The uremic toxin oxythiamine causes functional thiamine deficiency in end-stage renal disease by inhibiting transketolase activity

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Decreased transketolase activity is an unexplained characteristic of patients with end-stage renal disease and is linked to impaired metabolic and immune function. Here we describe the discovery of a link to impaired functional activity of thiamine pyrophosphate cofactor through the presence, accumulation, and pyrophosphorylation of the thiamine antimetabolite oxythiamine in renal failure. Plasma oxythiamine was significantly increased by 4-fold in patients receiving continuous ambulatory peritoneal dialysis and 15-fold in patients receiving hemodialysis immediately before the dialysis session (healthy individuals, 0.18 [0.11–0.22] nM; continuous ambulatory peritoneal dialysis patients, 0.64 [0.48–0.94] nM; and hemodialysis patients (2.73 [1.52–5.76] nM). Oxythiamine was converted to the transketolase inhibitor oxythiamine pyrophosphate. The red blood cell oxythiamine pyrophosphate concentration was significantly increased by 4-fold in hemodialysis (healthy individuals, 15.9 nM and hemodialysis patients, 66.1 nM). This accounted for the significant concomitant 41% loss of transketolase activity (mU/mg hemoglobin) from 0.410 in healthy individuals to 0.240 in hemodialysis patients. This may be corrected by displacement with excess thiamine pyrophosphate and explain lifting of decreased transketolase activity by high-dose thiamine supplementation in previous studies. Oxythiamine is likely of dietary origin through cooking of acidic thiamine-containing foods. Experimentally, trace levels of oxythiamine were not formed from thiamine degradation under physiologic conditions but rather under acidic conditions at 100°C. Thus, monitoring of the plasma oxythiamine concentration in renal failure and implementation of high-dose thiamine supplements to counter it may help improve the clinical outcome of patients with renal failure.

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Loss of clearance in chronic kidney disease (CKD) leads to accumulation of waste products from metabolism that increase to potentially damaging concentrations and thereby become uremic toxins. In end-stage renal disease (ESRD), potentially noxious metabolites may increase >10-fold, particularly preceding a dialysis session. Among classes of uremic toxins are catabolic and degradation products of essential nutrients and cofactors. Although similar in structure to their precursor but nonfunctional, uremic toxins may have potentially damaging function as antimetabolites.[1]

It is known that there is impaired function of the pentosephosphate pathway in uremia at the thiamine pyrophosphate (TPP)–dependent step catalyzed by transketolase.[2] The inhibition of transketolase was reversible, although the identity of the inhibitor was difficult to discern. The inhibitor was of low molecular weight and initially considered to be guanidinosuccinic acid. Low levels of guanidinosuccinic acid in the plasma of patients with decreased red blood cell transketolase activity, a lack of correlation of guanidinosuccinic acid concentration to inhibition of transketolase activity, and the failure of guanidinosuccinic acid to inhibit transketolase activity in red blood cells *ex vivo* suggested that other compound(s) are likely involved.[3–5] Disturbance of levels of pentosephosphate pathway metabolites in peripheral nerves *in vivo* regulated by transketolase activity and recovery of this by hemodialysis (HD) indicated reversible inhibition of transketolase. Transketolase activity was also decreased in patients with continuous ambulatory peritoneal dialysis (CAPD).[6] This occurred in the presence of normal levels of plasma thiamine and red blood cell TPP.[4,7,8] The mechanism of reversible inhibition of transketolase in renal failure has remained unresolved for more than 40 years.

We hypothesized that transketolase may be inhibited in renal failure by an antimetabolite of thiamine that is normally cleared but accumulates to inhibitory levels with the loss of clearance in ESRD. Oxythiamine (4-hydroxythiamine)[9] is pyrophosphorylated by thiamine pyrophosphokinase to

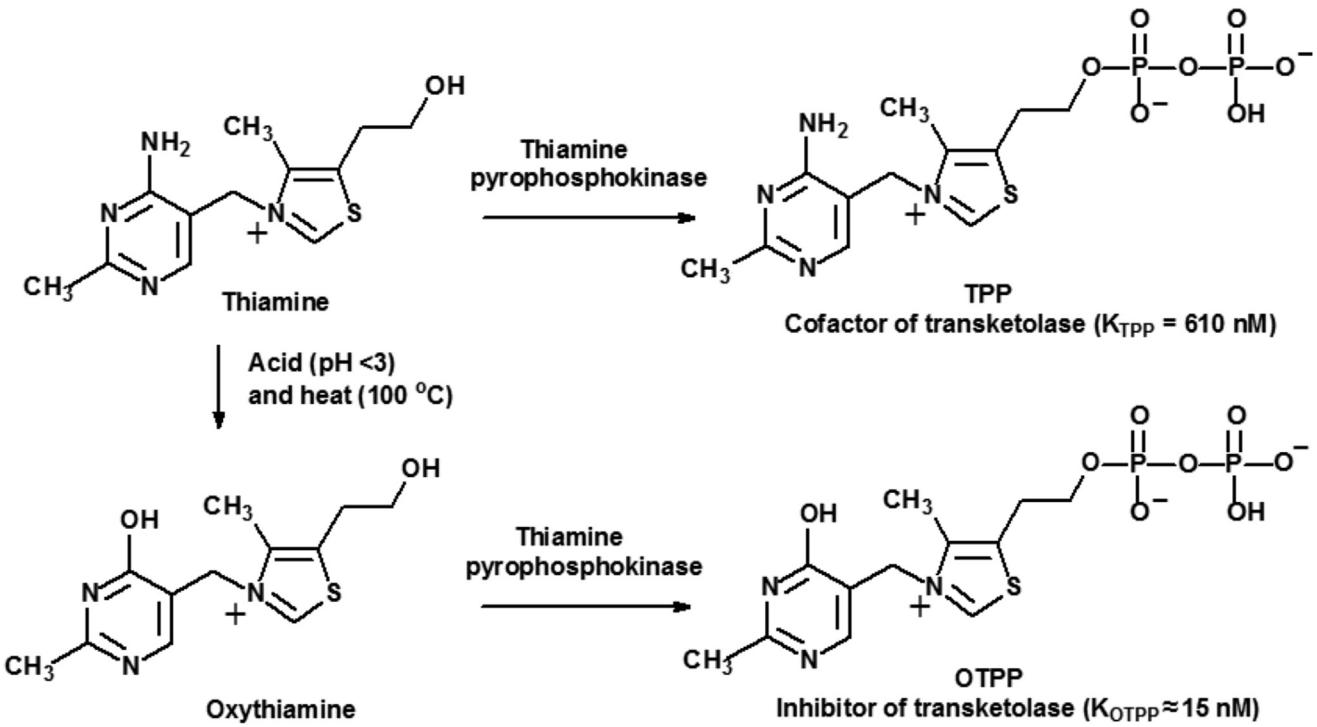

Figure 1 | Formation, metabolism, and antimetabolite activity of oxythiamine. Pathways of thiamine metabolism, oxythiamine formation, and metabolism to antimetabolite oxythiamine pyrophosphate. OTPP, oxythiamine pyrophosphate; TPP, thiamine pyrophosphate.

oxythiamine pyrophosphate (OTPP), which inhibits TPP-dependent enzymes[10–12] (Figure 1). Injection of oxythiamine into rats decreased tissue transketolase activity.[13] We report here the accumulation of oxythiamine in plasma and related OTPP in red blood cells of patients with ESRD that likely explains the inhibition of transketolase in renal failure.

RESULTS

We measured plasma thiamine concentration of healthy subjects and patients with ESRD receiving renal replacement therapy (CAPD and HD) by the conventional thiochrome assay[14] (Table 1). The plasma concentration of thiamine was within the normal range and even increased in CAPD and HD patients. Oxythiamine is not detectable by the thiochrome assay as it lacks the 4-aminopyrimidinyl moiety required for thiochrome formation.[15] We therefore developed a liquid chromatography–tandem mass spectrometry (LC-MS/MS) assay for oxythiamine and OTPP (Figure 2 and Table 2). Oxythiamine was analyzed in the plasma of ESRD patients receiving CAPD or HD. Oxythiamine was detectable at trace levels in the plasma of healthy individuals: median (lower–upper quartile), 0.18 (0.11–0.22) nM. In ESRD patients receiving CAPD and HD, however, this was increased 4-fold, 0.64 (0.48–0.94) nM and 15-fold, 2.73 (1.52–5.76) nM, respectively; $P < 0.001$, Mann-Whitney U test (Table 1). In a further group of HD patients, we measured plasma oxythiamine before and after a 4-hour dialysis session and found that oxythiamine decreased by 53% (34%–64%) during the dialysis session ($n = 12$, $P < 0.002$, Wilcoxon signed rank test).

We explored the metabolism of oxythiamine and its effect on thiamine metabolism in a subgroup of healthy control subjects and HD patients. We measured the concentration of TPP in red blood cells of healthy subjects and HD patients and found no significant difference. However, plasma oxythiamine was increased 19-fold in these patients, and the corresponding OTPP concentration in red blood cells was increased 4-fold (Figure 3). OTPP is a reversible competitive

Table 1 | Characteristics of healthy subjects and ESRD patients on dialysis

Variable	Healthy control subjects	CAPD	HD
Sex (M/F)	8/8	7/9	8/8
Age (yr)	48 ± 5	43 ± 15	49 ± 6
BMI (kg/m^2)	25.5 ± 3.2	25.6 ± 3.0	26.2 ± 3.6
Plasma creatinine (μM)	71 ± 17	655 ± 249	677 ± 268
Plasma albumin (mg/ml)	45.6 ± 3.2	35.6 ± 6.9	43.1 ± 3.5
Plasma thiamine (nM)	5.1 (4.3–9.7)	31.8 (18.0–53.5)[a]	51.1 (20.1–83.0)[a]
Plasma oxythiamine (nM)	0.18 (0.11–0.22)	0.64 (0.48–0.94)[a]	2.73 (1.52–5.76)[a,b]

Data are mean ± SD or median (lower–upper quartile). A second study group of HD patients had plasma analyzed for plasma oxythiamine before and after a dialysis session and characteristics were sex; male/female. 6/6; age, 49 ± 24 years; body mass index, 26.4 ± 7.9 kg/m^2; and plasma albumin, 40.5 ± 4.0 mg/ml ($n = 12$).
BMI, body mass index; CAPD, continuous ambulatory peritoneal dialysis; ESRD, end-stage renal disease; HD, hemodialysis, M/F, male/female.
[a]$P < 0.001$ with respect to healthy control subjects.
[b]$P < 0.001$ with respect to CAPD patients.