ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1985, p. 144–145
0066-4804/85/070144-02$02.00/0

Vol. 28, No. 1

# Effects of Elevation of Serum Cholesterol and Administration of Amphotericin B Complexed to Lipoproteins on Amphotericin B-Induced Toxicity in Rabbits

MALCOLM H. KOLDIN,[1] GEORGE S. KOBAYASHI,[1,2] JANINA BRAJTBURG,[1] AND GERALD MEDOFF[1]*

*Divisions of Infectious Diseases[1] and Laboratory Medicine,[2] Department of Medicine and Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri 63110*

Received 4 February 1985/Accepted 18 April 1985

**Amphotericin B was infused into normal rabbits or rabbits made hypercholesterolemic by diet. There was no difference in amphotericin B-induced toxicity between these two groups. Amphotericin B given in a mixture with human low-density lipoproteins was more toxic than when given without lipoproteins.**

Amphotericin B (AmB), a polyene antibiotic, is currently the preferred therapeutic agent in the treatment of most systemic fungal infections (2). The cytotoxicity of AmB is probably related to its binding to the sterols in the membranes of eucaryotic cells (6). Although AmB binds preferentially to ergosterol, the sterol in fungal cell membranes, it also binds to cholesterol, the sterol in mammalian cells (8, 9). Recent in vitro experiments done in our laboratory indicate that AmB also complexes with cholesterol in lipoproteins (3). The formation of these complexes has two consequences. (i) During brief incubations, the addition of lipoproteins protects erythrocytes against AmB-induced damage. (ii) In longer incubations, binding to cholesterol in lipoproteins protects AmB from inactivation and preserves its biological activity. These results suggest that levels of cholesterol in blood might also influence the cytotoxicity of AmB in vivo. In the work described here, we examined this possibility with an animal model. We studied the effects of elevation of serum cholesterol and administration of AmB complexed to lipoproteins on AmB-induced toxicity in rabbits.

AmB (Fungizone; E. R. Squibb & Sons, Princeton, N.J.) was given to three groups of uninfected New Zealand White rabbits (purchased from Eldridge Rabbitry, Barnhart, Mo.) weighing 2 to 3 kg each. In the first group, 10 rabbits were fed a diet of Rabbit Chow (Ralston Purina, St. Louis, Mo.) and received once each day, Monday through Friday for 2 weeks, 6.5 mg of AmB per kg in 50 ml of 5% glucose given over 4 h intravenously through a marginal ear vein. A maximum of 65 mg of AmB per kg was administered to each rabbit. A second group of 10 rabbits was made hypercholesterolemic by the procedure of Shore et al. (7). They were fed Rabbit Chow supplemented with 2% cholesterol for 2 weeks before the period of the AmB infusions. This supplementation was achieved by adding cholesterol (Sigma Chemical Co., St. Louis, Mo.) dissolved in diethyl ether to the chow and then removing the ether by evaporation. Total cholesterol (free and esterified) values in serum measured enzymatically (1) rose from the normal level of 43 ± 17 mg/100 ml to 923 ± 151 mg/100 ml just before the onset of AmB administration. The rabbits were then given AmB according to the regimen described previously. A third group of eight rabbits received the Rabbit Chow and the same regimen of the AmB, but AmB was complexed to low-density lipoproteins (LDL) before administration. The LDL, harvested by flotation (3) from serum obtained by plasmapheresis of a patient with Type IIa hyperlipidemia, was filtered through a 0.22-μm Nalgene filter and stored at 4°C in EDTA-saline. The total cholesterol concentration of the LDL preparation, measured by the same procedure as for cholesterol determination in serum, was 600 mg/100 ml. Before infusion into the rabbits, the daily dose of AmB was incubated with 8 ml of the LDL preparation for 30 min at room temperature. The AmB-LDL mixture was then made up to 50 ml with 5% glucose in water and infused into the rabbits.

Survival rates and elevations of creatine levels in serum served as measures of AmB-induced toxicity.

Of 10 animals in the group with normal serum cholesterol, 1 died 6 days after onset of AmB treatment, and 1 of 10 hypercholesterolemic animals treated with AmB in 5% glucose died 8 days after onset of AmB treatment. The results in these two groups were combined and compared with those in the third group. Four of the eight rabbits that received the AmB-LDL mixture died: one on day 3, one on day 4, and two on day 5 of AmB treatment. LDL without AmB given to a fourth group of five animals was without toxicity, and there were no deaths in this group. When the frequency of death in normal and hypercholesterolemic rabbits treated with AmB without LDL was compared with the frequency of death in normal rabbits treated with AmB-LDL by the Fisher exact test, the difference was statistically significant ($P < 0.02$).

Serum creatinine measurements (4) obtained before therapy, during therapy, and just after the last dose of AmB are shown in Table 1. Rabbits in all three groups had elevated creatinine levels in serum at the end of therapy. The creatinine levels in sera from normal and hypercholesterolemic rabbits were similar. The creatinine levels in serum of the four surviving rabbits in the AmB-LDL group were higher than those of the rabbits in the other two groups, but the differences were not statistically significant. However, the creatinine levels in serum in the rabbits that eventually died were much higher than those in surviving animals. In two rabbits treated with AmB-LDL, creatinine values in serum just before death were 4.8 and 6.8 mg/100 ml.

In summary, the incidence of fatal reactions and elevations of serum creatinine suggest that (i) hypercholesterolemia (the 20-fold increase in the level of cholesterol

* Corresponding author.

TABLE 1. Concentrations of creatinine in rabbit serum[a]

| Rabbit group and regimen | Creatinine levels in serum (mg/100 ml) | | | |
|---|---|---|---|---|
| | Pretherapy | AmB dose 2 | AmB dose 4 | Immediately posttherapy |
| Normal | | | | |
| AmB | 0.9 ± 0.1 | 2.2 ± 1.5 | 1.8 ± 0.3 | 2.6 ± 0.8 |
| AmB + LDL | 0.9 ± 0.1 ($P > 0.1$) | 2.9 ± 1.6 ($P > 0.1$) | 2.2 ± 1.2 | 3.5 ± 0.8 ($P > 0.1$) |
| Hypercholesterolemic (AmB) | 0.9 ± 0.1 ($P > 0.1$) | 1.2 ± 0.3 ($P > 0.1$) | 2.4 ± 1.2 | 2.4 ± 0.9 |

[a] Results are mean ± standard error from measurements done in duplicate on sera of all the surviving rabbits in each group. *P* values shown are determined by the Student *t* test comparing the creatinine values of the rabbits in the three groups on the same day of therapy.

circulating in blood) induced by diet does not influence the toxicity of AmB for rabbits, and (ii) AmB given with LDL is more toxic than AmB given alone. There are at least two possible explanations for the latter result. First, the LDL may have protected AmB from decomposing during administration; hence animals dosed with this mixture may have been given more active drug. This is unlikely because we found in separate experiments with *Saccharomyces cerevisiae* that the antifungal activities of the AmB-LDL mixture and AmB in 5% glucose at the conclusion of the infusions were equal. In addition, AmB levels in sera 1 and 24 h after infusion of 16 mg of AmB, determined by an agar diffusion assay with *Paecilomyces varioti* as the index organism (2), were similar in all three groups (1.5 to 3.0 μg/ml 1 h after the dose and 0.2 to 0.5 μg/ml 24 h after the dose). A second and more likely explanation is that the tissue distribution of AmB given with LDL was different from the distribution of AmB given alone, and more drug was delivered to critical host cells. Formation of aggregates comprised of AmB and LDL may have been responsible for this. Tissue distribution of AmB is known to be affected by the method of delivery. When AmB was given encapsulated in liposomes (5), its increased uptake into the reticuloendothelial system apparently decreased toxicity to the host. Utilization of LDL as a delivery system had the opposite effect, and toxicity was increased. Therefore, the in vitro protective effects of LDL against the toxicity of AmB for host cells which we have previously demonstrated were not reproduced in the in vivo rabbit model used in the present experiments.

We thank Richard Ostlund and Richard Levy for providing the LDL preparation and J. E. Bennett, National Institutes of Health, Bethesda, Md., for providing *Paecilomyces varioti* 5605.

This research was supported by Public Health Service grants AI 16228 and CA 15665 and training grants AI 07015 and AI 07172 from the National Institutes of Health.

## LITERATURE CITED

1. **Allain, C. C., L. S. Poon, C. S. G. Chan, W. Richmond, and P. C. Fu.** 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. **20**:470–475.
2. **Bindschadler, D. D., and J. E. Bennett.** 1969. A pharmacologic guide to the clinical use of amphotericin B. J. Infect. Dis. **120**:427–436.
3. **Brajtburg, J., S. Elberg, J. Bolard, G. S. Kobayashi, R. A. Levy, R. E. Ostlund, Jr., D. Schlessinger, and G. Medoff.** 1984. Interaction of plasma proteins and lipoproteins with amphotericin B. J. Infect. Dis. **149**:986–997.
4. **Jaffe, M.** 1886. Über den Niederschlag welchen Pikrinsäure in normalem Harn erzeugt und über eine neue Reaktion des Kreatinins. Z. Phys. Chem. **10**:391–400.
5. **Lopez-Berestein, G., R. Mehta, R. L. Hopfer, K. Mills, L. Kasi, K. Mehta, V. Fainstein, M. Luna, E. M. Hersch, and R. Juliano.** 1983. Treatment and prophylaxis of disseminated *Candida albicans* infection in mice with liposome encapsulated amphotericin B. J. Infect. Dis. **147**:939–945.
6. **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.
7. **Shore, V., B. Shore, and R. G. Hart.** 1974. Changes in apolipoproteins and properties of rabbit very low density lipoproteins on induction of cholesteremia. Biochemistry **13**: 1579–1584.
8. **Vertut-Croquin, A., J. Bolard, and C. M. Gary-Bobo.** 1984. Enhancement of amphotericin B selectivity by antibiotic incorporation into gel state vesicles. A circular dichroism and permeability study. Biochem. Biophys. Res. Commun. **125**: 360–366.
9. **Witzke, N. M., and R. Bittman.** 1984. Dissociation kinetics and equilibrium binding properties of polyene antibiotic complexes with phosphatidylcholine/sterol vesicles. Biochemistry **23**: 1668–1674.