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Chemotherapeutic bone-targeted bisphosphonate prodrugs with hydrolytic mode of activation

Rotem Erez,[a] Sharon Ebner,[b] Bernard Attali[b] and Doron Shabat[a,*]

[a]*Department of Organic Chemistry, School of Chemistry, Raymond and Beverly Sackler Faculty of Exact Sciences, Tel-Aviv University, Tel Aviv 69978, Israel*
[b]*Department of Physiology and Pharmacology, Sackler Faculty of Medical Sciences, Tel-Aviv University, Tel Aviv 69978, Israel*

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Abstract—Osseous tissues are considered to be limited as therapeutic target sites due to their biological properties. We have designed and synthesized two kinds of hydrolytically activated chemotherapeutic prodrugs containing bisphosphonate, a bone-targeting moiety. The first can be conjugated to drug molecules with an available hydroxy group; the drug is attached to the bisphosphonate component through an ester-labile linkage. The second is for use with drug molecules with amine functional group. In this case, a self-immolative linker is used to attach the drug to the bisphosphonate component through a carbonate-labile linkage. The concept was demonstrated using the drugs camptothecin, which has a hydroxy functional group, and tryptophan, which is a model molecule for a drug with amine functionality. Both prodrugs showed significant binding capability to hydroxyapatite, the major component of bone, and were hydrolytically activated under physiological conditions.

Chemotherapeutic prodrugs with capability to target certain tissues or cell types may enhance the potency or eliminate the side effects of drugs.[1–4] Although many prodrug approaches have been demonstrated, osseous tissues are considered to be limited as target sites due to their biological properties. In contrast to other tissues, the blood flow rate in bones is very low, because they mainly consist of an inorganic compound $Ca_{10}(PO_4)_6(OH)_2$, termed hydroxyapatite (HAP). Recently a promising drug delivery system with a bisphosphonate (BP) moiety for targeting osseous tissues was proposed.[5] Bisphosphonates have high affinity for HAP and calcified tissues are the main targets for accumulation after administration of bisphosphonates into the body.[6,7] Several examples of anticancer drugs linked to a bisphosphonate moiety have been reported in the scientific literature.[8] Most of them failed to show any improved anticancer effect, however, when tested in vivo in mice.[8,9] In these compounds, the chemotherapeutic drug was linked to the bisphosphonate through a stable chemical linkage that did not allow release of the active drug. Interestingly, when anti-inflammatory drugs were conjugated to a bisphosphonate moiety through an ester linkage, promising results were observed in animal-model experiments.[10–12] Therefore, we assume that a bisphosphonate moiety conjugated to a chemotherapeutic agent through a hydrolyzable linker will target the drug conjugate to the osseous tissue, where the active parent drug will be slowly released through the hydrolysis of the linkage. Here we report the design, synthesis, and in vitro evaluation of two new prodrugs, based on known chemotherapeutic drugs, attached to bisphosphonate moiety through a hydrolyzable linkage. The linkage was spontaneously hydrolyzed at a physiological pH (half-life time of hours to days) and active drug was released. Both prodrugs showed significant binding capability to hydroxyapatite, the major component of bone.

In order to demonstrate our concept, we chose to conjugate a bisphosphonate moiety to the known chemotherapeutic drug camptothecin.[13] In clinical trials, camptothecin was not effective due to its extremely low aqueous solubility and severe toxic side effects.[14] Several new chemical derivatives of camptothecin that overcome some of the disadvantages of the parent compound are now being evaluated in clinical trials.[2] Camptothecin is an ideal candidate for conjugation with bisphosphonate as this moiety will impart both water solubility and target the compound to bone.

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* Corresponding author. Tel.: +972 (0) 3 640 8340; fax: +972 (0) 3 640 9293; e-mail: chdoron@post.tau.ac.il

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Camptothecin can be esterified with bisphosphonate-butyric acid to form an esterolytic-activated prodrug, as illustrated in Figure 1. The prodrug is termed Camdronate; we chose the suffix 'dronate' since most bisphosphonate-based drugs follow this nomenclature.[5]

Camdronate was synthesized as illustrated in Figure 2. Bisphosphonate carboxylic acid **1** was prepared as previously described.[11] Chlorination of **1** with oxalyl chloride generated acylchloride-tetraethyl-bisphosphonate **2**. Then camptothecin was acetylated with acylchloride **2** to give ester **3**, which was deprotected with trimethylsilylbromide to afford Camdronate. The phosphonic acid moiety was transformed into its water-soluble sodium salt by the addition of an appropriate amount of sodium hydroxide (until the pH of the solution reached 9).

Next, we evaluated the hydrolytic stability of the ester linkage in Camdronate under physiological conditions. Camdronate was incubated in RPMI cell-medium (pH 7.4) at 37 °C and the release of free camptothecin was monitored by RP-HPLC. Camdronate was gradually hydrolyzed to release free camptothecin with a $t_{1/2}$ of 40 h (Fig. 3).

Camdronate was then evaluated by cell-growth inhibition assay using a cancerous acute lymphoblastic leukemia (ALL) Jurkat cell line (Fig. 4). Camdronate was 10-fold less active (IC_{50} 2.1×10^{-8}) than free camptothecin (IC_{50} 1.4×10^{-9}). This was expected since the masking of the 20-hydroxy group of camptothecin by an ester generates a prodrug with reduced anticancer activity. The prodrug can be recovered upon the hydrolysis of the ester linkage. No cytotoxicity was detected for the bisphosphonate-butyric acid moiety under the assay conditions.

Since Camdronate is designed for bone targeting, we sought for a simple model system to simulate bone tissue. Hydroxyapatite (HAP) is a commercially available calcium mineral. A suspension of HAP in aqueous media was previously used as a bone mod-

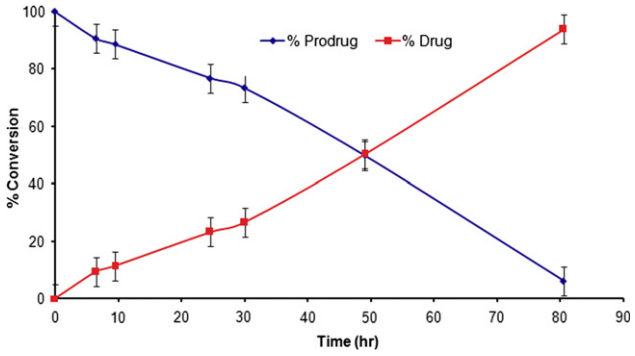

Figure 3. Hydrolysis of Camdronate (blue) to release camptothecin (red) in RPMI cell-medium, 37 °C.

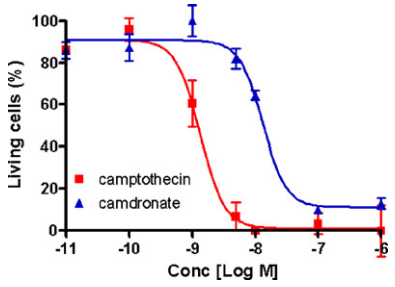

Figure 4. Growth inhibition activity of Camdronate (blue) and camptothecin (red) on Jurkat cells after a 96-h incubation over a range of concentrations of drug/prodrug.

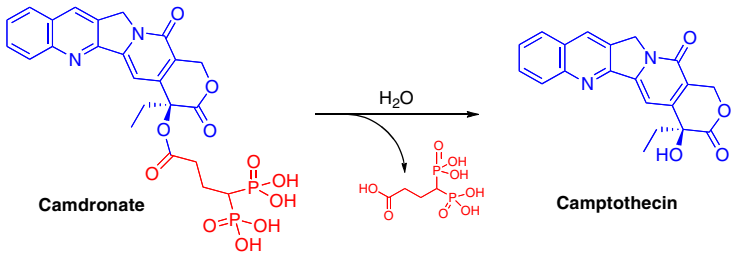

Figure 1. Hydrolysis of Camdronate under physiological conditions releases free, active camptothecin.

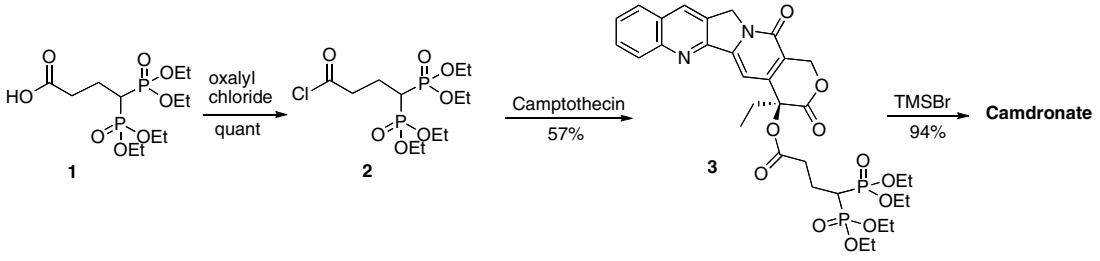

Figure 2. Chemical synthesis of Camdronate.