 1988). It is also feasible that hindrance of the permeability of antimicrobial agents and bacterial inhibitors by the polysaccharide outer layer is based on unknown functions rather than on physical and hydrophilic barriers (Saito and Tomioka 1988).

Bacterial cell wall structure and antibiotic susceptibility

With the exception of mycoplasmas, bacteria produce cell walls (Nikaido and Vaara 1985). Gram-negative bacteria are typically surrounded by an outer lipopolysaccharide (LPS) layer (Osborn 1969). This outer layer is very important in the physiology of Gram-negative bacteria, making them resistant to various host defence factors which are toxic to Gram-positive bacteria (Nikaido and Vaara 1985). This is particularly relevant in the case of enteric Gram-negative bacteria living in the intestinal tract of animals. The outer membrane of enteric and some other Gram-negative bacteria acts as a strong permeability barrier to many antibiotics that are effective against other bacteria (Nikaido and Vaara 1985). Even when the diffusion of the antibiotic is only slowed down by the presence of the outer membrane, the bacteria can then inactivate the small amount of penetrating antibiotic rather than attempt to inactivate the almost infinite amount of antibiotic present in the surrounding medium (Nikaido and Vaara 1985).

The LPS layer of Gram-negative bacteria prevents the entry and subsequent inhibition by intermediate and long-chain fatty acids (Sheu and Freese 1973). Sheu and Freese (1973) suggested that this protection would be essential for the survival of bacteria in the intestinal tract where such fatty acids are produced by the digestion of fats. However, these bacteria are not resistant to short-chain fatty acids up to hexanoate, so these fatty acids may therefore be useful for the treatment of infection by Gram-negative bacteria. Alakomi *et al*. (2000) reported that lactic acid, in addition to its antimicrobial property caused by lowering of the pH, also functions as a permeabiliser of the Gram-negative bacterial membrane, and may act as a potentiator of the effects of other antimicrobial substances.

After adding arachidonic acid to Gram-negative *N. gonorrhoeae* and *Haemophilus influenzae*, Knapp and Melly (1986) observed, using electron microscopy, a loss of ribosomes, increased irregularity of outer membranes, separation of outer and inner membranes, loss of cell shape and complete cell disorganisation and disruption. There were no morphological changes in *E. coli* resistant to the toxic effects of arachidonate (Knapp and Melly 1986). After treating the Gram-positive *S. aureus* with arachidonate, Knapp and Melly (1986) saw no visible alteration in cell wall structure, but the arachidonate-induced cell killing was associated with the appearance of peripheral cytoplasmic condensations similar to those seen in the susceptible Gram-negative organisms. The Gram-negative species sensitive to arachidonic acid were the same species known to have a more permeable outer membrane than other Gram-negative species (Knapp and Melly 1986). The presence of LPS or other components of the Gram-negative cell envelope is probably not the sole factor in determining arachidonic acid resistance (Knapp and Melly 1986). Knapp and Melly (1986) suggested the possibility that in the resistant species, arachidonic acid does not have access to critical cell struc-

tures or that potentially toxic metabolites of arachidonic acid are formed in a location or manner that causes less cell damage. According to Knapp and Melly (1986), the killing of *S. aureus* by arachidonic acid appears to involve a peroxidation of the fatty acid catalysed by bacterial Fe^{2+} and H_2O_2. A high concentration of arachidonic acid-derived free radicals could be expected to rapidly overcome local antioxidant defences and become involved in deleterious reactions with many bacterial macromolecules (Knapp and Melly 1986).

Effect of degree of saturation

The level of unsaturation of fatty acids and the resultant effect on antibacterial activity was comprehensively analysed by Kanetsuna in 1985. In this study, unsaturated fatty acids showed strong bactericidal activity against *Mycobacterium smegmatis* in low concentrations, whereas saturated fatty acids, except for lauric and myristic acids, were not very effective. Palmitic, arachidonic and linoleic acids displayed strong antibacterial activity, but the relationship between the degree of unsaturation of a fatty acid and its bactericidal activity was not clear. Among the saturated fatty acids, lauric and myristic acids showed mycobactericidal activity. Macrophages secrete mainly palmitic and stearic acids (saturated) along with the unsaturated oleic and linoleic acids. Kanetsuna (1985) did not observe an inhibition of bactericidal activity of the unsaturated fatty acids when examining the effect of co-existence with saturated fatty acids, and concluded that the co-existence of saturated fatty acids with unsaturated fatty acids in phagosomes should not interfere with the bactericidal effect of the latter acids. Besides free fatty acids, reactive oxygen metabolites (O^{2-} and H_2O_2) have been reported to be mycobactericidal substances secreted by macrophages (Kanetsuna 1985). Free fatty acids and reactive oxygen metabolites possibly cooperate as potent mycobactericidal substances in phagosomes. Depending on the kinds of lymphokines which activate the macrophages and also on the stage of activation of the macrophages, either fatty acids or H_2O_2 may play the main role in the intracellular killing of mycobacteria (Kanetsuna 1985). The susceptibilities of *M. tuberculosis*, *M. bovis* and *M. kansasii* were similar to that of *M. smegmatis*.

There are increasing indications that host fatty acids influence the types of bacteria that normally colonise in various sites within the host (Knapp and Melly 1986). Further studies on the exact mechanism of bactericidal effects of polyunsaturated fatty acids may allow the determination of the role this phenomenon plays in bacterial ecology and host-bacterial interactions (Knapp and Melly 1986).

Conclusions

The antimicrobial properties of fatty acids are well known and there is a close relationship between the structure of fatty acids and their ability to function as antimicrobial agents. Saturated fatty acids are effective against microorganisms at lower chain lengths, while monounsaturated and polyunsaturated fatty acids with longer chain lengths are more effective. The position of double bonds is significant for long chain fatty acids.

There exists some confusion as to the mechanisms of antibacterial action of fatty acids and further studies are necessary to clarify the situation. In general, fatty acid sensitivity is considered to be a characteristic of Gram-positive bacteria, with few Gram-negative species being susceptible. Gram-negative organisms are affected by short chain fatty acids of less than six carbons while long chain fatty acids are effective against Gram-positive species. Functional groups such as esters have a notable effect on antimicrobial activity, with esterification of fatty acids to polyhydric alcohols such as glycerol and sucrose increasing the antimicrobial properties of the parent fatty acid. The stereochemistry of unsaturated compounds has an important role as *cis*-isomers are more active than *trans*-isomers.

The therapeutic use of fatty acids, with particular regard to topical applications for the treatment of bacterial or fungal infections, should be further considered. One such example is the potential use of the long-chain polyunsaturated linolenic acid in treating skin infections. Future investigations of antibacterial activity of fatty acids should direct attention to the synergistic effects of these compounds against various species of micro-organisms.

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