Xanthocidin Derivatives as Topoisomerase IIα Enzymatic Inhibitors

Shuso Takeda,a,b Kentaro Yaji,b Kenji Matsumoto,c Toshiaki Amamoto,d Mitsuru Shindo,c and Hironori Aramakib,a

a Department of Molecular Biology, Daiichi University of Pharmacy; 22–1 Tamagawa-cho, Minami-ku, Fukuoka 815–8511, Japan; b Interdisciplinary Graduate School of Engineering Sciences, Kyushu University; c Institute for Materials Chemistry and Engineering, Kyushu University; d NEUES Corporation; Yaesu Center Building 3F, 1–6–6 Yaesu, Chuo-ku, Tokyo 103–0028, Japan. Received September 27, 2013; accepted November 23, 2013

Few studies have examined xanthocidin, a biotic isolated from Streptomyces xanthocidicus in 1966, because its supply is limited. Based on its chemical structure, xanthocidin has the potential to become a lead compound in the production of agrochemicals and anti-cancer drugs; however, it is unstable under both basic and acidic conditions. We recently established the total synthesis of xanthocidin using the FeCl3-mediated Nazarov reaction, and obtained two stable derivatives (#1 and #2). The results of the present study demonstrated that these derivatives exhibited the inhibitory activity of topoisomerase IIα, known as a molecular target for cancer chemotherapy, and this was attributed to the respective exo-methylene ketone group without DNA intercalation. The results obtained also suggest that these derivatives may have value as lead compounds in the synthesis of topoisomerase IIα inhibitors.

Key words xanthocidin; Streptomyces xanthocidicus; topoisomerase IIα; exo-methylene ketone group

Xanthocidin, isolated from Streptomyces xanthocidicus by Asahi et al. in 1966, was shown to have antibiotic activities against Escherichia coli, Bacillus agri, and Xanthomonas oryzae.1 Xanthocidin has a highly oxidized five-membered ring with contiguous cis-vinical diol (–OH), carboxylic acid (–COOH), and conjugated exo-methylene group substituents (Fig. 1, upper panel).2 Xanthocidin may be promising as a lead compound for the production of agrochemicals and anti-cancer drugs; however, it was shown to be unstable under both basic and acidic conditions.2 In addition, few studies have assessed its bioactivity due to its limited supply.3 We successfully established the total synthesis of xanthocidin using the FeCl3-mediated Nazarov reaction,3 and obtained two stable xanthocidin derivatives (#1 and #2) (Fig. 1, upper panel) that exhibited anti-proliferative effects on highly aggressive human breast cancer MDA-MB-231 cells.3 Although the biological activities of these derivatives imply their possible use as anti-proliferative agents for cancer cells, their action point(s) have not yet been resolved.

Catalytic inhibitors of topoisomerase IIα (Topo IIα), without DNA intercalating potential, were previously shown to be useful in targeting tumors that expressed markedly higher levels of Topo IIα (i.e., MDA-MB-231 cells) than those of normal cells. Using biochemical analyses, we and other researchers demonstrated that the catalytic activity of Topo IIα was sensitive to “Michael acceptors” due to the existence of nucleophilic thiol (–SH) residues in the active center.4–6) Because xanthocidin derivatives (#1 and #2) contain an exo-methylene ketone group (a possible reactive electrophilic moiety, see Fig. 1), whether they behaved as an inhibitor of Topo IIα through this group was investigated in the present study. We also analyzed their DNA intercalation potential. The results obtained indicated that the xanthocidin derivatives have inhibitory activity of Topo IIα as catalytic inhibitors of the enzyme, and that they did not have DNA intercalating potential.

MATERIALS AND METHODS

Reagents Xanthocidin derivatives (#1 and #2) were synthesized according to our previously established methods.2 These synthesized compounds were purified by HPLC or column chromatography, and their purity (>98%) was confirmed by 1H- and 13C-NMR spectroscopy. No ring-opened derivatives of the xanthocidins’ lactones were detected in our analyses.4,5) Amscarine (m-AMSA) and etoposide (VP-16) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Ellipticine was purchased from ChromaDex Inc. (Irvine, CA, U.S.A.). All other reagents were of the highest grade commercially available.

Topo IIα-Mediated DNA Relaxation and Cleavage Assays The human Topo IIα enzyme was obtained from Topogen (Columbus, OH, U.S.A.) and negatively supercoiled pHOT1 DNA was used as a substrate for Topo IIα (Topogen). The enzyme reaction and analysis of DNA relaxation/DNA cleavage were performed using the Topo II Drug Screening Kit according to the manufacturer’s protocol (Topogen). In brief, to detect topoisomerase that were relaxed DNA forms, reaction mixtures were subjected to 1% agarose gels cast in the absence of 0.5 µg/mL ethidium bromide (EtBr) (non-EtBr gel) and electrophoresed in the absence of EtBr. When linear DNA was analyzed (Cleavage assay), 1% agarose gels were cast and run in the presence of 0.5 µg/mL EtBr (EtBr gel). These gels were run at 50V for 60 min and either stained with EtBr (non-EtBr gel) or destained with water (EtBr gel).5)

DNA Intercalation Study An EtBr displacement fluorescence assay was employed to determine whether xanthocidin derivatives (#1 and #2) bind in the minor groove of DNA.5,7) Experiments using DNA-intercalating Topo II inhibitors (m-AMSA, a positive control) were performed.

Data Analysis IC50 values were determined using Sig-
RESULTS AND DISCUSSION

We recently reported that xanthocidin derivatives (#1 and #2), used in the present study at 100µM (Fig. 1, upper panel), significantly abrogated the proliferation of MDA-MB-231 breast cancer cells. In addition, a concentration of 100µM etoposide, a typical Topo IIα inhibitor known as Topo IIα poison, was required to significantly inhibit Topo IIα. Thus, we used the xanthocidin derivatives in combination with established Topo IIα inhibitors including etoposide at 100µM. Topo IIα has been shown to catalyze DNA relaxation after its transient introduction into DNA double-strand breaks (i.e., linear DNA). After incubation with the two xanthocidin derivatives in the presence of etoposide/m-AMSA, the inhibitory potentials following incubation with the xanthocidins, etoposide, m-AMSA, or ellipticine in the presence of EtBr. The appearance of LIN DNA was only detected with etoposide and m-AMSA and ellipticine, not with the xanthocidins Topo IIα and Topo IIα, respectively (Fig. S2), lanes 5 and 6 vs. 7–9 and see also the enlarged image placed below). Although etoposide exhibited its concentration-dependent production of LIN species (100µM, 500µM) (Fig. 2C, lanes 12 and 13), no LIN species were detected with xanthocidin derivative (#1) at concentrations up to 500µM (Fig. 2C, lanes 3–8 and see also the enlarged image placed below) (not shown for #2). In the absence of the Topo IIα enzyme, LIN was not detected either in the presence of etoposide/m-AMSA or xanthocidins/ellipticine (data not shown), which suggested that LIN DNA was produced as a result of the interaction between Topo IIα and etoposide or m-AMSA, and that the two xanthocidins may have an inhibition mode that differs from that of etoposide/m-AMSA (Topo IIα poisons) and ellipticine.

Certain chemicals that alter the gross structure of DNA were shown to markedly affect the catalytic activity of Topo IIα, while some –SH interacting agents had the ability to bind directly to DNA. Therefore, whether the two xanthocidins (#1 and #2) inhibition of Topo IIα may occur through direct interactions with DNA was examined. The ability of xanthocidins at 100µM to displace EtBr from the minor groove of DNA was determined by an established fluorescence emission assay, as DNA-bound EtBr has been shown to exhibit markedly stronger fluorescence emission than that of free EtBr. Although the DNA intercalator, m-AMSA (mA) at 100µM, was capable of displacing EtBr, neither xanthocidin (#1) nor xanthocidin (#2) significantly displaced EtBr (<1%) (Fig. 2D).

Taken together, these findings suggest that xanthocidins (#1 and #2) may be a catalytic inhibitor of Topo IIα, which is not mediated by an interaction with DNA. Compounds containing an exo-methylene ketone group/exo-methylene lactone group are widely considered to be toxic to humans and animals, and this has been attributed to these substances reacting non-selectively with cellular macromolecules; however, recent evidence strongly suggested that, for example, (−)-xanthatin, a component of Xanthium strumarium (Cocklebur) exhibited little or no toxicity to animals, with an LD₅₀ value of ca. 800mg/kg. Furthermore, parthenolide, a molecule containing an exo-methylene lactone group, which was identified as...
the major active component in Feverfew (Tanacetum parthenium), exhibited selective toxicity to human leukemic stem cells, but not to normal cells; however, its anti-proliferative mechanism(s) have not yet been fully resolved. Since Topo IIα is known to be highly expressed in rapidly proliferating human cancer cells, xanthocidins (#1 and #2) may cause selective toxicity to cancer cells by inhibiting Topo IIα. Further studies are needed to establish the biological effects of the xanthocidins in vivo.

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