Anti-Cancer Drugs 1997, **8**, pp. 413–418

Review paper

Fostriecin: a review of the preclinical data

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Fostriecin is a novel antitumor antibiotic. *In vitro* studies showed that fostriecin inhibits DNA topoisomerase II (Topo II) catalytic activity, protein phosphatases involved with cell-cycle control and histone phosphatases. The relative contribution of these mechanisms to the antitumor activity has not been elucidated, but Topo II inhibition seems to be the major mechanism of action at *in vitro* cytotoxic fostriecin levels. Tumor cell lines with decreased Topo II content showed similar or increased sensitivity to fostriecin, compared to the parent cell lines. The reduced-folate carrier is probably responsible for the cellular uptake of fostriecin. The possible clinical consequences of these *in vitro* observations are discussed.

Key words: Antibiotics, DNA topoisomerase, drug resistance, fostriecin, phosphoprotein phosphatases.

Introduction

The antitumor antibiotic fostriecin (CI-920) is a representative of a new class of type II DNA topoisomerase (Topo II)-directed anticancer drugs. The difference with classic Topo II inhibitors (Topo II poisons) is that the new drugs do not stabilize the cleavable complex, which is a reaction intermediate formed during the catalytic cycle of Topo II, but directly inhibit the catalytic activity of Topo II.[1] Topo II creates transient double-stranded DNA breaks in order to enable the passage of other double-stranded DNA, and in this way regulates DNA topology problems during DNA replication, transcription and chromosome segregation.[2,3] Stabilization of the cleavable complex interferes with the DNA relegation reaction and induces cell death.[4] The cytotoxic activity of the classic Topo II inhibitors, including most anthracyclines (e.g. doxorubicin) and the epipodophyllotoxins (e.g. etoposide and teniposide), is related to the rate of cleavable complex formation, and thus depends on Topo II level and activity. Reduction of the Topo II level is an important mechanism of drug resistance to these drugs and has been named atMDR (altered topoisomerase multi-drug resistance.[1,4] Because the activity of the Topo II catalytic inhibitors does not depend on cleavable complex formation, these agents might offer new treatment options for patients with intrinsic or acquired atMDR.[5]

Four phase I clinical trials with fostriecin, coordinated by the US National Cancer Institute (NCI), have been conducted recently and final reports can be expected in the coming year. The published preclinical data are reviewed in this article to facilitate the interpretation of the clinical studies and the further investigation of this interesting agent.

Biochemistry of fostriecin

Fostriecin was originally discovered in 1983 as a component of a fermentation product of the *Streptomyces pulveraceous* subspecies fostreus ATCC 31906, an actinomycete found in a Brazilian soil sample. It was obtained in pure form as a hydrated, amorphous sodium salt.[6,7] Fostriecin is a water-soluble polyene lactone with a phosphate ester. The biochemical structure is shown in Figure 1.[8] The intact lactone ring and presence of the phosphate group are necessary for antitumor activity and

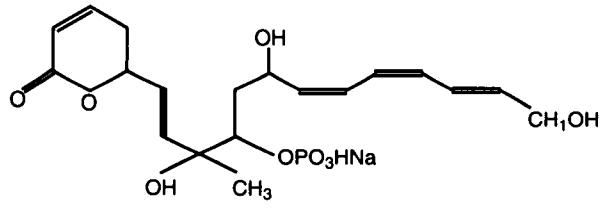

Figure 1. Fostriecin, $C_{19}H_{27}PO_9Na$, molecular weight: 452.36.[8]

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optimal interaction with the reduced-folate carrier (see below).[9–11] For clinical studies, fostriecin was formulated with sodium ascorbate to improve stability to oxidation. In addition to the antitumor activity discussed below, fostriecin showed antimycotic, but no antibacterial activity.[12]

Mechanism of action

Early biochemical studies with murine leukemia L1210 cells showed that fostriecin rapidly suppressed nucleic acid and protein synthesis, with 50% inhibition of DNA synthesis reached at a fostriecin concentration of 2.5 μM.[10,13] Because this process was not inhibited in cell-free systems, it was suggested that metabolic intracellular activation might be required.[13] The mechanism of nucleic acid synthesis inhibition was not elucidated. Nucleotide pools were not depleted, which means that fostriecin is not an antimetabolite, and fostriecin did not directly inhibit RNA or DNA polymerases. The latter was also found in human HeLa cells.[14] Because Topo II is part of the DNA replication and transcription machinery, it is likely that these observations were due to the subsequently demonstrated inhibition of Topo II by fostriecin.

Direct evidence for inhibition of Topo II catalytic activity by fostriecin was found in DNA relaxation and decatenation assays with purified Topo II from human Ehrlich ascites carcinoma cells.[15] Relaxation of supercoiled DNA by Topo II was almost completely inhibited at a fostriecin concentration of 50 μM (IC_{50}: 40 μM). Fostriecin was compared to the Topo II poisons teniposide and amsacrine in this study. In contrast to teniposide, fostriecin did not compete with ATP in the decatenation assay. Contrary to amsacrine, fostriecin (tested at 1–100 μM) did not induce DNA strand breaks and even partially inhibited the induction of strand breaks by amsacrine in L1210 cells.[10,15] Other investigators did not find such an inhibitory effect of fostriecin on cleavable complex formation in human CEM or K562 leukemia cell lines exposed to teniposide or etoposide, which indicates that Topo II activity was not significantly inhibited by fostriecin in these *in vitro* models.[16,17] Diverging observations were also reported from a study that showed strong inhibition of UV-damaged plasmid DNA, but no effect of fostriecin on DNA decatenating activity of mammalian cell extracts.[18] However, the latter might have been due to nonspecific binding of fostriecin to other proteins in the crude cell extracts.

Indirect evidence for inhibition of Topo II catalytic activity by fostriecin was found in studies that investigated the relation between the fostriecin sensitivity and Topo II activity of human GLC_4 lung cancer cell sublines.[19] GLC_4/ADR, which is resistant to the Topo II poisons doxorubicin and etoposide because of decreased Topo II activity, showed 3-fold increased sensitivity to fostriecin compared to the parent GLC_4 cell line (IC_{50}: 4.1 and 11.2 μM, respectively). A platinum-resistant subline with increased Topo II activity was less sensitive. A recent study confirmed this inverse relationship between fostriecin and Topo II activity in other Topo II poison-resistant GLC_4 sublines.[20] Other investigators also found no cross-resistance to fostriecin in cell lines with resistance to classic Topo II poisons due to altered Topo II. These studies were performed in a 400-fold teniposide-resistant human CEM leukemic cell line with Topo IIα gene mutations and very low Topo IIβ levels,[16] and in etoposide-resistant human K562 leukemia and human KB nasopharyngeal carcinoma cell lines with decreased Topo II levels.[17,21] However, in these cell lines no increased sensitivity to fostriecin was found, contrary to the observations in the GLC_4 cell lines. Interpretation of the results with the KB cell line was confounded by the presence of other resistance mechanisms and the fact that KB cells lack the reduced-folate carrier, which is required for fostriecin uptake (see below).

Other mechanisms of action were proposed by Roberge and coworkers who performed experiments with baby hamster kidney cells (BHK) and several human cell lines. They found that fostriecin inhibited the protein phosphatases PP2A and PP1 *in vitro*.[22] PP1 and PP2A regulate the phosphorylation state and activity of $p34^{cdc2}$ kinase, which is involved in the G_2 cell-cycle checkpoint.[23] Fostriecin exposure induced the inappropriate entry of cells blocked in S phase into mitosis and abrogated G_2 arrest induced by teniposide and camptothecin (a Topo I inhibitor). Fostriecin inhibited purified PP2A at an IC_{50} as low as 40 nM and PP1 at an IC_{50} of 4 μM, but the concentrations needed to achieve the described cell-cycle effects on intact cells were much higher (higher than 37.5–100 μM). The latter concentrations were also much higher than those needed to obtain significant cytotoxicity in the GLC_4 small cell lung cancer cell lines, and in the other cell lines and the human solid tumor screening assay which are discussed below. This suggests that inhibition of PP2A and PP1 might be less important than the effect on Topo II. Subsequent experiments in a mouse FT210 mammary tumor cell line showed that $p34^{cdc2}$ kinase activity was not always required for

these effects to occur and that fostriecin exposure at concentrations of 25 μM or more resulted in histone hyperphosphorylation and premature chromosome condensation.[24] Recently, the same group reported that exposure of intact BHK cells to fostriecin resulted in vimentin hyperphosphorylation. However, the latter was only observed at fostriecin concentrations of 50 μM or higher.[25]

The effects of fostriecin on cell-cycle distribution were also investigated in the initial *in vitro* studies.[10,15] Exposure of L1210 cells to low fostriecin concentrations (1.5–5 μM) resulted in delayed progression through the G_2 phase. Higher concentrations (15 μM) resulted in accumulation in S phase. Darzynkiewicz and coworkers observed similar effects on the cell-cycle distribution of human MOLT-4 promyelocytic and HL-60 lymphocytic leukemia cell cultures at fostriecin concentrations of 1–5 μM.[26–30] Etoposide and amsacrine had similar effects on the cell-cycle distribution compared to fostriecin. This is consistent with an effect of fostriecin on Topo II, although fostriecin was nearly two orders of magnitude less potent than the other agents on a molar base. At higher concentrations (5–550 μM) apoptotic DNA degradation occurred in HL-60, but not in MOLT-4. Necrotic cell death was observed in both cell lines at very high fostriecin concentrations (above 1 mM).

Transmembrane transport

Little is known about the cellular uptake mechanism of fostriecin, but a carrier mechanism is expected because of fostriecin's hydrophilic properties. Studies in the mouse L1210 leukemic cell model demonstrated an interaction with the reduced-folate carrier, although fostriecin is not structurally related to folates. Fostriecin inhibited methotrexate (MTX) uptake by L1210 cells at concentrations of 25–150 μM.[11,31] In contrast, there was relatively little influence of fostriecin on MTX uptake mediated by the folate receptor system.[31] The addition of folinic acid (leucovorin) or 5-methyltetrahydrofolate to the medium blocked the cytotoxic effect of fostriecin on L1210 cells and folic acid had no such effect.[13] Leucovorin administration also protected mice from lethal fostriecin doses.[10] Results of one study indicated very strong or even irreversible binding of fostriecin to the reduced-folate carrier.[11] This suggests that fostriecin might interfere with its own transport and may explain why activity of fostriecin decreased at high concentrations in a clonogenic assay.[32] The fostriecin concentrations at which impaired reduced-folate carrier function was observed were 5- to 10-fold higher than those needed for significant growth inhibition of L1210 and GLC_4/ADR cells. Thus impaired reduced-folate carrier function is probably not very important at cytotoxic fostriecin concentrations in sensitive tumor models. However, it might be a cause of acquired drug-resistance, because L1210 cell lines selected for resistance to fostriecin had a severely impaired MTX transport facility.[11,33]

Preclinical antitumor activity

Antitumor activity of fostriecin was first observed in mouse P388 and L1210 leukemias *in vivo* after i.p. administration.[6] This was followed by *in vitro* demonstration of activity in L1210 and in HCT-8 human colon cancer cell cultures.[9,10] Schedule dependency was investigated in the L1210 *in vivo* model. Once daily treatment for 5 or 9 days proved more effective than two or three single doses every 4 days. A schedule with multiple daily doses was more toxic in this mouse model, but less active. This suggested that continuous exposure might increase toxicity, but not activity. The *in vitro* IC_{50} in L1210 was 0.46 μM for continuous exposure to fostriecin, compared to 4.4 μM for a 1 h exposure. The IC_{50} in HCT-8 (5.1 μM) was almost similar to that of the GLC_4/ADR small cell lung cancer cell line discussed above.[10,19] *In vivo*, greatest activity was observed at an i.p. dose of 6.25 mg/kg/day on days 1–9, which was curative in L1210-bearing mice.[9]

Fostriecin was inactive against a range of solid tumors in mice.[9,10] This was attributed to the absence of the reduced-folate carrier, because these mouse tumors were also insensitive to MTX. However, in another mouse study rapid necrosis of s.c. murine Colon 38 tumor implants was found after i.p. fostriecin administration (65 mg/kg, single dose).[34]

Fostriecin was one of the most active agents in a clonogenic human solid tumor screening assay, compared to 17 clinically used anticancer drugs including doxorubicin and etoposide.[33] Tumor specimens derived from patients were exposed to fostriecin at 1 μg/ml (2.2 μM) for 1 h, a concentration based on the mouse LD_{50}. When 50% decrease in tumor colony forming units was used as the criterion of effect, responses were observed in 33% of the ovarian, 42% of the breast and 38% of the non-small cell lung cancer samples, whereas doxorubicin had response rates of 25% in the breast and 11% in the lung cancer samples.

Preclinical toxicity

Toxicity was studied in mice, rats, rabbits and dogs, but for the latter no published data are available. The mouse studies suggested that gastrointestinal toxicity might be dose limiting.[9] As discussed above, toxicity was much increased with multiple daily dosing. An interaction between fostriecin and X-rays has been observed in mice.[35] In this study fostriecin was a strong sensitizer to the toxic effect of X-ray irradiation on jejunal epithelium at a dose far below the LD_{10}. This suggested inhibition of DNA repair, which probably requires Topo II activity. Toxic changes of laboratory parameters and organ histology after fostriecin infusion were investigated in rats.[36] Morphological changes consisting of minimal to moderate vacuolization and/or necrosis of tubular epithelium were observed already 8 h after fostriecin administration. These changes were primarily localized at the corticomedullary junction. Similar abnormalities, together with diffuse necrosis of lymphoid tissue and bone marrow hypocellularity, were observed in animals euthanized 4 days after fostriecin administration. After 4 weeks only minor regenerative histological changes were present in these organs. In these studies the severity of the histological changes were dose related. Laboratory changes after 4 days included leuko-, neutro- and thrombocytopenia and dose-related increases of blood urea. Hematologic changes were less with the repeated dose schedule. In rabbits more than 50-fold increased liver transaminases and slightly elevated serum creatinine were observed within several hours after administration of a single 12 mg/m^2 i.v. dose. In the latter study a minor decrease of hemoglobin was the only hematologic change.[37]

Myelotoxicity was studied *in vitro* using human and murine bone marrow clonogenic assays.[38] Erythroid progenitor cells (CFU-e) were more sensitive to fostriecin than the granulocyte precursors (CFU-gm) and murine cells were more sensitive than their human counterparts. For human cells IC_{50} values of 0.6 μM for CFU-e and 5.0 μM for CFU-gm were found after 1 h exposure. However, in another study no inhibition of bone marrow progenitor colony formation was found.[39] These opposite results have not been explained.

Pharmacokinetics

An assay for the determination of fostriecin plasma levels was published in a paper by Pillon *et al.*[37] This high-pressure liquid chromatographic method uses sulfaquinoxaline as an internal standard and UV detection at 268 nM. Animal data on the pharmacokinetics of fostriecin are only available from experiments in rabbits, which revealed a very short mean plasma fostriecin half-line of 12 min after bolus infusion and a distribution space of 4.44 l/m^2. Fostriecin was stable in whole blood at room temperature for at least 2 h.[37] We confirmed the suitability of the assay described by Pillon *et al.* for the detection of fostriecin in human plasma and urine, and investigated the stability of fostriecin in these fluids (de Jong *et al.*[40] and unpublished data). Levels in urine did not decrease significantly when stored at room temperature for 48 h. When stored at −80°C, fostriecin is stable in plasma for at least 4 months and in water for at least 1 year. At −20°C, fostriecin is considerably less stable. *In vitro* protein binding of fostriecin in human plasma was 72.8 ± 1.7%.

Conclusions

From the reviewed studies it can be concluded that fostriecin may have several mechanisms of action, including inhibition of Topo II, the protein phosphatases PP1 and PP2A, histone phosphatases, and an effect on the phosphorylation state of cytoskeleton proteins. The relative contribution of these mechanisms to fostriecin's antitumor activity needs further investigation and the concentrations at which the different effects of fostriecin are observed should be related to clinically feasible levels. The inhibition of Topo II catalytic activity by fostriecin was demonstrated in studies with purified human Topo II, and supported by the observed inverse relation between Topo II activity and fostriecin activity in GLC_4 human small cell lung cancer cell lines. These data suggest a potential role for fostriecin in the treatment of tumors with drug-class-specific resistance to the classic topo II drugs. The implications of the observed effects on nuclear protein phosphatases are more difficult to perceive. Roberge *et al.* suggested that fostriecin may abrogate the G_2 phase arrest induced by DNA-damaging agents, such as etoposide, and drive the cells into a premature mitosis with lethal outcome. This possibility for a rational drug combination deserves further investigation. Some of the effects *in vitro*, especially those on vimentin phosphorylation, were only observed at very high fostriecin concentrations and might not be of clinical relevance.

It was found that fostriecin shares a membrane transporter with MTX, the reduced-folate carrier, but

not the transport facility mediated by folate receptors. This may have important implications, both for the interpretation of *in vitro* data and clinical studies. It should be noticed that the presence of the reduced-folate carrier has been well established in tumor cell lines, but that there is no definite proof of its presence on normal human tissue.[41] Only very recently evidence was found for the presence of this carrier in normal mouse tissue (kidney, liver, heart, brain and large intestine) and human intestine.[42] In addition, several tumor models have a non-functional reduced-folate carrier system, including the KB cell line used in one of the studies discussed above.[21,41] Therefore, such models are not very appropriate to study the effects of fostriecin. Investigation of the level of expression of this carrier in tumor and normal tissues is also needed for the interpretation of clinical data. It was found that the toxicity in mice was prevented by prior leucovorin administration, which is an argument in favor of the widespread presence of the reduced-folate carrier. It is questionable, however, whether leucovorin administration would be attractive to prevent drug toxicity, because it might also diminish the antitumor effect. Another important observation was the inhibition of reduced-folate carrier transport at high fostriecin concentrations, because this may have consequences for the optimal dose schedule.

The *in vitro* and *in vivo* studies have shown considerable antitumor activity of fostriecin, in particular in comparison to standard chemotherapeutic agents in a human tumor clonogenic assay. This and the novel chemical structure and mechanism of action of fostriecin were reasons to initiate further clinical development. Preliminary data confirmed the liver and renal toxicities observed in the animal studies, but the predicted hematological toxicities have been minimal.[40,43–45] Final reports have to be awaited before further conclusions are possible. Unfortunately, NCI has been forced to prematurely close the clinical studies due to drug production problems. However, evaluation of all available clinical data, in combination with an evaluation of the preclinical data as provided in this review, is needed before decisions about the future of fostriecin can be made.

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(Received 13 February 1997; accepted 3 March 1997)