than erythromycin (14, 23–26) which may explain their superior anti-oxidative properties. The relative insensitivity of PMA- and opsonized activated-neutrophils to the inhibitory effects of macrolides on superoxide generation has been described previously (11, 12, 25) and may reflect the potency of these stimuli of membrane-associated oxidative metabolism by comparison with FMLP and calcium ionophore.

The inhibitory effects of the macrolides on superoxide generation by FMLP- and calcium ionophore-stimulated neutrophils could not be attributed to superoxide-scavenging activity or to non-specific cytotoxicity. Moreover, we have previously reported that calcium metabolism in activated neutrophils, as well as the activity of protein kinase C and phospholipase A_2, enzymes which are critically involved in the activation of the superoxide-generating enzyme of phagocytes, NADPH-oxidase (27, 28) are unaffected by erythromycin and roxithromycin (11). Similarly, in the present study none of the test macrolides, at concentrations of up to 40 μg/ml, affected calcium-activated phospholipid metabolism in stimulated neutrophils. Taken together, these observations suggest that macrolides at concentrations of < 80 μg/ml do not interfere with transductional mechanisms involved in the activation of NADPH-oxidase, but may rather affect oxidase activity in neutrophils exposed to FMLP and calcium ionophore.

The physical state of the neutrophil membrane appears to modulate superoxide production by affecting the protein motions, both rotational and lateral, that must take place in the assembly and functioning of the multicomponent NADPH-oxidase (29, 30). Agents which increase membrane lateral mobility potentiate NADPH-oxidase activity, while membrane-stabilizing agents such as cholesterol and cholesteryl esters inhibit the production of superoxide (29). We are unaware of previous studies relating to the possible membrane-stabilizing activity of macrolides, although the lipophilicity of these agents (21) suggests that the plasma membrane is their probable site of anti-inflammatory action. Pretreatment of sheep erythrocytes with all four macrolides increased the resistance of these cells to the hemolytic effects of the bioactive lipids LPC, PAF and LPAF. We were unable to detect complex-forming interactions between the macrolides and LPC, suggesting that the observed protective effects of these agents are mediated exclusively by membrane-stabilizing mechanisms. Although high concentrations (20–100 μg/ml) of the macrolides were used for these experiments, it is reasonable to assume that lower concentrations of these agents would protect the cell-membrane against lower, non-cytotoxic concentrations of membrane-destabilizing lipids. Interestingly, there appeared to be a good correlation between the membrane-stabilizing and the anti-oxidative properties of the macrolides, with azithromycin, clarithromycin and roxithromycin being more effective than erythromycin. The apparent discrepancy between clarithromycin,

the most potent membrane-stabilizing macrolide, and roxithromycin, the most potent anti-oxidative agent, may be explained by the relative affinities of these agents for erythrocytes and phagocytes (21).

We also used LPC, LPAF and PAF to investigate possible relationships between the membrane-stabilizing and anti-oxidative properties of the macrolides. Pretreatment of neutrophils with low, non-cytotoxic, minimally-sensitizing concentrations (0.5 μg/ml) of these phospholipids prevented the inhibitory effects of roxithromycin, the most potent anti-oxidative macrolide, on the generation of superoxide by FMLP-activated neutrophils, clearly linking the membrane-stabilizing properties of these agents to their anti-oxidative activity. Interestingly, LPC, LPAF and PAF, which are generated by neutrophils during exposure of these cells to stimuli of membrane-associated oxidative metabolism (28, 31) have been reported to potentiate the activity of NADPH-oxidase (32, 33). Moreover, addition of exogenous, reagent lysophospholipids and PAF at concentrations of >0.5 μg/ml to neutrophils does not directly activate superoxide generation, but as observed in the present study (at least with PAF), induces a state of hyperresponsiveness (priming, sensitization) on subsequent exposure of the cells to stimuli of membrane-associated oxidative metabolism (34, 35). Importantly, these sensitizing effects of lysophosphatides on the prooxidative activities of neutrophils are highly correlated with their membrane-disruptive properties (35). While these membrane-stabilizing properties of macrolides may contribute to their anti-oxidative interactions with neutrophils, it is noteworthy that macrolides have also been reported to decrease the activity of the phospholipase D-phosphatidic acid phosphohydrolase pathway in neutrophils (36) and to induce accelerated apoptosis in these cells in vitro (37). It is conceivable that relationships may exist between the membrane-stabilizing properties of macrolides and their effects on apoptosis and neutrophil phospholipase D activity.

These membrane-stabilizing, anti-inflammatory properties of macrolides are unlikely to be restricted to neutrophils since PAF and LPC, both of which are thought to play key roles in the pathogenesis of bronchial asthma and rhinitis (31, 38, 39) are potent mediators of other inflammatory and cytotoxic processes. Apart from its prooxidative and cytotoxic properties, which are shared by PAF and LPAF, LPC also induces mast cell secretion by promoting influx of extracellular calcium (40), while exposure of respiratory epithlium to lysophospholipids and PAF is accompanied by ciliary dysfunction, cytotoxicity and impaired mucociliary clearance (41–43). Although many of the pro-inflammatory activities of PAF are clearly receptor-mediated (31, 38) some activities of this bioactive phospholipid, as well as those of LPC and LPAF, appear to be mediated by receptor-independent, membrane-disruptive mechanisms which may be amenable to pharmacologic control by membrane-stabilizing agents.

In conclusion, macrolide antibiotics at concentrations, which in the case of

clarithromycin (21) erythromycin (44) and roxithromycin (22) are either therapeutically relevant or close to being so, regulate the prooxidative activities of human neutrophils, possibly by antagonizing the proinflammatory, membrane-destabilizing activities of several bioactive lipids which have been implicated in the pathogenesis of bronchial asthma. Although the concentrations of azithromycin required to achieve these effects are somewhat higher than the peak serum concentrations achieved during chemotherapy with this agent, it must be emphasized that these are not representative of tissue levels, which are considerably higher (17). It is noteworthy that these anti-oxidative interactions of macrolides are stimulus-specific, with relative sparing of responses activated by opsonized particles. Selective modulation of superoxide generation by activated neutrophils exposed to pro-inflammatory stimuli such as leukoattractants with retention of oxidant-mediated antimicrobial potential, is likely to be a useful property of macrolides. These membrane-stabilizing, antiinflammatory activities are, however, clearly secondary properties of macrolides. Nevertheless, if operative in vivo, they may contribute to the anti-inflammatory properties of these agents (1–6).

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