Damage to bacteria by antibiotics \textit{in vitro} and its relevance to antimicrobial chemotherapy: a historical perspective

The advent of the antibiotic era brought about a radical change in the way we viewed the treatment of infections. At the time of the introduction of penicillin and \textit{Prontosil Rubrum} in the early 1940s, there was a strong awareness of the critical role of serum opsonins and phagocytic capacity in suppression of infection. The role of phagocytic cells in host defence, in cooperation with humoral factors, had been recognized at the turn of the century through the pioneering work of Ilya Metchnikoff and Louis Pasteur. Serotherapy—the use of heterologous immune serum to treat infection—was beginning to show results (Loughlin, Bennett & Spitz, 1938), just as penicillin and the sulphonamides were gaining widespread acceptance.

With the immense success of the new chemotherapeutic agents these tentative approaches to immunotherapy were largely abandoned and antibiotics were considered to be the primary factor responsible for 'curing' infections. This attitude was reinforced with the introduction of the concept of the 'therapeutic level of antibiotics', which arose from the application of microbiological and pharmacological principles derived from the study of the sulphonamides, and uncritically applied to penicillin (Tillett, Cambier & McCormick, 1944; Tompsett \textit{et al.}, 1949). The aim of such therapeutic dosage schedules was to provide 'continuous therapy' in which drug levels were maintained at above-minimal inhibitory concentrations throughout the dosage interval. Forty years ago, Altemeier (1948) expressed doubt about the need for continuous therapy, believing that the maintenance of a more or less constant blood level of penicillin was probably unnecessary for effective therapy in man. However, the 'continuous therapy' approach to dosing remains the basis for most currently-used antibiotic regimens. The relative contributions of antibiotics and host defence systems in recovery from infections are not precisely defined and are generally not taken into consideration when the choice of therapeutic agent and dosage regimen are made. Nonetheless, it is clear from clinical experiences with grossly immunosuppressed patients that the effectiveness of antimicrobial chemotherapy is largely dependent on the functional integrity of the host's defences.

As early as the 1940s, clinical experience showed that satisfactory therapy of pneumococcal pneumonia with aqueous crystalline penicillin could be achieved with a discontinuous dosage regimen supplying inhibitory levels of penicillin for only half the dosage interval (Meads, Harris & Finland, 1945; Tumulty & Zubrod, 1948; Tompsett \textit{et al.}, 1949). Experimental support \textit{in vivo} for these findings was clearly demonstrated by the work of Eagle, Fleischman & Levy (1953). The early work of Parker & Marsh (1946), describing the 'postpenicillin stationary phase' provided one explanation for the success of discontinuous antimicrobial therapy. The persistent suppression of bacterial growth after exposure to antibiotics has been more recently confirmed for several different classes of antibiotics, and described as the postantibiotic effect (McDonald, Craig & Kunin, 1977; Bundtzen \textit{et al.}, 1981).

While the postantibiotic effect confers advantage to the host, it is unlikely that it is a sufficient explanation for successful therapy with discontinuous antibacterial therapy. The therapeutic effectiveness of antibiotics depends on functional host defence mechanisms (Weinstein & Dalton, 1968). One of the earliest attempts to demonstrate an effect of host factors upon the interaction between antibiotic and bacteria was that of Rammelkamp & Keefer (1943). Using a semi-in-vivo method of sampling blood after injection of penicillin, they were able to show enhanced killing of haemolytic streptococci in whole human blood even when penicillin was not detectable. The involvement of host factors in suppression of bacteria after antibiotic exposure has been demonstrated \textit{in vitro} by several groups (Alexander & Good, 1968; Solberg, 1978; Horne & Tomasz, 1981; McDonald, Wetherall & Pruul, 1981). These studies employed suprainhibitory levels of antibiotics in the pretreatment phase. In the situation \textit{in vivo}, the actual time of contact of bacteria with suprainhibitory levels of antibiotic may be relatively short, especially with a
regimen of pulse dosing. Such brief exposure of bacteria to antibiotic may be important in determining the outcome of therapy. Exposure to therapeutic concentrations of antibiotics with different mechanisms of action has been shown to alter bacterial cell surface structure (Greenwood & O'Grady, 1973; Hirashima, Childs & Inouye, 1973; Iida & Koike, 1974; Klainer & Russell, 1974). These changes may bring about alterations in a variety of cell surface properties relevant to interaction with host defence components, including changes in cell surface antigens (Schultz, Dunne & Heist, 1969; Michel et al., 1982; Gemmell & O'Dowd, 1983; Milatovic, Braveny & Verhoef, 1983), hydrophobicity (van Oss, 1978), excretion of toxins and enzymes (reviewed by Lorian, 1986), release of lipopolysaccharide (Klainer & Russell, 1974; Lam et al., 1987) and changes in cell wall thickness (Efrati et al., 1976; Lorian, Atkinson & Kim, 1983). The adherence properties of bacteria exposed to subinhibitory levels of antibiotics has been recently reviewed (Chopra, 1986). While antibiotic-induced perturbations in bacterial outer membranes alter their interactions with opsonins and phagocytic cells, the mechanisms by which susceptibility to phagocytic killing is modulated is largely unknown. The enhanced leucocytic killing of Group A streptococci after brief exposure to erythromycin has been shown to be dependent on increased phagocytic uptake (Pruul, Wetherall & McDonald, 1986). In contrast, enhanced killing of Escherichia coli after exposure to chloramphenicol was dependent on increased intracellular killing (Pruul, Wetherall & McDonald, 1981). Enhanced opsonization has been shown to be the mechanism by which exposure of Gram-positive cocci to subinhibitory levels of clindamycin (Gemmell et al., 1981; Milatovic, Braveny & Verhoef, 1983) and other inhibitors of protein synthesis (Hand, King-Thompson & Johnson, 1984) enhance susceptibility to killing by phagocytic cells. Oxygen-dependent intracellular killing mechanisms have been implicated (Pruul, Wetherall & McDonald, 1981; Lam et al., 1982) as well as oxygen-independent mechanisms (Pruul, Wetherall & McDonald, 1982). Further work is needed to clarify the relationship between the organism and the bactericidal mechanisms operative in changed susceptibility caused by exposure to antibiotic.

The outcome of infection can be regarded as the result of the interaction between a combination of factors, in which antibiotic may interact benignly or antagonistically. The recognition that the bacterium the host defences encounter in the absence of antimicrobial therapy is likely to be quite different from that which it confronts during therapy has important implications in the assessment of current and newly developed antimicrobial agents. In search for extra benefits of one antibiotic over another, a manifestation of cooperation with host factors would present an immediate attraction. Attempts to classify antibiotics in relation to their ability to modulate the interaction of the pathogen with host defences have appeared (Milatovic, 1983; Pruul, Hill & McDonald, 1984; Daschner, 1985). With careful assessment, a comprehensive classification of antibiotics in these terms can yield information potentially useful in the clinical setting.

Finally, bacteria isolated from the physiopathological conditions provided within the infected host will be chemically, physically and biologically different from those grown at the bench. This provides serious difficulty in the application of findings in vitro to the complicated situation in the infected host. The most important function that in-vitro studies can provide is a precise description of interactions between antimicrobial agents, clinically relevant microorganisms and host factors in order to yield criteria for therapy that take into account the effect of antibiotic-induced modulation of bacteria and susceptibility to host factors.

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The chemoprophylaxis of meningococcal infection

The development of meningococcal disease depends on a virulent strain being transmitted to, being acquired by, and invading a susceptible host. Prevention is possible for serogroups A, C, Y and W135 by using purified capsular polysaccharide vaccines, but such an approach is not possible for the currently most prevalent group B strains because of the poor immunogenicity of the capsule. The use of antimicrobial agents to eliminate sources of infection, or interfere with acquisition is the only preventive strategy available.

Asymptomatic pharyngeal carriage has long been recognized as the major source of transmission. Eradication of this carrier state, with an attendant decline in case rates, was established soon after the introduction of sulphonamides (Fairbrother, 1940; Kuhns et al., 1943). Oral sulphadiazine in a dosage of 0.1 g bd for two days was effective and so simple that it became common practice to treat whole military populations and any case contacts irrespective of their risk of disease (Feldman, 1972). The recognition of sulphonamide resistance in 1963 (Millar et al., 1963) following failure of prophylaxis, and the current worldwide prevalence of such strains has made this approach no longer feasible and generated studies designed to define more clearly the risk of disease, and to devise alternative prophylaxis strategies. Although meningococcal disease is associated with poverty and overcrowding, the only clearly defined risk factor is being a close contact of an index case (Meningococcal Disease Surveillance Group, 1976). Such contacts include those eating and sleeping in the same dwelling (particularly if they are under the age of five years), those attending day care nurseries (Kaiser et al., 1974) and pre-elementary schools (De Wals et al., 1981) or close contacts of a case in a closed community such as a military barracks, or boarding school. Hospital staff attending cases are not at risk (Artenstein & Ellis, 1968) except following mouth to mouth resuscitation (Feldman, 1972). Maximal risk occurs in the week following recognition of the index case (De Wals et al., 1981).

How should such at-risk individuals be managed? Two conceptually different strategies have evolved. In Norway protection is offered to those close household contacts considered most susceptible (age under 15 years) for the period of maximal risk. Phenoxymethyl penicillin for seven days is offered to those close household contacts irrespective of their risk of disease (Haiby et al., 1986). This might be expected where continuing exposure to an unidentified source remains.

The alternative approach attempts to eradicate the meningococcus from the 'micro-environment' in which enhanced transmission and acquisition are occurring, by treating all individuals irrespective of their perceived susceptibility. Such eradication should also extend to the index case before return to the community. Although sulphadiazine remains the agent of choice for sensitive strains (MIC ≤ 1 mg/l), it may not eradicate strains of intermediate sensitivity (MIC 1–10 mg/l and will fail with strains where the MIC exceeds 10 mg/l (Feldman, 1967). Since prophylaxis should commence immediately after recognition of the index case to be maximally effective, at a time when sulphonamide sensitivity will be unknown, sulphonamides should not be used in current practice.

Many antibiotics have been assessed in the
search for a suitable replacement. Oral or parenteral penicillins, ampicillin, tetracycline, erythromycin and nalidixic acid have all failed to eliminate carriage (Millar et al., 1963; Dowd et al., 1966; Artenstein, Lamson & Evans, 1967; Feldman, 1967). Rifampicin is currently most widely used but is not without its disadvantages. Studies using 600 mg daily for four days (Beam & Sanders, 1969; Devine et al., 1970; Beam et al., 1971; Sivonen et al., 1978), 500 mg daily for four days (Weidmer et al., 1971) or 600 mg 12 hourly for two days (Munford et al., 1974) have reduced carrier rates by only 75–90% over one to two weeks, and in some studies carrier rates have then increased during more prolonged surveillance (Sivonen et al., 1978). Some of the failures of eradication resulted from the emergence of isolates resistant to rifampicin in up to 10% of the original carriers treated (Beam et al., 1971; Munford et al., 1974). Such strains may cause disease (Cooper et al., 1986). Minocycline given as 100 mg 12-hourly for five days has been marginally less effective than rifampicin in eradicating carriage but has not generated resistance (Guttler et al., 1971; Munford et al., 1974). Rifampicin and minocycline either together (Munford et al., 1974) or sequentially (Nicolle et al., 1982) have tended to be more effective at eradication but any use of minocycline has been compromised by the high frequency of vestibular side effects (Drew et al., 1976).

Are there alternatives? A single injection of ceftriaxone has undergone limited study (Judson & Ehret, 1984). The fluoroquinolone ciprofloxacin is highly active against Neisseria meningitidis in vitro (Felmingham & Wall, 1985); its long half-life and significant salivary concentrations suggest it would be a suitable candidate. Twenty-one carriers received ciprofloxacin 500 mg 12 hourly for five days (Pugsley et al., 1987). Apart from one transient positive culture on day three, all subjects were culture negative from day one of treatment until 13 days post treatment.

Gaunt & Lambert (1988) describe the use of a single dose of ciprofloxacin 500 mg. The eradication of carriage in 97% of carriers by four days and 93% after two months are encouraging findings. The use of single doses even in children is unlikely to generate significant adverse clinical or ecological problems. Further studies are warranted. Until then, rifampicin 12-hourly for two days, which has a lower frequency of side effects (Band & Fraser, 1984) must be the regimen of choice but with the following caveats. It is currently considered that identification of close contact carriers by nasopharyngeal culture should not be part of a preventive strategy. However, during the current resurgence of disease epidemiological patterns suggest that sources outside the household may be significant (Cartwright et al., 1986) emphasizing the need to establish clearly, where possible, the microenvironment in which transmission may be occurring. Where resources permit, the efficacy of eradication should be assessed, and since rifampicin resistant strains are being encountered in some cases of meningitis, all isolates should be tested for sensitivity to rifampicin, so that alternative chemoprophylaxis may be given and local policy modified if necessary. Secondly, chemoprophylaxis should be combined with surveillance, as there may be a significant frequency of co-primary cases (De Wals et al., 1981; Wall et al., 1986) or the source may not have been identified. Finally, since chemoprophylaxis will influence only a small number of the total cases, public education, and heightened diagnostic awareness are as important as drugs.

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