Glufosfamide as a new oxazaphosphorine anticancer agent

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Glufosfamide (β-D-glucose-isophosphoramide mustard, D-19575) belongs to the oxazaphosphorine class. Glufosfamide is a novel glucose conjugate of ifosfamide in which isophosphoramide mustard, the alkylating metabolite of ifosfamide, is glycosidically linked to the β-D-glucose molecule. Glufosfamide represents an attractive new agent for cancer therapy. Its mode of action on normal and pathological cells is still under experimental and clinical investigations. An assessment of the anticancer potential of glufosfamide is of key importance in therapy. The researchers reviewed the current knowledge available on glufosfamide tested in the preclinical studies/clinical trials, based on a collection of the original papers and conference abstracts published and relevant articles searched in the SCOPUS and MEDLINE database and websites. *Anti-Cancer Drugs* 22:488–493 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Oxazaphosphorine anticancer drugs

Oxazaphosphorines are a class of alkylating agents widely used in chemotherapy. Cyclophosphamide, ifosfamide, and trofosfamide represent an important group of oxazaphosphorines because of their anticancer activity against a variety of solid tumors and hematological malignancies. However, the clinical use of these chemotherapeutic drugs is limited by their cytotoxicity on normal cells and cancer cell resistance to their action. The dose-limiting toxicity and resistance to oxazaphosphorine agents, significantly hinder the effectiveness of chemotherapy [1–8].

Design of novel oxazaphosphorines

A potential approach to obtain more effective therapy is the design of novel analogs of alkylating agents [7,9,10]. Several oxazaphosphorine derivatives have been developed in an attempt to improve the therapeutic index. The new oxazaphosphorine agents, such as mafosfamide, *S*(–)-bromofosfamide, aldophosphamide perhydrothiazine, aldophosphamide tiazolidine, and glufosfamide, have been synthesized and tested [3,7,11].

Glufosfamide as a new oxazaphosphorine agent

Glufosfamide (D-19575, β-D-glucose-isophosphoramide mustard, β-D-Glc-IPM, CAS number: 132682-98-5) (Fig. 1) is the next generation glucose conjugate of ifosfamide. D-19575 is a novel oxazaphosphorine agent in which isophosphoramide mustard (IPM), the alkylating metabolite of ifosfamide, is covalently linked to β-D-glucose [12–14]. The development of this new drug was based on the rationale that cancer cells display an increased uptake and utilization of glucose [15–17].

Targeting potential of glufosfamide in cancer cells

The β-D-glucose moiety coupled to IPM leads not only to drug stabilization, but also uptake of the compound by rapidly proliferating cells. The increased glycolysis and accelerated rate of glucose transport are characteristic features of malignant transformed cells. Thus, it is accepted that glufosfamide is taken up preferably by cancer cells rather than by normal cells [15,18].

Owing to the hydrophilic properties of glufosfamide, its active transmembrane transport is required. It is known that cellular uptake of β-D-Glc-IPM is mediated by the transmembrane transport system of glucose. The glucose transporters mainly include the facilitative transporters (GLUT1-5) and the sodium-dependent glucose transporters (SGLT1-3), particularly the Na^+-D-glucose cotransporter SGLT3, formerly known as SAAT1. It has been found that the inhibitors of transmembrane glucose transporters, phlorizin and phlorentin, can reduce the action of β-D-Glc-IPM [4,15,19–21].

Glufosfamide represents an oxazaphosphorine analog with the potential to target the plasma membrane glucose transport system in cancer cells [3,18,20]. The difference between normal and cancer cells in terms of the expression of transmembrane sugar transporters may contribute to the selectivity of β-D-Glc-IPM. The accelerated metabolic rate and increased glucose consumption of neoplastic cells suggest that this oxazaphosphorine agent offers potentially enhanced cancer selectivity and introduces a novel concept for drug targeting [4,7,19].

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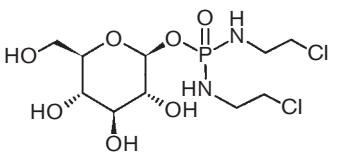
Fig. 1



Structural formula of glufosfamide.

Metabolism of glufosfamide

β-D-Glc-IPM is transported intactly into cells. On entry into cells, the active moiety IPM is thought to be released by either spontaneous hydrolysis or hydrolysis catalyzed by intracellular glucosidases [3,15,22,23]. Elucidating the metabolic pathway of glufosfamide in rats, two metabolites, IPM and monoaziridinyl derivative of IPM, were detected in their urine [24]. An interesting property of glufosfamide is the lack of release of acrolein because of the absence of the oxazaphosphorine ring in its structure. Moreover, β-D-Glc-IPM does not require metabolic activation by cytochrome P450 enzymes in the liver [4,7].

Glufosfamide appeared to be stable in neutral buffers, but it decomposed to form IPM under acidic and basic conditions, which was pH and temperature dependent. The stability of glufosfamide varied in different biological samples [25]. Moreover, it has been found that β-D-Glc-IPM is inherently biodegradable in the environment [26].

Pharmacokinetics and pharmacodynamics of glufosfamide

Glufosfamide transport and metabolism have a major impact on the pharmacokinetics and the pharmacokinetic–pharmacodynamic relationship. Pharmacokinetic and pharmacodynamic properties of glufosfamide have been studied in normal and tumor tissues [3,15]. Pharmacokinetic investigations and whole-body distribution of β-D-Glc-[^{14}C]-IPM in the rat have shown the highest level of [^{14}C]-D-19575 radioactivity in the liver, kidneys, thymus, thyroid gland, and the nervous system, including the brain [15,27]. Glufosfamide concentration in the rat plasma was also determined using liquid chromatography/tandem mass spectrometry [28]. Preclinical pharmacokinetic studies of β-D-Glc-IPM have shown low-protein binding, extensive tissue distribution, and rapid renal clearance [12]. Pharmacodynamic investigations carried out *in vivo* and *in vitro* have indicated the different response of various types of cells to glufosfamide action. For example, the in-vivo dose-dependent activity of β-D-Glc-IPM in terms of an increase in life span was found in murine leukemias and melanoma with different routes of administrations and schedules, with single versus repeated administrations [15]. In-vitro activity of glufosfamide was assessed in childhood acute leukemia and a very weak activity of this drug was observed against acute myeloblastic leukemia. However, in investigations carried out *in vitro*, this oxazaphosphorine agent showed a high activity against lymphoblasts both on diagnosis and on relapse. Moreover, glufosfamide cannot circumvent resistance to other oxazaphosphorines such as 4-HOO-ifosfamide, 4-HOO-cyclofosfamide, and mafosfamide cyclohexylamine salt [29,30].

During the clinical trials, plasma concentration–time profile of glufosfamide was determined. Pharmacokinetic parameters, such as terminal elimination half-life ($t_{1/2}$), area under concentration curve (AUC), clearance, and maximum concentration (C_{max}), were analyzed [Threshold Pharmaceuticals Inc. (*http://www.thresholdpharm.com*)] [31–36]. There was no effect of glufosfamide on C_{max} and plasma half-life observed in patients with recurrent glioblastoma multiforme (GBM) after surgery, radiotherapy, and no more than one line of chemotherapy [33]. In Japanese patients with advanced solid tumors, pharmacokinetic analysis showed a linear relationship between the AUC-versus-time curve and dose. The AUC values for IPM, the active metabolite of D-19575, were substantially greater than those achieved by bolus administration or continuous infusion of ifosfamide in conventional therapy [36]. The pharmacokinetic study of glufosfamide also indicated that hydration did not have a significant impact on the main pharmacokinetic parameters analyzed in patients with advanced non-small cell lung cancer (NSCLC), who had received one earlier line of platinum-based chemotherapy, [34] and in patients with advanced pancreatic cancer [32]. Moreover, the pharmacokinetic analyses carried out during the combined use of glufosfamide and gemcitabine in patients with solid tumors suggest no interaction between both these agents [35].

The pharmacological action together with pharmacodynamic and pharmacokinetic properties of glufosfamide results in its effects on pathological cells [15]. It should be emphasized that β-L-glucose-isophosphoramide mustard has no antineoplastic effects, whereas β-D-Glc-IPM shows anticancer activity [26].

Mechanisms of glufosfamide action

The mechanisms of the action of glufosfamide have not yet been fully elucidated. However, its effects on cells are accepted to be mainly dependent on its active alkylating agent, IPM. DNA molecule is known to be the main target of β-D-Glc-IPM action. Seker *et al.* [18] suggested that DNA crosslinks are the most critical lesions induced by β-D-Glc-IPM. Glufosfamide caused DNA strand breaks in Chinese hamster cells and in human histiocytic lymphoma U937 cells [37,38]. It has also been found that β-D-Glc-IPM impaired protein and DNA synthesis, and triggered the activation of poly(ADP-ribose)polymerase in breast carcinoma MCF7 cells [18]. Moreover, glufosfamide affected the level of Bcl-2 protein expression as well as activation of caspase 3, 8, and 9 in Chinese hamster

cells and human colon cancer cells [23,37]. A better understanding of the mechanisms responsible for the action of glufosfamide requires further investigations.

Induction of cell death by glufosfamide

DNA breakage, changes in Bcl-2 protein expression, and poly(ADP-ribose)polymerase and caspase activation are characteristic symptoms of cells undergoing programmed death. It has been shown that glufosfamide can trigger three forms of programmed cell death, namely, apoptosis, autophagy, and necrosis [37,39–42]. Mitotic catastrophe, a process preceding programmed cell death, was also observed in pathological hematopoietic cells [43]. Mitotic catastrophe and the programmed cell death-inducing potential of glufosfamide should be taken into consideration in cancer therapy.

Glufosfamide cytotoxicity in in-vitro and in-vivo preclinical studies

The cytotoxic effects of glufosfamide on normal cells and tissues were observed during the preclinical investigations carried out *in vitro* and *in vivo* [12,15,19,44,45]. The cytotoxicity of β-D-Glc-IPM for white blood cells and spleen colony-forming units CFU-S, seemed to be considerably lower, compared with ifosfamide [19]. Toxicity studies in rodents showed that glufosfamide was more toxic when given orally than when given intravenously, apparently because of a pronounced first-pass effect and an increased production of toxic metabolites. The intravenous infusion lethal dose for 50% of rats and mice was 1.7 and 3.8 times higher, respectively, compared with oral administration. Nevertheless, the acute and subacute toxicity profiles were similar for both routes of administration. The major toxicity targets were the bone marrow, kidney, skin, and the genital tract [3,4,7,12]. The extensive organ injury in Beagle dogs, after multiple intravenous administration of β-D-Glc-IPM was determined by Ding *et al.* [46]. Glufosfamide seemed to be genotoxic and cytotoxic to normal cells of the mouse erythropoetic system. In comparison with mafosfamide and 4-hydroperoxy-cyclophosphamide, it has been shown that D-19575, to a lesser degree, induced genotoxicity and cytotoxicity in the mouse erythropoietic system, triggering micronucleus formation and reducing the erythroblast proliferation rate [45]. The cytotoxic effects of glufosfamide on human leukemic cells were dependent on the dose of this agent applied, the time intervals after the exposure of cells to its action, and also on the cell line [40,41,44].

Side effects of glufosfamide application in clinical trials

During clinical trials, glufosfamide was generally well tolerated, with few drug-related serious adverse events [Threshold Pharmaceuticals Inc., Eleison Pharmaceuticals Inc. (*http://www.eleison-pharma.com*.)], [7,32–34,36,47]. Nausea, vomiting, and alopecia were reported to be the common side effects of glufosfamide treatment [Threshold Pharmaceuticals Inc., Baxter-Oncology (*http://baxter-oncology.com*)] [48]. Generally, it has been shown that hematological cytotoxicity was mild and nephrotoxicity was the major dose-limiting toxicity. In phase I and phase II trials, the adverse effects of glufosfamide on the bone marrow and the kidneys were observed mainly with a higher level dose of 6000 mg/m^2 (Threshold Pharmaceuticals Inc., Baxter-Oncology) [31–34,47]. Renal biopsies showed acute tubular necrosis and some degree of interstitial nephritis. The renal tubular damage was accepted to be related to the highly reactive IPM. This agent is supposed to be facilitated through active uptake of glufosfamide at the cell membrane of the proximal tubule, for reabsorption of filtered glucose [4,7]. The renal toxicity consisted of metabolic acidosis and an increased level of serum creatinine. Treatment-related metabolic and electrolytic abnormalities, such as the increased serum creatinine level, hypophosphatemia and hypokalemia, occurred in several patients. These occurrences indicated a possible renal toxicity in patients treated with glufosfamide [31,32,36,48]. Therefore, it was important to conduct intensive and close monitoring of renal function by regular detection of serum creatinine and electrolyte levels [4,31,32,47,49]. The renal toxicity of glufosfamide seemed to be unpredictable and not easily prevented by hydration [32,34].

A major advantage of glufosfamide over ifosfamide is that glufosfamide does not require mesna to protect from urothelial toxicity [1,34,50]. Glufosfamide action results in fewer side effects than other drugs in this class. These other oxazaphosphorine agents are known to cause hemorrhagic cystitis, a serious condition characterized by severe bladder bleeding. There are no reports of hemorrhagic cystis in patients treated with β-D-Glc-IPM (Threshold Pharmaceuticals Inc.). The adverse effects of glufosfamide in the urinary tract are avoided as no acrolein is formed. Free acrolein is responsible for the severe side effects in the urinary tract that are typical of alkylating agents [3,7].

Efficacy of glufosfamide in clinical trials

Several phase I and phase II clinical studies of glufosfamide were carried out. β-D-Glc-IPM was mainly used for the treatment of pancreatic adenocarcinoma, NSCLC, GBM, and head and neck squamous cell carcinoma [31–34,48,51].

During the phase I study, patients with refractory solid tumors were treated every 3 weeks with a two-step (fast/slow) intravenous infusion of glufosfamide over a 6-h period at doses of 800–6000 mg/m^2. The maximum tolerated dose was established as 6000 mg/m^2. The phase I trial with the 6-h infusion of glufosfamide, indicated enhanced selectivity for tumors that overexpressed transmembrane glucose transporters [31].

On the basis of promising antitumor activity of glufosfamide in the phase I studies, this oxazaphosphorine

agent was administered at a dose of 5000 mg/m^2 by a 1-h intravenous infusion regimen every 3 weeks, in the follow-up phase II studies. The glufosfamide first-line treatment of naïve patients with advanced or metastatic pancreatic cancer had modest activity [32]. The modest activity of β-D-Glc-IPM was also found in a multicenter phase II clinical trial in patients with advanced NSCLC, who received one earlier line of platinum-based chemotherapy. However, glufosfamide did not seem to have sufficient activity to support its further development as a second-line therapy for advanced NSCLCs [34,48]. During a phase II trial, β-D-Glc-IPM administered as a first-line chemotherapy showed antitumor activity in advanced colon carcinoma (Threshold Pharmaceuticals Inc.). In contrast, although carrying out a multicenter prospective phase II trial in patients with recurrent GBM, this oxazaphosphorine agent did not show significant clinical antitumor activity. Further investigations of β-D-Glc-IPM in recurrent GBM were not supported [33]. A phase II trial evaluated the activity and safety profile of glufosfamide administered as a second-line chemotherapy in patients with metastatic and inoperable breast cancer. β-D-Glc-IPM showed antitumor activity in patients with breast cancer, who had earlier received an alkylating agent [47]. The effectiveness of glufosfamide observed in the ex-vivo study, in patients with head and neck squamous cell carcinoma, suggested an equipotentiality of this drug in comparison with cis-platinum [51].

During the clinical trials, the anticancer potential of glufosfamide in patients with soft-tissue sarcoma and ovarian cancer was also determined. A phase II clinical trial for the treatment of patients with soft tissue sarcoma provided the evidence of the clinical activity of β-D-Glc-IPM. Enrollment was stopped in a phase II clinical trial in which β-D-Glc-IPM was used for the treatment of patients with platinum-resistant ovarian cancer because of lack of efficacy. Single-agent glufosfamide also did not seem to be an effective treatment option for women with ovarian cancer (Threshold Pharmaceuticals Inc.).

Recently, a phase I study determining the safety profile, pharmacokinetics, and antitumor activity of D-19575 in Japanese patients with refractory advanced solid tumors, has been carried out. The patients were treated with escalating doses of glufosfamide (3200, 4500, or 6000 mg/m^2), administered by a two-step (fast/slow) intravenous infusion for 6 h every 3 weeks. The data obtained indicate that D-19575 could be safely administered by infusion for 6 h at a dose of 4500 mg/m^2 every 3 weeks. The safety profile and antitumor activity of β-D-Glc-IPM show that the phase II studies of this drug are warranted [36].

Glufosfamide combined with other agents

Glufosfamide represents an attractive new agent for combination chemotherapy, which is now a recognized modality of cancer treatment. The effects of glufosfamide, in combination with gemcitabine, on in-vitro and in-vivo models of pancreatic cancer and other solid tumors, were studied [35,39,52]. The combined use of the alkylating agent glufosfamide and the DNA synthesis inhibitor gemcitabine resulted in an enhanced inhibition of tumor growth, increased level of apoptosis, and prolonged survival. These results have shown the usefulness of glufosfamide in combination with gemcitabine in the clinical treatment of pancreatic carcinoma [39]. A multicentered phase I/II trial determined the combined action of glufosfamide and gemcitabine as a first-line therapy in patients with advanced pancreatic cancer and other solid tumors. The data obtained indicated that glufosfamide at a dose of 4500 mg/m^2 could be safely given in combination with gemcitabine [35]. A dose-escalating study of glufosfamide and gemcitabine was also carried out during the phase II study. The combination of glufosfamide and gemcitabine seemed to be effective in pancreatic cancer. The safety and efficacy of this combination in chemotherapy-naïve pancreatic adenocarcinoma were evaluated [49]. In a randomized phase III trial, the effects of glufosfamide application were compared with the best supportive care in patients with metastatic pancreatic adenocarcinoma who were earlier treated with gemcitabine. This clinical study showed a low activity of glufosfamide in the refractory patient population [52].

Chemotherapy is also widely used in combination with other treatments such as ionizing radiation. Cancer control by radiotherapy can be improved with concurrent chemotherapy. Future agents for radiosensitization include glufosfamide with in-vitro activity against solid tumors such as sarcomas. The potential to treat the major causes of sarcoma treatment failure with concurrent chemotherapy during radiation should be considered [50].

Opportunity to improve glufosfamide efficacy

Clinical trials using glufosfamide are among the most recent clinical studies described in the current literature and presented at congresses [53–58]. Its mode of action is still under experimental and clinical investigations [36,38,42,49]. Data from clinical trials showed minor-to-modest antitumor activity, for example, in pancreatic cancer, NSCLC, whereas no activity was observed in GBM, and ovarian cancer (Threshold Pharmaceuticals Inc.) [32–34,48]. It is supposed that lack of substantial antitumor activity of glufosfamide is because of the degree of penetration of this agent into tumor cells, problematic dosing regimens, resistance, and/or fast elimination. New formulations for glufosfamide to slow down its elimination and change the dosing regimen, for example, longer infusion time, or combination with other antitumor agents with different mechanisms of action, can improve the therapeutic index. A better understanding of the action of glufosfamide on cancer and normal cells is important for its optional use in cancer therapy.

Glufosfamide development

Glufosfamide was originally developed from a research collaboration between Asta Medica and the Cancer Research Centre in Heidelberg, Germany in the 1990s. [12,14]. Further studies concerning the development of glufosfamide have been consequently conducted by Baxter Oncology GmbH in Germany and the European Organisation for Research and Treatment of Cancer Early Clinical Studies Group in the UK and Greece. Recently, glufosfamide has been under investigations with Threshold Pharmaceuticals Inc. in the USA, Latin America and Brazil, Eastern Europe and Russia, as well as in Japan and in certain Asian countries, in agreement with its development partner MediBIC. Threshold Pharmaceuticals Inc. focused on developing new cancer therapies by targeting the uptake and metabolism of glucose. Since 2009, Eleison Pharmaceuticals Inc., being under the agreement with Threshold Pharmaceuticals Inc., is responsible for the development, manufacturing, and marketing of glufosfamide (Threshold Pharmaceuticals Inc.) [14]. Developing more effective drugs is of key importance in chemotherapy and glufosfamide represents an attractive agent for cancer therapy.

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