Journal of Antimicrobial Chemotherapy (1987) **20**, *Suppl. A*, 87–93

Relevance of animal models to the prophylaxis of infective endocarditis

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Intravascular or cardiac endothelial lesions may become colonized during bacteraemic episodes and lead to the development of bacterial endocarditis (BE). It has therefore long been recommended that patients with known cardiac lesions receive prophylactic antibiotics before undergoing procedures that might release bacteria into the blood stream. Because clinical trials of antibiotic prophylaxis of endocarditis cannot be conducted in humans for ethical as well as for statistical reasons (Durack, 1985), the questions of which antibiotic, what dosage, and for how long are a matter of controversy. Unfortunately, these questions can only be studied in animals, with all the limitations that this type of approach brings with it. However, animal experimental studies have helped in understanding the conditions and, to some extent, the mode of action of antibiotics in preventing the development of endocardial infection, thus allowing some rationale for devising prophylactic recommendations for the various patients at risk of developing BE.

The animal model of endocarditis: observations on prophylaxis in rabbits and rats

In 1970, Garrison and Freedman reported that insertion of a polyethylene catheter in the rabbit heart led to development of small sterile vegetations at points of contact between the catheter and endocardium. If staphylococci were placed in the lumen of the catheter, staphylococcal endocarditis resulted. Modification of this model by injecting organisms intravenously provided a suitable in-vivo system for examining the efficacy of various antibiotic regimens for prophylaxis of endocarditis (Durack & Petersdorf, 1973). Under these circumstances, the time of onset of infective endocarditis is known exactly. Another important advantage is that the incidence of infection in untreated animals can be adjusted easily by altering the inoculum size; thus the problem of very low infection rates in patients can be overcome in animals by choosing an inoculum large enough to approximate the infective dose producing disease in 90% of the animals (ID_{90}) for the organism under investigation. Significant differences among antibiotic regimens can then be demonstrated with manageable numbers of animals in each group.

Durack & Petersdorf (1973) and Pelletier, Durack & Petersdorf (1975) have used the rabbit model to test the efficacy of various prophylactic antibiotic regimens against BE caused by streptococci. In these experiments, a catheter passed across the tricuspid or

Supported by grant no 3.847.083 from the Swiss Foundation for Scientific Research.
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0305–7453/87/20A087+07 $02.00/0

aortic valve produces sterile vegetations that serve as a nidus for microbial localization and growth after intravenous injection of 10^8 cfu of a strain of viridans streptococcus. Bacterial colonization of the catheter-induced vegetations was uniformly observed when the animals were killed 24 h after injection. Thirty minutes prior to injection of streptococci, various antibiotics were administered parenterally to evaluate their ability to prevent colonization and growth of bacteria in the vegetations.

Following a similar technique, we have recently developed a model in rats that is similar to the rabbit model (Héraïef, Glauser & Freedman, 1982). Briefly, a polyethylene catheter is inserted across the aortic valve through the right carotid artery, resulting in the production of sterile valvular vegetations. Twenty-four hours after catheterization, the tail vein is injected with a given bacterial inoculum of the test organism. The animals are sacrificed at various time intervals after bacterial challenge, quantitative blood cultures are drawn, the aortic vegetations excised, weighed, homogenized, serially diluted and cultured. Culture plates are counted after 48 to 72 h incubation.

Antibiotic prophylaxis is tested by injecting one group of animals with a given dosage of the selected antibiotic 30 min before bacterial challenge. The dosages of the various antibiotics tested in rabbits or rats are chosen to produce serum levels at the time the organisms are injected similar to those in humans after a recommended oral dose.

Experiments on prophylaxis in rabbits

Durack and his colleagues in their early rabbit experiments injected 10^8 cfu of viridans group streptococci and enterococci iv, an inoculum size that produced endocarditis in 100% of the animals. In fact, this number of bacteria was probably 10 to 100 times higher than the lowest infectious dose necessary to produce endocarditis in 90% of the rabbits (ID_{90}). Under these conditions, careful and extensive experiments, using various doses of prophylactic antibiotics given for various length of time, suggested that cell wall active antibiotics (the so called 'bactericidal' antibiotics) such as beta-lactams or vancomycin, were necessary for successful prophylaxis. In contrast, bacteriostatic antibiotics such as tetracycline, erythromycin or clindamycin failed to provide protection. Synergistic combinations of β-lactam and aminoglycoside were superior to single drug alone. For most antibiotics tested as single drug regimens, only prolonged serum levels such as those provided by long acting preparations were successful (Durack & Petersdorf, 1973; Durack, Starkebaum & Petersdorf, 1977; Pelletier *et al.*, 1975; Southwick & Durack, 1974)). Thus, these observations in rabbits strongly suggested that prophylaxis was mediated through bacterial killing and influenced the recommendation of the American Heart Association in 1977 for the prophylaxis of BE (Kaplin *et al.*, 1977), which suggested the parenteral administration of antibiotics over 24–48 h, preferably in combination.

Experiments on prophylaxis in rats

(i) *Prophylaxis of viridans streptococcal endocarditis.* Using the rat model of endocarditis, we first tested vancomycin for the prevention of endocarditis because it had been shown in the rabbit model to be the only effective antibiotic given as a single dose. In the rat, single dose vancomycin also prevented *Streptococcus sanguis*

endocarditis, but its efficacy was limited to rats challenged with a bacterial inoculum size equal or inferior to the ID_{90}, and vanished with higher inoculum sizes (Heraief, Glauser & Freedman, 1980). Further experiments disclosed that this effect was achieved in the absence of bacterial killing, since the *S. sanguis* strain used was demonstrated to be tolerant to vancomycin (Bernard, Francioli & Glauser, 1981). That successful endocarditis prophylaxis could be achieved by mechanisms other than bacterial killing was further demonstrated when clindamycin, and to a lesser degree erythromycin, were also shown to prevent endocarditis induced by several viridans streptococci in rats challenged with bacterial inoculum sizes equal to the ID_{90} (Glauser & Francioli, 1982). Using single dose amoxycillin for prophylaxis, and streptococcal strains of various susceptibilities to antibiotic killing for challenge, it became apparent that single dose amoxycillin prophylaxis was successful against inoculum sizes higher than the ID_{90}, only when the strain was rapidly killed by the antibiotic. Indeed, for single dose prophylaxis to be successful independently of the inoculum size, it appeared that the bacteria had to be killed rapidly enough so that bacterial death could occur during exposure to circulating antibiotic blood levels (Glauser *et al.*, 1983). Streptococcal strains with such a high level of sensitivity to killing by amoxycillin or vancomycin are rarely encountered in patients with bacterial endocarditis (Meylan, Francioli & Glauser, 1986). In contrast, when the bacteria were not rapidly killed, or were tolerant to amoxycillin, the efficacy of single-dose prophylaxis was limited to challenge with the ID_{90}, and failed in animals challenged with higher inoculum sizes. Similar observations were made after prophylaxis with penicillin G (Francioli & Glauser, 1985). Even single doses of the synergistic combination of amoxycillin plus gentamicin failed to protect against challenge with such tolerant strains when using inoculum sizes higher than the ID_{90}, possibly because antibiotic blood levels were not sustained for long enough to completely eliminate the bacteria that had attached to the vegetations (Francioli, Moreillon & Glauser, 1985). Against such high bacterial challenge, only multiple doses of amoxycillin given at 6-hourly intervals for 48 h after challenge were successful. Under these circumstances multiple doses of the in-vitro synergistic combination of amoxycillin plus gentamicin given at 6-hourly intervals was significantly superior to multiple doses of amoxycillin alone, in that only one or two additional doses were necessary for successful prophylaxis (Malinverni, Francioli & Glauser, 1986).

(ii) *Prophylaxis of enterococcal endocarditis.* Observations similar to those with viridans streptococci were made in rats challenged with various strains of *S. faecalis*, a bacterial species against which β-lactam antibiotics are notoriously bacteriostatic. Indeed, single dose amoxycillin prophylaxis was successful against challenge with inoculum sizes corresponding to the ID_{90}, but not against higher inocula (Francioli *et al.*, 1985). It should be noted that the ID_{90} of all *S. faecalis* strains tested in this model were 10- to 100-fold lower than those of the viridans streptococci tested (10^4 cfu/ml for *S. faecalis* versus 10^5–10^7 cfu/ml for various viridans streptococci). The bactericidal combination of amoxycillin plus gentamicin given as a single dose was not significantly superior to the single dose of amoxycillin alone. In rats challenged with inoculum sizes 1000–10,000 higher than the ID_{90}, only multiple doses of amoxycillin plus gentamicin given at 6-hourly intervals for >48 h were successful in preventing endocarditis, while multiple doses of amoxycillin alone were not (Malinverni *et al.*, 1986). This suggests that bactericidal combinations were necessary to prevent enterococcal endocarditis

induce by such high inocula, a known prerequisite for the treatment of established enterococcal endocarditis.

Comparison of observations on endocarditis prophylaxis in rabbits and in rats

While experiments in rabbits established the need for the prolonged administration of antibiotics (and preferably of the combination of synergistic antibiotics) to successfully prevent endocarditis (suggesting bacterial killing as a mechanism of prophylaxis), experiments in rats have shown that a single dose of antibiotic might prevent endocarditis, even in the absence of bacterial killing. However, the systematic observations in rats, using various inoculum sizes of each streptococcal strain tested, have provided a rationale to reconcile these apparent contradictions. Indeed, as previously mentioned, most experiments in rabbits have used an inoculum size for challenge that was largely higher than the ID_{90} (Pelletier *et al.*, 1975), a condition which has clearly been shown in rats to require multiple dose regimens for successful prophylaxis unless the strain used for challenge is very sensitive to bacterial killing.

Summary of the experimental observations on the antibiotic prophylaxis of streptococcal endocarditis

(a) Single doses of antibiotics such as amoxycillin, penicillin G, clindamycin (and to a lesser degree erythromycin) and vancomycin are successful in reliably preventing endocarditis induced by bacterial inocula corresponding to the ID_{90}.

(b) single doses of such antibiotics fail against bacterial challenges higher than the ID_{90}, unless the strain is exquisitely sensitive to bacterial killing, a rare phenomenon.

(c) Single doses of the synergistic combination of amoxycillin plus gentamicin are not superior to amoxycillin alone in animals challenged with inoculum sizes higher than the ID_{90}.

(d) To overcome this limited efficacy, multiple doses of amoxycillin alone for viridans streptococci and of amoxycillin plus gentamicin for enterococci, are necessary.

Relevance of the experimental model of endocarditis to the study of prophylaxis

There are three main objections to the animal model. The first objection is that it is an animal model; the second is the presence of the intra-cardiac catheter, and the third is the high bacterial inoculum used for challenge, which far exceeds those observed in humans after dental or urogenital procedures.

The animal model of endocarditis for the study of antibiotic prophylaxis

As clearly pointed out by Durack (1985), a controlled clinical study of endocarditis prophylaxis in human would be extremely difficult to perform. Indeed, ethical reasons would preclude the use of a placebo group of patients and the low frequency of developing endocarditis would require too large of a number of patients to be enrolled. Thus, the animal model becomes a realistic approach to clarify some of the conditions for successful endocarditis prophylaxis, and, more importantly, to help understand the mode of action of prophylactic antibiotics. There is little doubt that antibiotics prevent endocarditis *in vivo* by multifactorial mechanisms that are more complex than the simple pharmacokinetic behaviour of antibiotics in humans or the killing of bacteria as observed in the test tube.

The presence of the intracardiac catheter

In most experimental studies the catheter has been left *in situ* for the duration of the experiment, while there is usually no intravascular foreign body present in humans. There is experimental evidence however that the persistance of the catheter worsens the course of endocarditis (Perlman & Freedman, 1971; Francioli & Freedman, 1979) and reduces the effectiveness of antibiotic prophylaxis (Heraief *et al.*, 1980). Thus, experimental models for the prophylaxis of endocarditis with a catheter in place provide a very stringent test of an antibiotic to prevent infection of the cardiac vegetations, possibly giving a margin of safety when recommended in humans. Furthermore, the presence of a plastic catheter mimics the clinical situation of patients with prosthetic heart valves or other intravascular foreign bodies.

The high number of bacteria used for challenge

The magnitude of the bacterial inocula injected iv to the animal (10^4–10^8 cfu) has been considered unrealistic when compared with bacteraemia observed in humans after dental procedures. Indeed, the magnitude of bacteraemia observed in man after certain procedures such as dental extractions is generally of the order of 10^1–10^2 cfu/ml of blood for a given strain (Everett & Hischmann, 1977). However, it should be stressed that the number of bacteria injected iv to animals do not represent the number of bacteria that circulate, and that the bacterial numbers found in the heart of the animals after iv injections are far below the original inoculum, thanks to both a passive haemodilution phenomenon and an active clearance mechanism by the reticulo-endothelial system (Bernard *et al.*, 1981).

In addition, the relationship between the magnitude of the bacteraemia and the subsequent risk of developing endocarditis is unknown in humans. It might well be that those very few patients who develop endocarditis after dental procedures are precisely those with the highest numbers of circulating bacteria.

Lastly, recent studies on the production of endocarditis in rats after the extraction of periodontally diseased teeth have failed to demonstrate a relationship between the number of a given streptococcal species circulating immediately after dental extraction, and the likelihood for these streptococci to subsequently produce endocarditis (Moreillon *et al.*, 1985). Indeed, some streptococcal strains found in the blood at barely detectable levels, consistently produced endocarditis, while other viridans streptococci detected in much higher numbers in the blood only rarely infected the valves. In these experiments, the parameter that best predicted the likelihood to produce endocarditis was the in-vitro stickiness of a given bacterial species for platelet-fibrin matrices. Thus, the determination of the magnitude of bacteraemia alone after certain procedures might not provide reliable information on the risk for the subsequent development of BE.

Conclusions

The model of endocarditis both in rabbits and in rats has permitted extensive and systematic study of the various important parameters in animals when endocarditis is induced: firstly, the traumatized valvular endothelium necessary to induce such lesions; secondly, the bacterial strains and their virulence factors; and thirdly, with regard to

prophylaxis, the efficiency of numerous antibiotic regimens in preventing endocarditis under various conditions. Our present understanding indicates that single doses of several antibiotics are successful in preventing streptococcal endocarditis, but have limited efficacy. Under stringent conditions the best margin of safety is provided by multiple doses of amoxycillin (or penicillin) for viridans streptococci, and multiple doses of amoxycillin plus gentamicin for enterococci.

It is unlikely that a clinical trial of endocarditis prophylaxis will ever be performed in patients. Thus, the observations made in the animal model of endocarditis represent one useful approach in understanding the conditions necessary for successful endocarditis prophylaxis, and assist the physician and the dentist in recommending effective prophylactic regimens.

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