DNA Repair 43 (2016) 18–23

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

SCR7 is neither a selective nor a potent inhibitor of human DNA ligase IV

George E. Greco[a], Yoshihiro Matsumoto[b], Rhys C. Brooks[b], Zhengfei Lu[c], Michael R. Lieber[c], Alan E. Tomkinson[b,*]

[a] Department of Chemistry, Goucher College, Baltimore, MD, United States
[b] Departments of Internal Medicine and Molecular Genetics and Microbiology, and University of New Mexico Cancer Center, University of New Mexico, Albuquerque, NM 87131, United States
[c] Departments of Pathology, Biochemistry and Molecular Biology, Biological Sciences, and Molecular Microbiology and Immunology, University of Southern California Keck School of Medicine, Norris Comprehensive Cancer Center, Los Angeles, CA, United States

ARTICLE INFO

Article history:
Received 22 March 2016
Accepted 6 April 2016
Available online 7 May 2016

Keywords:
Human DNA ligases
DNA ligase inhibitors
DNA double strand break repair
Non-homologous end-joining

ABSTRACT

DNA ligases are attractive therapeutics because of their involvement in completing the repair of almost all types of DNA damage. A series of DNA ligase inhibitors with differing selectivity for the three human DNA ligases were identified using a structure-based approach with one of these inhibitors being used to inhibit abnormal DNA ligase IIIα-dependent repair of DNA double-strand breaks (DSB)s in breast cancer, neuroblastoma and leukemia cell lines. Raghavan and colleagues reported the characterization of a derivative of one of the previously identified DNA ligase inhibitors, which they called SCR7 (designated SCR7-R in our experiments using SCR7). SCR7 appeared to show increased selectivity for DNA ligase IV, inhibit the repair of DSBs by the DNA ligase IV-dependent non-homologous end-joining (NHEJ) pathway, reduce tumor growth, and increase the efficacy of DSB-inducing therapeutic modalities in mouse xenografts. In attempting to synthesize SCR7, we encountered problems with the synthesis procedures and discovered discrepancies in its reported structure. We determined the structure of a sample of SCR7 and a related compound, SCR7-G, that is the major product generated by the published synthesis procedure for SCR7. We also found that SCR7-G has the same structure as the compound (SCR7-X) available from a commercial vendor (XcessBio). The various SCR7 preparations had similar activity in DNA ligation assay assays, exhibiting greater activity against DNA ligases I and III than DNA ligase IV. Furthermore, SCR7-R failed to inhibit DNA ligase IV-dependent V(D)J recombination in a cell-based assay. Based on our results, we conclude that SCR7 and the SCR7 derivatives are neither selective nor potent inhibitors of DNA ligase IV.

1. Introduction

DNA ligation is required during DNA replication and to complete almost all DNA repair events. In human cells, the DNA ligases encoded by three *LIG* genes are responsible for joining interruptions in the phosphodiester backbone [1]. These enzymes have distinct but overlapping functions in cellular DNA metabolism. Interestingly, DNA ligase expression levels are frequently dysregulated in cancer. For example, the steady state levels of DNA ligase I (LigI) are usually elevated in cancer cell lines and tumor specimens [2,3]. This is presumed to reflect the increased proliferation that is a characteristic of cancer cells. In addition, a significant fraction of cancer cell lines have elevated levels of DNA ligase IIIα (LigIIIα) and reduced levels of DNA ligase IV (LigIV) [2]. Notably, this reciprocal change in DNA ligase levels has been shown to result in abnormal repair of DNA double-strand breaks in leukemia, breast cancer and neuroblastoma, with increased levels of LigIIIα correlating with reduced survival [4–6].

Given their dysregulation in cancer and almost ubiquitous involvement in DNA transactions, DNA ligases are potential therapeutic targets for the development of novel anti-cancer agents. There have been several attempts to identify DNA ligase inhibitors by screening of synthetic chemical and natural product libraries that have met with limited success. These have mainly involved radioactive-based assays and the screening of a relatively small number of compounds [7–9]. A series of small molecule inhibitors

* Corresponding author at: Cancer Research Facility, 915 Camino de Salud,1 University of New Mexico, Albuquerque, NM 87131, United States.
E-mail address: atomkinson@salud.unm.edu (A.E. Tomkinson).

http://dx.doi.org/10.1016/j.dnarep.2016.04.004
1568-7864/

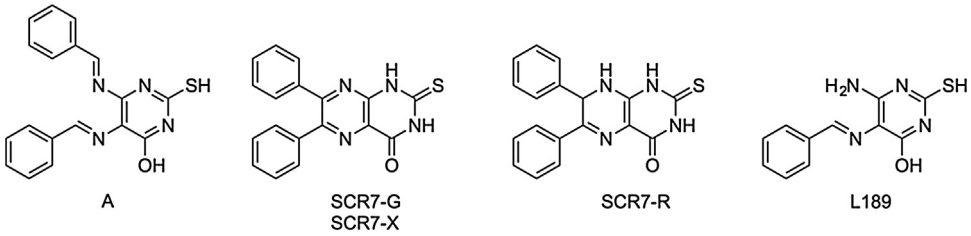

Fig. 1. Structures of SCR7 derivatives. In (A) is shown the structure of SCR7 as reported by Srivastava et al. [11]. The structure of the major product generated by the synthesis protocol described by Srivastava et al. [11] and by a different synthesis method [20] (SCR7-G) and the compound sold as SCR7 by XcessBio (SCR7-X) are shown. Our structure determination of the SCR7 provided by Dr. Sathees Raghavan (SCR7-R) and the pan human DNA ligase inhibitor (L189) described by Chen et al. [2] are also shown.

with differing specificities for the three human DNA ligases were identified by a structure-based approach using the atomic resolution structure of the DNA binding domain of human DNA ligase I complexed with nicked DNA [2,10]. As expected, several of these inhibitors were cytotoxic and, at subtoxic concentrations, they potentiated cell killing by DNA damaging agents [2]. Unexpectedly, this enhancement of cytotoxicity occurred in malignant cells, but not their non-neoplastic counterparts [2]. In further studies, a LigI/III inhibitor L67 was found to synergistically increase the cytotoxicity of a PARP inhibitor by inhibiting LigIIIα in therapy-resistant chronic myeloid leukemia and breast cancer cells lines with abnormal DNA repair characterized by elevated levels of LigIIIα and PARP-1 [5,6].

Using molecular modeling to predict the structure of the DNA ligase IV DNA binding domain with L189, the inhibitor of all three human DNA ligases identified in the previous structure-based approach [2], Raghavan and colleagues reported the identification of a derivative of L189, which they called SCR7 [11]. SCR7 appeared to selectively inhibit the repair of DSBs by the non-homologous end-joining (NHEJ) pathway in a DNA ligase IV-dependent manner as well as to both reduce tumor growth and increase the efficacy of DSB-inducing therapeutic modalities [11]. In attempting to synthesize SCR7 by the published procedure [11], we encountered problems with the synthesis procedures and discovered discrepancies in the reported structure of SCR7. Using three different preparations of SCR7, we found that it is a DNA ligase inhibitor with greater activity against DNA ligases I and III than DNA ligase IV and that it fails to inhibit DNA ligase IV-dependent V(D)J recombination in a cell-based assay.

2. Materials and methods

2.1. Purification of human DNA ligases

Human LigI and LigIIIβ were purified after expression in *Escherichia coli* as described [12,13]. Human LigIIIα/XRCC1 and LigIV/XRCC4 complexes were purified from insect cells infected with a single baculovirus expressing both subunits of the DNA ligase complex as described [12,14].

2.2. Preparation and purification of SCR7-G

A solution of benzaldehyde (466 mg, 4.4 mmol) in DMF (1.5 mL) and acetic acid (0.5 mL) was added to solid 4,5-diamino-6-hydroxy-2-mercaptopyrimidine (316 mg, 2.0 mmol). The reaction mixture was heated under reflux for 3 h, then cooled to room temperature, and added slowly to 10 mL of ice water. A yellow solid precipitated out which was collected by vacuum filtration and air dried. The solid was dissolved in 60 mL of chloroform, filtering the chloroform solution to remove insoluble material. The entire chloroform solution was loaded onto a silica gel column packed in dichloromethane. The column was eluted with 2:1 dichloromethane: ethyl acetate, and the first yellow band was collected. The solvent was removed to yield 257 mg (0.77 mmol, 39%). ^{1}H NMR (DMSO) δ 13.42 (br. s,) 12.82 (br. s, 1H), 7.43-7.34 (m, 10H). ^{13}C NMR (DMSO) δ 175.7, 158.5, 155.9, 149.1, 147.0, 137.7, 137.1,129.8 (C-H)129.7 (C-H), 129.5 (C-H), 128.8 (C-H), 128.3 (C-H), 128.2 (C-H), 127.2. HRMS Calcd. for $C_{18}H_{13}N_4OS$ ($M+H^+$) 333.0810. Found: 333.0802. IR (Nujol): 3407 (br), 1698, 1633, 1552, 1126, 722, 695, 665. M.P. 200–203 (d.).

2.3. Preparation and purification of SCR7-R

A solution of benzaldehyde (466 mg, 4.4 mmol) in DMF (2 mL) was added to solid 4,5-diamino-6-hydroxy-2-mercaptopyrimidine (316 mg, 2.0 mmol). The reaction mixture was heated under reflux under nitrogen and in the dark for 3 h, then cooled to room temperature and diluted with 3 mL of ethanol. The reaction mixture was added dropwise with stirring to 40 mL of diethyl ether to produce a yellow precipitate, which was collected. The solid was re-suspended in 20 mL of ethanol with heating and stirring to dissolve any oily residue that co-precipitated with the product. The ethanol-insoluble material was collected by vacuum filtration and dried under vacuum to yield pure product. Additional pure product crystallized out of the mother liquor over the course of 3 days, yielding a total of 150 mg (0.45 mmol, 22%) of free-flowing light yellow powder. ^{1}H NMR (DMSO)

δ 12.07 (br. s, 1H), 12.00 (s, 1H), 7.83 (dd, J = 7.6, 1.4 Hz, 2H), 7.52 (d, J = 3.3 Hz, 1H, N-H), 7.39-7.26 (m, 8H), 6.02 (d, J = 3.3 Hz, 1H, C-H). 13C NMR (DMSO)d 173.1, 157.6, 146.7, 143.2, 140.1, 136.1, 129.7 (C-H), 129.2 (C-H), 128.63 (C-H), 128.56 (C-H), 127.0 (C-H), 126.4 (C-H), 104.3, 52.3 (C-H). HRMS Calcd. for C18H15N4OS (M+H+) 335.0967. Found: 333.0959. IR (Nujol): 3381 (br), 1652, 1565, 1085, 693, 665, 612. M.P. 273–280 (d.).

2.4. DNA ligase assays

DNA nick ligation was measured using a fluorescence-based ligation assay described previously [15]. Briefly, 100 fmol of purified LigI, LigIIIα/XRCC1, or LigIV/XRCC4, SCR7, were incubated in the presence or absence of an SCR7 derivative with fluorescent nicked DNA annealed to an upstream quencher (200 fmol) in ligation buffer (60 mM Tris–HCl [pH 7.4], 10 mM $MgCl_2$, 5 mM DTT, 1 mM ATP, 50 μg/ml BSA, 4% DMSO, and 50 mM NaCl for LigI or 150 mM NaCl for LigIII and LigIV) at 25 °C in a total volume of 20 μL. Following incubation, reactions were further diluted to 200 μL with a 30-fold molar excess of the unlabeled competing oligonucleotide in annealing buffer (10 mM Tris–HCl [pH 7.4], 50 mM KCl, 1 mM EDTA and 5 mM $MgCl_2$) and heated to 95 °C for 5 min. After cooling to 4 °C at a rate of 2 °C/min, fluorescence read at 519 nm (excitation at 495 nm) was measured immediately using the Synergy H4 microplate reader (BioTek). Relative ligation efficiency was calculated as a percentage of the amount of product formed by each uninhibited ligase. This corresponds to 106 fmol (53% ligation), 104 fmol (52% ligation), and 9.4 fmol (4.7% ligation) of product formed for LigI, LigIII, and LigIV respectively. Additionally, the