Infective endocarditis — the effect of liposomes as carrier substance for α_1-antitrypsin and ampicillin

H. S. SCHAAF, W. D. BATES, C. HANEKOM, B. F. NEITELER, A. B. KRIEGLER, P.-L. VAN DER MERWE

Summary

Infective endocarditis has a high mortality and morbidity rate despite all available treatment. Little attention has been paid to the possible role of polymorphonuclear leucocytes in damage to the heart valves. It was postulated that if the elastases set free from these leucocytes could be neutralised, this would prevent damage to the heart valves. Alpha$_1$-antitrypsin (α_1-AT) in liposomes was used to neutralise elastases. This process on its own and in various combinations with ampicillin were compared in animal models. Evaluation was performed by measuring vegetation size, by blood and vegetation cultures, and by light microscopy of the damaged tissue. A statistically significant difference (*t*-test; $P < 0{,}005$, with Bonferroni's correction for multiple comparisons) was found in vegetation size in the groups receiving ampicillin in liposomes, but the hypothesis that α_1-AT might reduce valvular damage was not proven.

S Afr Med J 1991; **79:** 588-590.

Infective endocarditis (IE) is an uncommon but important disease with a rising incidence in children.[1-3] This rise is attributed to the longer survival of children with congenital heart defects, heart valve replacements and those who have undergone other cardiac surgery, intravenous drug abuse, and prolonged intravenous feeding through central venous lines.[4-6] Rheumatic heart disease complicated by IE has become a rarity in the Western world,[7,8] but the syndrome is still the major underlying lesion in Third-World countries.[9,10] Despite preventive measures, modified antibiotic therapy and surgical treatment, the mortality rate from IE is still 20-22% and the morbidity with sequelae 25-65%.[1]

Although the role of polymorphonuclear leucocytes (PMNs) in the pathogenesis of IE has not been fully investigated, Osler[11] mentioned in 1885 that PMNs might enhance valvular damage, while Freedman[12] recently discussed the possible role that the lysosomal contents of the PMNs could play in the destruction of heart valves in IE.

A preliminary investigation was undertaken to determine whether valvular damage from IE in rabbits could be reduced by the administration of human α_1-antitrypsin (α_1-AT) in liposomes and whether this therapy in combination with antibiotic-carrying liposomes could work synergistically.

Departments of Paediatrics and Child Health, Anatomical Pathology, Internal Medicine, Medical Microbiology and Pharmacology, University of Stellenbosch, Parowvallei, CP
H. S. SCHAAF, M.MED. (PAED.), D.P.H.
W. D. BATES, M.MED. (ANAT. PATH.)
C. HANEKOM, M.SC.
B. F. NEITELER, MED. TECH.
A. B. KRIEGLER, MED. TECH.
P.-L. VAN DER MERWE, M.D.

Accepted 9 Aug 1990.

Material and methods

The study was approved and supervised by the Ethical Advisory Committee of the Faculty of Medicine of the University of Stellenbosch and the South African Medical Research Council.

Experimental IE was established in 50 rabbits using a modified model described by Gutschik *et al.*[13] and Gutschik and Christensen.[14] The experimental animals were divided into five groups of 10 animals each. Eight animals died during the model-establishing phase, mainly from myocardial infarction. Group I ($N = 8$) — control group, no treatment; group II ($N = 7$) — ampicillin 100 mg/kg body weight/d in 2 divided doses (control for group III); group III ($N = 8$) — ampicillin 100 mg/kg body weight/d in 2 divided doses (10 mg in liposomes and 90 mg not in liposomes); group IV ($N = 10$) — α_1-AT in liposomes as a single dose daily; and group V ($N = 9$) — α_1-AT in liposomes as a single dose, and ampicillin in liposomes in 2 divided doses.

The experimental procedure consisted of anaesthetising the rabbits and inserting a polyethylene catheter in the left common carotid artery. The catheter was placed across the aortic valve under radiographic guidance and left in position for 3 days before removal. A bacterial suspension of a proteolytic strain of *Streptococcus faecalis* (variant *liquefaciens*, No. 2705) 1 ml was injected into a marginal ear vein. Treatment was started on the 4th day. The drugs were administered through a peripheral vein in all the groups.

Treatment was administered for 6 days. If the rabbits were alive on day 10, they were sacrificed and evaluated as follows: (*i*) surface area of vegetation (mm^2) was estimated by dividing the vegetations into smaller squares and measuring diameters with a small flexible standardised ruler; (*ii*) blood and vegetation tissue were taken from each experimental animal for culture (no quantitative colony counts were done); (*iii*) colour slides were taken of each dissected heart; and (*iv*) hearts were fixed in formalin and routine histological sections were made of each aortic valve, as well as at least one section per animal of the nearby aortic wall (a careful attempt was made to produce sections that showed the vegetations and the underlying valve or aorta in order to assess the damage); on each section the following stains were performed: haematoxylin and eosin, Verhoeff-Van Gieson (for elastic tissue and collagen) and a Gram stain.

Ampicillin was the antibiotic of choice because the *S. faecalis* strain used was sensitive to this antibiotic.

Liposomes were prepared according to the method of Finkelstein and Weissmann.[15] Purified lipids — i.e. phosphatidylcholine (PC); diacetyl phosphate (DCP); and cholesterol — were dissolved in chloroform 3 ml at a molar ratio of 7:2:1 in a round-bottomed flask, leaving a uniformly thin lipid film on the wall of the flask. All steps were performed at room temperature.

Ampicillin 450 mg or α_1-AT 1,55 mg or a combination of the two were dissolved in phosphate buffered saline (PBS) 3 ml. These aqueous solutions were added to the lipid films and vigorously agitated to form multilamellar liposomes.

By administering 1 ml of the α_1-AT solution per day to a rabbit of 3 kg, it received α_1-AT 385 μg, enough to inhibit 50% of the circulating neutrophil elastases.

In the groups receiving ampicillin in liposomes, 1 ml (150 mg ampicillin) was administered twice daily; i.e. 100 mg/kg ampicillin in a 3 kg rabbit per day. Group V received both drugs in liposomes, the doses were the same as above.

In a separate experiment with radioactive-labelled ampicillin it was determined that 10% of the ampicillin was trapped in the liposome carrier system. The α_1-AT administered was a predetermined dose to inactivate 50% of elastases in the circulating neutrophils. Ten per cent of the α_1-AT was trapped in the liposomes.

Results

The results are summarised in Table I. There was a statistically significant difference ($P < 0,005$; t-test with Bonferroni's correction for multiple comparisons) in the mean vegetation surface areas in the groups where ampicillin in liposomes (groups III and V) was used compared with the other three groups (groups I, II and IV).

Histological examination of the sections confirmed the frequency and the prominence of vegetations, as set out in Table I. The presence of Gram-positive cocci was also confirmed in all the cases with vegetations. As had been suspected at the outset of the project, the relatively small size of the rabbit aortic heart valve made assessment of damage to elastic fibres very difficult. The common appearance of vegetations on the aortic wall did, however, offer an alternative and more realistic opportunity to see whether there was a difference in the degree of elastic-fibre destruction independent of the size of the vegetation or prominence of neutrophils and other inflammatory cells (Fig. 1).

Although it must be conceded that it is difficult to quantify this type of difference, the nature of the vegetations and the degree of elastic damage in the aorta appeared similar in the different groups. The differences were in the frequency and the size of the vegetations.

Discussion

A statistical difference ($P < 0,005$; t-test with Bonferroni's correction for multiple comparisons) was found in vegetation size in the groups receiving ampicillin in liposomes. An explanation for this finding is not obvious. Three possible mechanisms are:

1. Ampicillin may be delivered at the site of infection via either capillary leakage or by transport by phagocytes of which there are a high concentration at the site of the lesions.[16,17]

2. Liposomes are sequestrated largely by the macrophages in the liver, spleen and bone marrow.[16–18] In the bacteraemic phase that follows injection with the organisms, many will be removed by the static macrophages in the organs mentioned. With the high concentration of both ampicillin-carrying liposomes as well as bacteria in these macrophages, the bacteraemia may be reduced to such an extent that a much smaller dose of bacteria is available for infecting the sterile vegetations, which may explain the smaller size of vegetations in these groups.

3. The incorporation of ampicillin in liposomes may influence the serum level of ampicillin due to a reduced excretion rate.

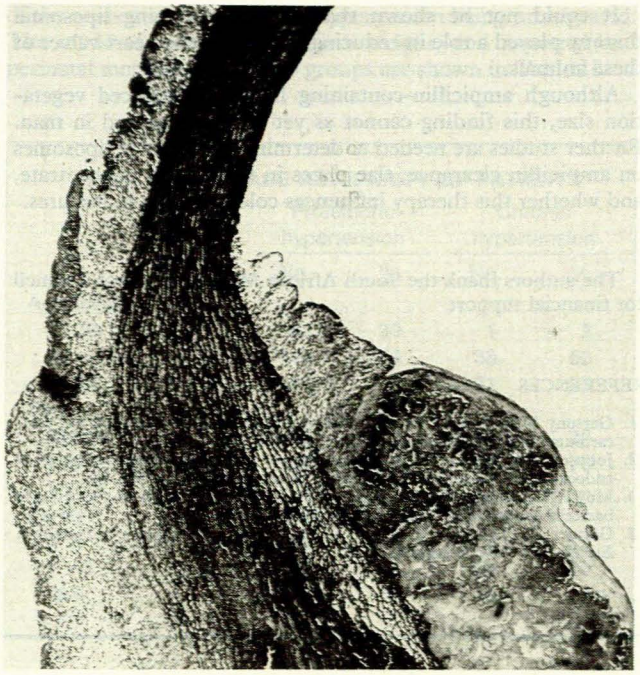

Fig. 1. An example of an aortic wall vegetation. On the left the normal elastic fibres of the aorta can be seen. On the right, beneath the vegetation, acute inflammatory cells are present in a widened aortic wall, which shows irregularity and disruption of elastic fibres.

In this preliminary study we could not show that α_1-AT reduced damage to the heart valves. This was possibly due to the sequestration of liposomes that takes place in the static macrophages of the liver, spleen and bone marrow but may also merely be a result of the sample size not being large enough to detect small, but scientifically meaningful, differences.

No significance as such should be attached to the apparent synergistic action of the two drugs owing to the small number of experimental animals used in each group.

Conclusions

In this study ampicillin-containing liposomal therapy reduced vegetation size in experimental infective endocarditis.

TABLE I. OBSERVATIONS OF VEGETATION SIZE AND BLOOD CULTURE RESULTS

Experimental group	No. of animals	Mean vegetation area (mm²)	Positive cultures		Both cultures negative
			Blood	Vegetations	
Group I	**8**	**48,78**	**8**	**8**	**0**
Group II	**7**	**44,5**	**6**	**7**	**0**
Group III	**8**	**9,58***	**2**	**6**	**2**
Group IV	**10**	**49,18**	**10**	**10**	**0**
Group V	**9**	**11,75***	**3**	**4**	**5**

* $P < 0,005$; t-test with Bonferroni's correction for multiple comparisons.

It could not be shown that α_1-AT-containing liposomal therapy played a role in reducing damage to the heart valves of these animals.

Although ampicillin-containing liposomes reduced vegetation size, this finding cannot as yet be implemented in man. Further studies are needed to determine the effect of liposomes on ampicillin clearance, the places in which they concentrate, and whether this therapy influences colony counts in cultures.

The authors thank the South African Medical Research Council for financial support.

REFERENCES

1. Gersony WM, Hordof AJ. Infective endocarditis and diseases of the pericardium. *Pediatr Clin North Am* 1978; **25**: 831-838.
2. Johnson DH, Rosenthal A, Nadas AS. A forty-year review of bacterial endocarditis in infancy and childhood. *Circulation* 1975; **51**: 581-588.
3. Moy RJD, George RH, De Giovanni JV, Silove ED. Improving survival in bacterial endocarditis. *Arch Dis Child* 1986; **61**: 394-399.
4. Garnier JL, Touraine JL, Colon S. Immunology of infective endocarditis. *Eur Heart J* 1984; **5**: suppl C, 1-20.
5. Kramer HH, Bourgeois M, Liersch R *et al*. Current clinical aspects of bacterial endocarditis in infancy, childhood and adolescence. *Eur J Pediatr* 1983; **140**: 253-259.
6. Gray IR. Infective endocarditis, 1937-1987. *Br Heart J* 1987; **57**: 211-213.
7. Schollin J, Bjarke B, Wesstrom B. Infective endocarditis in Swedish children. *Acta Paediatr Scand* 1986; **75**: 993-998.
8. Stanton BF, Baltimore RS, Clemens JD. Changing spectrum of infective endocarditis in children. *Am J Dis Child* 1984; **138**: 720-725.
9. Moethilalh R, Coovadia HM. Infective endocarditis in thirteen children: a retrospective study (1974-1981). *Ann Trop Paediatr* 1982; **2**: 57-62.
10. Cassel GA, Haitas B, Lakier JB, Barlow JB. Infective endocarditis at Johannesburg Hospital. *S Afr Med J* 1979; **55**: 624-627.
11. Osler W. The Gulstonian lectures on malignant endocarditis. *Br Med J* 1885: **1**: 467-579.
12. Freedman LR. The pathogenesis of infective endocarditis. *J Antimicrob Chemother* 1987; **20A**: 1-5.
13. Gutschik E, Moller S, Christensen N. Experimental endocarditis in rabbits. *Acta Path Microbiol Scand* 1979; **87B**: 353-362.
14. Gutschik E, Christensen N. Experimental endocarditis in rabbits. *Acta Path Microbiol Scand* 1978; **86B**: 215-221.
15. Finkelstein MC, Weissmann G. Enzyme replacement via liposomes. *Biochim Biophys Acta* 1979; **587**: 202-216.
16. Lopez-Berestein G. Liposomes as carriers of antimicrobial agents. *Antimicrob Agents Chemother* 1987; **31**: suppl. 5, 675-678.
17. Bakker-Woudenberg IAJM, Lokerse AF, Vink-Van den Berg JC, Roerdink FH, Michel MF. Effect of liposome-entrapped ampicillin on survival of Listeria monocytogeneses in murine peritoneal macrophages. *Antimicrob Agents Chemother* 1986; **30**: 295-300.
18. Torchillin VP. 1985 Liposomes as targetable drug carriers. *Crit Rev Ther Drug Carrier Syst* 1985; **2**: 65-114.