Psychotropic Drugs and Local Anesthetics Restore Cardiolipin-Inhibited Mammalian DNA Topoisomerase I Activity

Gulzar Hussain SHAH, Kazuhiro KATAOKA, Akiko UENO, Yoshihisa OHTSUKA,
Tohru MIZUSHIMA, and Kazuisha SEKIMIZU

Department of Microbiology, Faculty of Pharmaceutical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku,
Fukuoka 812-82, Japan. Received May 8, 1997; accepted August 19, 1997

The activity of mammalian topoisomerase I is inhibited by acidic phospholipids. We investigated the effect of psychotropic drugs and local anesthetics on cardiolipin-inhibited calf thymus DNA topoisomerase I activity. Chlorpromazine, promethazine, and imipramine, which interact with phospholipids, suppressed the inhibitory effect of cardiolipin. The present results suggest that relaxation of DNA supercoiling is involved in the cytotoxic action of these drugs.

Key words DNA topoisomerase I; acidic phospholipid; cardiolipin; chlorpromazine; cytotoxicity

Psychotropic drugs and local anesthetics interact with phospholipids and have a variety of effects on membrane-dependent biological processes. Studies have shown that these agents counteract the actions of phospholipids in some enzymes. We previously reported that chlorpromazine, a phenothiazine derivative used clinically as a tranquilizer, had potent cytotoxic effects on cancer cells. The cytotoxic action of chlorpromazine remains to be elucidated.

We also previously reported that the in vitro activity of DNA topoisomerase I from bacteria or mouse cells was sensitive to the acidic phospholipids cardiolipin and phosphatidylglycerol, suggesting that the enzyme activity in these cells is regulated by phospholipids. We also found that chlorpromazine-induced DNA relaxation in Escherichia coli cells was related to the function of the topA gene; chlorpromazine did not alter DNA supercoiling in the deletion mutant of the topA gene. These results suggest that chlorpromazine directly or indirectly stimulates the activity of E. coli DNA topoisomerase I, resulting in the relaxation of supercoiled DNA in cells.

In the present study, we examined the effects of chlorpromazine and other phospholipid-interacting agents on cardiolipin-inhibited calf thymus DNA topoisomerase I activity.

MATERIALS AND METHODS

Chemicals Chlorpromazine hydrochloride was generously provided by Shionogi Co. Impiramine hydrochloride (IPM) and promethazine hydrochloride (PMZ) were provided by Yoshitomi Pharmaceutical Industries, Ltd. Procaine hydrochloride was purchased from Sigma Chemical Co. Cardiolipin (bovine heart) was purchased from Lipid Products. Calf thymus DNA topoisomerase I was obtained from Gibco BRL.

Assay of DNA Topoisomerase I The relaxation of supercoiled plasmid DNA was investigated by agarose gel electrophoresis. The standard reaction mixture (40 µl) contained 50 mM Tris·HCl (pH 7.5), 50 mM KCl, 10 mM MgCl₂, 0.5 mM dithiothreitol, 0.1 mM EDTA, 1.2 µg bovine serum albumin, 0.3 µg pUC118 DNA, and 3 units calf thymus DNA topoisomerase I. The reaction was carried out at 37 °C for 15 min. Extraction of pUC118 DNA was performed by the phenol and chloroform method, followed by analysis using agarose gel electrophoresis in a buffer consisting of 100 mM Tris·acetate and 2 mM EDTA. Gels were stained with ethidium bromide and photographs were taken using a Polaroid camera.

RESULTS AND DISCUSSION

Inhibition of Calf Thymus DNA Topoisomerase I Activity by Cardiolipin Incubation of pUC118 DNA with calf thymus DNA topoisomerase I reduced the amount of negatively supercoiled DNA (Fig. 1). The addition of cardiolipin to the reaction mixture induced the appearance of negatively supercoiled DNA, indicating that cardiolipin inhibited the activity of the enzyme. The electrophoretic pattern of the substrate DNA (data not shown) was indistinguishable from that of the reaction products after incubation with 3 units DNA topoisomerase I and 100 µg/ml cardiolipin. The reaction induced by 3 units DNA topoisomerase I was almost completely inhibited by 50 µg/ml cardiolipin.

Effect of Chlorpromazine on Cardiolipin-Inhibited DNA Topoisomerase I Activity The addition of 50 µM chlorpromazine partially restored cardiolipin-inhibited DNA topoisomerase I activity (Fig. 2, lane 7). Supercoiled DNA

\[
\begin{align*}
\text{Form II} & \quad \text{Form I} \\
0 & \quad 0.4 \quad 2.0 \quad 10 \quad 50 \quad 100 \quad \mu g/ml \text{ CL} \\
\end{align*}
\]

Fig. 1. Cardiolipin Inhibition of Relaxation with Calf Thymus DNA Topoisomerase I Various amounts of cardiolipin (CL) were added to the standard reaction mixture without pUC118 and preincubated on ice for 10 min. After the addition of 0.3 µg pUC118 DNA, the preparation was incubated at 37 °C for 15 min. Positions of form I (negatively supercoiled molecules) and form II (relaxed molecules) are shown by arrows.

* To whom correspondence should be addressed.

1997 Pharmaceutical Society of Japan
Fig. 2. Effect of Chlorpromazine on Cardiolipin-Inhibited DNA Topoisomerase I Activity

Chlorpromazine (CPZ) was added to the standard reaction mixture with 3 units of DNA topoisomerase I and 50 µg/ml cardiolipin (CL). The preparation was preincubated on ice for 10 min and then with 0.3 µg pUC118 DNA.

Fig. 3. Effect of Phospholipid-Interacting Drugs on Cardiolipin-Inhibited DNA Topoisomerase I Activity

IPM or PMZ was added to the standard reaction mixture with 3 units of DNA topoisomerase I and 50 µg/ml cardiolipin (CL). The preparation was preincubated on ice for 10 min and then with 0.3 µg pUC118 DNA.

was completely eliminated by 200 µM chlorpromazine (lane 9), indicating that this concentration of chlorpromazine completely restored enzyme activity. Chlorpromazine itself did not affect the activity of DNA topoisomerase I (Fig. 2, lanes 11 to 16).

Effect of Other Phospholipid-Interacting Reagents on Cardiolipin-Inhibited Calf Thymus DNA Topoisomerase I Activity

IPM (Fig. 3, lanes 4 to 6) and PMZ (lanes 7 to 9) restored cardiolipin-inhibited DNA topoisomerase I activity, but did not affect the activity of DNA topoisomerase I in the absence of cardiolipin (data not shown). Procaïne (25 mM) and lidocaine (200 mM) did not influence cardiolipin-inhibited DNA topoisomerase I activity. Organic solvents, methanol (10% (v/v)) and dimethyl sulfate (10% (v/v)), did not restore cardiolipin-inhibited DNA topoisomerase I activity.

Cytotoxic Action of Psychotropic Drugs and Local Anesthetics

We previously reported that the activity of DNA topoisomerase I from Escherichia coli cells was inhibited by cardiolipin, and that chlorpromazine restored this activity.7) We also showed that chlorpromazine induced relaxation of plasmid DNA in E. coli. In this report, we showed that chlorpromazine also restored the activity of mammalian DNA topoisomerase I inhibited by cardiolipin. The drug may alter the physical structure of cardiolipin liposomes and abolish the inhibitory action. We propose that the activity of DNA topoisomerase I, either in bacteria7) or in eukaryotic cells,8) is inhibited by cardiolipin or by other acidic phospholipids in biological membranes, and that phospholipid-interacting reagents such as chlorpromazine restore this enzyme activity. We believe that the cytotoxic action of phospholipid-interacting reagents9) may be caused by stimulation of DNA topoisomerase I activity resulting in relaxation of chromosomal DNA.

Acknowledgement This work was funded by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES