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Comparative Anticancer Activity in Human Tumor Xenograft Models, Preclinical Pharmacology and Toxicology for 4-Hydroperoxyifosfamide (HOOI): A Potential Neuro-Alkylating Agent for Primary and Metastatic Cancers Involving the Central Nervous System

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Additional information is available at the end of the chapter

Abstract

Background: 4-Hydropeoxyifosfamide (HOOI) is a hydroperoxy derivative of ifosfamide that was developed as an anticancer agent that can penetrate the blood-brain barrier (BBB), which can be potentially useful in the management of brain tumors.

Methods: A novel synthetic scheme for HOOI is presented and verified. HOOI and an HOOI L-lysine salt were prepared and mice implanted intracranially (IC) and in the mammary fat pad with human U251 glioblastoma, D54 glioblastoma, and MX-1 breast tumor xenografts and treated with HOOI IP once daily for 1–5 days. The animals were monitored for responses, increased long-term survival (ILS) and long-term survival (LTS). Mice, rats, and dogs received single IV doses of HOOI in a wide range of concentrations and results are compared and presented herein.

Results: HOOI has been synthesized as per a new route in 67% yield. The drug is stable when frozen in the absence of moisture; however, as a lysine salt the drug is stable in solution and as a lyophilized product. HOOI produced complete responses with improved long-term survival against IC implanted U251 glioblastoma, D54 glioblastoma, and MX-1 breast tumor xenografts in mice. The drug was superior to 4-demethyl-4-cholesteryloxycarbonylpenclomedine (DM-CHC-PEN) and BCNU vs. IC implanted tumor models. The HOOI lysine salt demonstrated equal activity to that of HOOI alone. Over

all, the drug was well tolerated. Predictions for human pharmacokinetic parameters and dosing are made from allometric analysis using the above three species. Data predicted an acceptable starting dose of 39 mg/m^2 with a clearance of 11 L/h +/− 2.75 and a $T_{1/2\alpha}$ 15 min and $T_{1/2\beta}$ 5.30 h for a 70 kg human patient. The presented toxicity data plus strong antineuro-oncology activity supports DM-CHOC-PEN's proposed use as a treatment for CNS malignancies. The drug is being prepared for Phase I trial studies in the US–IND pending.

1. Introduction

Isophosphoramide mustard (IPM) (**Figure 1**) is the active metabolite of ifosfamide (IFOS) and a bifunctional DNA alkylator that generates guanine-cytosine interstrand cross-linking in G-X-C sequences producing cell death [1, 2]. Although IPM is the ultimate alkylator that is derived from IFOS, it has been removed from clinical trials because of lack of sufficient anticancer activity in clinical trials [3–5]. IFOS is still the phosphoramide mustard that is most used in sarcoma therapy; however, its use is hampered by requirement for hepatic activation and release of extracellular acrolein (ACR) and chloroacetaldehyde (CAA)—resulting in dose limiting cystitis, renal toxicity, and neurotoxicity, plus myelosuppression [6, 7].

4-Hydroperoxyifosfamide (HOOI, **Figure 1**) is a peroxide derivative of IFOS that spontaneously undergoes ring cleavage releasing acrolein and chloroacetaldehyde primarily *in situ* in cancer cells, not extracellularly in the general circulation as does IFOS [8–10]. The support data for HOOI's anticancer activity and toxicity is reviewed here.

DEKK-TEC's interest in HOOI was to document its potential usefulness as an anticancer agent and if it possesses any of IFOS' toxicities – cystitis, renal tubular necrosis, and CNS alterations, all of which hamper the usefulness and utilization of IFOS [6, 7]. A secondary goal was to develop a stable form of HOOI for clinical use [11, 12].
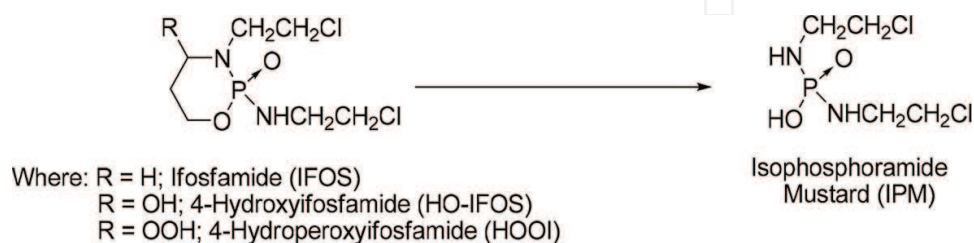

Where: R = H; Ifosfamide (IFOS)
R = OH; 4-Hydroxyifosfamide (HO-IFOS)
R = OOH; 4-Hydroperoxyifosfamide (HOOI)

Isophosphoramide Mustard (IPM)

Figure 1. Ifosfamide (IFOS) and derivatives.

2. Chemistry, formulations and analyses

HOOI and the L-lysine salt have been synthesized at DEKK-TEC, Inc., using GLP/GMP guidelines, previously described in detail [8, 9, 13–16]. The HOOI-L-lysine salt is a very stable chemical in the solid state under ambient conditions, soluble in water or saline and can be administered to animals in a saline solution (10%); the elemental analysis, NMR, mass spectra, and X-ray crystallography all agreed with the structure given in **Figure 2** [9].

Bulk HOOI and the L-lysine salt are stable as a lyophilized powder and can be stored at 20–23°C for up to 1.6 years without deterioration [16].

HOOI is a weak acid which in prolonged contact with water undergoes hydrolysis resulting in IPM and deterioration; thus, the stability of HOOI has always been an issue [16]. The HOOI structure can be stabilized with L-lysine, a basic amino acid. The optimized structure for HOOI as the L-lysine salt is a ternary (three-molecule) HOOI-2-Lys complex—as described from molecular mechanics and semiempirical computational analysis of the HOOI-Lys complexes (**Figure 2**) [16]. Two lysine molecules aligned themselves "above" and "below" HOOI. L-lysine stabilizes the HOOI through hydrogen bonding between the –NH-PO-NH- moiety and the ammonium group of the lysine. In this way, the ternary complex prevents water molecules from approaching the acidic –NH–P=O moiety, thus protecting HOOI from hydrolysis [9, 11, 12, 16].

HOOI and its L-lysine salt (bulk, as well as, in aqueous solutions – including biological) can be assayed/monitored with standard high-pressure liquid chromatography (HPLC) analysis (**Figure 3**) [16].

However, the most useful method to monitor HOOI in biological samples (blood, urine, etc.) is with GC/MS [8, 16, 17]. HOOI can be derivatized with t-butyl dimethylsilyl-N-methyl-tri-
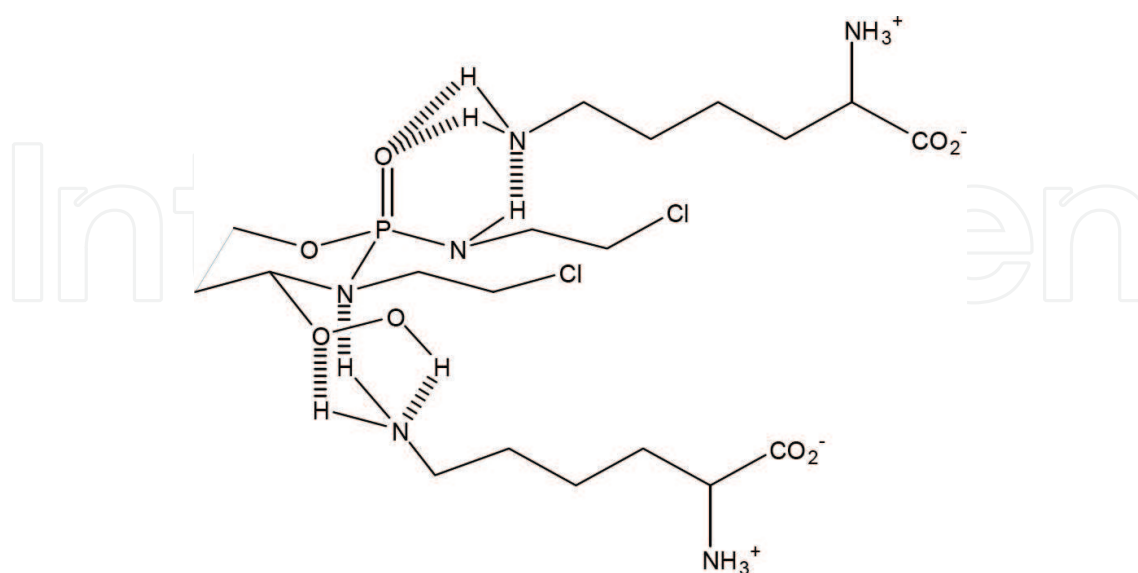

Figure 2. 4-HOOI/L-lysine.

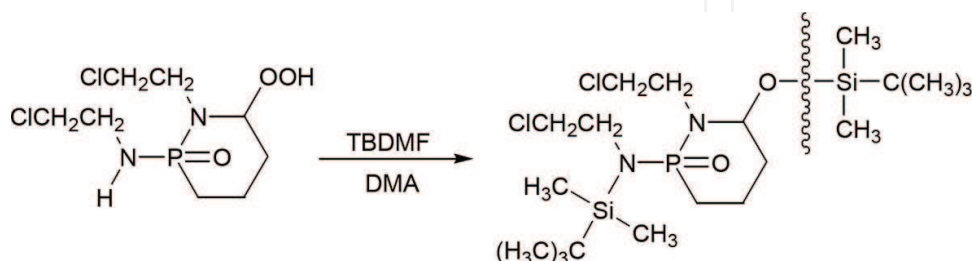**Figure 3.** HPLC: retentions – L- lysine-4.018 and HOOI-7.535.

Figure 4. Derivatization of HOOI.

fluoroacetamide (TBDMF), which is very stable, easy to prepare, and reproducible in GC/MS assays (**Figure 4**) [8, 16, 17].

The GC/MS chromatogram for pure HOOI-TBDMF had a unique peak at 15 minutes. HOOI's quantification can be performed by selected ion monitoring (SIM) at m/z = 406 amu (m = 520–114) of fragments corresponding to the mass spectrum of the *t*-butyl dimethylsilyl derivatized compound revealing the loss of the well-known *t*-butyl dimethyl *Si* group (m-114) (**Figure 4**). Differences between HOOI and 4-HO-IFOS (a metabolite of IFOS, **Figure 1**) cannot be made on GC/MS, but this is resolved with HPLC [8, 9, 16].

The TBDMF derivatization of HOOI yield is 90% and limits of quantitation are 10 ng/mL; the extraction coefficients from plasma and saline are 75% and 98%, respectively [16]. Validation of the assay was conducted using GLP guidelines, with reference to the reported values per Struck et al. [12].

The GC/MS assay also allows identification of IPM (**Figure 1**), the ultimate degradation product and active anticancer species generated from both IFOS and HOOI [16].

3. Antitumor evaluation *in vivo*

Antitumor evaluations for HOOI were performed employing standard GLP guidelines at Southern Research Institute and DEKK-TEC [8, 9, 16, 18]. Human xenograft tumors (U251 or D54 human glioma) were implanted intracranially (IC) into athymic *NCr-nu/nu* and the 9L rat glioma implanted subcutaneously (SC) into *Hsd:SD* rats, respectively in concentrations of 10^6 cells per animal [16, 18]. All approved and monitored under the respective IACUCs.

Drug	Dose (mg/kg)	Life span (% ILS)	Long-term survival (% LTS)
Control	Vehicle	0	0/9
HOOI	100	+54	89% (8/9 CR)
HOOI·lys	125	+54	100% (9/9 CR)
IFOS	400	5	0
TMZ	120	+54	60% (3/5 CR)

Treatment Schedule: 8 days post-SC implant admin.
HOOI, IFOS—IP once; TMZ—PO q 4 day × 3. Species: Hsd:SD rats—female, Harlan rats; study termination at 54 days.

Table 1. Activities of HOOI and HOOI-Lys vs. 9L rat glioma in rat implant: 10^6 cells SC.

HOOI and the L-lysine salt were prepared as 10% saline solutions and evaluated against the above rodent tumor models per IP administered in doses ranges of 25–300 mg/kg per dose/day × 5 days, which included the maximum tolerated dose.

Of significance is that HOOI was curative at 90 mg/kg/day × 5 days (84% long-term survival, LTS, with 20% CR) against the human U251 glioblastoma implanted IC. In contrast BCNU—the gold standard for years in the treatment of gliomas produced—no CRs, while temozolamide (TMZ) [120 mg/kg/d × 3 days], the current standard produced identical responses to HOOI [16, 18, 19].

HOOI vs. HOOI-L-lysine salt possessed similar activity in a rat glioma model. No weight loss or hematuria was noted with either HOOI or the L-lysine salt. In contrast, IFOS produced gross hematuria; both HOOI and the L-lysine salt were well tolerated (**Table 1**).

4. Pharmacology and toxicity

The results for the acute IV toxicity studies for HOOI in mice and dogs are presented in **Tables 2** and **3**, which includes the median lethal dose values observed. Two separate single IV mouse-dosing studies calculated an $LD_{10/50}$ of 200/385 mg/kg (for both sexes; with 95% confidence limits) [16].

Route	Dose (mg/kg)	Number and sex	Observations
IV	0	5 M 5 F	No death
	50	5 M 5 F	No death
	100	5 M 5 F	No death
	200	5 M 5 F	0 M and 3 F—died
	300	5 M 5 F	4 M and 4 F—died
	400	5 M 5 F	5 M and 5 F—died

Table 2. Acute IV toxicity for HOOI in the mouse – DEKK-TEC study (Single dose).

Route/schedule	Dose (mg/kg)	Number and sex	Observations
IV once	0	2 M	No death
	10	2 M	No death
	15	2 M	No death
	20	2 M	All died
	30	2 M	All died

Table 3. Acute IV toxicity for HOOI in the dog.

Clinical deterioration occurred in both sexes of mice and rats post-HOOI dosing in a dose-dependent manner. No seizures or loss of coordination were observed [16].

4.1. Acute studies in mice

Table 2 reviews the acute toxicity for HOOI when administered intravenously in single doses of 50, 100, 150, 250, and 400 mg/kg to adult male and female mice, 10 animals per sex per dose level [16, 17, 19].

No animals died at 0, 100, or 150 mg/kg, then a dose vs. lethal response occurred (**Table 2**). The cause of death was generally from cardiovascular collapse. No seizures or CNS toxicities were reported. No macroscopic findings were reported in any of the animals [6, 19].

Based on the conditions and findings of this study, the intravenous LD_{10} of HOOI was calculated to be 200 mg/kg (95% confidence limits could not be calculated) in mice (combined sexes). Acute intravenous toxicity study results are presented in **Table 2**.

4.2. Acute intravenous toxicity in dogs [16]

A dog study evaluated the acute toxicity of HOOI, when administered via a single intravenous (bolus) injection to dogs (**Table 3**). Male beagle dogs, in groups of two (2) were administered HOOI at dose levels–10, 15, 20, and 30 mg/kg. One additional group of two male animals served as the control and received the vehicle, 0.9% sodium chloride, administered once on Day 1 via intravenous single bolus injection, at a dose volume of 1 mL/kg. Following administration, all animals were maintained on study for up to a 14-day observation period. Blood work was obtained for complete chemistry, hematological, and urine analyses, plus timed-blood samples for pharmacokinetic studies throughout the study.

No treatment-related effects were noted on coagulation, clinical chemistry, and urinalysis evaluations, or on macroscopic and organ weight evaluations during the study. Treatment related mortality was noted during the study, but was limited to the 20 and 30 mg/kg dose groups. All animals in these groups were euthanized *in extremis* on Day 8 due to their deteriorating physical condition and following veterinary consultation. The respective groups that were administered the vehicle control, HOOI—10 and 15 mg/kg, survived to their scheduled necropsy (Day 15).

Treatment-related clinical findings noted during the study were most prominent at 30 mg/kg and included decreased activity, feces few/absent, yellow discharge from the eyes, emesis/vomit, with decreased activity, and salivation which were also noted at 20 mg/kg. However,

the animals in both these dose groups generally stopped (or nearly stopped) eating over time and would not respond to attempts to stimulate their appetite with canned food supplementary diet, leading to their continued deterioration and the need for subsequent early euthanasia *in extremis* on Day 8. At 15 mg/kg, some similar signs were seen (decreased activity, inappetence, and thinness in one animal; feces few/ absent in the other). The conditions of the latter dosed animals did not deteriorate over time (they responded to attempts to stimulate appetite with canned food supplementary diet), allowing them to survive to the scheduled necropsy on Day 15, as did the animals at 10 mg/kg, whose only noteworthy clinical finding was feces few/absent in one of the two animals [16]. Treatment-related body weight loss and correlated decreases in food consumption were noted at all HOOI dose levels, and exhibited a dose-response pattern of effect [16].

Alterations in hematology were noted as follows: erythrocytes, hemoglobin, and hematocrit decreased similarly in all treatment groups by Day 3, while the control animals tended to increase slightly. The erythrocytes and hematocrit tended to continue to decrease through Day 15 with the 15 mg/kg dose. At 10 mg/kg, there was little change in erythrocyte numbers of hemoglobin, but hematocrit continued to decrease slightly through Day 15. These changes in red cell parameters were accompanied by marked reductions of reticulocytes in treated animals, again with no dose-dependency. These reticulocytes were beginning to rebound at Day 15 in the surviving animals at 10 and 15 mg/kg. Total leukocytes tended to decrease slightly at 15 mg/kg by Day 3, and slightly more at 20 and 30 mg/kg. This was due primarily to dose-dependent decreases of lymphocytes, although neutrophils also decreased at Day 3 at 20 mg/kg. By Day 15, the lymphocytes were beginning to rebound at 10 and 15 mg/kg.

Treatment-related microscopic findings from necropsies (all animals) were limited to the bone marrow (femur, rib, and sternum), spleen, thymus, lymph nodes (mandibular and mesenteric), Peyer's patch, and sublingual salivary gland. Treatment-related depletions in the hematopoietic and lymphoid tissues were noted throughout the body. Bone marrow from of the femur, rib, and sternum had mild to moderate mixed depletion at 20 and 30 mg/kg. Mixed bone marrow depletion was characterized by decreased numbers of hematopoietic cells in erythroid, myeloid, lymphoid, and megakaryocytic lineages [16].

The spleen had minimal to moderate generalized lymphoid depletion in males at 10, 15, 20, and 30 mg/kg. Generalized lymphoid depletion in the thymus was considered to be increased in severity compared to controls in males at 15, 20, and 30 mg/kg. The mandibular and/or mesenteric lymph nodes had minimal to moderate generalized lymphoid depletion in males at 20 and 30 mg/kg. The Peyer's patch (gut-associated lymphoid tissue) had mild generalized lymphoid depletion in males at 20 and 30 mg/kg. Stress and/or an overall impairment of health potentially contributed to the development of hematopoietic and lymphoid depletion; however, these findings may indicate a direct test article effect in these animals. The sublingual salivary gland had minimal to moderate atrophy in males at 30 mg/kg. Salivary gland atrophy was potentially associated with a decrease in food intake; however, a direct test article-related effect cannot be ruled out [16]. The kidneys were normal [16].

The $LD_{10/50}$ for dogs was calculated to be 17.24/17.32 mg/kg. The experimental design is presented in **Tables 3** and **4**.

Study	Species and strain	No./sex	Age and wt	Route and mode	Pretest conditioning, dose and regimen	Results	Date/ laboratory
HOOI-12	Mice CD2F1	30 M 30 F	10 wks 19–24 g	IV Bolus	Single doses of 50–400 mg/kg of HOOI with observation × 15 days	LD_{10}—200 mg/kg	2007/ DEKK-TEC
793-017	Dogs Beagle	10 M	6 months 7.75–9.8 kg	IV Bolus	Single dose of 10–30 mg/kg of HOOI in a 24 day observation	LD_{10}—17.24 mg/kg LD_{50}—17.32 mg/kg	2008/MPI Research
Bevh-011	Hd Rats	20 F	10 wks 160–180 g	IP	Single doses of 100–150 mg/kg of HOOI and obs'd x 7 days	No evidence of impaired memory or learning	2008/ DEKK-TEC

Table 4. Acute toxicity summary study.

Table 4 summarizes the toxic effects of single IV dose administrations of HOOI in mice and dogs, which includes investigations on the acute toxicity performed in mice, rats, and dogs. The intravenous studies were conducted under FDA GLP guidelines [16].

4.3. Pharmacokinetics

The bioavailability for HOOI in one dog dosed once with IV HOOI 30 mg/kg is presented in **Figure 5**. The plasma HOOI was assayed employing the GC/MS method. In **Table 6**, all of the PK parameters are reviewed for all the dogs treated [16]. Overall, PK profile for HOOI in dogs revealed a two-compartment model with AUCs linear for all doses evaluated. The assay is sensitive to 20 ng/mL of HOOI [16]. Calculations were made as per methods previously reported [14, 15, 17, 21–23].

Model parameters were estimated using Micropharm software and nonlinear least squares regression was performed using Simplex and Gauss-Newton fitting algorithms (Statistical software available from Stat soft, Tulsa, OK) [17, 21]. An open two-compartment model provided the best fit. Clearance, volume of distribution, and half-lives were derived from estimates of the model parameters. Data analysis was performed on all plasma studies and analyzed via non-linear regression using a non-weighed quasi-Newtonian/simplex [17, 20, 21].

4.4. Brain/tumor penetration (CNS accumulation of drug)

Female athymic NCr-*nu/nu* mice were IC implanted with U25I glioma cells (10^6 cells) and divided into groups of 5 animals and administered HOOI (135 mg/kg/ day) in saline or saline (vehicle) (0.5 mL) IP daily for two consecutive days (qd × 2) beginning 4-day post inoculation
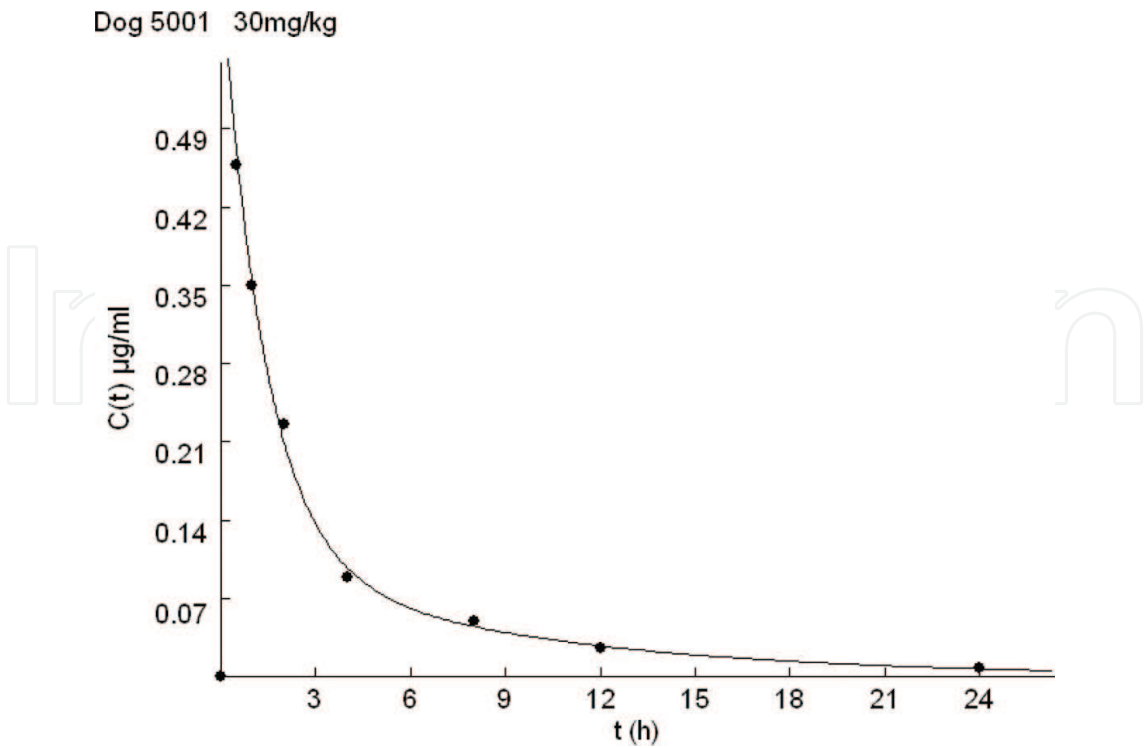
Figure 5. Bioavailability and pharmacokinetic profile for HOOI in dogs [16].

Parameter	15 mg/kg	20 mg/kg	30 mg/kg
AUC (mg·h/L)	0.49	0.96	1.66
	0.39	1.49	1.14
Cl (L/h)	30.35	20.63	18.01
	38.64	13.36	26.2
$T_{1/2}$ (h)	1.23	1.22	0.9
	0.68	1.51	0.24
$T_{1/2}\beta$ (h)	3.19	15.6	6.05
	3.09	13.56	1.85

Table 5. PK parameters in dogs treated with HOOI.

Drug	Species	Acute IV LD_{10}	Comparable human IV dosage[*]
HOOI	Mouse	200 mg/kg (600 mg/m^2)	60 mg/m^2 (10% of LD_{10})
HOOI	Dog	17.2 mg/kg (344 mg/m^2)	57 mg/m^2/d (1/6th of LD_{10})

[*]Standard conversion per FDA guidelines [26].

Table 6. Estimated comparable human intravenous dosages[*].

of cells. Four hours after the second treatment of each group, the animals were sacrificed and the brains removed intact and flash frozen in liquid nitrogen for storage [16, 17].

The encapsulated gliomas were easily identified and separated from normal brain with a scalpel and both homogenized separately in 10 mL 0.6 M phosphate buffer, pH 7.4 at 5°C [16, 17]. The cold homogenates were extracted with 10 mL chloroform, the organic layer separated and evaporated to dryness. HPLC and GC/MS analyses revealed HOOI in 100–126 ng/g glioma tissue. No drug was identified in the normal brain tissue homogenates. No chemicals or substances that could have interfered with the above extraction assays were noted in any of the tumors or normal brain tissues [16, 17].

4.5. Acute rat behavioral studies

HOOI vs. ifosfamide was evaluated in a modified rat neurobehavioral Morris water maze (18–20). Adult Sprague Dawley female rats (160–180 g) in groups of 3–6 rats per dosing were treated with single IP doses (MTD) of IFOS (400 mg/kg) vs. HOOI (200 mg/kg) vs. HOOI-Lys (300 mg/kg) and monitored with repeated timed swimming in the maze to find a hidden stage [17, 22–25].

The acute behavioral studies (latency to find a hidden platform in a Morris water maze—**Figures 6** and **7**) was analyzed by variance (ANOVA) [17, 18].

Body weights and water temperature—prior to each dosing and during each assessment were monitored. Necropsies were performed on all rats [17].

There were no significant differences in behavior between the animals that received saline (controls), HOOI, and its lysine salt on the memory and learning time intervals. The IFOS-treated animals had shakes and tremors for >7 h. (secondary to chloroacetaldehyde), but demonstrated normal learning behavioral patterns. Five days later, all rats treated with IFOS demonstrated hemorrhagic cystitis with gross bleeding, and bone marrow evaluations revealed pancytopenia. HOOI- and the lysine salt-treated animals did reveal hemorrhagic cystitis, but renal tubular necrosis was not observed in any animals. Histological examinations confirmed the gross observations.

A control memory agent, MK-801, and 5-flurouracil (5-FU) were included to demonstrate complete and temporary impalement, respectively. Neither HOOI nor the lysine salt had any influence on memory or learning, in contrast to IFOS which produced long lasting impairment [8].

4.6. Plasma levels of 4-HOOI, chloroacetaldehyde and acrolein

Adult, female C3H mice were dosed with a single IV MTD of cyclophosphamide (CPA) (250 mg/kg), IFOS (400 mg/kg), or HOOI (100 mg/kg); dogs were dosed with IV HOOI (30 mg/kg). Blood was collected and measured for HOOI, chloroacetaldehyde, and acrolein.

IFOS released CAA and ACR during hepatic metabolism and GC/MS assays were employed to quantitate the plasma HOOI, CAA, and ACR generated. For ACR and CAA, the Kobayashi et al. procedure (involving a pentafluorophenylhydrazine derivatization) was modified, validated with pure CAA and ACR and biological samples [16, 21, 25].

HOOI did not generate any detectable plasma levels of CAA in mice, in contrast to IFOS [16]. There is a striking inefficiency in the metabolic activation of IFOS to IPM *in vivo* [supported in the observed IV single dose LD_{10} for HOOI vs. IFOS (200 vs. 470 mg/kg) in mice]. The highest tolerated single IV dose tolerated for IFOS was 400 mg/kg and for HOOI is 100 mg/kg. Ideally we would have liked to use HOOI at 400 mg/kg to equalize doses, but that would be too toxic for HOOI and to reduce IFOS would have made it too low [7, 8, 11, 16].

ACR plasma levels were lower for HOOI in both dogs and mice. Mice, rats, and dogs dosed with HOOI did not demonstrate urinary hemorrhagic cystitis, in contrast to the IFOS treated mice, in which hematuria occurred 5-days post dosing. The results support our hypothesis that subjects treated with HOOI vs. IFOS would be exposed to lower plasma levels of ACR and no CAA with potentially reduced risks of developing hemorrhagic cystitis and no neu-rotoxicity. This is in agreement with Carlson et al. who measured 2.12 µg/mL for CAA @ 4 h after 1-h infusion of IFOS (400 mg/kg) in a clinical study [10].
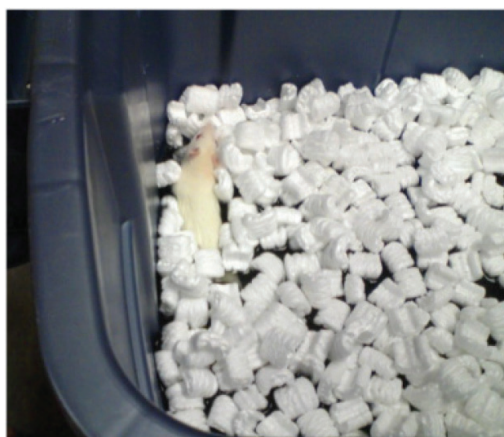

Figure 6. A rat swimming through the peanuts.

Figure 7. A rat on the hidden water maze platform.

5. Statistical analyses

Data analyses were performed on all plasma/tissue studies and analyzed with nonlinear regression methods using a nonweighed quasi-Newtonian/simplex fitting algorithms (Statistical software from Stat Soft, Tulsa, OK) [16, 17].

6. Discussion

The rationale for the preclinical development of HOOI was based on observed antitumor activity vs. intracranially implanted human tumor xenografts growing in mice and an anticipated reduction in renal toxicity and encephaloneuropathy that occur with standard IFOS and IPM therapy (1, 8, 9).

We review here the anticancer activities for HOOI in mice bearing intracranially implanted human xenografts and the results of acute toxicity and pharmacology studies with single intravenous injections in groups of mice and dogs. The end-point of all the studies was to document anticancer activity and drug toxicity for HOOI and an acceptable starting dose for a Phase I clinical trial in humans with advanced cancer. The anticancer activities, toxicology, and pharmacology studies reported in **Tables 1–4** support the clinical development of HOOI.

Human subjects treated with IFOS develop CNS toxicities which appear to be due to the CAA that is generated from the dechloroethylation of either of the two 2-chloroethyl moieties [2, 7, 10].

These cyclic mono-dechloroethylated metabolites of IFOS undergo 4-hydroxylation, resulting in a 4-hydroxyl dechloroethylated IFOS metabolite in which the ring opens resulting in the corresponding aldehydes with subsequent elimination of ACR. The use of HOOI would bypass the dechloroethylation step seen with IFOS. The ACR formation would be significantly reduced and therefore lead to a significant reduction in the incidence of hemorrhagic cystitis and renal tubular necrosis, which are common toxicities associated with IFOS therapy [2].

The observations presented in the **Table 5** support DEKK-TEC's hypothesis that subjects treated with HOOI vs. IFOS would be exposed to lower plasma levels of acrolein and no chloroacetaldehyde with potentially reduced risks of developing renal tubular necrosis and with no neurotoxicity. There is a striking inefficiency in the metabolic activation of IFOS to IPM *in vivo* (3). This is noted in the observed LD_{10} for HOOI vs. IFOS (200 vs. 470 mg/kg) in mice. Because of this, an effective clinical IFOS dose is harder to achieve because of intrasubject metabolism variability. Since HOOI does not require hepatic activation *in vivo*, a reduced intrasubject variability in the clinic is another potential advantage of administering HOOI.

Renal tubular necrosis and CNS toxicity have not been noted with HOOI in the animal studies. In the mouse study, HOOI did not generate any detectable chloroacetaldehyde and only 20% of the acrolein produced by equivalent doses of IFOS. The latter difference is because HOOI does not appear to be a substrate for microsomal metabolism and enters cancer cells intact and releases IPM and ACR *in situ*. Neither proximal tubular necrosis nor Fanconni syndrome was observed in the rat or dog studies at final necropsy [1–3, 11, 13].

Specific emphasis has been placed on documenting potential toxicities associated with IV administered HOOI, a peroxide—capable of producing convulsions, renal damage, hemolysis, arterial gas emboli-pulmonary damage, and neurological pathology—none of which have been noted.

In dog studies, animals that received HOOI, leukocytes, and neutrophils were moderately variable—decreased on Day 2 and at termination. Predominant organ(s) defect at autopsy was depletion of splenetic lymphocytes. Bone marrow was microscopically minimally to moderately depressed with acceptable ratios of blood elements. No other evidence of toxicity was noted at autopsies, including careful complete examinations of the bone marrow and brain.

All dogs in the 10 and 15 mg/kg dosage groups survived to the scheduled necropsy on Day 15, while groups administered 20 and 30 mg/kg were euthanized *in extremis* on Day 8 following veterinary consultation due to their deteriorating physical condition, primarily a worsening lack of appetite and associated/expected physical deterioration over time. At the gross necropsy of animals euthanized *in extremis*, three of the four animals had no findings while one animal exhibited mild red discoloration of the duodenum and mucosa of the small intestine. Other than spleens devoid of blood element precursors other pathology was noted.

Based on the results and outcomes of the dog HOOI study, the LD_{10} was calculated to be 17.2 mg/kg and the LD_{50} was 17.3 mg/kg. Thus, the intravenous LD_{10} single-dose value for mice and dogs (sexes combined) were calculated as 200 and 17.2 mg/kg, respectively, and agreed well.

Clinical studies have revealed very high urinary levels of ACR and CAA in subjects dosed with IFOS [10]. In one study, 48% of the dose was excreted as the dechloroethylated metabolites, while unchanged drug and carboxyifosfamide, the other major metabolite, accounted for only 4.7% and 2.2%, respectively [21]. Consequently, circulating levels of the dechloroethylated metabolites of IFOS could conceivably be a secondary source of CAA, in addition to the parent drug, which is very likely the primary source.

In the present study, the metabolism of HOOI *in vivo* was not observed to be a source of CAA. HOOI is probably less likely to be a substrate for cytochrome P450 oxidation to generate an exocyclic hemiacetal at an α-carbon in either of the two 2-chloroethyl moieties (with subsequent elimination of CAA), but is readily converted directly to carboxy-IFOS and IPM.

It is also reasonable to expect that clinical doses of HOOI would be significantly less than that for an equivalent amount of IFOS from the experimental studies (e.g., single MTD for HOOI 100 mg/kg vs. 400 mg/kg for IFOS), thus less drug would be available to form CAA. The bioavailability profile supports the single dose schedule, which is acceptable with the FDA [26].

HOOI possesses two (2) 3-high energy atom chains [**-OOH** and **-O-P->O**] (**Figure 1**), thus the drug more than enough 'fits' the criteria proposed earlier that high energy drugs like HOOI have a propensity to enter cancer cells- that are low in energy, but high in energy requirements [17]. Furthermore, the drug's lipophilicity and the ability to accumulate in glioblastomas growing in the brain, make it a very desirable drug to develop for the treatment of brain tumors [16, 17].

No CNS/behavioral alterations or toxicities have been noted for HOOI or its L-lysine salt [16, 19].

Thus, preclinical studies, conducted under GLP guidelines are reviewed and are supportive for HOOI's entry into Phase I clinical trials as treatment for advanced cancer with CNS involvement. **Table 6** reviews calculated starting doses, and data that satisfied the FDA's requirements for an IND [26]. The initial level of dosing in the Phase I clinical trial has been established as 60 mg/m^2 [9, 16, 19, 26].

7. Conclusion

Over all, the drug was well tolerated. Predictions for human pharmacokinetic parameters and dosing are made from allometric analyses using the above three species. Data predicted an acceptable starting dose of 60 mg/m^2 (from mouse and dog studies). The presented toxicity data plus strong antineuro-oncology activity supports HOOI's proposed use as treatment for CNS malignancies. The drug is being prepared for the US–IND pending Phase I trial studies [26].

Acknowledgement

Disclaimer

Multiple patents have been filed based on the information obtained for the drug, 4-hydroperoxyifosamaide (HOOI), from DEKK-TEC's group. Portions of that information plus techniques and results generated from our group that have been published are included in this chapter and appropriately referenced. All of the previously published studies and reports presented are referenced and are from DEKK-TEC's laboratories and contract facilities.

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