Silylation of Deoxynucleotide Analog Yields an Orally Available Drug with Antileukemia Effects

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ABSTRACT

DNA methyltransferase inhibitors have improved the prognosis of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). However, because these agents are easily degraded by cytidine deaminase (CDA), they must be administered intravenously or subcutaneously. Recently, two orally bioavailable DNA methyltransferase inhibitors, CC-486 and ASTX727, were approved. In previous work, we developed 5-O-trialkylsilylated decitabines that resist degradation by CDA. However, the effects of silylation of a deoxynucleotide analog and enzymatic cleavage of silylation have not been fully elucidated. Enteric administration of OR21 in a cynomolgus monkey model led to high plasma concentrations and hypomethylation, and in a mouse model, oral administration of enteric-coated OR21 led to high plasma concentrations. The drug became biologically active after release of decitabine (DAC) from OR21 following removal of the 5′-O-trisilylate substituent. Toxicities were tolerable and lower than those of DAC. Transcriptome and methylome analysis of MDS and AML cell lines revealed that OR21 increased expression of genes associated with tumor suppression, cell differentiation, and immune system processes by altering regional promoter methylation, indicating that these pathways play pivotal roles in the action of hypomethylating agents. OR21 induced cell differentiation via upregulation of the late cell differentiation drivers *CEBPE* and *GATA-1*. Thus, silylation of a deoxynucleotide analog can confer oral bioavailability without new toxicities. Both *in vivo* and *in vitro*, OR21 exerted antileukemia effects, and had a better safety profile than DAC. Together, our findings indicate that OR21 is a promising candidate drug for phase I study as an alternative to azacitidine or decitabine.

Introduction

Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) are characterized by impaired differentiation of hematopoietic stem cells (HSCs) or hematopoietic progenitor cells (1, 2). Somatic mutations and aberrant DNA hypermethylation that silence cancer-regulating genes (e.g., tumor-suppressor genes) in these cells (3) are involved in pathogenesis and progression of MDS and AML (4–8). Epigenetic alterations lead to reduced hypermethylation, which can in turn cause reexpression of silenced tumor-regulating genes. These alterations represent novel therapeutic targets for both of these cancers (9).

Although the clinically available DNA methyltransferase (DNMT) inhibitors azacitidine (AZA) and decitabine (DAC) have improved the prognosis of MDS and AML (10, 11), they are easily degraded by cytidine deaminase (CDA), limiting their bioavailability after oral administration; consequently, they must be administered intravenously or subcutaneously. The efficacy and safety of oral AZA or DAC, as well as the combination of these drugs with CDA inhibitors, has been evaluated in clinical trials (12–14), resulting in the approval of CC-486 (oral AZA) and ASTX727 (DAC plus cedazuridine, a CDA inhibitor; refs. 13, 14).

Resistance to CDA is crucial for achieving elevated plasma concentrations of these drugs, which would in turn increase efficacy (15). Silylation of glycoproteins is known to influence their stabilities and therapeutic effects (16, 17); however, the effects of silylation of deoxynucleotide analogs and enzymatic cleavage of silylation have not been fully elucidated. In previous work, we developed 5-O-trialkylsilylated DACs that were resistant to CDA and exerted anticancer effects against solid tumors and adult T-cell leukemia/lymphoma (18, 19). In this study, we assessed the oral bioavailability and efficacy of 5-O-trialkylsilylated DAC for treatment of MDS and AML both *in vitro* and *in vivo*.

Materials and Methods

Reagents

5-O-trialkylsilylated DAC (OR2100, 2003, 2007, 2008, 2009, 2010, 2102, 2103, 2104, and 2201) was obtained from Ohara Pharmaceutical. AZA and DAC were purchased from Sigma-Aldrich. All reagents were dissolved in DMSO and stored at −20°C.

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Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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