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ET-743: More than an innovative mechanism of action

Kathleen W Scotto
Department of Pharmacology, Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA.

Ecteinascidin-743 (ET-743), an anti-tumor agent derived from the marine tunicate, *Ecteinascidia turbinata*, is active against various solid tumor cell lines, including soft tissue sarcoma, breast, ovarian, non-small-cell lung and prostate cancers and melanoma, and has a broad spectrum of anti-cancer activity *in vivo*. For reasons as yet unclear, sarcoma cell lines are exquisitely sensitive to ET-743. The drug has a unique mechanism of action that makes it a novel anti-tumor agent. ET-743 is a DNA-binding agent that covalently interacts with the minor groove of the DNA double helix to bend the molecule towards the major groove. Defects in DNA repair pathways have paradoxical effects on the anti-tumor activity of ET-743: loss of mismatch repair does not affect its toxicity; loss of DNA-dependent protein kinase activity enhances its toxicity; defects in transcription-coupled nucleotide excision repair confer resistance to ET-743. As a DNA repair capability appears to be necessary for at least one mechanism of ET-743-mediated cytotoxicity, the drug may interact with the DNA repair machinery to induce lethal strand breaks. One of the most novel aspects of ET-743 is its effect on RNA polymerase II-mediated gene transcription. ET-743 selectively inhibits activation of the multidrug resistance gene, while leaving constitutive gene expression relatively unaffected. Preliminary studies of other genes and transcriptional inducers indicate that ET-743 may be a more general inhibitor of activated, but not basal, transcription. [© 2002 Lippincott Williams & Wilkins.]

Key words: Ecteinascidin-743, anti-tumor agents, natural products, minor groove binders, DNA repair, transcriptional control.

Correspondence to Kathleen W Scotto, Department of Pharmacology, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111, USA.
Tel: (+1) 215 728 4077; Fax: (+1) 215 728 4333;
E-mail: KW_Scotto@fccc.edu

Introduction

Ecteinascidin-743 (ET-743) is an anti-tumor agent derived from the colonial tunicate, *Ecteinascidia turbinata* – a sea squirt found in the Caribbean and Mediterranean seas.[1] The drug is cytotoxic against various solid-tumor cell lines, including soft-tissue sarcoma, breast, ovarian, non-small-cell lung, prostate and renal cancers and melanoma, and has a broad spectrum of anti-cancer activity *in vivo*.[1] ET-743 has a unique mechanism of anti-tumor action, yet to be fully elucidated, that makes it a novel anti-tumor agent (Table 1).

Interactions of ET-743 with DNA

ET-743 is a carbinolamine-containing DNA-binding agent that consists of three tetrahydroisoquinoline rings. Structural modeling studies indicate that two of these rings (the A- and B-subunits) provide the framework for covalent interaction with the minor groove of the DNA double helix, whereas the third ring (the C-subunit) protrudes from the DNA duplex, apparently allowing it to interact with adjacent macromolecules (nuclear proteins or nucleic acids).

DNA-binding agents frequently cause structural perturbation of the DNA molecule, with the direction of bending being dictated by their site of interaction: binding in the minor groove causes bending of DNA towards the minor groove, whereas binding in the major groove results in bending towards the major groove. One of the first features of ET-743 to be noted, and one that distinguishes it from currently available DNA-binding agents, is that it interacts with the minor groove of DNA, but bends the molecule towards the major groove[2] (Figure 1). This pattern of interaction with DNA may account for some of its unique properties.

Table 1. Summary of pharmacodynamic properties of ET-743

ET-743 interacts with DNA and proteins
ET-743 blocks cell cycle progression at G_2/M
Cells in G_1 are hypersensitive to ET-743
Cells deficient in TC-NER are resistant to ET-743
ET-743 is a unique inhibitor of activated transcription
Sarcoma cell lines are exquisitely sensitive to ET-743

TC-NER = transcription-coupled nucleotide excision repair.

Figure 1. Interaction of ET-743 at the minor groove of DNA results in bending of the macromolecule towards the major groove.[2]

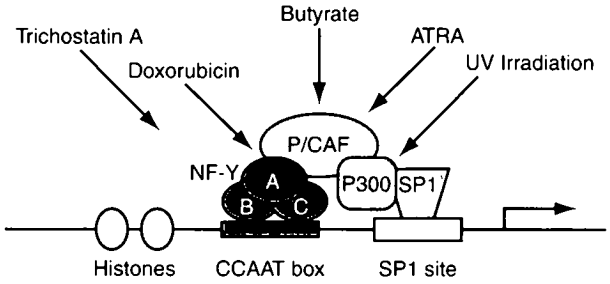

Figure 2. Activation of the *MDR1* promoter by different inducers. ATRA = all *trans*-retinoic acid; UV = ultraviolet; P/CAF = histone acetyltransferase; NF-Y = nuclear factor-Y.

ET-743 and DNA repair mechanisms

Defects in DNA repair pathways (mismatch repair and nucleotide excision repair) have paradoxical effects on the anti-tumor activity of ET-743: loss of mismatch repair does not affect the toxicity of ET-743; loss of the DNA double-strand repair pathway enhances its toxicity; defects in transcription-coupled nucleotide excision repair actually confer resistance to ET-743.[3,4]

The observation that impairment of a critical DNA repair mechanism results in resistance to ET-743 sets this drug apart from other DNA-damaging agents such as cisplatin, because resistance to this latter agent is often attributable to an increase in DNA repair proteins. Of the two types of nucleotide excision repair mechanism, the first type (global nucleotide excision repair) involves the entire gene, but is particularly important to those parts of the gene that are not undergoing active transcription. In contrast, the second type (transcription-coupled nucleotide excision repair, TC-NER) occurs on actively transcribing genes, which tend to be more susceptible to DNA damage. These two nucleotide excision repair mechanisms share many protein components, but the transcription-coupled repair process also makes use of several specific subunits: XPC, CSA and CSB. By analyzing cells deficient in these subunits, Takebayashi and coworkers[4] identified the TC-NER pathway as a mediator of ET-743 cytotoxicity. Thus it would appear that a DNA repair capability is a prerequisite for at least one type of ET-743-mediated cytotoxicity, suggesting that ET-743 may interact with the DNA repair machinery to induce lethal DNA strand breaks.[3–5]

Inhibition of activated gene transcription

Another novel aspect of ET-743 is its effect on RNA polymerase II-mediated gene transcription. In our laboratory, we have investigated the effect of ET-743 on transcription of the human P-glycoprotein gene, *MDR1*, which is involved in multidrug resistance. P-glycoprotein has an important role in certain tumor cells, removing drugs before they have a chance to exert their cytotoxic effects, and *MDR1* gene overexpression has been associated with poor clinical prognosis in some tumor types.[6] P-glycoprotein expression can be induced very rapidly: in solid tumors isolated from patients undergoing chemotherapy, we have observed activation of P-glycoprotein expression within 20 min of exposure to doxorubicin.

Transcriptional activation of the *MDR1* gene involves the MDR1 enhanceosome, which is a complex of transcription factors including nuclear factor-Y (NF-Y), Sp1/Sp3 and the histone acetyltransferase (P/CAF) in association with the DNA enhancer sequence. The *MDR1* promoter is readily activated by several different inducers, including the histone deacetylase inhibitors trichostatin A (TSA) and sodium butyrate, in addition to doxorubicin, ultraviolet radiation and certain hormones (Figure 2).

Identification of the *MDR1* gene in human tumors has prompted the search for approaches to blocking its activation. In theory, prevention of *MDR1* gene activation abrogates the need to reverse P-glycoprotein function as a later event. Our initial screening efforts failed to identify a compound that could inhibit activation of *MDR1* gene transcription without also affecting constitutive gene expression. However, in ET-743 we found a compound that selectively inhibited activation in response to many of the above-mentioned inducers, but did not appreciably alter constitutive gene expression.

Transcriptional analysis using promoter constructs linked to luciferase (a reporter gene that is not normally present in mammalian cells) has provided information on the effects of ET-743 on inducible *MDR1* promoter activity in SW620 colon carcinoma cells. In this test system, luciferase activity serves as a marker of *MDR1* promoter activity. In the absence of inducers such as TSA, sodium butyrate or ultraviolet radiation, there is only a basal level of *MDR1* gene expression, and this constitutive expression

is essentially unmodified by ET-743. However, in the presence of inducers there is a pronounced increase in promoter activity, which is largely abolished by concomitant incubation with ET-743. Therefore, ET-743 specifically inhibits promoter activation through the *MDR1* enhanceosome, without affecting basal expression.

A total of more than 20 DNA-binding compounds, including minor groove binders, major groove binders, intercalators and alkylating agents, have been screened for inhibitory activity at the *MDR1* promoter, but none have been found to mirror the effects of ET-743. For example, cisplatin, distamycin, mitoxantrone, actinomycin D or mitomycin C did not differentially affect basal or TSA-induced *MDR1* promoter activity. Thus, to date, ET-743 is the only drug that has been shown to inhibit activated transcription while leaving basal transcription relatively unaffected.

Our studies of the *MDR1* promoter, taken in conjunction with the findings of Mantovani[7] that ET-743 inhibited heat-shock induction of the hsp70 promoter, led us to propose that NF-Y, a transcription factor involved in induction of both the *MDR1* and *hsp70* genes, was the target for ET-743. However, ET-743 does not appear to alter the NF-Y-dependent *MDR1* promoter-associated histone hyperacetylation induced by TSA, suggesting that the drug acts at a molecular target downstream of, or independent of, NF-Y binding. Our recent attempts to identify this target have been directed at other genes that are regulated by the same inducers that regulate *MDR1*.

On the basis of experimental findings obtained with these genes and other DNA-binding transcription factors,[8] it would appear that ET-743 blocks activated gene transcription, and that this effect is generally independent of the transcription factor and the DNA binding site (major or minor groove). Preliminary indications suggest that this inhibitory effect of ET-743 may be mediated through interaction with chromatin.

It is interesting to speculate as to whether the above-mentioned effects of ET-743 on activated gene transcription and TC-NER may in some way be inter-related. Both processes involve the transcription factor, TFIIH, and the fact that components of this multisubunit protein are required both for polymerase-II-mediated gene transcription and for TC-NER raises the possibility that TFIIH may link these seemingly disparate actions of ET-743.

ET-743 is highly active against several different tumor cell lines, in particular sarcoma cells.[9,11] Against many sarcoma cell lines, including those resistant to methotrexate, doxorubicin, VP-16 and paclitaxel, ET-743 is cytotoxic at sub-picomolar concentrations.[11] The reason for this exquisite sensitivity of sarcoma cells to the drug is unclear. In addition, ET-743 has cell-specific effects on the cell cycle. At sub-toxic concentrations, it causes growth arrest at the G_2/M transition in some tumor cell lines, and these cells show hypersensitivity to ET-743 during the G_1 phase.[8] Moreover, ET-743 shows synergy with the P-glycoprotein substrate, doxorubicin,[12] and this effect is apparently enhanced by prior exposure to ET-743. This is consistent with the ability of ET-743 to prevent activation of *MDR1* transcription by the chemotherapeutic agent, thereby enhancing the anti-tumor activity of the latter.

Conclusions

ET-743 is a novel anti-tumor agent. It interacts with DNA and possibly with proteins. Most unusually, ET-743 binds in the minor groove of DNA to bend the helix towards the major groove, resulting in significant physical distortion. It can block cell cycle progression at the G_2/M phase, making cells hypersensitive in G_1. Paradoxically, cells deficient in TC-NER mechanisms are resistant to the cytotoxic action of ET-743. Uniquely, ET-743 selectively blocks activated transcription of different genes by different inducers. Finally, for reasons that are as yet unclear, sarcoma cell lines are exquisitely sensitive to this drug.

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