TOXICITY OF AMPHOTERICIN B EMULSION TO CANINE KIDNEY CELLS IN MONOLAYER CULTURE

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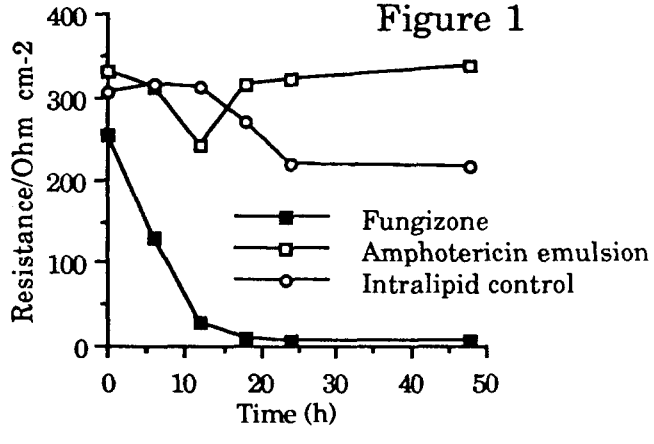
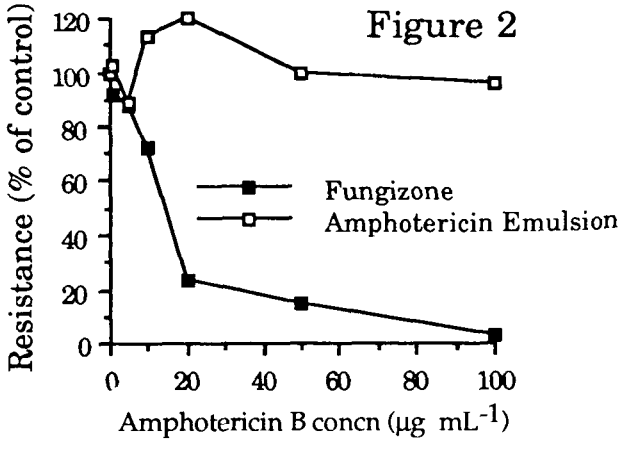
Amphotericin B is a powerful antifungal agent with a wide spectrum of activity. It is toxic to fungi since it forms a complex with ergosterol in the cell membrane, leading to the formation of pores, increased membrane permeability and cell lysis. Unfortunately it displays marked toxicity in vivo due to its similar ability to bind to cholesterol in mammalian membranes, the main site of damage being the kidney. Liposomal amphotericin B displays a considerably lower toxicity, but poses severe manufacturing problems, and so we have developed an emulsion formulation of amphotericin B as an alternative technology. This has been shown to have low toxicity and high efficacy in a murine model (Davis et al. 1987) and has undetectable toxicity to human erythrocytes at amphotericin concentrations up to 200 $\mu g\ ml^{-1}$.

We have now measured the toxicity of the formulation to canine kidney cells in monolayer culture for extended periods. The cell line (MDCK NBL-2) was established in a modified MEM medium and grown as a confluent monolayer on Millicell HA filters. The integrity of the monolayer was measured via its resistance. The amphotericin B emulsion was prepared as described previously (Davis et al. 1987). The cell monolayers were transferred to calcium and magnesium-free Hanks' balanced salt solution (HBSS) to avoid emulsion flocculation, concentrations of amphotericin B formulations up to 100 $\mu g\ mL^{-1}$ were added, and the resistance measured over a period of 48 hours. Control experiments were performed with an amphotericin-free emulsion (Intralipid 20%) and a commercial amphotericin formulation (Fungizone, Squibb). A typical plot of resistance vs. time is shown in Fig. 1 (amphotericin concentration 10 $\mu g\ mL^{-1}$). The loss of confluence on addition of Fungizone is evident within 6 hours, and is demonstrated by a severe drop in monolayer resistance. Only a small decrease is observed using either the Intralipid control or the amphotericin emulsion formulation, and we believe this to be due to minor changes in cell viability after changing to low-salt HBSS medium. The dose-response curve, calculated as a percentage of the control resistance after 6 hours, is shown in Figure 2. The low toxicity of the emulsion formulation is maintained up to an amphotericin concentration of 100 $\mu g\ mL^{-1}$.





The results clearly demonstrate the low toxicity to kidney cells of the amphotericin B emulsion formulation, paralleling previous results for red cell toxicity. We believe that this formulation has potential as a systemic antifungal agent.

Davis S.S. et al. (1987) Ann. N.Y. Acad. Sci. 507: 75-88