Short Communication

Polygodial-induced Sensitivity to Rifampicin and Actinomycin D of *Saccharomyces cerevisiae*

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Amphotericin B, which is used to treat various deep-seated mycoses, is known to interact with sterol-containing cell membranes of eukaryotic cells, ultimately leading to the leakage of cellular constituents with concomitant loss of cell viability.^{1,2)} Even at nontoxic concentrations, amphotericin B is capable of

altering the cell membranes of several fungal and animal cells enough to increase their permeability to antibiotics such as rifampicin and actinomycin D.^{3,4)} As a result, synergistic effects based on increased uptake of second antibiotics into cells on a variety of cellular functions have been demonstrated.^{3~6)}

In a previous paper,⁷⁾ we reported that the fungicidal action of the sesquiterpene dialdehyde, polygodial, was the result of the structural disruption of the cell membrane. Its activity *in vitro* was even stronger than that of the polyene antibiotic, amphotericin B; the minimum inhibitory concentrations (MICs) of polygodial and amphotericin B against *Saccharomyces cerevisiae* IFO 0203 were 0.78 and 1.56 $\mu\text{g/ml}$, respectively. Here, we wish to report the synergistic effects of polygodial with rifampicin or actinomycin D on the growth and RNA synthesis of *S. cerevisiae* cells.

When applied alone rifampicin showed no activity against yeast growth at concentrations up to 100 $\mu\text{g/ml}$ (Fig. 1A). Polygodial alone extended the lag period by several hours when applied in amounts below its MIC, but the growth measured in terms of turbidity and cell viability was restored to approximately the same level as that of the control within 48 hr.⁷⁾ However, when polygodial at 0.39 $\mu\text{g/ml}$ was

FIG. 1. Growth Inhibition by Rifampicin (A) and Actinomycin D (B) in Combination with Polygodial. *S. cerevisiae* was cultured with shaking at 25°C in 2.5% malt extract medium containing various concentrations of rifampicin, actinomycin D and polygodial. After 48 hr, growth was determined as the optical density at 660 nm. x, without polygodial; O, with 0.10 $\mu\text{g/ml}$ polygodial; ●, with 0.39 $\mu\text{g/ml}$ polygodial.

FIG. 2. Inhibition of RNA Synthesis by Rifampicin (A) and Actinomycin D (B) in Combination with Polygodial.

$[2\text{-}^{14}\text{C}]\text{Uracil}$ was added to a log-phase culture of *S. cerevisiae* in GYE medium.⁸⁾ After further incubation at 25°C for 10 min, rifampicin, actinomycin D and polygodial were added singly and in combination. At intervals of 10 min, 1-ml samples of the culture were treated with 1 ml of ice cold 10% (w/v) trichloroacetic acid. Radioactivity of the acid-insoluble fraction was counted as described previously.⁹⁾ $\times$, none; $\circ$, 0.39 $\mu\text{g/ml}$ polygodial; Δ , 100 $\mu\text{g/ml}$ rifampicin; $\bullet$, 100 $\mu\text{g/ml}$ rifampicin plus 0.39 $\mu\text{g/ml}$ polygodial; $\blacktriangle$, 12.5 $\mu\text{g/ml}$ actinomycin D; $\odot$, 12.5 $\mu\text{g/ml}$ actinomycin D plus 0.39 $\mu\text{g/ml}$ polygodial.

combined with rifampicin at 50 or 100 $\mu\text{g/ml}$, growth inhibition was observed.

Polygodial also increased the inhibitory effect of actinomycin D on yeast growth. Actinomycin D alone was observed to be slightly inhibitory toward yeast, with complete growth inhibition at 100 $\mu\text{g/ml}$ (Fig. 1B). When actinomycin D at 50 $\mu\text{g/ml}$ was combined with polygodial at 0.10 $\mu\text{g/ml}$, complete growth inhibition was observed. Complete inhibition was also seen at a lower concentration of actinomycin D (12.5 $\mu\text{g/ml}$) when a larger amount of polygodial (0.39 $\mu\text{g/ml}$) was used.

A similar synergistic effect was observed on RNA synthesis. The combination of polygodial at 0.39 $\mu\text{g/ml}$ with rifampicin at 100 $\mu\text{g/ml}$ (Fig. 2A) or actinomycin D at 12.5 $\mu\text{g/ml}$ (Fig. 2B) caused marked inhibition of $[^{14}\text{C}]\text{uracil}$ incorporation into the acid-insoluble fraction of growing cells of *S. cerevisiae*, whereas the drugs alone had little effect at similar concentrations. These results suggest that the synergistic effect of polygodial is due

FIG. 3. Effects of Polygodial and Amphotericin B on Liposomes Prepared from Extracted Yeast Lipids.

Cell suspensions of *S. cerevisiae* were heated for 3 min in boiling water and then sonicated twice for 4 min at 20 kc in an ice bath. Lipids were then extracted from the aqueous suspension by the procedure described by Bligh and Dyer.¹⁰⁾ Preparation of liposomes containing glucose and assaying of glucose release from the liposomes were carried out according to the methods of Kinsky *et al.*¹¹⁾ $\times$, none; $\bullet$, 10 $\mu\text{g/ml}$ polygodial; $\circ$, 10 $\mu\text{g/ml}$ amphotericin B.

to increased permeability of the cell membrane as to rifampicin and actinomycin D.

Polygodial seems to exhibit an action on the membrane permeability barrier similar to that of amphotericin B. However, the effects of these two compounds on liposomes prepared from lipids extracted from cells of *S. cerevisiae* were different. As shown in Fig. 3, polygodial at 10 $\mu\text{g/ml}$ had little effect on liposomal glucose release during incubation for over 2 hr. In contrast, amphotericin B was quite effective in inducing liposomal glucose release at a similar concentration (Fig. 3). Polygodial is known to react with compounds containing sulfhydryl groups¹²⁾ and methylamine¹³⁾ under biomimetic conditions, but is not known to interact readily with sterols.¹⁴⁾ Detailed studies on the site of action of polygodial at the molecular level are now in progress.

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