Short Communication

Inactivation of Phage ϕ X174 by Anthracycline Antibiotics, Aclacinomycin A, Doxorubicin and Daunorubicin

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Many anthracycline antibiotics have been found in *Streptomyces* cultures. Among them, doxorubicin (adriamycin) and daunorubicin (daunomycin) have been studied most in detail, because of their strong antitumor activities,¹⁾ but their clinical usefulness is limited by side effects. Aclacinomycin A, isolated from the culture of *Streptomyces galilaeus* MA144-M1, is becoming more interesting for its antitumor activity and low cardiac toxicity.^{2,3)}

It has been reported that daunorubicin inhibits the multiplication of double-stranded DNA phages, but not single-stranded DNA phages.¹⁾ Daunorubicin cannot, however, inactivate free T series phages,¹⁾ and the effect of doxorubicin or aclacinomycin A on the infectivities of phages is unknown.

In this communication, we report on the inactivation of phage ϕ X174 by daunorubicin, doxorubicin and aclacinomycin A. Daunorubicin and doxorubicin alone inactivated free phage ϕ X174 containing single-stranded DNA, but aclacinomycin A did not. Cu^{2+} was necessary for the ϕ X174 inactivation by aclacinomycin A.

Phage ϕ X174 was prepared as described earlier.⁴⁾ Aclacinomycin A is a product of Sanraku-Ocean Co. Daunorubicin, HCl and doxorubicin·HCl were supplied by Farmitalia, Milan. Daunorubicin·HCl from Meiji-Seika

Co., Tokyo and doxorubicin·HCl from Kyowa Hakko-Kogyo Co., Tokyo were also used. The drugs were dissolved at a rate of 10 mg/ml in redistilled water and stored at -20°C in the dark before use. The reaction of ϕ X174 with anthracyclines was carried out as follows; The reaction mixture contained 0.1 ml of anthracycline solution and 0.1 ml of ϕ X174 (1×10^9 plaque-forming units/ml) in 0.05 M Tris-HCl (pH 8.1) and 0.8 ml of 0.05 M Tris-HCl (pH 8.1). The reaction mixture was incubated at 37°C with gentle shaking. An aliquot (0.1 ml) was withdrawn and diluted 100-fold with ice-cold 0.05 M Tris-HCl (pH 8.1) to stop the reaction. The infectivity of phage was assayed by the agar overlayer method of Adams.⁵⁾

Daunorubicin and doxorubicin inactivated single-stranded DNA phage ϕ X174 (Fig. 1). Daunorubicin inactivated ϕ X174 more effectively than doxorubicin. Aclacinomycin A alone little affected the infectivity of ϕ X174. Transfection ability of ϕ X174 DNA was also inactivated by daunorubicin and by doxorubi-

FIG. 1. Antiphage Activity of Aclacinomycin A, Doxorubicin and Daunorubicin.

A, ϕ X174 (1×10^9 p.f.u.) was incubated with 0.35 mM aclacinomycin A (○), doxorubicin (●) or daunorubicin (△) at 37°C as described in the text. B, ϕ X174 (1×10^8 p.f.u.) was incubated with aclacinomycin A (○), doxorubicin (●) or daunorubicin (△) at the indicated concentrations for 180 min at 37°C .

TABLE I. EFFECTS OF SEVERAL AGENTS ON PHAGE INACTIVATION BY ANTHRACYCLINES

ϕ X174 (1×10^8 p.f.u.) was incubated for 180 min at 37°C with 0.35 mM anthracyclines with or without additions, and assayed for infectivity as described in the text. Survival ratio (%) is the ratio of the number of plaque-forming units at 180 min to that of drug-free control.

Additions	Survival ratio (%)		
	Dauno-rubicin	Doxo-rubicin	Aclacino-mycin A
None	13.5	50.6	93.6
0.2 mM Cu ²⁺	35.0	1.9	30.0
0.35 mM NADH	14.0	33.8	84.8
0.2 mM Cu ²⁺ , 0.35 mM NADH	6.3	0.17	6.4
10 mM EDTA	75.6	—	—
10 mM EDTA (added after reaction)	70.3	—	—

cin, but not by aclacinomycin A (unpublished results). The effects of several agents on the phage inactivation by anthracyclines are shown in Table I. Cu²⁺ stimulated the inactivation reaction by doxorubicin, but inhibited the reaction of daunorubicin. Aclacinomycin A was found to inactivate ϕ X174 in the presence of Cu²⁺. Neither Fe²⁺ nor Fe³⁺ could substitute for Cu²⁺. NADH did not affect the degree of ϕ X174 inactivation by anthracyclines, although it was reported that the reduction of anthracyclines, was necessary for *in vitro* DNA strand scission of phage PM2.^{6,7)} Other reducing agents such as NADPH, NaBH₄, Na₂S₂O₄ or thiol compounds also exhibited no effect. The ϕ X174 inactivation by anthracyclines was much enhanced in the presence of both Cu²⁺ and reducing agents, as shown in mitomycin C-induced ϕ X174 inactivation.⁸⁾

Antiphage activity of anthracyclines alone seems to be in accordance with the mutagenicity of anthracyclines; daunorubicin and doxorubicin were highly mutagenic, but aclacinomycin A was not.⁹⁾ It was suggested that amino moiety of anthracycline glycosides was essential for mutagenicity of anthracyclines.⁹⁾ Antiphage activity of anthracyclines may also be influenced by the glycoside moiety. The effect of Cu²⁺ on the antiphage activity is interesting, because the activity of doxorubicin and

aclacinomycin A is stimulated, while that of daunorubicin is inhibited. The results of transfection suggest that anthracyclines such as daunorubicin or doxorubicin can bind to ϕ X174 DNA in the virion. The reduction of quinone moieties of anthracyclines does not seem to affect the binding of anthracyclines to ϕ X174 DNA. The drug-DNA complex seems to be stable against dilution under our experimental conditions. The drugs are probably removed from DNA by EDTA because ϕ X174 inactivation was reversible by the addition of EDTA (Table I). The drug-DNA complex may penetrate into host cells, but inhibit the nucleic acid synthesis of phage. The drug bound to DNA may be reduced by a process of intracellular chemical or enzymatical reduction and cause DNA strand scission, as ϕ X174 inactivation was much enhanced in the presence of both reducing agents and Cu²⁺ (Table I). ϕ X174 DNA in the virion inactivated by anthracyclines in the presence of both reducing agents and Cu²⁺ was found to be nicked, although ϕ X174 DNA inactivated by anthracyclines alone was not (unpublished results). It was reported that strand scission of phage PM2 double-stranded DNA was enhanced in the presence of both reducing agents and Cu²⁺.⁷⁾

The detailed mechanism of ϕ X174 inactivation and the effect of anthracyclines on other phages are under investigation.

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