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# INHIBITION OF DNA TOPOISOMERASES I AND II BY G3 PAMAM-$NH_2$ DENDRIMER-MODIFIED DIGOXIN AND PROSCILLARIDIN A CONJUGATES IN A CELL FREE SYSTEM

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**Abstract:** Two modified glycosides – digoxin and proscillaridin A conjugated to a generation 3 of polyamidoamine dendrimer (G3 PAMAM-$NH_2$) were evaluated as DNA topoisomerase II inhibitors. The ability of these compounds (PAMAM-Dig and PAMAM-Prosc) to inhibit topoisomerase I and II activity was quantified by measuring the action on supercoiled DNA substrate as a function of increasing concentration of the test compounds by the use of agarose gel electrophoresis. The obtained results suggest that a conjugation of the modified glycosides with G3 PAMAM-$NH_2$ significantly improved the ability of the parent compounds to an inhibition of DNA topoisomerases.

**Keywords**: PAMAM-$NH_2$ dendrimer, cardiac glycosides, digoxin, proscillaridin A, DNA topoisomerases

One specific way to overcome the side effects of cancer chemotherapy and to achieve improved therapeutic effects in the treatment of cancer is to develop drug delivery systems that enhance tumor cytotoxicity and cellular entry (1). Dendrimers, due to their controllable size and monodispersity, can act as excellent carriers for a wide range of molecules, which can be encapsulated in the interior of the dendrimer or interact with the dendrimer's terminal groups (2). Because dendrimers are synthesized from branched monomer units in a stepwise manner, it is possible to conduct a precise control on molecule size, shape, dimension, density, polarity, and solubility by choosing different branching units and surface functional groups (3).

Cardiac glycosides are a class of natural products that are used to increase cardiac contractile force in patients with congestive heart failure and cardiac arrhythmias (4). Epidemiological studies showed that digitalis has also anti-cancer effects (5–8). Over the last 10 years, interest in developing cardiotonic steroids as anti-cancer agents has grown progressively (5–8). The potential use of cardiac glycosides for the treatment of cancer was abandoned because of the inherent cardiotoxicity of these compounds and a narrow therapeutic index. The studies on the structure–activity relationship revealed that a lactone in position 17β is crucial for the cardiotoxicity of cardiac steroids (9, 10). Therefore, we synthesized two compounds Dig and Prosc (Figure 1), derivatives of these glycosides containing the carboxylic group instead of the lactone moiety (11). Dig and Prosc, the carboxylic acid containing drugs, were conjugated to G3 PAMAM dendrimers (with 32 primary amino groups on surface) *via* amide linkage (Figure 2) (12). It was shown that the cytotoxic and antiproliferative effects of PAMAM dendrimer conjugates were significantly higher than free drugs in breast cancer cells (12). In the present study, the ability of PAMAM-Dig and PAMAM-Prosc to inhibit DNA topoisomerases was examined.

## EXPERIMENTAL

### Reagents and chemicals

An amine-terminated G3 PAMAM dendrimer, digoxin, proscillaridin A, calf thymus DNA, ethidium bromide were provided by Sigma-Aldrich. Topoisomerase I and II, supercoiled pHOT1 DNA, supercoiled pRYG DNA, etoposide, camptothecin were purchased from TopoGEN.

### Relaxation assay of topoisomerase I

Supercoiled pHOT1 DNA (0.5 mg) was incubated with 4 units of human topoisomerase I in

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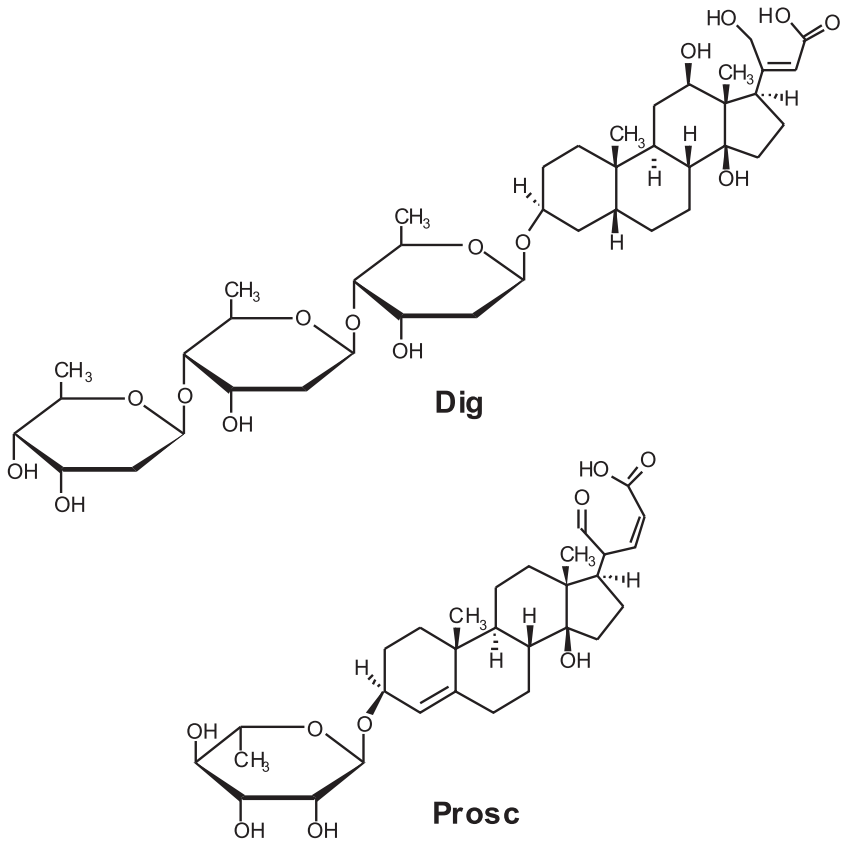


Figure 1. Chemical structures of the modified digoxin (Dig) and the modified proscillaridin A (Prosc).

relaxation buffer (10 mM Tris-HCl (pH 7.8), 1 mM EDTA, 0.15 M NaCl, 0.1% BSA, 0.1 spermidine, 5% glycerol), in the presence of varying concentrations of the test compounds. Reactions were carried out at 37°C for 1 h and then terminated by the addition of sodium dodecyl sulfate (SDS) to the final concentration of 0.25% and proteinase K to the final concentration of 250 mg/mL. The reaction mixture was subjected to electrophoresis through a 0.8% agarose gel containing 0.5 mg/mL of ethidium bromide in TAE buffer (40 mM Tris-borate and 1 mM EDTA). The gels were stained with ethidium bromide and photographed under UV light.

**Relaxation assay of topoisomerase II**

Supercoiled pRYG DNA (0.5 mg) was incubated with 4 units of human topoisomerase II in the cleavage buffer (30 mM Tris-HCl (pH 7.8), 50 mM KCl, 10 mM $MgCl_2$, 3 mM ATP, 15 mM mercaptoethanol), in the presence of varying concentrations of the test compounds. Reactions were carried out at 37°C for 1 h and then terminated by the addition of 2 mL of 10% sodium dodecyl sulfate (SDS) and 2 mL of 50 µg/mL proteinase K. The reaction mixture was subjected to electrophoresis through a 0.8% agarose gel containing 0.5 mg/mL ethidium bromide in TBE buffer (90 mM Tris-borate and 2 mM EDTA). The gels were stained with ethidium bromide and photographed under UV light.

## RESULTS AND DISCUSSION

G3 PAMAM dendrimer is stable, nonimmunogenic, and contains 32 primary amines on the surface. It is advantageous to utilize low generation PAMAM dendrimers for drug delivery because low generations (generation 4.0 or below) appear to have relatively low or negligible toxicity and immunogenicity as well as favorable biodistribution (2). In PAMAM-Dig and PAMAM-Prosc, only half of the

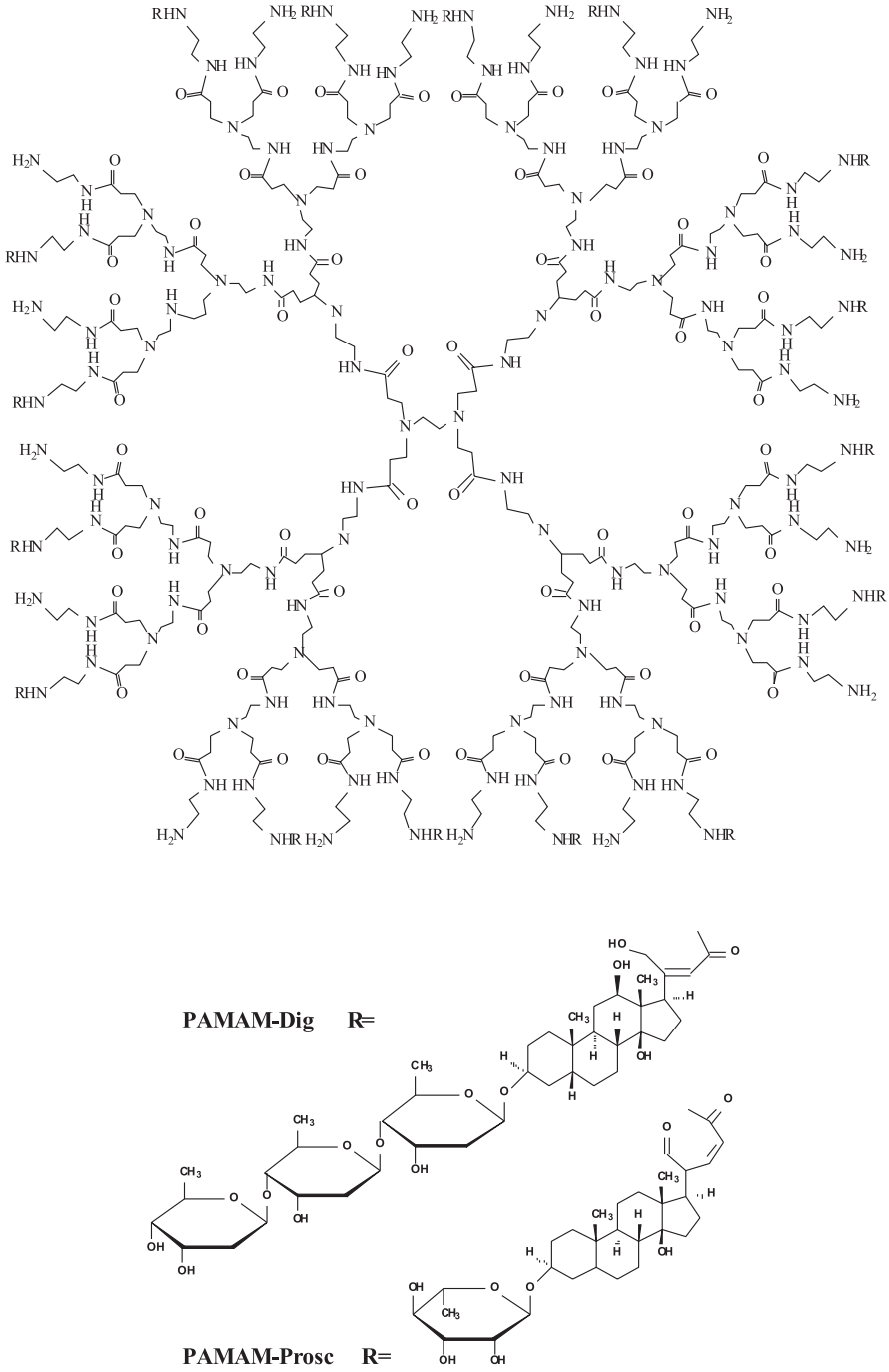


Figure 2. Chemical structures of PAMAM-Dig and PAMAM-Prosc.

amine terminal groups (16 of 32) was covalently bound to the modified digoxin or proscillaridin A (Figure 2). Our previous experimental studies have demonstrated that these compounds prevented the growth and decreased the number of viable cells in both estrogen-dependent MCF-7 and estrogen-independent MDA-MB-231 breast cancer cells (12). DNA processing enzymes are important targets for anticancer drugs. A large number of agents bind to duplex DNA in a covalent or non-covalent fashion,

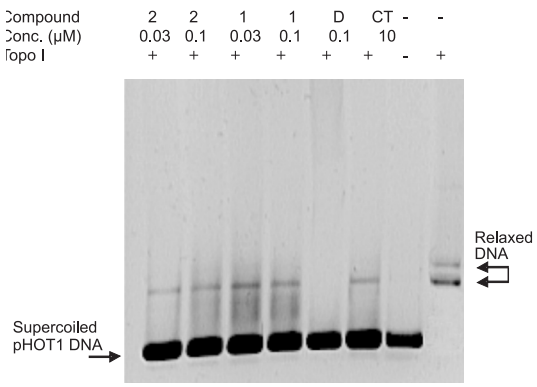


Figure 3. Inhibition of topoisomerase I-mediated DNA supercoiling in the presence of PAMAM-Dig (**1**), PAMAM-Prosc (**2**), G3 PAMAM-$NH_2$ (D) and camptothecin (CT). Supercoiled pHOT1 DNA (lane 7) was incubated with topoisomerase I in the absence (lane 8) or in the presence of drug at the indicated concentration. The DNA was analyzed by 0.8% agarose gel electrophoresis. The gels were stained with ethidium bromide and photographed under UV light.

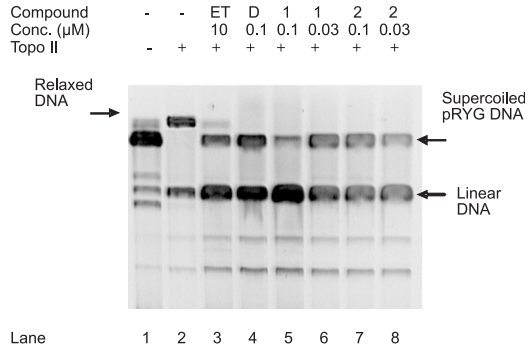


Figure 4. Inhibition of topoisomerase II-mediated DNA supercoiling in the presence of PAMAM-Dig (**1**), PAMAM-Prosc (**2**), G3 PAMAM-$NH_2$ (D) and etoposide (ET). Supercoiled pRYG DNA (lane 1) was incubated with topoisomerase II in the absence (lane 2) or in the presence of drug at the indicated concentration. The DNA was analyzed by 0.8% agarose gel electrophoresis. The gels were stained with ethidium bromide and photographed under UV light.

thereby interfering with its role as a template in replication and transcription. To test whether cytotoxic properties of PAMAM-Dig and PAMAM-Prosc were related to DNA topoisomerases action, these compounds were evaluated in a cell-free system. Topoisomerase I/II was incubated with increasing concentrations of PAMAM-Dig and PAMAM-Prosc in the presence of supercoiled plasmid DNA, and the products were subjected to electrophoresis in the presence of ethidium bromide. It can be seen unambiguously that PAMAM-Dig and PAMAM-Prosc at concentrations of 30 nM strongly promote DNA cleavage by human topoisomerase I (Figure 3). To examine whether the synthesized compounds stimulate the stabilization of the cleavable complex, the supercoiled RYG DNA was incubated with 4 units of topoisomerase II in the presence of PAMAM-Dig and PAMAM-Prosc and etoposide as a control compound (Figure 4). It was shown that while Dig inhibited only topoisomerase II at concentration 100 nM (11), PAMAM-Dig inhibited both topoisomerase II at 30 nM concentration and additionally it inhibited topoisomerase I at the same 30 nM concentration (Figure 3 and 4). Prosc was a potent poison of topoisomerase I and II at 100 nM and 30 nM, respectively (11), whereas PAMAM-Prosc inhibited topoisomerase I and II at concentration of 30 nM (Figure 3 and 4).

The obtained results suggest that a conjugation of the modified glycosides Dig and Prosc with G3 PAMAM-$NH_2$ significantly improved the ability of the parent compounds to an inhibition of DNA topoisomerases. High levels of expression of topoisomerases were observed in a number of tumor cells including breast, lung, and pancreatic cancers (13–15). A number of studies have demonstrated that the overexpression of topoisomerases associated with transformation and metastatic progression of neoplastic cells and the expression level exhibits a correlation with the degree of malignant transformation (15). Several widely used anticancer agents, including camptothecins, doxorubicin and other anthracyclines, amsacrine, etoposide and mitoxantrone target topoisomerases are thought to be cytotoxic by virtue of their ability to stabilize a topoisomerase-DNA intermediate (16). Therefore, drugs able to interact with both DNA topoisomerase types may show selectivity for cancer cells. Further biological evaluation of PAMAM-Dig and PAMAM-Prosc is underway.

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