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Discodermolide analogues as the chemical component of combination bacteriolytic therapy

Amos B. Smith, III,[a,*] B. Scott Freeze,[a] Matthew J. LaMarche,[a] Jason Sager,[b] Kenneth W. Kinzler[b] and Bert Vogelstein[b,*]

[a]*Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, USA*
[b]*The Howard Hughes Medical Institute, Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins School of Medicine, Baltimore, MD 21231, USA*

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Abstract—The marine natural product (+)-discodermolide (**1**) and several simplified analogues of this microtubule-stabilizing agent have proven to be potent in vitro cell growth inhibitory agents in several human cancer cell lines. Here, we demonstrate the in vivo efficacy of discodermolide and several simplified congeners, both as stand-alone anti-tumor agents and, in the case of (+)-2,3-anhydrodiscodermolide (**3**), as a chemical component of the combination bacteriolytic therapy. A single intravenous injection of (+)-**3** plus genetically modified *Clostridium novyi*-NT spores caused rapid and complete regressions of tumors in mice bearing HCT116 colorectal cancer xenografts.

The treatment of large, poorly vascularized tumors is a longstanding problem in clinical oncology. The limited oxygenation renders such tumors resistant to radiation therapy,[1] and the lack of blood flow impedes delivery of conventional chemotherapeutics.[2] Although neoangiogenesis is ultimately necessary for prolonged tumor growth, fast-growing tumors often develop at rates that outpace vascularization. This results in large hypoxic regions, and, in many cases, significant necrosis of the poorly oxygenated areas. Taken together, these observations led to the development of anaerobic bacteria as agents that selectively target the hypoxic regions of these fast-growing tumors.[3] When administered along with a chemotherapeutic agent, such combination bacteriolytic therapy, termed COBALT, can result in the destruction of both the vascularized and avascularized tumor compartments.[4]

The strict anaerobe *C. novyi*-NT is one of the most promising microbiological agents for use in COBALT.[4] When *C. novyi*-NT is administered along with specific microtubule interacting agents, complete tumor regression in athymic nude mice bearing HCT116 xenografts is observed.[5] Of the compounds tested, the microtubule-stabilizing marine natural product (+)-discodermolide (**1**, Fig. 1) was among the agents shown to have a significant effect against HCT116. We now present the results of a broader study that evaluates (+)-discodermolide in conjunction with several simplified congeners as a chemical counterpart of the COBALT therapeutic approach.

Discodermolide (**1**) was isolated from the marine sponge *Discodermia dissoluta*[6] in 1990 and has been shown to be a potent cell growth inhibitory agent. The primary mechanism of action, akin to that of the clinically proven anti-cancer agent paclitaxel (Taxol), entails the

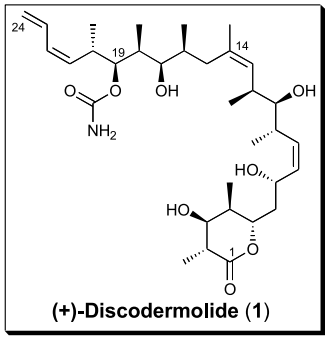

Figure 1. The structure of (+)-discodermolide.

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* Corresponding authors. Tel.: +2158981445; fax: +2158985129; e-mail: freeze@sas.upenn.edu

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binding and stabilization of microtubules.[7] Related studies indicate that paclitaxel and discodermolide likely share a common or overlapping tubulin binding site.[7] Importantly, discodermolide retains activity against paclitaxel-resistant cell lines;[8] furthermore, there exists a highly synergistic cytotoxic cooperativity between these two mitotic inhibitors.[9] Subsequent exploration of the latter effects led to the discovery, in collaboration with Horwitz and co-workers, that discodermolide possesses a second significant mechanism of tumor cell growth inhibition, specifically the powerful induction of an accelerated senescence phenotype.[10]

Our own studies with discodermolide (**1**) have focused largely on the evolution of a preparatively useful synthesis of discodermolide,[11] as well as the synthesis of numerous analogues of the natural product.[12]

Selection of specific compounds to be explored in the current study was primarily guided by the in vitro cytotoxicity profile of these analogues, based on two criteria. First, the compound should display anti-proliferative activity comparable to that of the natural product. Second, the analogue should possess a skeleton that is less complex than the parent (+)-discodermolide. The cytotoxicity data reported by the Kosan Biosciences Inc. and our laboratories are illustrated in Figure 2. Ultimately, three analogues were chosen for this study: (+)-14-normethyldiscodermolide (**2**),[12a,c,d] (+)-2,3-anhydrodiscodermolide (**3**)[12a,c,d], and (+)-2-normethyl-2,3-anhydrodiscodermolide (**4**).[12e]

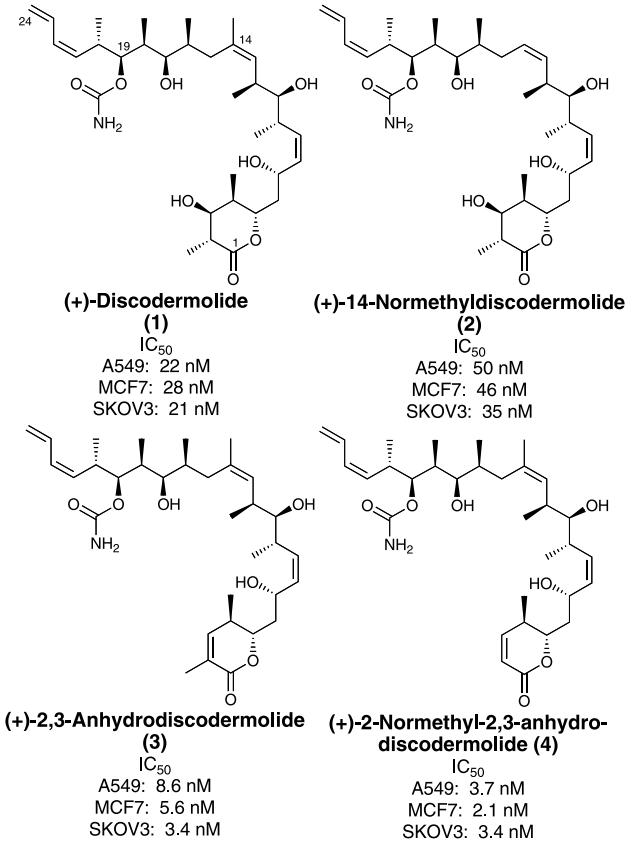

Figure 2. Analogues of (+)-discodermolide.

To begin, each of the analogues was evaluated for general toxicity at 10–20 mg/kg dose levels. Female nu/nu athymic nude mice[13] were treated with a single intravenous dose and the resultant weight fluctuations were recorded for a period of 28 days. Temporary weight loss was noted in all four cases (Fig. 3a). The only other clinically observed toxicity was that of transient seizure activity upon administration of the drug, from which the mice quickly recovered. Interestingly, compounds **1** and **2**, which were administered at lower dosage levels, resulted in a more pronounced, longer-lasting weight loss, despite being less potent in the in vitro studies.

To gauge the efficacy of the discodermolide analogues as stand-alone anti-tumor agents, athymic nude mice bearing HCT116 xenografts were injected intravenously (tail vein) with a single administration of each of the compounds and the effect on tumor volume was recorded. Based on the weight loss data presented in Figure 3a and other preliminary experiments, dosage levels of 10 mg/kg were chosen for compounds (+)-**1** and (+)-**2**, and 20 mg/kg for compounds (+)-**3** and (+)-**4**. All the compounds tested proved to inhibit tumor growth, relative to untreated animals, for a period of at least 15 days, at which point the tumors began to regrow (Fig. 3b and data not shown). The response noted for the α,β-unsaturated congener (+)-**3** was particularly dramatic; no tumor growth was noted for a full 15 days, following a single intravenous administration of the drug. For this reason, the α,β-unsaturated congener (+)-**3** was chosen to represent the discodermolide as the chemical component of the COBALT.

A combined administration of the drug and *C. novyi*-NT was then carried out, utilizing a mixture of 3×10^8 bacterial spores and 20 mg/kg of (+)-2,3-anhydrodiscodermolide (**3**) in a total volume of 400 μL of delivery vehicle. This mixture was used as a matter of conve-

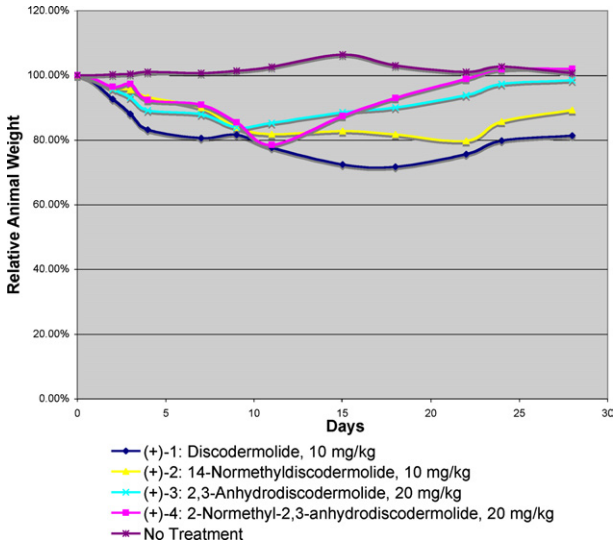

Figure 3a. Relative weight following treatment.

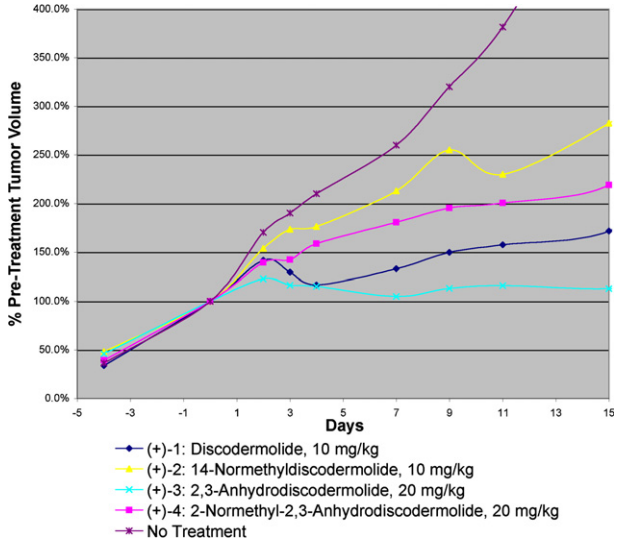

Figure 3b. Relative tumor volume following treatment.

nience, as treatment with the drug within a few hours before or after the spores had been demonstrated to have similar effects in the COBALT protocol. Toxicity associated with this protocol was acceptable, with animals showing a maximum average weight loss of 20% at 7 days and a full recovery of the initial weight by 22 days.

Remarkably, the tumors underwent massive hemorrhagic necrosis within one day following treatment. These lesions gradually scarred over and healed during the next three weeks (Fig. 4).

Necrosis developed in all treated animals, so that the tumors completely regressed in the five mice treated with a combination of (+)-2,3-anhydrodiscodermolide (**3**) and *C. novyi*-NT spores (Fig. 5).

Four of the five mice were apparently cured, as no tumors were observed in these mice at 90 days, when the experiment was terminated. Interestingly, responses noted in this study contrast with those observed with other microtubule-stabilizing agents.[5] In general, microtubule-stabilizing agents prompted a slow regression in tumor volume with little or no necrosis, while microtubule-destabilizing agents were found to induce hemorrhagic necrosis.

In conclusion, discodermolide and related microtubule-stabilizing discodermolide congeners have been demonstrated to be tumor growth suppression agents in vivo. Additionally, use of (+)-2,3-anhydrodiscodermolide (**3**) plus *C. novyi*-NT spores in combination bacteriolytic therapy (COBALT) has proven to be highly successful, leading to a robust and rapid obliteration of HCT116 xenograft tumors. The mechanism of action of this response, however, seems to more closely resemble that seen previously with microtubule-destabilizing agents, a phenomenon that is currently being investigated.

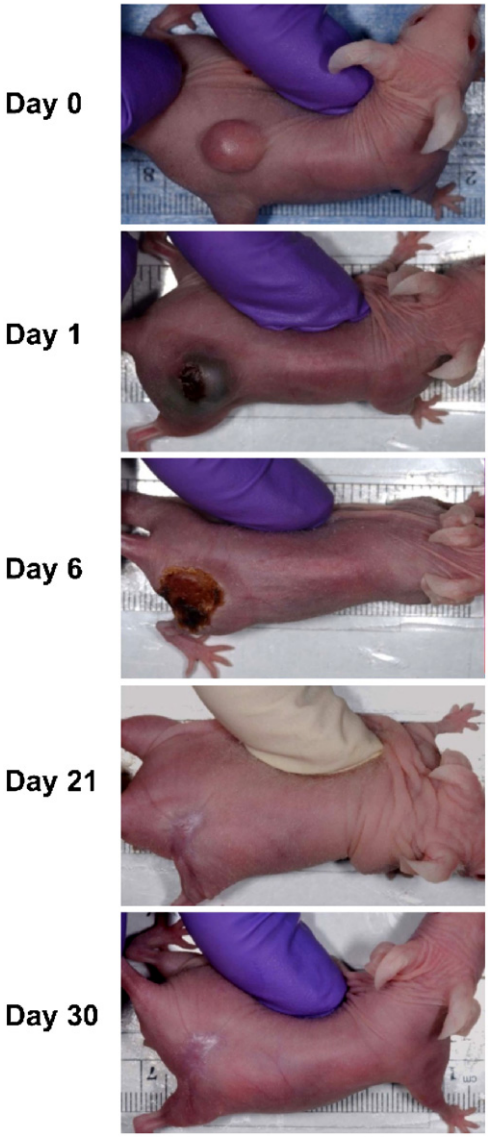

Figure 4. Tumor regression following adminstration of COBALT.

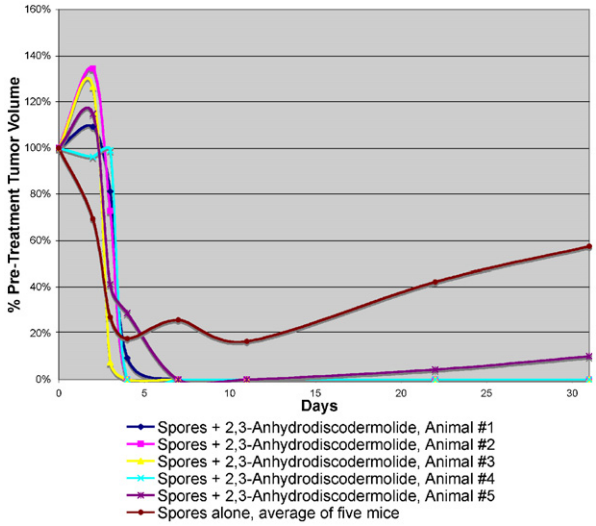

Figure 5. Relative tumor volume following COBALT.

Acknowledgments

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13. All animal protocols were designed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication 86-23) and were approved by the Johns Hopkins University Institutional Animal Care and Use Committee. Six- to eight-week-old nude mice were implanted subcutaneously on one flank with 3–6 million HCT116 cells. Formation of tumors of 250–400 mm^3 took 8–21 days. (+)-2,3-Anhydrodiscodermolide (**3**) was dissolved at 50 mg/ml in ethanol, added to an equal volume of Cremophore EL (Sigma C-5135), and then diluted with phosphate-buffered saline (PBS) to a final concentration of 2 mg/ml. The other drugs were dissolved at 50 mg/ml in ethanol and diluted with PBS to 2 mg/ml. *C. novyi-NT* spores (300×10^6 in 200 μl) and drugs were administered by a single intravenous injection through the tail vein in a total volume of 400 μl. Quantitative assessment of tumor growth and other procedures were performed as described in Ref. 5. Four mice were used for each treatment arm in the experiments described in Figures 3a and b, and five mice were used for each treatment arm in the experiments described in Figures 4 and 5. A representative mouse is illustrated in Figure 4.