The Janus Kinase 2 Inhibitor Fedratinib Inhibits Thiamine Uptake: A Putative Mechanism for the Onset of Wernicke’s Encephalopathy

Qiang Zhang, Yan Zhang, Sharon Diamond, Jason Boer, Jennifer J. Harris, Yu Li, Mark Rupar, Elham Behshad, Christine Gardiner, Paul Collier, Phillip Liu, Timothy Burn, Richard Wynn, Gregory Hollis, and Swamy Yeleswaram

Incyte Corporation, Wilmington, Delaware

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ABSTRACT

The clinical development of fedratinib, a Janus kinase (JAK2) inhibitor, was terminated after reports of Wernicke’s encephalopathy in myelofibrosis patients. Since Wernicke’s encephalopathy is induced by thiamine deficiency, investigations were conducted to probe possible mechanisms through which fedratinib may lead to a thiamine-deficient state. In vitro studies indicate that fedratinib potently inhibits the carrier-mediated uptake and transcellular flux of thiamine in Caco-2 cells, suggesting that oral absorption of dietary thiamine is significantly compromised by fedratinib dosing. Transport studies with recombinant human thiamine transporters identified the individual human thiamine transporter (hTHTR2) that is inhibited by fedratinib. Inhibition of thiamine uptake appears unique to fedratinib and is not shared by marketed JAK inhibitors, and this observation is consistent with the known structure-activity relationship for the binding of thiamine to its transporters. The results from these studies provide a molecular basis for the development of Wernicke’s encephalopathy upon fedratinib treatment and highlight the need to evaluate interactions of investigational drugs with nutrient transporters in addition to classic xenobiotic transporters.

Humans and other mammals must obtain thiamine from diet because they cannot synthesize thiamine. Two human thiamine transporters (hTHTR1 and hTHTR2) have been demonstrated to actively transport thiamine across the cell membrane and are widely expressed in various tissues including the intestine, liver, brain, and kidney (Dutta et al., 1999; Eady et al., 2000; Said et al., 2004; Bukhari et al., 2011; Larkin et al., 2012). Based on the structural similarity between thiamine and a substrate of fedratinib, it was hypothesized that fedratinib interferes with the oral absorption of thiamine via inhibition of thiamine transport. We conducted in vitro and in vivo experiments to test this hypothesis and present the results in this article.

Materials and Methods

[3H]thiamine and [3H]thiamine pyrophosphate (TPP) were purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO). Thiamine hydrochloride, amprolium, oxythiamine, amiloride, and Hanks’ balanced salt solution were purchased from Sigma-Aldrich (St. Louis, MO). Dulbecco’s modified Eagle’s medium, fetal bovine serum, nonessential amino acids, penicillin, and streptomycin were purchased from Mediatech (Manassas, VA). Fedratinib was purchased from Selleck Chemicals, LLC (Houston, TX) (Fig. 1). Ruxolitinib and tofacitinib were synthesized by Incyte Corporation (Wilmington, DE) (Fig. 1). Rabbit anti-human THTR1 polyclonal antibody was purchased from Abcam (Cambridge, MA), and rabbit anti-human THTR2 polyclonal antibody was purchased from Pierce Biotechnology (Rockford, IL). Recombinant human thiamine pyrophosphokinase 1 (TPK1) was purchased from Origene (Rockville, MD). The Transcreener AMP®/GMP® kit was purchased from Life Technologies (Carlsbad, CA).

Cell and Culture Conditions. Caco-2 cells were purchased from American Type Culture Collection (Rockville, MD), whereas GriPro HEK293 MSR

ABBREVIATIONS: hTHTR, human thiamine transporter; JAK, Janus kinase; MTPPT, mitochondrial thiamine pyrophosphate transporter; PCR, polymerase chain reaction; TG101348, N-tert-butyl-3-[(5-methyl-2-[4-(2-pyrrolidin-1-yl-ethoxy)-phenylamino]-pyrimidin-4-ylamino]-benzenesulfonamide; TPK1, thiamine pyrophosphokinase 1; TPP, thiamine pyrophosphate; WE, Wernicke’s encephalopathy.
cells were obtained from Life Technologies. Both cell lines were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 mg/ml streptomycin. For GripTite HEK293 MSR cells, both penicillin and streptomycin were not included, and 0.1 mM nonessential amino acids and 600 mg/ml Geneticin (Life Technologies) were also added in the culture medium. Cells were maintained at 37°C in an atmosphere of 5% CO₂. Caco-2 cells were seeded at a density of 4 × 10⁴ cells/well in 24-well microplates for thiamine uptake studies and 4 × 10⁵ cells/well in Transwell 24-well plates on membrane filters (BD Biosciences, San Jose, CA) for transcellular transport studies. To determine the uptake of thiamine in GripTite HEK293 MSR cells, these cells were seeded at a density of 2 × 10⁵ cells/well in 12-well microplates.

Uptake Studies of [³H]Thiamine. The uptake experiments in Caco-2 cells were performed 11–17 days after seeding. Cells were incubated with Krebs Henseleit buffer (Taub et al., 2011) (142 mM NaCl, 23.8 mM NaHCO₃, 4.83 mM KCl, 0.96 mM K₂HPO₄, 12.5 mM HEPES, 1.53 mM CaCl₂, and 1.2 mM MgSO₄, pH 7.4) containing 15 nM [³H]thiamine at 37°C for 7 minutes in the absence or presence of inhibitors. Unlabeled thiamine, amprolium, and oxythiamine were employed as the positive control inhibitors. The uptake was stopped by aspirating off the uptake solution and rapidly washing the cells twice with 500 μl ice-cold phosphate-buffered saline. Cells were then solubilized in 0.2 N NaOH, and all of the lysate was transferred to a 24-well plate and then 350 μl Supermix scintillation cocktail (PerkinElmer, Waltham, MA) was added to each well for liquid scintillation counting in a MicroBeta scintillation counter (PerkinElmer).

Real-Time Polymerase Chain Reaction Analysis. Real-time polymerase chain reaction (PCR) analysis was performed using the total RNA isolated from Caco-2 cells to assess expression levels of hTHTR1 and hTHTR2. cDNA synthesis was performed with the Advantage RT-PCR kit (Clontech Laboratories, Inc., Mountain View, CA) according to the manufacturer’s instructions using random hexamers and DNase I–treated total RNA. hTHTR1 and hTHTR2 primers and probes were synthesized and purified by Biosearch Technologies, Inc. (Novato, CA) with the 18S rRNA probe/primers being obtained from Applied Biosystems (Foster City, CA). hTHTR1 was analyzed using primers TGCACCTCATCGCTGTGGTTT and TGGATCTTCCAGTTTCTACATTTC and probe FAM-CTGGCCAGTGTGACGTAGTGTATGA-BHQ1. hTHTR2 was detected with primers GAGGTGGCCTACTACGCCTACATA and CAG-TAGCCGCTACTCTGGTAAA and probe FAM-ACAGCGTGGTCAGC-CCCGAG-BHQ1. Real-time PCR was performed on an Applied Biosystems ABI 7900 as recommended by the manufacturer. Reactions performed in the absence of reverse transcriptase were used to confirm lack of genomic DNA contamination. All expression measurements were performed in duplicate.

Western Blot. Protein samples (30 μg) from the Caco-2 cell lysate were resolved on a 4–12% SDS-polyacrylamide gel and electroblotted onto nitrocellulose membranes. The blots were blocked for 1 hour with 5% nonfat dry milk in phosphate-buffered saline/0.05% Tween, and were incubated overnight at 4°C with rabbit anti-human THTR1 polyclonal antibody (1:500 dilution) and rabbit anti-human THTR2 polyclonal antibody (1:500 dilution). The membranes were washed and then incubated with horseradish peroxidase–conjugated secondary antibodies (Cell Signaling Technology, Danvers, MA) and signals were detected using a SuperSignal West Dura extended duration substrate kit (Thermo Fisher Scientific, Waltham, MA). Images were captured by the FluorChem M system (ProteinSimple, Santa Clara, CA).
Transcellular Transport Studies of [3H]Thiamine in Caco-2 Cells. Cell membrane confluencc was confirmed by measuring transepithelial electrical resistance. Caco-2 cell monolayers with transepithelial electrical resistance values ≥ 300 Ω·cm² were used for transport experiments. In addition, nadolol, a low passive permeability benchmark, was also used to confirm the integrity of the monolayers of Caco-2 cells. The permeability values of nadolol were within the acceptable range of the in-house data (on file at Incyte Corporation) and were also consistent with the values reported in the literature (Yang et al., 2007). All transport experiments were conducted between 22 and 25 days after seeding. Using Hank's balanced salt solution containing 20 nM of [3H]thiamine in the absence or presence of inhibitors in the apical side, Caco-2 cells were incubated for 120 minutes at 37°C. Unlabeled thiamine, amprolium, and oxtixthiamine were utilized as the positive control inhibitors. After incubation, samples were removed from the donor and the receiver side, respectively. Radioactivity was counted on a MicroBeta scintillation counter (PerkinElmer).

Mitochondrial Transport of [3H]TPP. HEK-293T cells (Thermo Fisher Scientific) were transfected with a mitochondrial thymaine pyrophosphate transporter (MTPPT) using the Maxyce STX scalable transfection system (Maxcyte, Gaithersburg, MD). Mitochondria were purified from HEK-293T and HEPG2 cells (American Type Culture Collection) following the protocol in the kit. The freshly purified mitochondria were suspended in the storage buffer from the kit to achieve the protein concentration of 10–12 μg/ml. The assay was carried out as reported elsewhere (Hopfer et al., 1973; Subramanian et al., 2013). Briefly, mitochondria (5 μl) were added to the uptake buffer (20 μl) containing 0.1 μM [3H]TPP and then incubated at 37°C. The uptake reaction was terminated at various time points by adding the ice-cold stop buffer followed by rapid filtration. The membranes were washed two times with the stop buffer and the radioactivity was counted in a TopCount NXT scintillation counter (PerkinElmer).

TPK1 Activity. TPK1 (0.1 μM) was incubated with 1 μM thiamine and 1 mM ATP at 37°C for 30 minutes in 50 mM HEPES, 1 mM MgCl₂, 1 mM EGTA, and 0.01% Tween 20. The reaction was stopped by the addition of 10 mM EDTA, AMP²/GMP² Alexa Fluor tracer, and AMP²/GMP² antibody (Life Technologies). The plates were read on a BMG Labtech PHERAstar microplate reader (Ortenberg, Germany).

Brain Uptake Studies in Rats. Three groups of male Sprague-Dawley rats (n = 4/group) were given either fedratinib, ruxolitinib, or tofacitinib via intravenous dosing to reach steady-state plasma concentrations of approximately 1 mg/kg per hour forfedratinib, 2 mg/kg per hour for ruxolitinib, and 2 mg/kg per hour for tofacitinib. Blood samples were collected at 10, 20, 30, and 60 minutes after dosing. Radioactivity was counted on a MicroBeta scintillation counter (PerkinElmer). The kinetic parameters for uptake (K_{up}, V_{max}, and K_{diff}) were estimated according to the following equation (Greenwood et al., 1982; Izumi et al., 2013):

\[ v = \frac{V_{max}}{C + K_{up}} + K_{diff} \]

where v, C, K_{up}, V_{max}, and K_{diff} represent the uptake velocity, the substrate concentration, the Michaelis constant, the maximum uptake velocity, and the transfer constant for diffusion, respectively. GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA) was used for the statistical analysis and estimation of the kinetic parameters and the apparent IC₅₀ values. The permeability coefficient (P_app) was determined as described elsewhere (Yee, 1997). Statistical analysis was conducted using a t test for comparing two treatments. A P value < 0.05 was considered significant.

Results

Studies of Thiamine Uptake by Caco-2 Cells. The human-derived intestinal epithelial cell line, Caco-2, is commonly used as an in vitro model for oral absorption and has been demonstrated to have functional transporter-mediated thiamine uptake with expression of both human thiamine transporters, hTHTR1 and hTHTR2 (Said et al., 2004). Real-time PCR analysis was performed to assess expression of hTHTR1 and hTHTR2 in Caco-2 cells. Consistent with data by Said et al. (2004), the expression of both transporters at the mRNA level in Caco-2 cells was observed. The mean cycle thresholds were 26.4 and 26.0 for hTHTR1 and hTHTR2, respectively, suggesting moderate expression of these transporters at the mRNA level (Fig. 2). The efficiencies of the two reactions were nearly identical (data not shown). The results from Western blot analysis confirmed the protein expression of hTHTR1 and hTHTR2 in Caco-2 cells used in this study (Fig. 3). The predicted

![Fig. 2](image-url)
molecular masses of native hTHTR1 and hTHTR2 protein are approximately 55 kDa (Larkin et al., 2012). hTHTR1 and hTHTR2 protein bands were detected at approximately 55 and 60 kDa, respectively, which are similar to the predicted molecular masses of these transporters. A band corresponding to a molecular mass of approximately 75 kDa displayed in the blot of hTHTR1 may be a form of glycosylated hTHTR1 protein.

Fig. 4 shows the concentration dependency of [3H]thiamine uptake by Caco-2 cells. The uptake of thiamine consisted of both active and passive transport of thiamine, and the apparent $K_m$ and $V_{max}$ of the active uptake as well as the transfer constant for diffusion $K_{diff}$ were 3.06 ± 0.76 μM, 10.7 ± 1.41 pmol/mg protein per minute, and 0.156 ± 0.040 μmol protein per minute, respectively. The observed $K_m$ is consistent with that reported (3.18 μM) (Said et al., 1999).

To confirm the specificity of thiamine uptake by Caco-2 cells, two thiamine analogs, amprolium and oxythiamine (Casirola et al., 1988), were used. As indicated in Fig. 5, amprolium was a potent inhibitor of thiamine uptake with an IC$_{50}$ value of 0.80 μM, whereas oxythiamine at 200 μM, the highest concentration studied, inhibited thiamine uptake by 44%, indicating weak inhibition of thiamine transport. Fig. 6 indicates that fedratinib potently inhibited thiamine uptake with an IC$_{50}$ of 2.1 μM, whereas the marketed JAK inhibitors, ruxolitinib and tofacitinib, did not exhibit any significant inhibition.

Studies of Transcellular Transport of Thiamine in Caco-2 Cells.

To extend the observations regarding inhibition of thiamine uptake by fedratinib, transcellular transport of thiamine, which captures both uptake and passive diffusion, was evaluated in Caco-2 cells. As shown in Fig. 7, the transcellular transport of [3H]thiamine decreased with increasing concentrations of unlabeled thiamine, consistent with the presence of active transport.

As shown in Fig. 8, unlabeled thiamine and the thiamine analog, amprolium, inhibited the transcellular transport of [3H]thiamine with maximal inhibitions observed at 30 μM and 100 μM, respectively. The other thiamine analog, oxythiamine, was less effective in inhibiting the transcellular transport of thiamine. A concentration-dependent decrease of the flux of [3H]thiamine across the monolayer of Caco-2 cells was observed with fedratinib with an apparent IC$_{50}$ value of 6.5 μM, comparable to the inhibitory potency of fedratinib for the uptake of thiamine as discussed above. No significant inhibitory effects were observed with ruxolitinib (100 μM) or tofacitinib (100 μM), consistent with the results from the uptake studies described above. The maximum inhibition of transcellular transport of [3H]thiamine by unlabeled thiamine, amprolium, or fedratinib plateaued at approximately 50%.

**Effects of Fedratinib on Recombinant Proteins Involved in Thiamine Transport and Metabolism.** To further understand the mechanistic basis of the effect of fedratinib, each of the four proteins that play a key role in the uptake and metabolism of thiamine, including hTHTR1, hTHTR2, TPK1, and MTPT, was evaluated. The time course and kinetics of thiamine uptake in the hTHTR1- and hTHTR2-transfected HEK293 MSR cells are shown in Fig. 9. The apparent $K_m$ and $V_{max}$ values of the thiamine uptake in the hTHTR1- and hTHTR2-transfected cells were 1.85 μM and 7.29 pmol/mg protein per minute, and 3.28 μM and 35.0 pmol/mg protein per minute, respectively. Fedratinib potently inhibited hTHTR2 with an IC$_{50}$ of 1.2 μM but did not inhibit hTHTR1 (Fig. 10), indicating that fedratinib specifically affected uptake of thiamine by inhibiting hTHTR2. By contrast, both ruxolitinib and tofacitinib did not inhibit the function of hTHTR1 or hTHTR2 (Fig. 10). The effects of the three JAK inhibitors at various concentrations (up to 30 μM) on the activity of TPK1 were determined. In the presence of fedratinib, ruxolitinib, or tofacitinib at 30 μM, the percentages of TPK1 activity compared with the dimethylsulfoxide-treated control were 97.3 ± 1.06%, 93.8 ± 3.37%, and 102.8 ± 1.31%, respectively. The effects of these JAK inhibitors at 10 μM on the mitochondrial transport of [3H]TPP were also determined. The percentages of mitochondrial transport of [3H]TPP compared with the dimethylsulfoxide-treated control in the presence of fedratinib, ruxolitinib, or tofacitinib at 10 μM were 91.1 ± 12.4%, 90.2 ± 5.14%, and 106.3 ± 9.77%, respectively. These results
Fig. 6. The effects of JAK inhibitors on thiamine uptake by Caco-2 cells. Caco-2 cells were incubated at 37°C for 7 minutes in the presence of 15 nM [3H]thiamine and different concentrations of fedratinib (circles), ruxolitinib (squares), or tofacitinib (triangles). Inhibitory effects are reported as the percentage of dimethylsulfoxide-treated controls. Results are shown as the mean ± S.D. (n = 3).

demonstrate that the three JAK inhibitors did not inhibit the function of TPK1 and MTPPT under current study conditions.

**Brain Exposure of JAK Inhibitors in Rats.** The brain uptake of JAK inhibitors was evaluated in rats to identify any differences in brain exposure among the three compounds. After intravenous administration of fedratinib, tofacitinib, or ruxolitinib in rats to achieve steady state, the unbound brain to unbound plasma ratio of fedratinib was 0.26, whereas those of ruxolitinib and tofacitinib were only 0.035 and 0.026, respectively, demonstrating a 7- to 10-fold higher brain uptake for fedratinib compared with ruxolitinib or tofacitinib (Fig. 11). The unbound fractions of fedratinib, tofacitinib, and ruxolitinib in the plasma were 0.11, 0.65, and 0.18, and the corresponding unbound fractions in the brain were 0.02, 0.22, and 0.08, respectively. A 2-hour plasma sample was also collected during the infusion, and for each compound the 2 hour plasma concentration (data not shown) was similar to the 4 hour plasma concentration confirming that steady state was achieved.

**Discussion**

The chemical structure of fedratinib includes a 4-aminopyrimidine group and it is this structural similarity to thiamine that prompted this investigation. It has been reported that this substructure is important in the recognition of thiamine by thiamine transporters such that oxythiamine, a close structural analog of thiamine whose 4-amino group is replaced by a hydroxyl group, is not a good substrate or potent inhibitor of thiamine transporter at the blood–brain barrier (Greenwood and Pratt, 1985). Because the Caco-2 cell line is widely used as a cell model to evaluate transport of molecules across human intestinal epithelium, this cell system was chosen to study the uptake and transport of thiamine and the effect of fedratinib on that process. The expression and subcellular localization of hTHTR1 and hTHTR2 have been reported in Caco-2 cells, with hTHTR1 protein expressed on both the apical and basolateral sides and hTHTR2 protein exclusively expressed on the apical side (Said et al., 2004). The expression of these two thiamine transporters in Caco-2 cells was also confirmed in our laboratories at both mRNA and protein levels (Figs. 2 and 3).

Thiamine uptake by Caco-2 cells follows typical Michaelis–Menten kinetics, consistent with saturable carrier-mediated transport, with a $K_{m}$ of 3.06 μM (Fig. 4). This uptake process was potently inhibited by amprolium ($IC_{50} = 0.8$ μM), a structural analog of thiamine, but not by oxythiamine (Fig. 5), in agreement with the published literature as mentioned above. Next, the effects of fedratinib and two other JAK inhibitors (ruxolitinib and tofacitinib) on thiamine uptake were evaluated. As shown in Fig. 6, only fedratinib potently inhibited thiamine uptake with an inhibitory activity ($IC_{50} = 2.1$ μM) comparable to that of amprolium while ruxolitinib and tofacitinib did not. This striking difference in the inhibitory activity of thiamine uptake is consistent with the acknowledged criticality of the 4-aminopyrimidine substructure, absent in ruxolitinib and tofacitinib (Fig. 1). The intestinal concentrations of fedratinib (calculated using a default intestinal volume of 250 ml) after an oral dose greater than 100 mg are expected to exceed the apparent $IC_{50}$ for thiamine uptake determined in this study by a few orders of magnitude; this in turn may inhibit transporter-mediated uptake of dietary thiamine in patients.

Fedratinib also inhibited the apical-to-basolateral transport of thiamine across Caco-2 cells with inhibitory potency ($IC_{50} = 6.5$ μM) similar to that observed in the thiamine uptake assay. However, the inhibition of transcellular transport of thiamine by unlabeled thiamine, amprolium, and fedratinib was incomplete (Fig. 8) and this may reflect the underlying contribution of passive diffusion, which is not expected to be affected by inhibitors of thiamine uptake.

To further understand the mechanistic basis of interaction between fedratinib and thiamine, each of the four proteins that play a key role in thiamine uptake and metabolism were investigated (hTHTR1, hTHTR2, TPK1 and MTPPT) and the interaction between thiamine and fedratinib was evaluated. Fedratinib did not inhibit TPK1 or MTPPT activities, indicating that it does not interfere with the function of thiamine in cellular metabolism. Fedratinib did not inhibit hTHTR1 but potently inhibited the uptake of thiamine in HEK293 MSR cells transfected with hTHTR2 in a concentration-dependent manner with an $IC_{50}$ of 1.2 μM (Fig. 10). These results indicate that fedratinib directly affects the uptake of thiamine via hTHTR2, which is expressed at the intestinal epithelium and other tissues including the brain (Eudy et al., 2000). Although both hTHTR1 and hTHTR2 are expressed on the apical side of Caco-2 cells (Said et al., 2004), hTHTR2 appears to play a more important role in thiamine uptake than hTHTR1 based on the report that the intestinal thiamine uptake was significantly reduced in the THTR2-deficient mice but was preserved in the THTR1-deficient mice (Reidling et al., 2010). Thus, fedratinib, by virtue of potent inhibition of hTHTR2, can be expected to inhibit uptake of dietary thiamine.

The brain uptake of JAK inhibitors was evaluated in rats to look for any differences in profile among the three compounds. Based on the unbound brain to unbound plasma ratio, the distribution of fedratinib into the brain was high in rats. Therefore, the possibility that thiamine...
uptake by neurons and astrocytes might be also inhibited by fedratinib after this compound enters the brain may not be ruled out. This unbound brain to unbound plasma ratio is much higher than expected based on the polar surface area of the molecule, which would suggest low passive diffusion, and the brain uptake of fedratinib was also much higher compared with that of ruxolitinib and tofacitinib. Although the higher-than-expected brain uptake of fedratinib in rats is interesting and may suggest that this compound might undergo active uptake at the blood–brain barrier, the mechanism is unknown. The mean steady-state maximum plasma concentration of fedratinib in patients was 5–10 μM for a 400–500 mg daily dose (Pardanani et al., 2011), which raises the possibility of fedratinib inhibiting thiamine transport into the brain via hTHTR2, in addition to inhibition of thiamine uptake at the intestine. Further experiments are required to quantify these effects.

In summary, this study was carried out to discern the mechanism underlying fedratinib-induced WE. The results clearly demonstrate that fedratinib is a potent inhibitor of thiamine uptake and transport via specific inhibition of hTHTR2. It is important to note that nausea, diarrhea, and vomiting are the major adverse events of fedratinib (Pardanani et al., 2011) and these effects may also contribute to or exacerbate the direct inhibition of thiamine uptake by fedratinib, leading to a more pronounced thiamine deficiency. Assessment of interactions of a new molecular entity with drug metabolizing enzymes

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**Fig. 8.** Inhibitory effects of thiamine analogs and JAK inhibitors on the transcellular transport of [3H]thiamine in Caco-2 cells. Caco-2 cells were incubated at 37°C with 20 nM [3H]thiamine in Hanks’ balanced salt solution (pH 7.4) for 120 minutes in the presence of various concentrations of unlabeled thiamine (3, 30, and 500 μM), amprolium (30, 100, and 300 μM), oxythiamine (300 μM), fedratinib (10, 50, and 100 μM), ruxolitinib (100 μM), or tofacitinib (100 μM) in the apical side. Results are shown as the mean ± S.D. (n = 3). Inhibitory effects are reported as the percentage of dimethylsulfoxide-treated controls. Significance was determined by a t test when an inhibitor treatment was compared with dimethylsulfoxide-treated controls (*P < 0.05; **P < 0.01).

**Fig. 9.** Time and concentration dependence of thiamine uptake in hTHTR1- and hTHTR2-transfected HEK293 MSR cells. (A and C) Time dependence of thiamine uptake in transfected cells. Cells expressing recombinant hTHTR1 (A) or hTHTR2 (C) were incubated for various periods of time in the presence of 20 nM [3H]thiamine. Results are shown as the mean ± S.D. (n = 3). As a control for background, uptake in mock transfected HEK293 MSR cells is plotted. (B and D) Concentration dependence of thiamine uptake in transfected cells. HEK293-MSR cells transiently transfected with hTHTR1 (B) or hTHTR2 (D) were incubated in the presence of increasing concentrations of thiamine. Results are shown as the mean ± S.D. (n = 3). The kinetic parameters, $K_m$ and $V_{max}$, were estimated using GraphPad Prism 6.0.
also serve to discern compound-specific versus class-specific effects in the pharmacology and toxicology of inhibitors of JAK enzymes, which are being studied in a wide array of oncological and inflammatory indications.

**Authorship Contributions**

*Participated in research design:* Q. Zhang, Y. Zhang, Diamond, Boer, Behshad, Liu, Burn, Wynn, Hollis, Yeleswaram.

*Conducted experiments:* Q. Zhang, Y. Zhang, Boer, Harris, Li, Rupar, Behshad, Gardiner, Collier.

*Performed data analysis:* Q. Zhang, Y. Zhang, Diamond, Boer, Harris, Li, Behshad, Collier, Liu, Burn, Wynn, Hollis.

*Wrote or contributed to the writing of the manuscript:* Q. Zhang, Y. Zhang, Diamond, Harris, Behshad, Hollis, Yeleswaram.

**References**


**Address correspondence to:** Dr. Qiang Zhang, Incyte Corporation, Route 141 and Henry Clay Road, Wilmington, DE 19880. E-mail: qizhang@incyte.com