

Focus on Fotemustine

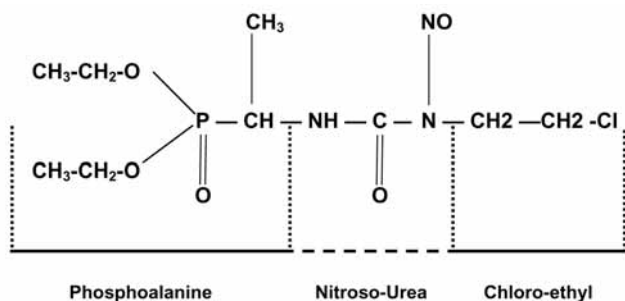
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Fotemustine is a cytotoxic alkylating agent, belonging to the group of nitrosourea family. Its mechanism of action is similar to that of other nitrosoureas, characterized by a mono-functional/bi-functional alkylating activity. Worth of consideration is the finding that the presence of high levels of the DNA repair enzyme O⁶-methylguanine-DNA-methyltransferase (MGMT) in cancer cells confers drug resistance. In different clinical trials Fotemustine showed a remarkable antitumor activity as single agent, and in association with other antineoplastic compounds or treatment modalities. Moreover, its toxicity is generally considered acceptable. The drug has been employed in the treatment of metastatic melanoma, and, on the basis of its pharmacokinetic properties, in brain tumors, either primitive or metastatic. Moreover, Fotemustine shows pharmacodynamic properties similar to those of mono-functional alkylating compounds (e.g. DNA methylating drugs, such as Temozolomide), that have been recently considered for the management of acute refractory leukaemia. Therefore, it is reasonable to assume that this agent could be a good candidate to play a potential role in haematological malignancies.

Key Words: Fotemustine, Melanoma, Brain tumor, Chemotherapy, Brain metastases

Fotemustine or diethyl{1-[3-(2-cloroethyl)-3-nitrosoureido]ethyl}phosphonate, is an alkylating cytotoxic agent, belonging to the group of antitumor nitrosoureas:



This molecule has been synthesized to obtain a nitrosourea endowed with more selective antineoplastic activity than that of other alkylating agents. Actually, this compound shows more attractive pharmacokinetic properties than those of the other classical nitrosoureas, in terms of blood-brain barrier passage

and of cell penetration coefficient, especially in neoplastic cells. This has been reached by adding a phospho-alanine group, with a carrier function, to the chloro-nitroso-urea molecule (1).

All these structural changes have a precise rationale. It is known that aminoacids are actively transported inside the cell. Moreover, neoplastic cells are characterized by a greater permeability to aminoacids compared to that of normal cells. Thanks to its structural analogy with alanine, phospho-alanine increases the penetration coefficient of the drug into malignant cells.

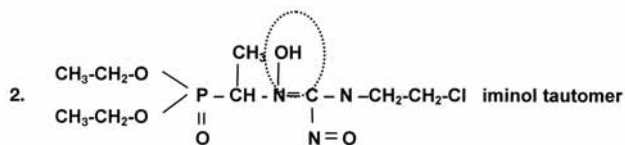
The elevated lipophilic properties of Fotemustine contribute to facilitate its passage through the blood-brain barrier. In fact, Fotemustine is more lipophilic than other important nitrosoureas, such as Carmustine (BCNU) and Lomustine (CCNU) (2). Moreover, high brain permeability coefficient of this compound was demonstrated in rats subjected to drug administration into the right external carotid artery (3). This is accompanied by the finding that Fotemustine shows greater diffusion in neuronal cells and glia, than Carmustine (4).

The elevated lipophilicity and cell transport capacity of Fotemustine is also responsible for its high tissue distribution volume. All these biochemical characteristics make the molecule remarkably active towards different kinds of tumor, especially those of the central nervous system (5).

Mechanism of action

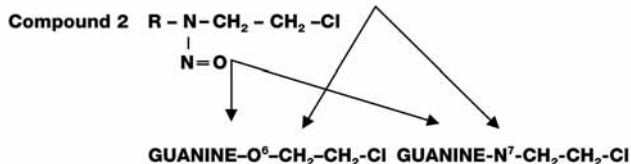
Although the mechanism of action of Fotemustine is similar to that of other nitrosoureas, it has not yet been conclusively defined. Antitumor activity of this agent shows also similarities with that of triazene compounds (i.e. Dacarbazine and Temozolomide). However, unlike triazenes, that are well known mono-functional DNA methylating agents, Fotemustine is a mono-functional/bi-functional agent containing a chloroethylating group. As described by Hayes et al. (1), Fotemustine decomposes quickly in aqueous solution, yielding at least two DNA-reactive species relevant to its biological activity. In particular, they appear to be a short-lived compound, 2-chloroethyl-diazohydroxide, and a long-lived iminol tautomer of the parent drug:

1. $\text{HO}-\text{N}=\text{N}-\text{CH}_2-\text{CH}_2-\text{Cl}$ 2-chloroethyl-diazohydroxide



Compound 1, which is rapidly inactivated within 5 minutes in aqueous solution, and the long-lived compound 2 produce a cytotoxic damage through a double and intriguing mechanism based on mono and bi-alkylating process as well. If the compounds bind DNA O⁶-guanine from the molecule end opposite to the alkylating chloroethyl group, O⁶-chloroethyl-guanine is generated. On the other hand, if the same end group of the molecules binds the N⁷ atom of the guanine, the N⁷-chloroethyl-guanine is produced.

Compound 1 $\text{HO}-\text{N}=\text{N}-\text{CH}_2-\text{CH}_2-\text{Cl}$



However, N⁷-alkyl-guanine has a minor role in

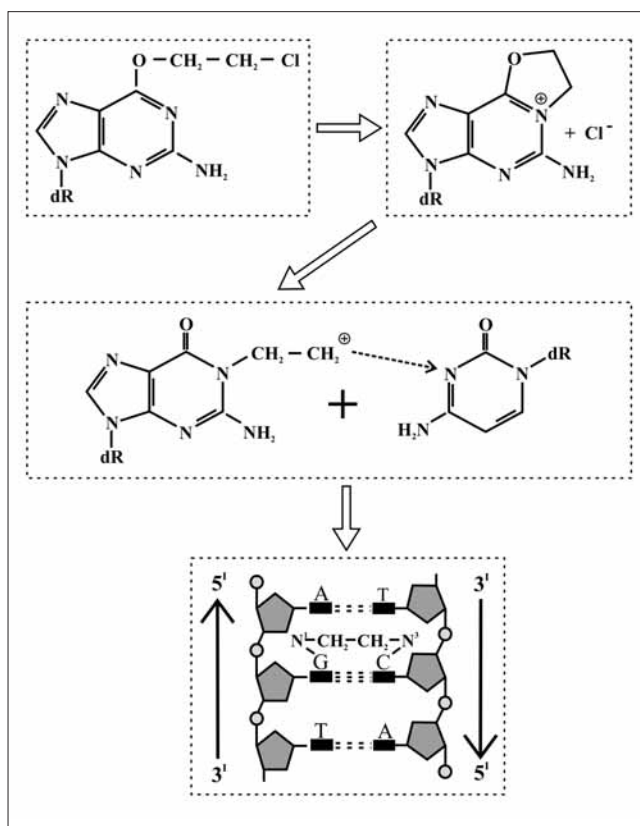


Fig. 1 - Fotemustine: alkylating mechanism of action.

Fotemustine cytotoxicity. The chloroethyl adduct at O⁶-guanine, instead, is able to behave as a classical alkylating moiety, producing an intramolecular N¹-O⁶-ethanoguanine intermediate which subsequently reacts with the nitrogen-3 of the adjacent cytosine, mainly of the opposite DNA strand, to form GC cross-link (Fig. 1). However, the alkylating compound can alternatively bind O⁶-oxygen of DNA guanine by the chloroethyl moiety.



In this case, the new molecule (O⁶-hydroxyethyl-guanine) is not able to produce a cross linking, and the compound behaves as a less toxic mono-functional alkylating agent.

Another important pathway of nitrosourea metabolism, and in particular of Fotemustine, concerns the carbamoylation process. Fotemustine produces the diethyl-ethyl-phosphonate isocyanate derivative (6) that is characterized by a less carbamoylating activity than that of BCNU (7). It was demonstrated that the car-

bamoylation process is able to activate the HSP70 gene transcription, leading to a reduction of the pro-apoptotic effects of antineoplastic agents. In fact, HSP70 reduces caspase activation (8) blocking of the Apaf-1 attachment to the cytochrome C (9). Therefore, the reduced carbamoylating effects of Fotemustine could represent an attracting aspect of its antineoplastic mechanism.

The effects of Fotemustine and BCNU on cell cycle pattern have been compared in different mouse and human cell lines by tritiated thymidine capture and flow cytometry. The results have shown that Fotemustine is more active than BCNU, and that Fotemustine inhibits DNA synthesis at a more advanced stage of cell cycle progression, probably at S and G2-M phase (7, 10).

Mechanism of resistance

As previously described, the rapid alkylation of oxygen-6 of DNA guanine is the first biochemical step mainly involved in the cytotoxic activity of Fotemustine. This step is followed by high frequency of DNA two-strand cross-linking events. However, at the early stage of drug activity, corresponding to the formation of alkyl adducts at oxygen-6 of DNA guanine, DNA is subjected to mono-functional alkylation. Therefore, the presence of high-levels of O⁶-methylguanine-DNA-methyltransferase enzyme (OGAT/MGMT) (11) confers drug resistance. The MGMT enzyme is able to remove alkyl adducts at oxygen-6 of DNA guanine, transferring them on cysteine residues of MGMT itself, with a mechanism that has been defined as "suicidal activity". This process can take place only before the formation of cross linking events, since alkyl residues bound to oxygen 6 of guanine and to another reactive site of a DNA base is not recognized as suitable substrate by MGMT (1, 6, 12). In any case, MGMT inhibitors [e.g. O⁶-Benzylguanine or of O⁶-(4-Bromothienyl) guanine (PaTrin-2), (13)], are able to reduce substantially resistance to Fotemustine in neoplastic cells endowed with high levels of MGMT activity (12, 14). It is well known that another DNA repair system, the mismatch repair system (MMRS), is involved in tumor cell resistance to antineoplastic DNA alkylating agents, such as triazene compounds (15). In this case, MMRS-deficient target cells are resistant to triazenes (15). In contrast, no conclusive data are available on the possible role of DNA mismatch repair system on tumor cell resistance to Fotemustine (16), considering that, differently from triazenes, Fotemustine acts through a mechanism largely based on DNA inter-

strand cross-linking effect.

Pharmacokinetics in animal models

Preclinical studies in mice, rats, monkeys and dogs provide essential information on the pharmacokinetic profile of Fotemustine (17). After intravenous bolus administration (100 mg/m²), the analysis of main pharmacokinetic parameters in these four animal species showed that: (a) peak plasma concentrations were in the range of 9-18 µg/mL (28-57 µM), equivalent to *in vitro* antitumor activity; (b) half-life of unmodified drug distribution and elimination was very short, thus indicating that Fotemustine is rapidly degraded and metabolized; (c) plasma clearance was very high; (d) distribution volume was also found to be high, thus confirming that the drug is highly lipophilic.

Tissue distribution

Binding to plasma proteins, studied *in vitro* with labelled [¹⁴C]Fotemustine in equilibrium dialysis, is low and not saturable. The main binding plasma proteins are α1-acid glycoprotein and human serum albumin. Moreover, 30% of drug binds erythrocytes.

Tissue distribution was studied by autoradiography methods using [¹⁴C]fotemustine in healthy mouse and in animals bearing subcutaneous B16 melanoma. These studies demonstrated rapid and high distribution volume. Most of injected [¹⁴C]Fotemustine radioactivity was observed in the liver, kidneys, lung and brain. Furthermore, high radioactivity was detected in the tumor, mostly in peripheral non-necrotic areas, being tumor/plasma ratio of 2:1 at 96 hrs. A study on tissue distribution of Fotemustine, conducted on normal rats or in animals bearing Walker A and B carcinoma, confirmed these results (18).

Metabolism and elimination

Fotemustine undergoes a rapid and extensive metabolism in all animal species studied (mouse, rat and monkey). The unmodified drug has not been detected in the urine. The urinary metabolic [¹⁴C]Fotemustine profile determined by HPLC with radiochemical analysis, was found to be similar in mouse and rat, whereas it is more complex in monkey (17). Two main metabolites were revealed in the urine of three animal species, i.e. acetic acid and 1-idantoin-ethyl-diethylphosphonate that was identified by mass spectrometry analysis.

The urinary excretion of [¹⁴C]Fotemustine in mouse, either normal or bearing B6 melanoma,

proved that both rate and excretion modality are similar in the two animals groups. Furthermore the peak of radioactivity was found 24 hrs after administration of the labelled drug. Faecal excretion appeared to be very low (3-5%) and less than 1% of the agent was detected in pulmonary breathing as chloroethanol whereas a variable fraction of 4-12% was identified as $^{14}\text{CO}_2$, indicating that the chloroethyl group was detached from the molecule. Similar results were obtained in a study on urinary excretion in monkeys treated with a single intravenous injection of 100 mg/m² of the drug (19).

Pharmacokinetics in humans

Intravenous administration (standard or high dose protocol)

The pharmacokinetic profile of Fotemustine was analysed in a multicentric study (20) that was performed in 66 patients treated with Fotemustine, administered intravenously for 1 hr. Two protocols were adopted i.e. 100 mg/m² once a week, for three weeks (44 patients) or high single dose (600-1000 mg/m²) for two days, followed by autologous bone marrow transplantation (22 patients).

When administered as intravenous infusion for 1 hr, the plasma concentration reached the steady-state in 45 min. After intravenous infusion the plasma concentration varied between 1 and 14 µg/mL, and declined quickly; so that the unmodified drug disappeared in the blood within three hours.

Administration through hepatic arterial infusion

Fotemustine was given as a standard dose by iv infusion for 1 hr (15 patients with colon carcinoma) or by hepatic arterial infusion for 4 hrs (10 patients with liver metastases). The comparative study on pharmacokinetic parameters proved that the area under the curve following intra-arterial infusion was much lower than that detectable after intravenous infusion, thus indicating a manyfold increase in drug concentration at the liver tumor site (21).

Excretion

Further studies on pharmacokinetics of the agent have been performed in two cancer patients inoculated with [^{14}C]Fotemustine. The results confirmed that (a) the excretion of metabolized Fotemustine is mainly urinary (50-60%); (b) faecal excretion is minimal (5%); (c) no unmodified drug can be detected in the urine (22).

Diffusion in cerebral parenchyma

Patients with intermediate or advanced stage of Hodgkin's or non-Hodgkin's lymphoma, were treated with a single high dose of Fotemustine (300 mg/m²). In this case, pharmacokinetic investigation to determine the fraction of the drug able to cross the blood-brain barrier showed that Fotemustine concentration in the cerebrospinal fluid reached 23% of plasma level (23).

Metabolism

The most important Fotemustine metabolites identified in the plasma after treatment with [^{14}C]Fotemustine have been found to be chloroethanol and N-nitrous-1-Imidazolidone-1-ethyl-diethylphosphonate (NIEDP). These metabolites were identified also *in vitro* as degradation products of the drug. Fotemustine catabolism appears to be mainly a chemical decomposition, associated with a minor enzymatic inactivation process (20-22).

Toxicology

Acute single-dose toxicity

In the mouse, several studies have shown that LD10 and LD50 are 20 and 50 mg/kg, respectively. In the rat it was impossible to determine LD50 because the drug is essentially insoluble at doses higher than 50 mg/kg (24, 25).

Acute and sub-acute toxicity

The sub-acute toxicity was studied in the rat, dog and monkey, with a weekly intravenous administration of the drug for 3-5 weeks. The chronic toxicity was studied, instead, with intraperitoneal and intravenous administration for 6 to 12 months. The most important toxic events were dose-dependent, retarded and cumulative, and concerned haematological parameters (thrombocytopenia, leukopenia and, less frequently, anemia). Liver and kidney toxicity was moderate, and less frequent when compared with that found in animals treated with BCNU (26).

Mutagenesis and Carcinogenesis

Nitrosoureas, including Fotemustine, as alkylating agents, have toxic effects on DNA, accompanied by mutagen and carcinogenic activity. However, Fotemustine shows reduced damage to DNA and possibly less mutagenic effects when compared with that of the other alkylating nitrosoureas of clinical interest (27). This has been later confirmed by Ashby J. et al. (28) who showed that Fotemustine is less mutagenic than BC-

NU, as revealed by the ad hoc assays performed on Salmonella, Drosophila and mouse bone marrow.

Therapeutic Activity

Preclinical Studies

The activity of Fotemustine was investigated in a number of tumor animal models. In particular, the agent was found to be highly active against L1210 and P388 leukemia inoculated intraperitoneal in histocompatible (H-2^d/H-2^d) recipient mice (18). In this case, the therapeutic efficacy was comparable to that of the classical BCNU nitrosourea. In additional studies conducted in rodents, Fotemustine showed appreciable activity against primary and metastatic M5076 reticular cell sarcoma injected intramuscularly in mice, and against subline A of the Walker carcinoma of the rat (18). However, cross-resistance between Fotemustine and BCNU was detected in experiments performed with BCNU-resistant L1210/BCNU leukaemia and ICIG-Ci4 fibrosarcoma in mice and Walker carcinoma subline B in the rat (18). Further studies were performed using a single intraperitoneal injection of Fotemustine in seven types of human brain tumor models, in nude mice (29, 30). In these studies Fotemustine activity was compared with that of BCNU, by assessment of tumor growth and volume, and Overall Survival (OS).

A single dose of Fotemustine (50 mg/kg) showed remarkable therapeutic effects in three out of four medulloblastomas. In particular, in animals inoculated with two of these tumors (i.e. IGRM34 e IGRM57), drug treatment resulted in 100 % of complete response rate (CR) and, respectively, 37 % and 100 % of long term survival. Additional experiments performed with IGRG88 glioma showed that treatment with Fotemustine produced 100% CR and 37% of long term survivors. Moreover, comparative studies between the antitumor effects of Fotemustine and BCNU against IGRM34 and IGRG88 medulloblastoma, showed that Fotemustine provided better results than those obtainable with BCNU treatment. Additional studies have been carried out with Fotemustine, BCNU and Temozolomide *in vivo*, in two human glioma models transplanted in nude mice (29). These drugs, administered intravenously, increased the survival time of the Hs683 oligodendroglioma-bearing mice, whereas temozolomide only induced a weak, although statistically significant, increase in U373 glioma-bearing mice.

Clinical Studies

Melanoma

In a number of phase I, II and III clinical trials, Fotemustine, either as a single agent or in association with other antineoplastic drugs, showed appreciable activity against metastatic melanoma that is generally considered highly resistant to chemotherapy (31- 33).

In phase II trials, Fotemustine alone showed a Response Rate (RR) of 26% and a RR of 25% at brain sites. Moreover, RR was found to be higher in those patients who have not been treated previously (30%) (34-37). However, the majority of clinical studies with this agent did not show therapeutic results significantly higher than those obtainable with Dacarbazine. On the other hand, Fotemustine could have some advantages over Dacarbazine, since it does not require metabolic activation by the liver, crosses the blood-brain barrier and should be more active than the triazene compound against MMRS-negative target tumor cells (see the previous paragraph on mechanisms of drug resistance). Actually, in phase III study on single drug treatment with Fotemustine compared with Dacarbazine in metastatic melanoma (33), a trend of longer survival was found in patients subjected to Fotemustine, if compared to those treated with Dacarbazine, although the difference did not reach statistical significance.

Fotemustine has also been studied in association with other drugs to improve chemotherapeutic efficacy. The combination of Fotemustine with Dacarbazine produced a 27% of overall RR and 21.5 weeks of response duration. These results are similar to those obtained with Fotemustine alone (38). However, better results were obtained with the combination of Fotemustine, Dacarbazine and Vindesine, with 32% of overall RR and 15% of CR (39). A subsequent study, in which Fotemustine was used in association with Dacarbazine, Cisplatin and Interferon- α , showed an overall RR of 38%, and a CR of 18% (40). Fotemustine in association with interferon- α gave an overall RR of 28% and 30 weeks of median response duration (41).

Because of the high risk of brain metastasis in melanoma, Fotemustine was studied, in a recent clinical trial, in association with whole brain irradiation versus Fotemustine alone. The results were evaluated in terms of cerebral response and time to cerebral progression in melanoma patients with brain metastases (42). The results did not show any significant difference between the two arms of the study (the best objective RR was 11% in the arm containing patients treat-

ed with Fotemustine alone and 17% in the combination arm). Nevertheless, it is not excluded that further trials enrolling a higher number of patients could provide sound evidence of a potential preventive activity of Fotemustine on brain metastases.

Fotemustine is likely to play a significant role also in malignant melanoma of the uvea that is considered more chemoresistant than cutaneous melanoma. In addition, patients bearing uveal melanoma are at higher risk of liver metastases, than those affected by the cutaneous disease. Compared to dacarbazine, that provided no more than 10% of RR, Fotemustine, in a multicenter study, induced 35.5% of RR, with a median survival from liver metastases diagnosis of 14.8 months (22, 43).

Finally, since in aqueous solution the drug is converted spontaneously into its active derivatives, Fotemustine can play an appreciable role in local perfusion of limb melanoma, as shown by relatively recent clinical trials (44, 45).

Primary and Metastatic Brain Tumors

Large clinical evidence is available showing that alkylating agents can have a role in the treatment of primary or metastatic malignancies at CNS level. Recently, particular attention has been given to Temozolomide for its noticeable efficacy in a variety of brain tumors (46). However, Fotemustine has been found to be effective in the treatment of medulloblastoma, malignant glioma, and brain metastasis of other solid tumors (30, 47). The drug was used as single agent, in association with radiotherapy (48-50), or with other antitumor compounds, such as Dacarbazine (51), Procarbazine (52), Temozolomide (53, 54) and other antineoplastic (55) and differentiating agents (56). All these studies have shown that Fotemustine is endowed with an appreciable antitumor activity at CNS level, along with acceptable toxicity and tolerability.

Hematological Malignancies

Little information is available from the literature about the possible use of Fotemustine in haematological malignancies. However, in a relatively recent clinical trial (57), this agent was found to be active and moderately toxic in relapsed multiple myeloma. In addition, in the last few years, increasing interest has been devoted to the role of mono-functional alkylating agents, such as triazene compounds, in the treatment of acute leukaemia, especially in case of leukaemia cells refractory to conventional chemotherapy (13, 58-66). Therefore, on the basis of some pharmacodynamic similarities between triazene compounds and Fotemus-

tine, it is reasonable to consider this agent as a good candidate to be investigated in acute leukaemia and lymphomas.

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