vanadate and the related compounds including molybdate, tungstate and peroxocompounds may be useful drugs for diabetes mellitus in man. Nevertheless, further studies are required to elucidate the detailed mechanism of these agents and to establish a new class of drugs for managing diabetes mellitus.

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Department of Pediatrics
Ehime University School of Medicine
Shigenobu
Ehime 791-02, Japan

YOSHINORI GOTO*
KAICHI KIDA
MASAHITO IKEUCHI
YUKIKAZU KAINO
HIROSHI MATSUDA

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* Corresponding author. Tel. (81) 899-64-5111 ext. 2121; FAX (81) 899-64-9131.

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Inhibition of transketolase and pyruvate decarboxylase by omeprazole

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Abstract—Omeprazole inhibited two thiamin diphosphate-dependent enzymes, pyruvate decarboxylase (EC 4.1.1.1, PDC) from *Zymomonas mobilis* and transketolase (EC 2.2.1.1, TK) from human erythrocytes. Inhibition of PDC was competitive with the coenzyme with a K_i value of $42 \pm 3\ \mu M$, whereas inhibition of TK was complex.

Omeprazole (Fig. 1B) is a compound which blocks gastric acid secretion by inhibiting the membrane $(H^+ + K^+)$ATPase [1,2]. When protonated, omeprazole is converted to the sulfenamide which is able to react with the sulfhydryl groups of cysteine residues and, it has been proposed, thereby inactivate the enzyme [3,4]. Additionally, Brown [5] has argued that H^+ transport involves a "thiamin shuttle" and that the inhibition by omeprazole depends upon its structural similarity to the tricyclic form (Fig. 1A) of thiamin (Fig. 1C), raising the suggestion of competition between omeprazole and thiamin for binding to the $(H^+ + K^+)$ATPase.

Apart from its postulated role in membrane transport, thiamin (as its diphosphate, ThDP*) is better known as a cofactor for a number of enzyme-catalysed reactions. The similarity of omeprazole to thiamin raises the possibility that this compound may interact with ThDP-dependent enzymes. Here we examine the effect of omeprazole on two of these: transketolase (EC 2.2.1.1, TK) from human erythrocytes and pyruvate decarboxylase (EC 4.1.1.1, PDC) from *Zymomonas mobilis*.

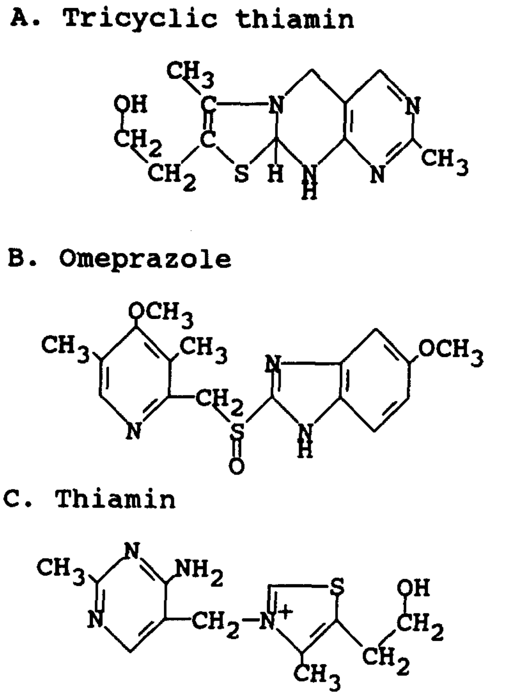

Fig. 1. Structures of tricyclic thiamin (A), omeprazole (B) and thiamin (C).

Materials and Methods

Human erythrocyte TK was purified and resolved of coenzyme using modifications of methods described previously [6,7]. Omeprazole inhibition was studied by adding apotransketolase (apoTK) to a reaction mixture containing 100 mM Tris–HCl buffer, pH 7.6, 50 mg/mL polyethyleneglycol 600, 20 mM $MgCl_2$ and various concentrations of omeprazole. After incubation for 5 min at 30°, various concentrations of ThDP were added and incubation was continued for a further 5 min before completion of the assay mixture by addition of xylulose 5-phosphate, ribose 5-phosphate, NADH, triosephosphate isomerase (EC 5.3.1.1) and glycerol phosphate dehydrogenase (EC 1.1.1.8) to respective final concentrations of 1 mM, 10 mM, 0.2 mM, 8 IU/mL and 0.8 IU/mL, in a final volume of 700 μL. TK activity was measured by the rate of change of A_{340}.

PDC was purified from *Z. mobilis* and converted to the apoenzyme as described elsewhere [8]. Omeprazole inhibition was studied by adding pyruvate decarboxylase apoenzyme (apoPDC) to 2 mL of 5 mM $MgCl_2$ in 50 mM Mes-KOH buffer, pH 6.5, containing various concentrations of ThDP and omeprazole. After incubation at 25° for 30 min, PDC activity was measured by the rate of change of A_{340} on addition of 60 μL of 172 mM sodium pyruvate, 5.15 mM NADH and 340 IU/mL alcohol dehydrogenase (EC 1.1.1.1).

Table 1. Inhibition of TK by omeprazole

Omeprazole (μM)	ThDP (μM)	Activity*	
		Initial	Steady state
0	100	91, 108	147, 173
50	100	68	95
200	100	38	119
0	2	34	66
10	2	20	68
50	2	20, 20	65, 55
200	2	0	2

* 1000 × ΔA_{340}/min.

Results and Discussion

When human erythrocyte apoTK was preincubated with mixtures of 100 μM ThDP and various amounts of omeprazole, the rate of catalysis measured immediately after addition of substrates was inversely related to the omeprazole concentration (Table 1). At 200 μM omeprazole, there was approximately 60% inhibition. At a lower ThDP concentration (2 μM), the inhibition was more pronounced with all activity abolished by 200 μM omeprazole. The inclusion of mercaptoethanol at 14 mM abolished the inhibition by omeprazole, and the activity of holoTK was not affected by omeprazole concentrations up to 200 μM.

There is a complicating factor in these assays; the rate progressively increased with time, whether or not omeprazole was present (Table 1). Moreover, the length of the lag period depended on the concentrations of both omeprazole and ThDP, and prolonged incubation appeared to partially reverse the inhibition by omeprazole. Owing to the difficulty of investigating such a complex hysteretic system, the inhibition of TK was not characterized further. However, the following points can be noted. First, the inhibitory effect of omeprazole on the final (steady state) reaction velocity was much less than its effect on the initial velocity. Given the sequence of addition of reactants (preincubation with omeprazole before addition of ThDP), this is consistent with reversal of inhibition by the addition of ThDP, albeit slowly. Second, the abolition of omeprazole inhibition by mercaptoethanol raises the possibility that it might be reacting with an essential sulfhydryl group, exposed in the apoenzyme but not in the holoenzyme. this would be similar to the mechanism of its inactivation of the $(H^+ + K^+)$ATPase [2,3], but would require the unlikely activation of omeprazole to the sulfenamide [3] at pH 7.6 and 6.5, and would be inconsistent with the apparent reversal of the inhibition by ThDP.

* Abbreviations: ThDP, thiamin diphosphate; TK, transketolase; apoTK, apotransketolase; PDC, pyruvate decarboxylase; and apoPDC, pyruvate decarboxylase apoenzyme.

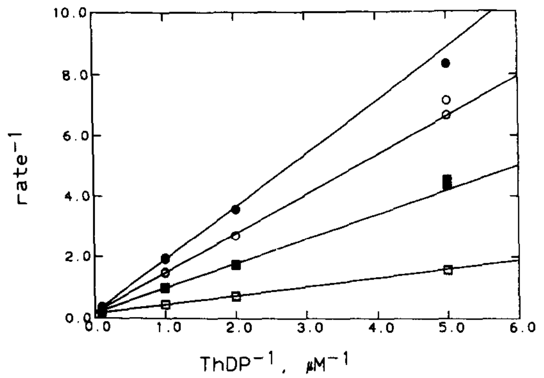

Fig. 2. Inhibition of PDC by omeprazole. ApoPDC was incubated with ThDP concentrations as shown, and omeprazole concentrations of 0 (□), 60 μM (■), 150 μM (○) and 300 μM(●). After 30 min, substrate was added and the activity was measured as described in Materials and Methods. Duplicate determinations were made and both are plotted; in most cases duplicates were so similar that the points are superimposed. The lines represent separate fits of the Michaelis–Menten equation to the data obtained at each omeprazole concentration.

Assays of *Z. mobilis* apoPDC after preincubation with mixtures of ThDP and omeprazole did not show any lag period, but there was a marked inhibition (Fig. 2). The inhibition was competitive with ThDP with a K_i of 41.6 ± 3.3 μM, approximately 24 times the K_m for ThDP (1.72 ± 0.12 μM) measured in the same experiment.

These results clearly demonstrate that omeprazole is a thiamin analog, although they do not test Brown's postulate for the action of omeprazole on gastric acid secretion. They do suggest that the drug may have secondary effects on metabolism by inhibiting ThDP-dependent enzymes and that such metabolic effects may be of some significance in patients with marginal thiamin nutrition. Accurate prediction of the consequences of therapeutic doses of omeprazole upon thiamin-dependent enzymes from human sources will require formal measurement of the K_i values for the interactions of omeprazole with those enzymes, together with assays of tissue concentrations of omeprazole.

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Department of Biochemistry
The University of Queensland
St. Lucia, Queensland 4072
Australia

Peter F. Nixon*
Russell J. Diefenbach†
Ronald G. Duggleby

* Corresponding author. Tel. 61-7-365-4613; FAX 61-7-365-4699.

† Present address: Department of Biochemistry, University of Toronto, Toronto M5S 1A8, Canada.

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