Journal of Antimicrobial Chemotherapy (1987) **20**, 719–724

A comparison of the efficacy of itraconazole, amphotericin B and 5-fluorocytosine in the treatment of *Aspergillus fumigatus* endocarditis in the rabbit

L. P. Longman and M. V. Martin

School of Dental Surgery, University of Liverpool, Pembroke Place, Liverpool L69 3BX, U.K.

The efficacy of amphotericin B, 5-fluorocytosine and itraconazole was compared for the treatment of experimental rabbit *Aspergillus fumigatus* endocarditis. Therapy with amphotericin B or 5-fluorocytosine, at dosages of 3·0 and 35 mg/kg body weight respectively, failed to eradicate aspergillus from the cardiac vegetations in all but one of the animals tested; none of these animals survived for longer than nine treatment days. When similar doses of amphotericin and 5-fluorocytosine were administered concomittantly, 30% of the animals had sterile vegetations. Itraconazole at 2·5 and 3·5 mg/kg body weight was not successful; all the animals tested had infected vegetations and did not survive beyond nine days of therapy. In contrast, itraconazole at 5·0 mg/kg sterilised the endocardial vegetations and all these animals survived for 14 days. It is concluded that itraconazole may be useful in the treatment of aspergillus endocarditis.

Introduction

Aspergillus endocarditis is a life threatening condition frequently only recognised at autopsy. It is usually associated with a history of cardiac surgery, and particularly recipients of prosthetic heart valves (Carrizosa *et al.*, 1974; Kammer & Utz, 1974; Rubinstein *et al.*, 1975; McLeod & Remington, 1978). Untreated endocarditis is usually fatal, and even with prompt therapy has a high mortality (*ca* 87%, Rubinstein *et al.*, 1975). Once the infected cardiac vegetations became established radical treatment is required, involving prolonged antimycotic therapy and surgical removal of the vegetations. Amphotericin B in combination with 5-fluorocytosine is commonly used clinically. These two antifungal agents do not penetrate well into the bulky cardiac vegetations and hence long-term therapy and follow-up is necessary (Rubinstein *et al.*, 1975).

The aim of this study was to compare the efficacy of amphotericin B and 5-fluorocytosine with itraconazole in the treatment of experimental rabbit aspergillus endocarditis. Itraconazole is a triazole with potent activity against aspergillus (Van Cutsem *et al.*, 1983).

Materials and methods

Micro-organism

Aspergillus fumigatus strain LL1 was used throughout this study. This fungus was isolated *post mortem* from the mitral valve of a patient with aspergillus endocarditis, and maintained on Sabouraud's agar slopes until required.

0305–7453/87/110719+06 $02.00/0

Inocula for the production of infective endocarditis were prepared from the growth of the fungus on Sabouraud's agar slopes for 56 h at 30°C. The slopes were covered with 2 ml of sterile distilled water and gently agitated prior to aspiration of the fungal suspension. A drop of Tween 80 was then added to the aspirate and the mycelium allowed to settle out for 18 h at room temperature. The conidia in the supernatant were harvested by centrifugation (20,000 g for 10 min) and then washed three times with 0·85 (w/v) sodium chloride solution. The resultant spore suspension was then adjusted with sterile 0·85 (w/v) saline to contain approximately 10^4 spores/ml after counting in a haemocytometer.

Production of endocarditis

Left-sided sterile thrombotic endocarditis was established in New Zealand White rabbits weighing 2–3 kg. The rabbits were anaesthetized with a mixture of 0·5 ml/kg Hypnorm (Janssen Pharmaceuticals, Wantage, Oxford), 300 μg benzodiazepine (Roche Pharmaceuticals, Welwyn Garden City, Herts) and 120 μg atropine sulphate (Evans Pharmaceuticals, Liverpool). The right common carotid artery was exposed through a 2 cm skin incision, to the right of and parallel to the trachea. A 0·83 mm (external diameter) sterile polythene cannula (Portex, Hythe, Kent) was introduced into the exposed artery and passed inferiorly into the ventricle. The catheter was secured with silk sutures and the wound closed. Seventy-two hours after catheterization the cardiac vegetations were infected with a single 1 ml inoculation of approximately 10^4 *A. fumigatus* spores. The micro-organisms were introduced intravenously through a marginal ear vein. Treatment of the endocarditis with antifungal drugs began three days after inoculation.

Assessment of the endocarditis

The rabbits were killed by cervical dislocation at the end of the study and the heart removed complete with pericardium and catheter. The heart was sectioned in a superior to inferior direction through the left ventricle. The visible vegetations were harvested aseptically and put into 1 ml of Sabouraud's broth with a Mickle homogenizer (Mickle, Middlesex). The homogenate was then inoculated on to Sabouraud's agar and incubated at 30°C for 10 days. If any colonies of *A. fumigatus* were recovered the animal was regarded as infected.

Animal groups

A total of 75 infected animals was used in this study. Three groups of ten animals received daily doses of either 2·5, 3·5 or 5 mg/kg body weight intraconazole for 14 days. Three other groups of ten animals received either 3·0 mg/kg body weight amphotericin B, 35 mg/kg 5-fluorocytosine or a combination of both drugs daily for 14 days. One final group of ten animals was left untreated and five further animals received the vehicle (polyethylene glycol) used to dissolve the itraconazole. All agents were administered by intraperitoneal injection. After 14 days of therapy any remaining animals were killed.

In-vitro susceptibility tests

The susceptibility of *A. fumigatus* LL1 to itraconazole was measured by the method of Graybill, Kaster & Drutz (1983). The inhibitory concentration was the concentration of itraconazole required to achieve an inhibition of 50% of the growth of a drug-free control. Amphotericin B and 5-fluorocytosine singly and in combination were measured in a similar fashion.

Antifungal agents

Itraconazole was obtained from Janssen Pharmaceutica, Belgium. The drug was dissolved in polyethylene glycol at a concentration of 10 mg/ml. Amphotericin B deoxycholate (Fungizone, Squibb, Moreton, Merseyside) and 5-fluorocytosine (Alcobon, Roche, Welwyn Garden City, Herts) were both dissolved in sterile distilled water.

Results

In-vitro sensitivities

Growth of *A. fumigatus* LL1 *in vitro* was inhibited by itraconazole. The 50% inhibitory concentration was 2·6 mg/l. The concentration of amphotericin B required to achieve the same inhibition was 6·0 mg/l and for 5-fluorocytosine 16 mg/l. In combination 50% inhibition could be achieved by a mixture of 3·0 mg/l amphotericin B and 9 mg/l 5-fluorocytosine.

Animal experiments

Table I shows the results of treatment of aspergillus endocarditis. The survival time of the animals was recorded, counting day 1 as the day that antimycotic therapy commenced and not measuring beyond day 14.

The two control groups were 10 animals given no antifungal agent and five animals given polyethylene glycol only, and they showed similar survival times. Itraconazole at a concentration of 5·0 mg/kg sterilized the cardiac vegetations, and no aspergillus was harvested from the heart on day 14. Therapy with less than 5·0 mg/kg body weight of itraconazole did not sterilize the vegetations, and none of these animals survived the full 14 days duration of the experiment. Amphotericin B was also unsuccessful in eliminating the fungus from the cardiac lesions, although the mean survival time (seven days) of these rabbits was greater than that of the controls. 5-Fluorocytosine alone was successful for only one animal but proved to be 30% successful when used with amphotericin B.

Discussion

This investigation has used the rabbit model for aspergillus endocarditis (Carrizosa, Kohn & Levison, 1975) to investigate the efficacy of treatment with three antifungal agents. The assessment of endocarditis differed from that reported previously (Carrizosa *et al.*, 1975) in that no attempt was made to estimate the quantity of aspergillus in the vegetations. In an extensive series of initial investigations (M. V. Martin & L. P. Longman, unpublished work) the number of colony-forming units

Table I. Treatment of aspergillus endocarditis

Antifungal agent	Concentration	No.	Mean no. days survival[a] from start of therapy	No. of animals surviving to day 14[b]	No. with infected vegetations	% successful therapy
Itraconazole	2·5 mg/kg body weight	10	6·3 (5–8)	0	10	0
Itraconazole	3·5 mg/kg body weight	10	8·1 (6–9)	0	10	0
Itraconazole	5·0 mg/kg body weight	10	14	14	0	100
Amphotericin B	3·0 mg/kg body weight	10	7 (6–9)	0	10	0
5-Flurocytosine	35 mg	10	5·1 (4·0–7·0)	0	9	10
Amphotericin B + 5-fluorocytosine	3·0 mg/kg +35 mg	10	8·0 (7–14)	1	7	30
Untreated	—	10	4·5 (3–5)	0	9[c]	0
Polyethylene glycol	1 ml/kg body weight	5	4·8 (3–5)	0	5	0

[a]Range of survival days is shown in parentheses.
[b]Day 1 is the day antimycotic therapy commenced.
[c]One animal had no vegetations present.

recovered from infected vegetations was not proportional to thrombus weight. The presence or absence of aspergillus in the vegetations was a reliable and sensitive indicator of infection. Carrizosa *et al.* (1975) initiated antifungal therapy 6 h after infection. We preferred to investigate the treatment of an established infection as this is more analogous to the clinical situation.

The strain of *A. fumigatus* LL1 was found to reproducibly infect the cardiac thrombi. This strain was sensitive to amphotericin B, 5-fluorocytosine and to itraconazole by the criteria of Graybill *et al.* (1983). This latter assay, although only measuring 50% inhibition rates, is reproducible and avoids problems associated with azoles in the determination of minimum inhibitory concentration (Odds, 1985).

Amphotericin B at 3·0 mg/kg body weight prolonged the life of some animals but none survived the proposed 14 day duration of therapy. 5-Fluorocytosine alone at a daily dose of 35 mg/kg, the maximum that could be tolerated by the animals was not successful as the survival rates were the same as untreated controls. A combination of amphotericin B and 5-fluorocytosine did sterilize the cardiac vegetations of three rabbits but the remaining seven were infected. Previous workers have used shorter treatment periods with the combination of amphotericin B and 5-fluorocytosine and although survival for up to three days has been reported cardiac vegetations have been infected (Carrizosa *et al.*, 1975).

Itraconazole at a dose of 5 mg/kg body weight was successful in sterilizing cardiac vegetations and all animals survived the 14 days treatment. Treatment of the rabbits with concentrations of less than 5·0 mg/kg body weight was not successful and although survival time increased slightly at 3·5 mg/kg body weight all vegetations were infected.

We concluded that itraconazole at a dose of 5 mg/kg body weight is effective in sterilizing rabbit cardiac vegetations. Although this treatment does eradicate *A. fumigatus* infection, the vegetations remain. Such structures may form circulatory emboli and occlude end arteries leading to ischaemia of vital organs. The cardiac vegetations are always potential sites for reinfection should a bacteraemia or fungaemia occur, so surgery is still required for their removal (Seelig *et al.*, 1974).

Acknowledgements

The authors would like to acknowledge the expert technical assistance of Mrs H. M. Dunn, Miss P. McGowan and Mr R. Connell in these studies.

References

Carrizosa, J., Kohn, C. & Levison, M. E. (1975). Experimental aspergillus endocarditis in rabbits. *Journal of Laboratory and Clinical Medicine* **86**, 746–53.

Carrizosa, J., Levison, M. E., Lawrence, T. & Kaye, D. (1974). Cure of *Aspergillus ustus* endocarditis on a prosthetic valve. *Archives of Internal Medicine* **133**, 486–90.

Graybill, J. R., Kaster, S. R. & Drutz D. J. (1983). Treatment of experimental murine aspergillosis with BAY n7133. *Journal of Infectious Diseases* **148**, 898–906.

Kammer, R. B. & Utz, J. P. (1974). Aspergillus species endocarditis. The new face of a not so rare disease. *American Journal of Medicine* **56**, 506–21.

McLeod, R. & Remington, J. S. (1978). Fungal endocarditis. In *Infective Endocarditis* (Rahimtoola, S. H. Ed.), pp. 211–90. Grune & Stratton, New York.

Odds, F. C. (1985). Laboratory tests for the activity of imidazole and triazole antifungal agents, *in vitro*. *Seminars in Dermatology* **4**, 260–70.

Rubinstein, E., Noriega, E. R., Simberkoff, M. S., Holzman, R. & Rahal, J. J. (1975). Fungal endocarditis: analysis of 24 cases and review of the literature. *Medicine* **54**, 331–44.

Seelig, M. S., Speth, C. P., Kozinn, P. J., Taschdjian, C. L., Toni, E. F. & Goldberg, P. (1974). Patterns of Candida endocarditis following cardiac surgery: Importance of early diagnosis and therapy (an analysis of 91 cases). *Progress in Cardiovascular Diseases* **17**, 125–60.

Van Cutsem, J., Van Gerven, F., Zaman, R., Heeres, J. & Janssen, P. A. J. (1983). Pharmacological and preclinical results with a new oral and topical broad-spectrum antifungal R51,211. *Proceedings 13th International Congress of Chemotherapy*, (Spitzy, K. H. & Karrer, K., Eds), pt 40, pp. 32–9. Verlag H. Egermann, Vienna.

(*Manuscript accepted 24 June 1987*)