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Comparative pharmacokinetic profile of carboxyamidotriazole and carboxyamidotriazole-orotate

Research Article

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Abbreviations: area under the curve, (AUC); Carboxyamidotriazole, (CAI); distribution/absorption, (D/A); intraperitoneal, (i.p.); intravenous, (i.v.)

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Summary

Carboxyamidotriazole (CAI) is a novel antineoplastic agent in clinical development with limited oral bioavailability, but has significant gastrointestinal and neurotoxicities. In contrast, when CAI was converted to CAI-orotate, it was found to enter the bloodstream faster and achieved higher plasma concentrations while maintaining a similar elimination half-life relative to CAI. This comparative pharmacokinetic analysis was performed in rats. CAI-Orotate produced an increase in area under the curve (AUC) and Cmax and the time to reach Cmax was shortened. This may allow smaller dosages of CAI needed for inhibition of tumor cell proliferation with the added advantage of reduced toxicity. The clinical utility of such an improved delivery of CAI as an orotate derivative is being explored.

I. Introduction

Carboxyamidotriazole (CAI) was originally developed as an anti-parasitic agent but has since been shown to have anti-angiogenic effects, probably due to inhibition of voltage-independent calcium signal transduction necessary for VEGF-induced endothelial proliferation (Kohn et al, 1995; Ge et al, 2000; Antoniotti et al, 2003; Oliver et al, 2003; Franklin et al, 2004). This has led to investigation of this agent as an inhibitor of tumor growth due to interference with the formation of an adequate microvasculature to support such growth. Such anti-angiogenic activity has been shown in chick chorioallantotic membrane assay and human umbilical vein endothelial cells in vitro (Kohn et al, 1995; Ge et al, 2000; Antoniotti et al, 2003; Franklin et al, 2004). CAI inhibits proliferation characteristics in several tumor cell lines in vitro such as prostate, glioblastoma, breast cell lines and others (Kohn and Liotta 1990; Kohn et al, 1992; Enfissi et al, 2004; Migneno et al, 2005; Guo et al, 2006) and in cancer patients ((Figg et al, 1995; Kohn et al, 2001). Orally administered CAI showed a decrease in tumor voume and blood vessel density in tumors from nude mouse xenografts of human melanoma implants (Oliver et al, 2003).

CAI is a small molecule inhibitor of non-voltage gated channels, calcium influx and intracellular calcium signaling and regulation. It inhibits transmembrane calcium flux in nonexcitable cells including endothelium and cancer cells (Felder et al, 1991; Kohn et al, 1994; Wasilenko et al, 1996; Wu et al, 1997; Alessandro et al, 1998). CAI down regulates inhibits production of basic fibroblast growth factor signaling, matrix metalloproteinase-2 production and VEGF-stimulted

proliferation and motility (Kohn et al, 1994; Alessandro et al, 1998; Neufeld et al, 1999). CAI also reduces production of VEGF and IL-8 from tumor and endothelial cells (Wu et al. 1997). The effects on calcium flux center around disruption of thapsigargin induced calcium entry, G-protein coupled recetor mediated calcium influx and influx downstream of IP3 (Kohn et al, 1995; Alessandro et al, 1998; Masiero et al, 1999).

Clinical trials have been done characterizing its pharmacokinetic and pharmacodynamic profile with variable results, perhaps due in part to the variable pharmacokinetic profile and poor bioavailability of CAI (Berlin et al, 1997; Bauer et al, 1998; Dutcher et al, 2005). A recent study published by Desai et al, 2004, employed ketoconazole to inhibit CYP3A4-mediated metabolism of CAI to achieve high enough therapeutic levels of CAI. Several early phase clinical trials with CAI have been done including Kohn et al, (1996) showing efficacious blood levels could be achieved with oral administration with the most common side-effect being nausea. Kohn et al. (2001) also showed dose-dependent CAI concentrations in blood and showed an positive interaction with paclitaxel in terms of efficacy, while the most common adverse effect again was nausea. Somewhat similar results were found in other clinical trials using micronized CAI, although nausea was not as frequently observed. (Berlin et al, 2002; Kohn et al, 1997)

Due to its hydrophobic properties, oral absorption of CAI is variable and low, most likely <57%. Increasing aqueous solubility by synthesizing the orotate salt form of CAI (**Figure 1** for chemical structures of CAI and CAI-orotate) may increase oral bioavailability and reduce the time of appearance of CAI into the bloodstream. Studies in animals and man show CAI to have an unusually long time to maximal plasma concentrations (Tmax) showing a slow rate of absorption (Figg et al, 1995). The goal of this study was to determine whether the increased hydrophilicity of CAI-orotate would improve oral bioavailability and rate of absorption compared to CAI in rats.

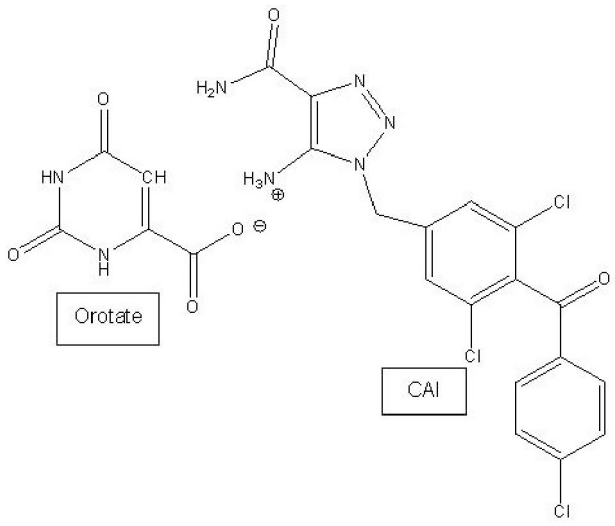

Figure 1. Chemical structure for CAI and CAI-orotate.

II. Materials and methods

A. Oral and intravenous CAI or CAI-orotate animal studies

Male Sprague-Dawley rats (150-200 g) were used for these studies. All studies were done in accordance with the Helsinki accords and were in compliance with the Eurofins animal use and care committee, which was where all animal studies were done. For CAI and CAI-orotate, we used separate animals for oral gavage (p.o.) studies (n = 6 per group) and intravenous (i.v.) injection studies (n = 2 per group). In the p.o. study, the animals were orally gavaged with CAI or CAI-orotate in equimolar doses (100 mg/kg and 137 mg/kg for CAI and CAI-orotate respectively). Both compounds were suspended in trioctanoin (Sigma Chemical Co.) and given at a volume of 0.5 mL per kg. Blood was then collected (into EDTA) via the retroorbital plexus at 0.33, 1, 2, 4, 16, and 48 hours after gavage. Plasma was collected from the blood for analysis of CAI levels using a previously validated LC/MS/MS method described below.

In the i.v. studies, CAI or CAI-orotate in DMSO was administered via the tail vein at equimolar doses of 10 and 13.7 mg/kg, respectively. Blood samples were collected into EDTA from the retroorbital plexus at 0.08, 0.25, 0.5, 1, 2, 4, 8, 12, 16, 24, and 48 hours. Plasma was collected from the blood for bioanalytical analysis. A sample size of 2 was selected because the i.v. study was only used as a reference to determine oral bioavailability.

1. Bioanalytical analysis

The concentration of CAI in plasma was determined using an high-performance liquid chromatography mass spectrometric assay, with the lower limit of quantitation of 20 ng/mL (Figg et al,; 1995; D'Argenia et al, 1979). All samples were assayed in duplicate and the mean concentration was used for pharmacokinetic analysis.

2. Pharmacokinetic analyses

Data for the oral or intravenous studies were analyzed using Summit Software PK Solutions Pharmacokinetic Software by Summit Software (Montrose, CO). The pharmacokinetic parameters calculated were: time to maximal concentration (T_{max}, both observed and calculated, hrs), maximal plasma concentration (C_{max}, both observed and calculated, ng/mL plasma), elimination half-life ($T_{1/2}$, hrs), area under the curve (AUC ∞, ng-hr/mL) using the trapezoid rule. Calculated values for C_{max} and T_{max} were only used for bi-exponential oral values. The slope was expressed as the rate or 2.303 x slope (1/hr) calculated from the linear fit of absorption phase. Oral bioavailability was calculated by AUC ∞ for oral dosing/ AUC ∞ for intravenous dosing x 10.

B. Evaluation of CAI and CAI-Orotate Compared with cisplatin-induced emesis in ferrets

The objective of this study is to evaluate the effect of CAI and CAI-orotate on inducing emesis in the ferret, compared with cisplatin as described by Wong et al, 1997). A group of *Mustela putorius furo* ferrets was received from Marshall Farms. The animals were singly housed in suspended stainless rabbit cages that conform to the size recommendations in the most recent Guide for the Care and Use of Laboratory Animals DHEW (NIH). Litter paper was placed beneath the cages and was changed at least three times per week during acclimation. The animal room was temperature controlled and had a 12-hour light/dark cycle. The animals were fed Purina Ferret Lab Diet and filtered tap water was supplied ad libitum by an automatic watering system.

Following acclimation to the laboratory for 9 days, 18 healthy male ferrets weighing between 803 and 1067 grams were selected for test and distributed into the following four groups of animals (**Table 1**).

Prior to dosing, each group of animals was fasted overnight by removing feed from their cages. During the fasting period, the ferrets were examined for health and weighed (initial). Individual doses were calculated based on these fasted bodyweights, taking into account the concentration of the suspension. Each test group animal (Groups 3 and 4) received a single administration of the test article by oral intubation using a gavage cannula. Group 2 animals received a single intraperitoneal (i.p.) injection of cisplatin and 2 mL/kg of trioctanoin by oral intubation. The test articles (Groups 3 and 4) were administered as w/w suspensions in trioctanoin (medium chain triglyceride), as described above. All animals were dosed at a constant volume of 2 mL/kg. The control group (Group 1) received 2 mL/kg of trioctanoin by oral intubation and was maintained under similar environmental conditions.

After administration, each test animal was observed for emetic responses for five hours. Observations included, but were not limited to, retching, vomiting, lip licking, drooling, general malaise and any other sign that may have been considered atypical ferret behavior. During observations, the number of times the animal retched or vomited was counted. All ferrets were euthanized using an overdose of isofluorane following the observation period.

C. Statistics

All comparisons between group with respect to time were done using a one way analysis of variance. A Newman-Keuls post-hoc test was used to determine differences between specific groups. All data are shown as mean $\pm$ standard error of the mean

III. Results

A. Pharmacokinetic studies

The mean plasma values of CAI with respect to times after treatment with CAI or CAI-orotate are shown in **Figure 2.** The pharmacological parameters of interest for the oral study are shown in **Table 2**. CAI was detected in the plasma sooner. CAI-orotate caused significantly greater plasma concentrations of CAI at all times except 48 hours when drug was eliminated. The faster rate of appearance is shown in **Table 2** as a significantly greater distribution/absorption (D/A) slope. The elimination half-life, however, is similar between groups, suggesting similar drug disposition.

Table 1. Acclimation to the laboratory for 9 days

Group #	No. of Animals	Test/Control Article	Dose Level (mg/kg), Route	Test Suspension Concentration (%)
1	3	Vehicle Control (trioctanoin)	0, PO	-
2	5	Cisplatin + Vehicle (trioctanoin)	10, IP + 0, PO	0.5
3	5	CAI	250, PO	12.5
4	5	CAI-Orotate	343, PO	17.15

PO – oral intubation, IP – intraperitoneal injection

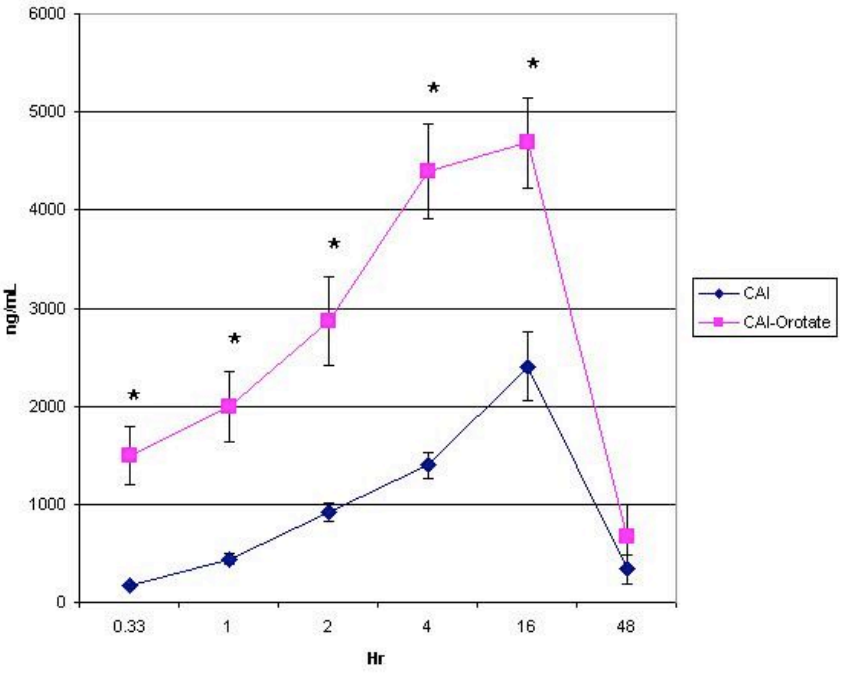

Figure 2. Mean plasma concentration of CAI with respect to time after oral CAI or CAI-orotate treatment. *denotes significance from its respective CAI-orotate value, $p < 0.05$.

Consistent with the data shown in **Figure 2**, Cmax (both observed and calculated, **Table 2**) was significantly greater for CAI-orotate compared to CAI. The time to peak plasma concentrations was shorter for CAI-orotate compared to CAI. The AUC for CAI-orotate was significantly greater compared to CAI. These data were used to calculated % oral bioavailability from the i.v. data shown in **Table 3**. When normalized for the i.v. AUC, CAI-orotate had a 20% higher oral bioavailability compared to CAI.

The i.v. data for CAI-orotate and CAI are shown in **Table 3**. The elimination half-life for CAI-orotate i.v. was double that for CAI, although the relevance of this is unknown. Statistical analysis was not done due to a small sample size of 2 for the respective groups.

B. Observations of ferret responses

1. Group 1 - Trioctanoin (medium chain triglyceride) - 2 mL/kg, PO

The vehicle at a dose of 2 mL/kg PO produced lip-licking, a sign of pre-emesis or nausea, in all three ferrets during the first 15 minute observation period. Mild retching was observed in one ferret during the second observation period. No other signs or symptoms were observed during the five hour period.

2. Group 2 - Cisplatin - 10 mg/kg IP + Trioctanoin - 2 mL/kg PO

Mild to moderate retching was noted in four of five ferrets. The onset was variable. Lip-licking was noted in the same four of five ferrets. One ferret not showing signs of pre emesis or retching did show signs of significant malaise throughout the observation period. Cisplatin produced typical behavioral effects in all ferrets that retched.

3. Group 3 - CAI - 250 mg/kg PO

CAI produced mild retching in four of five ferrets. Lip-licking was noted in all ferrets. Retching, when observed was delayed with an onset greater than 60 minutes. No significant behavioral effects were noted other than possible drowsiness.

4. Group 4 – CAI-Orotate - 343 mg/kg PO

Mild retching was only noted in one ferret and occurred during the 3-4 hour observation period. Lip-licking was observed in three ferrets. No other abnormal behavior was noted and the ferrets appeared normal other than possible drowsiness.

IV. Discussion

A. Pharmacokinetic studies

CAI has anti-tumor efficacy thought to be due to several mechanisms including anti-angiogenic activity and anti-proliferative activity. CAI has no known cytotoxic effects that contribute to anti-tumor efficacy. It has been shown to be effective in several models of tumor growth, both *in vivo* and *in vitro*. Like many anti-neoplastic agents, the therapeutic window of efficacy vs side effects is not perfect with one of the most common side effects being nausea and vomiting. In addition, CAI has variable pharmacokinetic characteristics, further complicating its effective use. Its poor aqueous solubility leads to variable if not poor bioavailability. Synthesis of the orotate salt form of CAI is hypothesized to increase oral bioavailability and increase the rate of absorption. Both CAI and CAI-orotate have long plasma half lives when given orally and this was seen in the present study in rats. CAI is highly protein bound and is primarily metabolized in the liver (Figg et al, 1995).

The results of the present study show that the orotate salt of CAI gets into the bloodstream faster than CAI. In addition to a faster rate of absorption, oral bioavailability is significantly increased, leading to higher plasma concentrations. This may have distinct advantages as lower doses may need to be delivered and more consistent blood levels can be obtained. This will make it possible to use lower total doses and also make it potentially easier to use as adjunctive therapy with cytotoxic anti-neoplastic agents. It is not clear how CAI-orotate will affect the side effect profile but preliminary studies performed suggest a modest reduction in emesis and pre-emetic behavior. In this study, ferrets were treated with i.p. 10 mg/kg cisplatin, p.o. 250 mg/kg CAI or p.o. 335 mg/kg CAI-orotate (equimolar dose to CAI) and 4 out of 5 ferrets showed mild to moderate retching for CAI and cisplatin while only 1 out of 5 animals showed mild retching for CAI-orotate. These preliminary data suggest that CAI-orotate produces less nausea, perhaps by rapidly exiting the gastrointestinal tract.

In summary, CAI-orotate enters the bloodstream faster and achieves higher plasma concentrations while maintaining a similar elimination half-life relative to CAI. This may allow smaller dosages needed for inhibition of tumor cell proliferation with the added advantage of reduced toxicity. Use of orotate salts may also be useful for other anti-neoplastic drugs, therefore reducing the doses necessary for efficacy. In the present study, neither CAI or CAI-orotate appeared to have the acute toxic effects seen in patients (Figg et al, 1995; Berlin et al, 1997; Desai 2004). They tolerated the dose well with no obvious effects other than mild retching over the 5 hour observation period. CAI-orotate appeared to have less potential for producing pre-emetic behavior and retching

Table 3. Pharmacokinetic parameters for intravenous CAI-orotate or CAI.

	CAI-Orotate	CAI
Elimination half-life, hr	4.1 + 0.06	2.41 + 0.01
AUC ∞, ng-hr/mL	22272 + 743	14748 + 783

than CAI. It should be stated that it these studies are preliminary and further testing is needed to show that there are therapeutic advantages vs side effects for the orotate salt. It is also important to note that the most common side effects of CAI in liquid formulations and gelatin capsules are nausea and vomiting and the dose-limiting toxicities were cerebellar ataxia and with micronized CAI reversible cerebellar ataxia and confusion were the most common side-effects (Berlin et al, 2002; Kohn et al, 1996, 1997). Perhaps using lower doses of CAI-orotate will reduce these side-effects.

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Table 2. Pharmacokinetic parameters for oral CAI-orotate and CAI.

CAI Orotate	CAI
D/A Phase rate 1/hr	
0.22 + 0.02	0.15 + 0.01**
Elimination half-life hr	
12.5 + 2.5	12.8 + 3.3
Cmax (obs) ng/ml	
4683 + 461	2408 + 358**
Tmax (obs) hr	
12.0 + 2.5	14.5 + 1.5
Cmax (calculated) ng/ml	
6418 + 857	2940 + 419**
Tmax (calculated) hr	
8.3 + 0.9	8.7 + 1.0
AUC □, ng-hr/ml	
158354 + 10233	84234 + 9756*
Vd (area)/kg ml/kg	
14480 + 2857	24131 + 7514*
% oral bioavailability	
74%	57%

*denotes significance from its respective CAI orotate value, $p < 0.05$; ** denotes significance from its respective CAT orotate value, $p < 0.01$.

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