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CHARTREUSIN, AN ANTITUMOR GLYCOSIDE ANTIBIOTIC, INDUCES DNA STRAND SCISSION

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SUMMARY

The interaction of chartreusin with covalently closed circular PM2 phage DNA was studied. The antibiotic caused a single strand scission in the presence of reducing agents, such as dithiothreitol, ascorbic acid or $NaBH_4$. The degree of DNA breakage was dependent upon the drug concentration. The DNA-cleaving activity was enhanced by ferrous ion; but was completely blocked by catalase and partially by superoxide dismutase. The results suggest that reduction, chelate formation and auto-oxidation of the antibiotic, presumably the 5,12-dione moiety, produce free radicals, including $O_2^{\cdot}$ and $^{\cdot}OH$, which are capable of inducing DNA strand scission.

Chartreusin, produced by Streptomyces chartreusis, is a greenish-yellow crystalline antibiotic, showing antimicrobial activity against Gram-positive bacteria and Mycobacteria[1]. The structure of the antibiotic was elucidated[2,3] (Fig. 1). The drug was recently demonstrated to exhibit a significant antitumor activity against murine tumors: ascitic P388 and L1210 leukemia and B16 melanoma[4,5]. Biochemical investigations showed that the antibiotic binds to DNA and inhibits RNA and DNA polymerase reactions[16].

We have isolated chartreusin from culture broth of a strain of Streptomycetes, and found that the antibiotic is a potent DNA-cleaving agent. The results are presented in this communication.

MATERIALS AND METHODS

PM2 phage DNA was purchased from Boehringer Mannheim, Germany. Superoxide dismutase was a product of Miles Lab. (PTY), South Africa, and catalase was that of Sigma Chemical Co., St. Louis, Mo., U.S.A. All other chemicals were of the highest grade available commercially.

Chartreusin was prepared from culture broth of a strain of Streptomycetes by solvent extraction[1], and purified by silica gel chromatography. It gave a single spot on TLC (Rf 0.27), using a solvent system of benzene : acetone (1:2). The antibiotic was identified with chartreusin by ultraviolet, visible and infrared spectra, elementary analysis, and antibacterial spectrum.

The strand scission of PM2 phage DNA was detected by agarose gel electrophoresis, following the procedure described previously[6].

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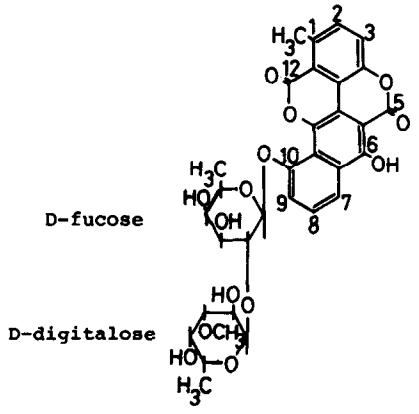

Fig. 1. Structure of chartreusin.

RESULTS

A single strand scission of PM2 phage DNA induced by chartreusin --- Dependency on antibiotic concentrations:

As illustrated in Fig. 2 lane 1, PM2 phage DNA showed three bands on agarose gel electrophoresis. According to Aaij and Borst[7], the fastest moving band corresponded to the native form of covalently closed circular (CCC)DNA, the most slowly moving one the open circular form, and the intermediate the linear form of the DNA. The electrophoretic analysis of the products of PM2 DNA and chartreusin, incubated in the presence of a reducing agent (1 mM dithiothreitol) revealed an antibiotic concentration-dependent decrease of the cccDNA and increase of open circular form DNA (Fig. 2). The results indicated that the drug produced a single strand scission of the cccDNA. The DNA cleavage was observed even at 7 μM of chartreusin, and more breaks at higher concentrations. The DNA strand scission was not detected at concentrations lower than 7 μM by the method employed (Data are not shown).

Requirement of a reducing agent and ferrous ion for chartreusin-produced DNA cleavage:

As shown in Fig. 3, chartreusin caused DNA strand scission in the presence of 1 mM dithiothreitol, but not significantly in the absence of reducing agents (lanes 2 and 3). The DNA cleavage was also observed with 0.1 mM ascorbic acid or 1 - 10 mM $NaBH_4$ (Data are not shown).

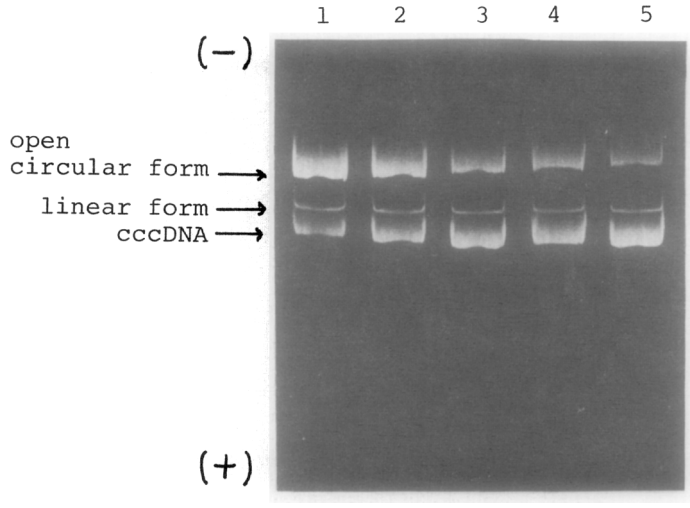

Fig. 2. Chartreusin concentration dependency of the PM2 DNA breakage in the presence of 1 mM dithiothreitol.

Chartreusin 56 μM (1), 28 μM (2), 14 μM (3), 7 μM (4), and 0 μM (5).

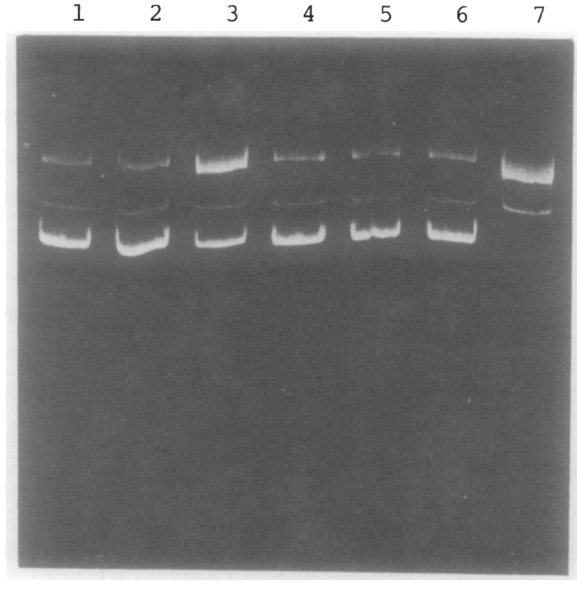

Fig. 3. Effects of a reducing agent and ferrous ion on chartreusin-induced DNA cleavage.

(1): Control, (2): + 28 μM chartreusin, (3): + 28 μM chartreusin and 1 mM DTT (dithiothreitol), (4): + 28 μM chartreusin, 1 mM DTT and 20 mM EDTA, (5): + 28 μM chartreusin and 5 μM $FeSO_4$, (6): + 5 μM $FeSO_4$ and 1 mM DTT, (7): + 28 μM chartreusin, 5 μM $FeSO_4$ and 1 mM DTT.

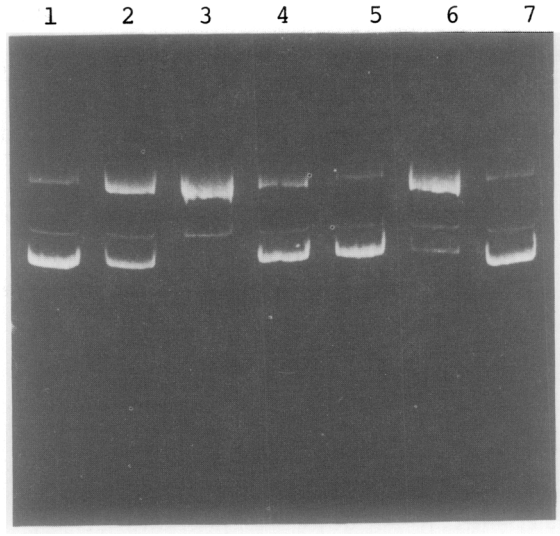

Fig. 4. The inhibition of chartreusin-produced DNA degradation by catalase and/or superoxide dismutase.

(1): Control, (2): + 28 μM chartreusin and 1 mM DTT, (3): + 28 μM chartreusin, 5 μM $FeSO_4$ and 1 mM DTT, (4): + 5 μM $FeSO_4$ and 1 mM DTT, (5): + 28 μM chartreusin, 5 μM $FeSO_4$, 1 mM DTT and 0.2 mg/ml catalase, (6): + 28 μM chartreusin, 5 μM $FeSO_4$, 1 mM DTT and 0.1 mg/ml superoxide dismutase, (7): + 28 μM chartreusin, 5 μM $FeSO_4$, 1 mM DTT, 0.2 mg/ml catalase and 0.1 mg/ml superoxide dismutase.

Addition of 20 mM EDTA to the reaction mixture abolished the DNA-cleaving activity of chartreusin, showing requirement of metal ion for the DNA strand scission (Fig. 3 lane 4). Of metal ions tested, ferrous ion (5 μM) was found to the most potent in stimulating the DNA breakage (Fig. 3 lane 7). The DNA cleavage was induced by ferrous ion itself at higher metal concentrations, but not at 5 μM with or without dithiothreitol (Fig. 3 lanes 5 and 6).

Effects of catalase and superoxide dismutase on chartreusin-induced DNA strand scission:

The DNA cleavage, caused by chartreusin in the presence of dithiothreitol and ferrous ion, was completely reversed by catalase and partially by superoxide dismutase (Fig. 4 lanes 5 - 7).

DISCUSSION

Several antitumor antibiotics have been reported to produce DNA breakage under appropriate conditions. They include bleomycin[8,15], tallysomycin[9], streptonigrin[10], anthracyclines[11,12], hedamycin[13],

neocarzinostatin[14], macromomycin[6], and auromomycin[6]. The current study shows that chartreusin is a new member of DNA-cleaving antitumor antibiotics.

The mechanism of DNA breakage by chartreusin is not certain. The requirement of a reducing agent and ferrous ion for chartreusin-induced DNA strand scission suggests that reduction, chelate formation and auto-oxidation of the drug, which result in the production of H_2O_2, $O_2^{\cdot-}$ and $^{\cdot}OH$, participate in the DNA cleavage, as in the case of streptonigrin and anthracyclines[9-12]. The inhibition of the DNA breakage by catalase and superoxide dismutase seems to support the assumption. Since reduction and auto-oxidation occur in the quinone moiety of streptonigrin and anthracyclines, the 5, 12-dione portion of chartreusin may be reduced and oxidized, forming a free radical, which sequentially produces $O_2^{\cdot-}$ and $^{\cdot}OH$. These free radicals are capable of inducing DNA strand scission. Since the production of $^{\cdot}OH$ from H_2O_2 is stimulated by Fe^{2+} (Haber-Weiss reaction), the greater effect of catalase than that of superoxide dismutase (Fig. 4) suggests that hydroxyl radical ($^{\cdot}OH$) plays a more important role in the DNA breakage than superoxide radical ($O_2^{\cdot-}$). A similar mechanism of DNA strand scission has been reported with bleomycin[15].

Li et al.[16] have observed that chartreusin preferentially inhibits RNA synthesis over DNA synthesis, and also causes damage to DNA in cultured L1210 cells. Although the mechanism of action of the antibiotic cannot be precisely pinpointed, its effects on the DNA structure may play a major role in determining its antitumor activity.

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REFERENCES

1. Leach, B.E., Calhoun, K.M., Johnson, L.E., Teeters, C.M., and Jackson,W.G. (1953) J. Am. Chem. Soc. 75, 4011-4012.
2. Simonitsch, E., Eisenhuth, W., Stamm, O.A., and Schmid, H. (1964) Helv. Chim. Acta 47, 1459-1475.
3. Eisenhuth, W., Stamm, O.A., and Schmid, H. (1964) Helv. Chim. Acta 47, 1475-1484.

4. McGovren, J.P., Neil, G.L., Crampton, S.L., Robinson, M.I., and Douros, J.D. (1977) Cancer Res. 37, 1666-1672.
5. Takai, M., Uehara, Y., and Beisler, J.A. (1980) J. Med. Chem. 23, 549-553.
6. Suzuki, H., Miura, K., and Tanaka, N. (1979) Biochem. Biophys. Res. Commun. 89, 1281-1286.
7. Aaij, C., and Borst, P. (1972) Biochim. Biophys. Acta 269, 192-200.
8. Suzuki, H., Nagai, K., Yamaki, H., Tanaka, N., and Umezawa, H. (1969) J. Antibiotics 22, 446-448.
9. Strong, J.E., and Crooke, S.T. (1978) Cancer Res. 38, 3322-3326.
10. Cone, T., Hasan, K., Lown, J.W., and Morgan, A.R. (1976) Canad. J. Biochem. 54, 219-223.
11. Lown, J.W., Sim, S.-K., Majumdar, K.C., and Chang, R.-Y. (1977) Biochem. Biophys. Res. Commun. 76, 705-710.
12. Someya, A., and Tanaka, N. (1979) J. Antibiotics 32, 839-845.
13. Mong, S., Strong, J.E., and Crooke, S.T. (1979) Biochem. Biophys. Res. Commun. 88, 237-243.
14. Beerman, T.A., and Goldberg, I.H. (1974) Biochem. Biophys. Res. Commun. 59, 1254-1261.
15. Takita, T., Muraoka, Y., Nakatani, T., Fujii, A., Iitaka, Y., and Umezawa, H. (1978) J. Antibiotics 31, 1073-1077.
16. Li, L.H., Clark, T.D., Murch, L.L., Wooden, J.M., Pschigoda, L.M., and Krueger, W.C. (1978) Cancer Res. 38, 3012-3018.