ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1991, p. 2046–2048
0066-4804/91/102046-03$02.00/0

Vol. 35, No. 10

Antifungal Activity of HWA-138 and Amphotericin B in Experimental Systemic Candidiasis

KISHOR M. WASAN,[1,2] KIUMARS VADIEI,[1,2] DAVID R. LUKE,[1,2,3] AFSANEH KEYHANI,[2] R. ALLEN WHITE,[4] TERESA J. MCQUEEN,[2] REETA MEHTA,[2] AND GABRIEL LOPEZ-BERESTEIN[1,2]*

Immunobiology and Drug Carriers Section[2] and Department of Biomathematics,[4] The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, and Department of Pharmaceutics, University of Houston, Texas Medical Center, 1441 Moursund Street,[1] Houston, Texas 77030, and Clinical Pharmacology Unit, Hoffmann-La Roche Inc., Newark, New Jersey 07112[3]

Received 3 May 1991/Accepted 18 July 1991

HWA-138, a pentoxifylline analog, has been shown to increase yeast urinary clearance and to reduce yeast counts in the kidneys of rats infected with *Candida albicans*. Furthermore, HWA-138 has also been shown to prevent amphotericin B-induced acute renal failure in rats. We report here on the effects of HWA-138 alone and in combination with amphotericin B in the treatment of systemic candidiasis in mice. When single doses of HWA-138 were administered intravenously (10, 25, or 50 mg/kg of body weight) into infected mice, no significant improvement in survival was observed. In infected mice treated intravenously with multiple doses of HWA-138 (10, 25, or 50 mg/kg once daily for 5 consecutive days), a significant increase in survival time was seen only in animals also receiving 25 mg of HWA-138 per kg (14 ± 3 days test versus 9 ± 1 days control; $P < 0.05$). The coadministration of subtherapeutic doses of amphotericin B and HWA-138 resulted in increased survival time. Combination therapy with amphotericin B (0.1-mg/kg single dose) and HWA-138 (10-, 25-, or 50-mg/kg multiple doses) resulted in a significant increase in survival time over controls (19 ± 4, 19 ± 5, and 21 ± 9 days, respectively, versus 9 ± 3 days; $P < 0.05$). Combination therapy with amphotericin B (0.2-mg/kg single dose) and HWA-138 (10-, 25-, or 50-mg/kg multiple doses) also resulted in a significant increase in survival time over controls (24 ± 6, 24 ± 6, and 24 ± 6, respectively, versus 9 ± 3 days; $P < 0.05$). Variance analysis of these findings indicate synergistic activity between amphotericin B and HWA-138 in the treatment of experimental candidiasis in mice.

Amphotericin B (AmpB) is the drug of choice for treatment of systemic fungal infections, but it causes adverse changes in renal hemodynamics and loss of tubular integrity (6). AmpB binds to membrane sterols, leading to increased permeability and the production of oxygen radicals that cause the lysis of sensitive cells (16, 20). The pathogenesis of AmpB nephrotoxicity is not well understood; however, recent studies suggest that renal vascular congestion may play a role (26). The AmpB-induced increases in neutrophil and erythrocyte congestion and platelet aggregation may lead to decreased perfusion of the hypoxic region (24).

The methylxanthines have been shown to induce hemodynamic changes in drug-associated nephropathies (10, 11). Theophylline, a methylxanthine derivative and an adenosine receptor antagonist, has been demonstrated to improve renal function in acute renal failure induced by cisplatin, AmpB, cyclosporine, radiocontrast agents, and glycerol (2, 3, 10, 11, 21). Theophylline diuretic and vasodilator effects may explain in part the improvement of acute renal failure (12).

Pentoxifylline (PTX), another methylxanthine, has been shown to be nephroprotective in AmpB-, cyclosporine-, glycerol-, and endotoxin-induced acute renal failure (5, 14, 25, 25a). Vascular decongestants like PTX have been shown to preserve ATP stores (most likely at the level of the adenosine receptor), increase erythrocyte deformability, and reduce platelet and neutrophil aggregation (4, 7, 9, 15, 15a, 19, 22). Furthermore, coadministration of each of PTX's intravenous (i.v.) analogs, HWA-138 and HWA-448, with AmpB resulted in increased renal clearance and decreased renal tissue concentrations of *Candida albicans* (15, 15a). This latter finding prompted us to explore the antifungal activity of HWA-138 alone and in combination with AmpB.

MATERIALS AND METHODS

Animals and drugs. ICR mice, 6 to 8 weeks old and weighing 21 to 25 g, were purchased from Harlan Breeders (Indianapolis, Ind.). AmpB with deoxycholate was purchased from E. R. Squibb (Princeton, N.J.), reconstituted with sterile water, and diluted to final concentrations of 0.1 and 0.2 mg/ml. HWA-138 was kindly supplied by William Novick (Hoescht-Roussel Pharmaceuticals, Inc., Somerville, N.J.); it was dissolved in physiologic saline and diluted to final concentrations of 10, 25, and 50 mg/ml.

Model for murine *C. albicans* infection. *C. albicans* 336, isolated from a patient with disseminated candidiasis, was used to infect mice. The inoculum was cultivated at 37°C for 18 h in Sabouraud dextrose agar and then prepared in saline. The animals were injected with 1×10^6 CFU via the tail vein. This concentration produces a disseminated seeding of *C. albicans*. All studies were conducted by injecting mice with the test drugs 48 h after inoculation, since at this time a well-established systemic infection is present (13).

Treatment studies. i.v. doses, either single or multiple (once daily for 5 consecutive days) of HWA-138 (10, 25, or 50 mg/kg of body weight) or an equivalent volume of saline (0.2 ml) were injected via the tail vein into one group of infected mice (10 mice per treatment). Another group of infected mice (also 10 per treatment) were administered single i.v. doses of AmpB, either 0.1 or 0.2 mg/kg, or an equivalent volume of sterile water (injection volume, 0.2 ml).

* Corresponding author.

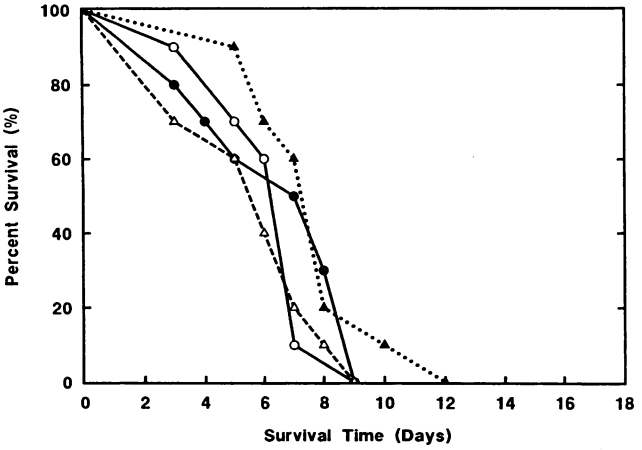

FIG. 1. Survival times of mice infected with *C. albicans* and administered a single i.v. dose of 10 (○), 25 (●), or 50 (▲) mg of HWA-138 per kg or with a 0.9% NaCl solution (△).

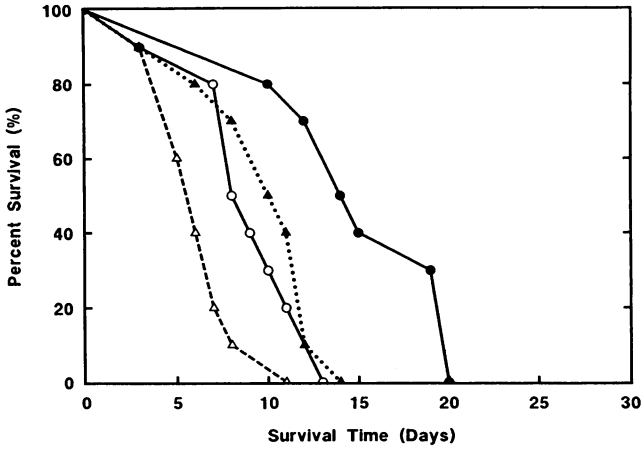

FIG. 2. Survival times of mice infected with *C. albicans* and treated daily for five days (i.v.) with 10 (○), 25 (●), or 50 (▲) mg of HWA-138 per kg or with a 0.9% NaCl solution (△).

The mice in the AmpB-receiving group were then further randomized to receive multiple i.v. doses of HWA-138 (10, 25, or 50 mg/kg) or an equivalent volume of physiologic saline once daily for 5 consecutive days.

Statistical analysis. Mean survival times for all groups were compared by two-way analysis of variance. Critical differences were determined by the post-hoc Newman-Keuls test (27). A difference was considered significant if the probability of chance explaining the results was reduced to less than 5% ($P < 0.05$). The term synergism has been clarified according to the statistical considerations used. The analysis of variance used takes into account two major aspects: (i) whether the increase in life expectancy is due to chance and (ii) whether the interaction of two drugs is statistically significant to prolong life. Synergy was taken to mean that the prolongation of life caused by the drug combination was longer than would be predicted by adding the effects of the individual drugs.

RESULTS

Single- and multiple-dose studies of HWA-138. All animals given single i.v. doses of saline died by day 9. There was no significant difference in mean survival time of mice administered single i.v. doses of HWA-138 (10, 25, or 50 mg/kg) compared with that of the saline-treated group (Fig. 1).

All mice treated with saline once daily for five consecutive days (Fig. 2) died by day 11. Mice treated on the same schedule with 10 mg/kg of HWA-138 died by day 13, those treated with 25 mg/kg died by day 20, and those treated with 50 mg/kg died by day 14. The mean survival time of mice administered multiple i.v. doses of HWA-138 at 25 mg/kg was significantly higher than that of the saline-treated group (15 ± 4 versus 9 ± 1 days; $P < 0.05$). There were, however, no significant differences in the mean survival times of mice administered HWA-138 at 10- and 50-mg/kg doses and those administered saline.

Combination of HWA-138 and AmpB. In the combination regimen, mice were injected with either a single dose of AmpB (0.1 or 0.2 mg/kg) or with a single dose of AmpB (0.1 or 0.2 mg/kg) plus five daily multiple doses of HWA-138 (10, 25, or 50 mg/kg) (Fig. 3). Combinations of AmpB at higher doses (0.3 to 0.8 mg/kg) and HWA-138 at lower or higher doses (1.0 to 100 mg/kg), although they led to significant increases in survival times, did not result in additive or synergistic responses (data not shown). Mice injected with a single dose of AmpB at 0.2 mg/kg were all dead by day 23, and those injected with a single dose of AmpB at 0.1 mg/kg were all dead by day 13. Animals injected with a single dose of AmpB at 0.1 mg/kg and multiple doses of HWA-138 at 10 mg/kg all died by day 24; when the HWA-138 dose was changed to 25 or 50 mg/kg, all died by day 30. Animals injected with a single dose of AmpB at 0.2 mg/kg and multiple doses of HWA-138 at 25 or 50 mg/kg all died by day 30; when the HWA-138 dose was changed to 10 mg/kg, all died by day 27. Single-dose administration of AmpB (0.2 mg/kg) significantly increased mean survival time compared with the saline group (19.4 ± 8.0 versus 9.3 ± 2.5 days; $P < 0.05$); single-dose administration of AmpB at 0.1 mg/kg, however, did not significantly increase mean survival time. Coadministration of AmpB at 0.1 mg/kg plus HWA-138 at 10,

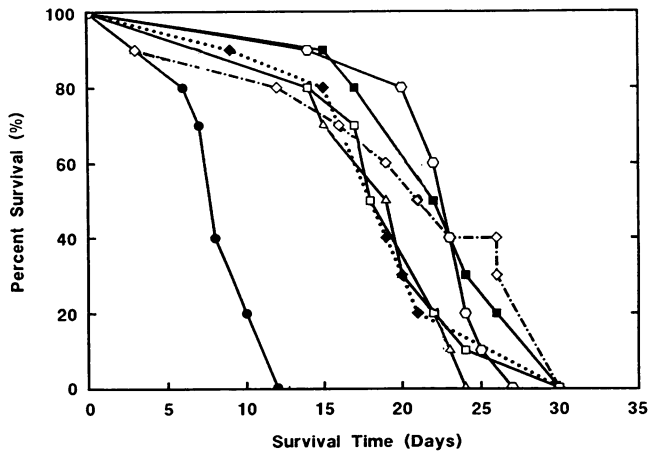

FIG. 3. Survival times of mice infected with *C. albicans* and receiving coadministration of a single dose of AmpB and multiple doses of HWA-138. The following groups were studied: 0.1 mg of AmpB per kg + 10 mg of HWA-138 per kg (△), 0.1 mg of AmpB per kg + 25 mg of HWA-138 per kg (◆), 0.1 mg of AmpB per kg + 50 mg of HWA-138 per kg (◇), 0.2 mg of AmpB per kg + 10 mg of HWA-138 per kg (○), 0.2 mg of AmpB per kg + 25 mg of HWA-138 per kg (■), 0.2 mg of AmpB per kg + 50 mg of HWA-138 per kg (□), and 0.9% NaCl solution (control) (●).

25, and 50 mg/kg significantly prolonged mean survival time compared with that of mice treated with saline only (19 ± 3.6, 18.8 ± 5.3, and 21 ± 8.9, respectively, versus 9.3 ± 2.5 days; $P < 0.05$), as did coadministration of AmpB at 0.2 mg/kg plus HWA-138 at 10, 25, and 50 mg/kg (24.4 ± 5.5, 24.1 ± 5.7, and 23.1 ± 7.4, respectively, versus 9.3 ± 2.5 days; $P < 0.05$). Analysis of variance showed the drug interaction to be significant (the level of F probability was <0.001).

DISCUSSION

HWA-138 alone prolonged the survival time of mice infected with *C. albicans*, and its coadministration with subtherapeutic doses of AmpB led to synergistic antifungal activity. The antifungal activity observed with HWA-138 may comprise two effects; reduction of renal congestion and dysfunction associated with systemic candidiasis and reduction of the nephrotoxic effects of AmpB in infected animals (15). Neither pentoxifylline nor HWA-138 were found to have antifungal activity in vitro (unpublished data).

The release of inflammatory cytokines secondary to inflammatory processes from infections may lead to tissue damage and impediments to local circulation (17, 18). It has been recently shown that PTX increases the whole-blood filtration rate of polymorphonuclear neutrophils, thus improving microvascular function (1). Furthermore, PTX inhibits the inflammatory action of interleukin-1 and tissue necrosis factor so that the increased adherence of neutrophils mediated by these cytokines is blocked (23). This suggests that certain methylxanthines, such as PTX and HWA-138, may prevent the accumulation of neutrophils and polymorphonuclear neutrophils in the vasculature, thus restoring normal blood flow to damaged tissue. However, another methylxanthine, HWA-448, when injected intravenously led to improved renal function in a rat infected with candida without effecting renal vascular decongestion (15a). This further implies that other methylxanthines, such as HWA-448, may have alternative pathways of activity. For example, HWA-448 may prevent the formation of ATP degradation products, thus reducing the production of superoxide anions that cause direct renal tubular damage (15a).

ACKNOWLEDGMENT

This work was supported in part by National Institutes of Health grant NIAID-NO1-72639.

REFERENCES

1. **Armstrong, M., D. Needham, D. L. Hatchell, and R. S. Nunn.** 1990. Effect of pentoxifylline on the flow of polymorphonuclear leukocytes through a model capillary. Angiology **41**:253–262.
2. **Bakris, G. L., and J. C. Burnett.** 1985. Theophylline attenuates radiocontrast induced intrarenal vasoconstriction. Kidney Int. **27**:227–230.
3. **Bidiani, A. K., P. C. Churchill, and W. Packer.** 1987. Theophylline-induced changes in myoglobinuric acute renal failure: further characterization. Can. J. Physiol. Pharmacol. **65**:42–49.
4. **Bilto, Y. Y., J. C. Ellory, M. Player, and J. Stuart.** 1988. Binding of oxpentoxifylline to the erythrocyte membrane and effects on cell ATP, cation content and membrane area. Clin. Hemorheol. **8**:901.
5. **Brunner, L. J., K. Vadiei, L. V. Iyer, and D. R. Luke.** 1989. Prevention of cyclosporine-induced nephrotoxicity with pentoxifylline. Renal Failure **11**:97–104.
6. **Burgess, J. L., and R. Birchall.** 1972. Nephrotoxicity of amphotericin B with emphasis on changes in tubular function. Am. J. Med. **53**:77–84.
7. **Crocket, K. L., J. M. Lackie, and A. A. Rogers.** 1988. Effect of pentoxifylline on neutrophil behaviour: stimulation of movement without adhesion changes. Biomed. Pharmacother. **42**:117–125.
8. **Dinarello, C. A.** 1984. Interleukin-1. Rev. Infect. Dis. **6**:51–95.
9. **Hammerschmidt, D. E., D. Kotasek, T. McCarthy, P. Huh, G. Freyburger, and G. M. Vercellotti.** 1988. Pentoxifylline inhibits granulocyte and platelet function, including granulocyte priming by platelet activating factor. J. Lab. Clin. Med. **112**:254–260.
10. **Heidemann, H. T., J. F. Gerillens, E. K. Jackson, and R. A. Branch.** 1983. Effect of aminophylline on renal vasoconstriction produced by amphotericin B in the rat. Arch. Pharmacol. **324**:148–153.
11. **Heidemann, H. T., S. Muller, L. Mertins, G. Stepan, K. Hoffmann, and E. E. Ohnhaus.** 1989. Effect of aminophylline on cisplatin nephrotoxicity in the rat. Br. J. Pharmacol. **97**:313–318.
12. **Hendeles, L., and M. Weinberger.** 1983. Theophylline: "a state of the art" review. Pharmacotherapy **3**:2–24.
13. **Lopez-Berestein, G., R. Mehta, R. L. Hopfer, K. Mills, L. Kasi, K. Mehta, V. Fainstein, M. Luna, E. M. Hersh, and R. Juliano.** 1983. Treatment and prophylaxis of disseminated infection due to *Candida albicans* in mice with liposome encapsulated amphotericin B. J. Infect. Dis. **147**:939–945.
14. **Luke, D. R., K. L. Berens, and R. R. Verani.** 1989. Role of vascular decongestants in ischemic acute renal failure defined by post insult administration of pentoxifylline. Renal Failure **11**:187–194.
15. **Luke, D. R., K. M. Wasan, T. J. McQueen, and G. Lopez-Berestein.** 1990. Enhancement of the treatment of experimental candidiasis with vascular decongestants. J. Infect. Dis. **162**:211–214.

15a. **Luke, D. R., K. M. Wasan, R. R. Verani, K. L. Berens, L. J. Brunner, K. Vadiei, and G. Lopez-Berestein.** 1991. Attenuation of amphotericin-B nephrotoxicity in the candidiasis rat model. Nephron **59**:139–144.

16. **Medoff, G., J. Brajtburg, G. S. Kobayashi, and J. Bolard.** 1983. Antifungal agents useful in therapy of systemic fungal infections. Annu. Rev. Pharmacol. Toxicol. **23**:303–330.
17. **Movat, H. Z., M. I. Cybulsky, I. G. Colditz, M. K. W. Chan, and C. A. Dinarello.** 1978. Acute inflammation in gram-negative infection: endotoxin, interleukin-1, tumor necrosis factor, and neutrophils. Fed. Proc. **46**:97–104.
18. **Myerowitz, R. I., G. T. Pazin, and C. M. Allen.** 1977. Disseminated candidiasis. Changes in incidence, underlying disease and pathology. Am. J. Clin. Pathol. **68**:29–34.
19. **Nishio, T., Y. Toshima, and Y. Matsuno.** 1982. Effects of pentoxifylline on cell shape, ATP content, and deformability in rabbit erythrocytes under hyperosmolar conditions. Int. J. Biochem. **14**:915–922.
20. **Norman, A. W., A. M. Spielvogel, and R. G. Wong.** 1976. Polyene antibiotic sterol interaction. Adv. Lipid Res. **14**:127–170.
21. **Rob, P. M., J. Fandrey, and W. Jelkmann.** 1989. Theophylline: a new concept of nephroprotection in acute cyclosporine A nephrotoxicity? Klin. Wochenschr. **67**:648–654.
22. **Sinzinger, H.** 1983. Pentoxifylline enhances formation of prostacyclin from rat vascular and renal tissue. Prostaglandins Leukotrienes Med. **12**:217–220.
23. **Sullivan, G. W., H. T. Carper, W. J. Novick, Jr., and G. L. Mandell.** 1988. Inhibition of the inflammatory action of interleukin-1 and tissue necrosis factor (alpha) on neutrophil function by pentoxifylline. Infect. Immun. **56**:1722–1730.
24. **Tolins, J. P., and L. Raij.** 1988. Adverse effect of amphotericin B administration on renal hemodynamics in the rat: neurochemical mechanisms and influence of calcium channel blockade. J. Pharmacol. Exp. Ther. **245**:594–599.
25. **Vadiei, K., L. J. Brunner, and D. R. Luke.** 1989. Effects of pentoxifylline in experimental acute renal failure. Kidney Int. **36**:466–470.

25a. **Vadiei, K., G. Lopez-Berestein, and D. R. Luke.** Unpublished data.

26. **Wasan, K. M., K. Vadiei, G. Lopez-Berestein, and D. R. Luke.** 1990. Pentoxifylline in an amphotericin B toxicity rat model. Antimicrob. Agents Chemother. **34**:241–244.
27. **Zar, J. H.** 1984. Two sample hypothesis, p. 122–150. *In* J. H. Zar (ed.), Biostatistical analysis. Prentice-Hall, Englewood Cliffs, N.J.