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Pivalate-Generating Prodrugs and Carnitine Homeostasis in Man

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Abstract*——Prodrugs that liberate pivalate (trimethylacetic acid) after hydrolysis have been developed to improve the bioavailability of therapeutic candidates. Catabolism of pivalate released by activation of a prodrug is limited in mammalian tissues. Pivalate can be activated to a coenzyme A thioester in cells. In humans, formation and urinary excretion of pivaloylcarnitine generated from pivaloyl-CoA is the major route of pivalate elimination. Because the total body carnitine pool is limited and can only slowly be replenished through normal diet or biosynthesis, treatment with large doses of pivalate prodrugs may deplete tissue carnitine content. Animal models and long-term treatment of patients with pivalate prodrugs have resulted in toxicity consistent with carnitine depletion. However, low plasma carnitine concentrations after pivalate prodrug exposure may not reflect tissue carnitine content and, thus, cannot be used as a surrogate for potential toxicity. The extent of tissue carnitine depletion will be dependent on the dose of pivalate, because carnitine losses may approximate the pivalate exposure on a stoichiometric basis. These concepts, combined with estimates of carnitine dietary intake and biosynthetic rates, can be used to estimate the impact of pivalate exposure on carnitine homeostasis. Thus, even in populations with altered carnitine homeostasis due to underlying conditions, the use of pivalate prodrugs for short periods of time is unlikely to result in clinically significant carnitine depletion. In contrast, long-term treatment with substantial doses of pivalate prodrugs may require administration of carnitine supplementation to avoid carnitine depletion.

I. Introduction

The use of prodrugs (drugs which undergo covalent modification after administration to yield biologically active molecules) is a well accepted strategy for improving the pharmaceutical, pharmacokinetic, or pharmacodynamic characteristics of a therapeutic agent. Prodrug activation may involve a structural modification to the single administered molecule, such as the conversion of the lactone ring of lovastatin to the active hydroxyacid. Alternatively, the prodrug may be broken down into two molecules, one of which is the active therapeutic moiety, and the other an inactive byproduct. The latter scenario

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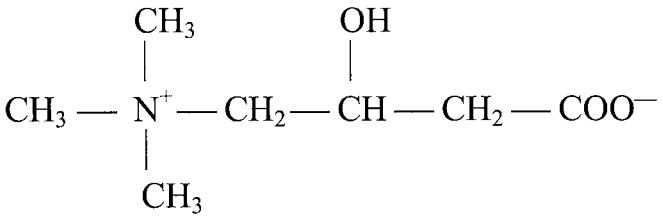
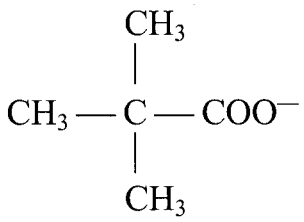
occurs for prodrugs that are esters of the active drug with a carboxylic acid. These derivatives are intended to enhance oral absorption and systemic delivery of the active chemical entity. In vivo hydrolysis of the ester then yields the active drug and the carboxylic acid. The carboxylic acid is then further broken down through endogenous intermediary metabolism.

Pivalate (trimethylacetic acid, Fig. 1) has been used to generate prodrugs to increase oral bioavailability (for example cefetamet pivoxil and pivampicillin). After hydrolysis of the prodrug, pivalate like most carboxylic acids can be activated inside cells for further metabolism by acyl-CoA synthases to form pivaloyl-CoA. However, unlike the coenzyme A thioesters of acetate or isobutyrate, pivaloyl-CoA cannot be oxidized to carbon dioxide in mammalian cells. As a result, pivaloyl-CoA accumulates in cells and is a substrate for a variety of acyl-CoA transferases. These reactions generate pivaloyl conjugates that can be excreted, usually by the kidney. In humans, formation and urinary excretion of pivaloylcarnitine is the dominant route of pivalate elimination. The formation of pivaloylcarnitine, and its excretion in the urine, has the potential to perturb normal cellular function due to the important roles of carnitine in cellular homeostasis.

The current manuscript critically examines the theoretical, experimental, and clinical bases of the pivalate-carnitine interaction in humans. The review indicates that although long duration therapy with high doses of pivalate-containing prodrugs may result in clinically important changes in carnitine homeostasis, normal carnitine metabolism protects humans from pivalate-associated toxicity except under extreme conditions. Additionally, plasma or urinary carnitine concentrations are a poor predictor of potential pivalate-associated toxicity.



CARNITINE



PIVALATE

FIG. 1. Chemical structures of carnitine and pivalate. The chemical structures of carnitine and pivalate are shown at physiologic pH.

II. Overview of Carnitine Homeostasis

A. Functions of Carnitine

Carnitine (Fig. 1) is an endogenous molecule with important functions in normal intermediary metabolism and cellular physiology (Bremer, 1983). Biochemical reactions involving carnitine all involve the reversible transfer of a carboxylic acid moiety (acyl group) from a coenzyme A thioester (acyl-CoA) to form the corresponding carnitine ester (acylcarnitine). This reaction is catalyzed by a family of enzymes, the carnitine acyltransferases (Bieber, 1988). These enzymes differ based on their subcellular localization and acyl group structural specificity.

The formation of acylcarnitines is a critical step in the mitochondrial oxidation of long-chain fatty acids (Bremer, 1983). Long-chain acyl-CoAs cannot cross the inner mitochondrial membrane to reach the site of β-oxidation. In contrast, the acylcarnitine is the substrate for a transmembrane carrier, which moves the molecule into the mitochondrial matrix where a second acylcarnitine transferase regenerates the acyl-CoA for further oxidation. Thus, carnitine is an obligate for normal mitochondrial fatty acid oxidation, and loss of tissue carnitine may compromise cellular bioenergetics.

The coenzyme A pool of the cell is important in a large number of catabolic and anabolic biochemical reactions. As the cellular total coenzyme A pool is small and cannot be increased quickly, the cell is dependent on continued turnover of acyl-CoAs to make coenzyme A available for other reactions. Thus, if a specific acyl-CoA accumulates in a cell, multiple biochemical pathways may be impaired. The formation of acylcarnitines under these conditions serves to buffer the coenzyme A pool from transient acyl-CoA accumulation and make coenzyme A available for other reactions (Bieber, 1988). In pathologic conditions of acyl-CoA accretion, this detoxification function of carnitine may be extremely important and require large amounts of carnitine (Chalmers et al., 1983). However, this interchange between the coenzyme A and carnitine pools is ongoing and dynamic. Under a variety of physiologic conditions, including fasting (Hoppel and Genuth, 1980) or exercise (Hiatt et al., 1989), the carnitine pool is redistributed between carnitine and acylcarnitines to reflect a change in the status of the coenzyme A pool and metabolic state. Thus, such redistribution cannot be viewed as intrinsically detrimental and must be viewed in the context of the overall metabolic status of the cell or tissue. Furthermore, definition of carnitine status requires knowledge of the total carnitine availability (the sum of carnitine and all acylcar-

nitines) and the distribution of the carnitine pool between carnitine and acylcarnitines.

B. Carnitine Balance in Humans

All tissues that use fatty acids as a fuel source, or require coenzyme A for cellular reactions, require availability of carnitine for normal function as discussed above. In humans, carnitine is derived from dietary sources and endogenous biosynthesis, and biosynthesis is adequate to meet total body carnitine needs (Rebouche and Seim, 1998). Meat and dairy products are important dietary sources of carnitine, and variations in dietary carnitine intake can impact plasma and urinary carnitine contents without any physiologic or functional impact on the individual (Lennon et al., 1986; Cederblad, 1987). Muscle carnitine content is also independent of dietary carnitine intake (Cederblad, 1987), again illustrating the dissociation between tissue stores and urine or plasma carnitine concentrations.

Carnitine biosynthesis involves a complex series of reactions involving several tissues (Hoppel and Davis, 1986). The carbon backbone for carnitine is derived from lysine. Lysine in protein peptide linkages undergoes methylation of the ϵ-amino group to yield trimethyllysine, which is released upon protein degradation. Muscle is the major source of trimethyllysine. The released trimethyllysine is further oxidized to butyrobetaine and ultimately hydroxylated to form carnitine. The last reactions of carnitine biosynthesis occur primarily in the liver and the kidney.

There are no catabolic reactions involving carnitine in mammalian cells, and the only route of elimination is through urinary elimination. Both carnitine and acylcarnitines appear in the urine. In normal kidneys, greater then 90% of the filtered carnitine is reabsorbed (Engel et al., 1981; Rebouche et al., 1993). This reabsorption is saturable, with a transport maximum of 60 to 100 μM. Urinary acylcarnitines derive from both filtered plasma acylcarnitines and products of renal metabolism. Filtered acylcarnitines are reabsorbed to varying degrees based on the specific acyl moiety present.

TABLE 1
Carnitine pool in humans

Tissue/ Compartment	Total Carnitine Concentration[a]	Total Compartment Content[b]	Turnover
		mmol/kg	
Plasma	40 μM	<0.001	
Extracellular fluid	40 μM	0.007	Fast
Liver	0.94 mmol/kg	0.019	Fast
Kidney	0.5 mmol/kg	0.003	Intermediate
Heart	1.3 mmol/kg	0.005	Slow
Skeletal muscle	4.2 mmol/kg	1.8	Slow
Total body carnitine content:		1.84	

[a] Data from Suzuki et al., 1982; Tripp and Shug, 1984; Lennon et al., 1986; Brass, 1992.
[b] Total carnitine contents were calculated based on the total carnitine concentrations and estimated size of the compartment per kilogram of body weight in humans taken from Diem and Lentner (1970). Values are per kilogram of body weight.

C. Tissue Carnitine Content

The total body carnitine pool is extremely dynamic, with carnitine and acylcarnitines moving between the gastrointestinal tract (after absorption), the liver (after biosynthesis), the kidney (for elimination), and tissues such as heart or skeletal muscle that require carnitine for function. Within a tissue, the carnitine pool is redistributed between carnitine and acylcarnitines as metabolic shifts occur. Neither carnitine nor acylcarnitines can efficiently diffuse across plasma membranes, and tissue-specific transport systems exist to move carnitine into and out of tissues (Brass, 1992; Kerner and Hoppel, 1998). As a result, tissues vary enormously in their total carnitine content and the kinetics of carnitine homeostasis. Furthermore, dramatic changes in carnitine homeostasis may occur in one biologic compartment, which are not reflected in other compartments. The specific gene and corresponding protein primarily responsible for renal and muscle carnitine transport have been characterized (Wang et al., 1999; Lahjouji et al., 2001).

Plasma serves only to carry carnitine and acylcarnitines between tissues, and as such its concentrations are relatively low (Table 1). As carnitine serves no metabolic function within plasma, changes in plasma carnitine concentrations can only be interpreted in the context of other metabolic or tissue-specific information. Kidney, liver, heart, and skeletal muscle all contain carnitine at concentrations in excess of those found in plasma (Table 1). Due to the large amount of skeletal muscle, most of the total body carnitine is present in the skeletal muscle compartment, with very little in the plasma or extracellular compartments (Table 1).

Tissue carnitine turnover rates also vary widely (Brooks and McIntosh, 1975; Rebouche and Engel, 1984). The liver appears to rapidly equilibrate with the plasma compartment, both in respect to total carnitine content (albeit at concentrations higher than plasma) and carnitine-acylcarnitine distribution (Brass and Hoppel, 1980). In contrast, the large skeletal muscle carnitine pool interacts only sluggishly with plasma. Thus, very large changes may occur acutely in the plasma compartment through acute carnitine administration (Brass et al., 1994) or acute carnitine depletion during hemodialysis (Guarnieri et al., 1987; Wanner et al., 1987) without any significant perturbation in skeletal muscle carnitine content or function.

TABLE 2
Response to plasma carnitine depletion

Plasma total carnitine concentration[a]	
Pre-dialysis	73 μM
Immediately post-dialysis	37 μM
Net loss of carnitine by patient[b]	0.63 mmol
Percentage of tissue carnitine pool shifted to extracellular to replenish losses[b]	0.5%

[a] Data from Guarnieri et al., 1987.
[b] Based on 70 kg of body weight, 25% body weight as extracellular fluid and tissue carnitine content per kilogram (see Table 1). Assumes no carnitine intake or biosynthesis over dialysis interval.

Processes affecting net carnitine balance (biosynthesis, elimination, absorption) will most immediately affect the plasma compartment. However, the plasma carnitine pool interacts with the discrete carnitine pools of specific organs, as discussed above. Thus, changes in any aspect of carnitine homeostasis eventually will be reflected in all tissues in the body. The relative impact at steady state will be dependent on the size of the perturbation and the total body carnitine pool. Before steady-state redistribution of the carnitine pool, individual compartments may show large changes, as is the case with the plasma carnitine depletion with hemodialysis. In the case of hemodialysis, the plasma carnitine pool is restored from tissue stores within 1 to 2 days after a hemodialysis session (Bartel et al., 1981; Guarnieri et al., 1987; Wanner et al., 1987). Due to the large amount of carnitine in tissue compared with extracellular fluid, the extracellular compartment may be replenished with only a small physiologically irrelevant decrement of the tissue stores (Table 2).

III. Impact of Pivalate-Generating Prodrugs on Carnitine Homeostasis

A. Metabolism of Pivalate and Cellular Impact of Pivaloylcarnitine Production

Pivalate generated from prodrug administration can enter cells for further metabolism. As for other organic acids, formation of a thioester with coenzyme A is a critical step in cellular metabolism of pivalate. The ability to generate pivaloyl-CoA has been demonstrated directly in rat hepatocytes (Ruff and Brass, 1991) and indirectly in rat heart cells (Diep et al., 1995b). This reaction most likely occurs in most mammalian cells. However, in contrast to most organic acids, pivaloyl-CoA cannot be further metabolized in any mammalian cells. As pivaloyl-CoA accumulates, the pivaloyl moiety can be transferred from coenzyme A to carnitine to form pivaloylcarnitine. The cellular coenzyme A and carnitine pools appear to be in equilibrium. Thus, the degree of pivaloyl-CoA accretion will be reflected in the carnitine pool as pivaloylcarnitine. In animal models, administration of pivalate is associated with pivaloylcarnitine accumulation and a shift in the carnitine pool toward acylcarnitines (Bianchi and Davis, 1991; Diep et al., 1995a).

Despite the accumulation of pivaloyl-CoA and pivaloylcarnitine in cells, cellular metabolism is well preserved in in vitro model systems. A build-up of acyl-CoAs may be toxic if the compound inhibits other cellular pathways directly or through depletion of coenzyme A (Brass, 1994). Similarly, the resultant shift of the carnitine pool toward acylcarnitines may decrease carnitine availability. However, even at very high concentrations (greater than 1 mM), pivalate does not have major effects on glucose or lipid metabolism (Ruff and Brass, 1991; Ji and Tremblay, 1993). This most likely reflects the lack of direct toxicity of pivaloyl-CoA, and the preservation of adequate free coenzyme A and carnitine under the kinetics of the synthase and transferase reactions in the cell. Thus, direct or acute cellular toxicity of pivalate is not a major concern with pivalate-containing prodrugs.

Cellular accumulation of acylcarnitines leads to export of the compounds to the plasma, from which the acylcarnitine can be transported to other tissues for metabolism or transported to the kidney for elimination in the urine. Specific transport mechanisms may facilitate the cellular export of acylcarnitines across plasma membranes, with carnitine cotransported from the plasma into the cell through an exchange mechanism (Sartorelli et al., 1985). Net elimination occurs through urinary excretion of pivaloylcarnitine because no mammalian tissue can further catabolize pivaloyl-CoA.

B. Impact of Net Pivaloylcarnitine Excretion

1. Theoretical Considerations. As discussed above, once generated, pivaloylcarnitine will move from tissues to plasma, and ultimately be excreted in the urine. At high pivalate doses, the loss of pivaloylcarnitine may exceed normal physiological carnitine excretion. The net loss will reflect the stoichiometry of the pivalate dose and the percentage of the pivalate eliminated as pivaloylcarnitine. Pivalate may be excreted as the glucuronic acid conjugate in animals (Vickers et al., 1985), whereas pivaloylcarnitine is the dominant route of pivalate elimination in humans. Thus, for each mole of pivalate or pivalate-generating prodrug administered, there is the potential to lose one mole of carnitine as pivaloylcarnitine in the urine. This may be offset by decreased excretion of carnitine or other acylcarnitines, by increased synthesis or dietary intake, or by non-pivaloylcarnitine elimination of pivalate. However, this construct allows worst-case estimates to be made and clearly emphasizes that any impact of pivalate prodrug administration on carnitine homeostasis will be dependent on the dose of prodrug given and the duration of therapy.

The effect of pivaloylcarnitine excretion on total body carnitine stores can be readily estimated. For example, each 500 mg of pivampicillin contains 1.08 mmol of drug, and hence may yield 1.08 mmol of pivalate. A 70-kg patient receiving 2 g of pivampicillin per day would be at risk of losing 4.32 mmol of carnitine per day or approximately 3.5% of total body stores. In contrast, a 70-kg patient receiving 9 mg/kg per day of cefditoren pivoxil (0.0016 mmol of pivalate per milligram) is at risk of losing 1.01 mmol of carnitine or 0.8% of body stores (Fujii et al., 1993). Daily carnitine biosynthesis is approximately 0.07 mmol, and dietary intake may be estimated as 0.28 mmol per day (Lombard et al., 1989). Thus, unless therapy was maintained for an extended period of time, it would be predicted that due to the large carnitine stores, pivalate prodrug administration would

not adversely affect metabolism. This theoretical conclusion is supported by experiments in animals and clinical experience, as detailed below.

2. Animal Models. The effect of pivalate administration on carnitine homeostasis and metabolism has been studied in animals. Animal models will not extrapolate directly to humans due to species differences in carnitine and pivalate metabolism, but nonetheless provide insight into the in vivo pivalate-carnitine interaction.

Bianchi and Davis (1991) administered sodium pivalate to rats for 8 weeks via incorporation into their drinking water. Within 4 days, urinary total carnitine elimination was markedly elevated, and plasma total carnitine concentration was depressed to less then 40% of control values. In contrast, total carnitine content of skeletal muscle was unaffected after 4 days. This illustrates the large pool size and slow turnover of the skeletal muscle carnitine pool, and that plasma concentration is not indicative of tissue stores. With 8 weeks of treatment, plasma total carnitine concentrations fell to 25% of control values, and total carnitine content of skeletal muscle was approximately 50% of control values. Thus, cumulative dose is critical in defining the impact of pivalate administration on carnitine stores. Liver and heart carnitine content fell to variable degrees during the 8 weeks of treatment. Despite these apparently dramatic changes in carnitine levels, animals on pivalate ate and gained weight at the same rate as controls, and their metabolic response to starvation was unaffected, except for an exaggerated ketosis. As ketone bodies are the products of fatty acid oxidation, this accelerated ketosis is surprising since decreased carnitine content might have been predicted to decrease fatty acid oxidation and ketosis. In contrast, liver fatty acid oxidation was apparently preserved despite the 8 weeks of pivalate exposure and carnitine losses.

Diep et al. (1992) administered large doses of pivampicillin (630 mg/kg) orally to rats for 24 days. Plasma total carnitine concentrations fell by 60%, liver content by 70%, but heart total carnitine content was only 35% less than controls and skeletal muscle content was 30% less than controls. The hepatic response to fasting as assessed by plasma β-hydroxybutyrate concentrations was mildly accentuated, as seen by Bianchi and Davis (1991). Nakajima et al. (1996) also demonstrated relatively preserved hepatic metabolic function despite pivalate-induced carnitine depletion. Adaptive compensatory mechanisms, including an increase in carnitine biosynthesis, may further limit the net impact of pivaloylcarnitine losses on tissue carnitine stores (Nakajima et al., 1999).

Broderick et al. (1995) administered pivalate (approximately 192 mg of pivalate/kg of body weight) to rats for up to 28 weeks. This protracted exposure resulted in a 60% decrease in heart total carnitine content. In vitro assessment of these hearts revealed impairment in fatty acid oxidation that limited cardiac work. In contrast, treatment of rats with pivalate for two weeks was associated with a 24% reduction in heart carnitine content but no change in cardiac function under control conditions (Broderick et al., 2001). Two weeks of pivalate exposure was associated with impaired recovery of cardiac function after a no-flow ischemic insult in this rat model (Broderick et al., 2001).

Rat pups born with mothers who were treated with pivalate throughout pregnancy demonstrated decreased plasma and tissue carnitine contents (Ricciolini et al., 2001). This is consistent with the observation in humans that neonatal plasma carnitine concentrations are influenced by maternal carnitine concentrations (Novak et al., 1981). It is not clear if maternal pivalate concentrations are high enough to result in fetal pivalate exposure. If rats born from pivalate treated mothers are continued on pivalate treatment for the first four months of life, the animals develop hyperglycemia, insulin resistance and increased body mass (Ricciolini et al., 2001). The relevance of this extreme pivalate exposure to other models or potential therapeutic use of pivalate prodrugs is unclear.

Thus, studies of pivalate administration to animals confirm the loss of carnitine as pivaloylcarnitine. Net losses occur preferentially in the plasma and liver compartments. The degree of depletion is a function of cumulative dose exposure. The large tissue carnitine stores allow preservation of tissue function except under extreme degrees of pivalate exposure and associated carnitine depletion.

3. Human Studies. The formation of pivaloylcarnitine following pivalate-containing prodrug administration has been confirmed in human studies, and these reports demonstrate that urinary pivaloylcarnitine excretion is the dominant route of pivalate elimination (Vickers et al., 1985; Melegh et al., 1987, 1990; Konishi and Hashimoto, 1992). Melegh et al. (1987), studying children receiving a 7-day course of pivampicillin (2 g/day), demonstrated that the pivaloylcarnitine excretion was associated with a 39% decrease in plasma total carnitine, and a 73% decrease in plasma carnitine concentration. This study also demonstrated that urinary pivaloylcarnitine clearance approximated creatinine clearance, implying lack of pivaloylcarnitine reabsorption in the kidney and explaining the rapid fall in plasma carnitine concentrations.

Holme et al. (1989) confirmed the acute effects of pivampicillin and pivmecillinam to increase urinary pivaloylcarnitine excretion and decrease plasma carnitine concentrations. They studied a group of children on long-term treatment with a combination of pivampicillin and pivmecillinam for urinary tract infections. These patients had been on the antibiotics for as long as 30 months, and demonstrated dramatic decreases in their plasma carnitine concentrations. Furthermore, in two patients who underwent muscle biopsy, muscle carnitine content was decreased more then 75% compared

with reference values. Although no specific signs or symptoms were associated with these low carnitine contents, the authors note that they had previously seen two patients with hypoketotic hypoglycemia with pivaloylcarnitine in their urine. Furthermore, patients on the pivalate prodrugs frequently complained of being tired, and two subjects had discontinued antibiotic therapy due to nonspecific symptoms. Thus, long-term treatment with pivalate prodrugs may cause substantial depletion of muscle carnitine stores. However, the degree of depletion required to produce clinical symptoms or signs could not be identified. Of note, in patients with inherited muscle carnitine deficiency due to a mutation in the plasma membrane transport system, carnitine therapy is effective in relieving symptoms despite yielding only very low muscle carnitine contents (Schulte et al., 1990; Kerner and Hoppel, 1998).

Diep et al. (1993) studied an additional 6 patients who had been on pivaloyl-containing antibiotics for as long as 24 months. As expected, these patients all showed low serum carnitine concentrations, and with discontinuation of therapy, serum total carnitine concentrations did not return to the reference range until periods ranging from 5 to 12 months. Only one of the six patients demonstrated symptoms or signs potentially related to carnitine deficiency. This subject, an 82-year-old woman who had received pivmecillinam for 8 months, reported muscle weakness, anorexia, and cognitive impairment. This patient had blunted ketosis with starvation and dicarboxylic aciduria. The metabolic abnormalities and symptoms resolved ten months after pivmecillinam therapy was discontinued. The patient had no signs or symptoms of cardiac dysfunction.

Holme and colleagues (1992) studied 17 children who had been treated with a combination of pivmecillinam and pivampicillin for at least 1 year. All had low serum carnitine concentrations (average 2.0 μM). Several patients were described as having nonspecific symptoms such as tiredness, behavioral problems or weakness, which resolved after discontinuation of the antibiotic or with carnitine supplementation. Four patients had muscle biopsies for carnitine quantitation and demonstrated carnitine contents that were less then 20% of control values. Six of the patients underwent a fasting test to assess the metabolic status of the liver. Three of these patients developed hypoglycemia during the fast and were characterized by the authors as having a blunted ketogenic response. Blunted ketosis and hypoglycemia would be consistent with impaired liver fatty acid oxidation. The authors emphasized the relationship between cumulative dose and carnitine depletion, and estimated that half the body stores would be lost after 5 months of therapy.

The effects of 7 to 8 weeks of treatment with pivmecillinam (1200 mg/day) were studied in seven adults (Abrahamsson et al., 1995b). Muscle total carnitine content fell by 55% during the treatment period. Serial echocardiography demonstrated a 10% decrease in left ventricular mass, but a number of functional assessments, including left ventricular ejection fraction and fractional shortening, were unaffected by the treatment.

In contrast to the dramatic effects of long-term pivalate-prodrug administration, Abrahamsson et al. (1994) demonstrated less dramatic effects of short-term treatment. Six patients were given 1200 mg of pivmecillinam for 12 days. Carnitine concentrations fell from an average 42.8 μM to 11.6 μM. In contrast, muscle total carnitine content did not change, emphasizing the compartmentalization of carnitine homeostasis. Two subjects had a blunted ketosis during a 36-h fast post-treatment as compared with pretreatment, which was interpreted by the authors to reflect clinically significant hepatic carnitine deficiency. However, two subjects showed equivalent ketotic responses, while two subjects did not demonstrate ketosis during the pre- or post-treatment fast, making generalizing the results difficult. In a follow-up study, the same authors (Abrahamsson et al., 1995a) demonstrated decreases in serum carnitine concentrations after 7 days of pivmecillinam therapy.

Ito et al. (1995) also observed a decrease in blood carnitine concentrations with short-term (3–14 days) treatment with a pivalate prodrug, and suggested that the hypocarnitinemia was associated with increased ammonia concentrations. However, the blood ammonia concentrations were within the normal range, no patient developed any symptoms, and the ammonia concentrations pretreatment were not different from those during drug therapy. Statistically significant changes in ammonia concentration were only demonstrated when comparing post-treatment to concentrations during therapy, raising the possibility that the acute infection may have contributed to the differences noted. Melegh et al. (1997) suggested that short-term pivampicillin therapy for the treatment of bacterial pharyngitis was associated with a decrease in the use of fat as an energy source and an increase in the respiratory exchange quotient.

Therapy with carnitine during a course of treatment with pivalate prodrugs can correct the hypocarnitinemia associated with pivaloylcarnitine excretion (Holme et al., 1992; Melegh et al., 1997; Pap et al., 1999). However, the clinical significance of this correction is unknown. In potentially symptomatic patients with severe carnitine depletion secondary to long-term pivalate exposure, carnitine supplementation may accelerate symptom relief (Holme et al., 1992).

Thus, the clinical experience with pivalate-containing prodrugs is congruent with both the theoretical considerations and the animal models. Short-term exposure (less then 2 weeks) and molar pivalate exposures equivalent to less then 10 to 20% of total body carnitine stores are associated with hypocarnitinemia, but no significant decrease in muscle carnitine stores and no clinical signs or symptoms. Treatment for periods of 1 to 2 months are associated with decreases in muscle carnitine content,

but subjects remain asymptomatic due to the extent of body carnitine stores. In contrast, long-term treatment (greater then 6 months) with pivalate prodrugs may result in dramatic depletion of carnitine stores and signs or symptoms consistent with carnitine deficiency.

4. Considerations in Disease Populations. The effect of pivalate-prodrug administration in humans may be accentuated by underlying disease or metabolic conditions. As carnitine depletion is stoichiometric with the pivalate dose, the impact may be increased if body carnitine stores are significantly below normal. A rare inherited mutation in a critical carnitine transport will result in systemic carnitine deficiency and inadequate carnitine stores (Stanley, 1995; Pierpont et al., 2000; Lahjouji et al., 2001). These patients, estimated as fewer then 100 worldwide, present with severe metabolic disturbances in childhood. Similarly, patients with inherited organic acidurias characterized by acyl-CoA accretion may develop carnitine insufficiency, or secondary carnitine deficiency, due to increased acylcarnitine excretion (Chalmers et al., 1983). These patients will be under the care of a physician skilled in metabolic disorders and will often be on carnitine supplementation as part of their therapeutic regimen.

Diseases associated with decreases in muscle carnitine stores might also be predisposed to pivalate-induced carnitine depletion. Patients with end-stage renal disease on maintenance hemodialysis for extended periods may have muscle carnitine stores 50% lower then healthy controls (Moorthy et al., 1983; Hiatt et al., 1992). Muscle carnitine stores (per gram of muscle) have been shown to be preserved in diabetes (Cederblad et al., 1977), alcohol abuse (de Sousa et al., 1988), patients with protein-calorie malnutrition (Wennberg et al., 1992), liver disease (Wennberg et al., 1992), and in woman compared with men (Lennon et al., 1986; Opalka et al., 2001). Muscle skeletal content has been reported to be unaffected by aging (Cederblad et al., 1976), or to be unaffected in women with men showing a small decline with aging (Opalka et al., 2001). Thus, these diverse populations would not represent substantially increased theoretical risk during short-term exposure to pivalate prodrugs.

Carnitine stores will also be relatively reduced if muscle mass is decreased. This decrease may result from small stature, with normal muscle per body weight, or with a decrease in muscle mass per body weight (for example, in paraplegics). Thus, children will have smaller carnitine stores then adults. However, if pivalate exposure is normalized per kilogram of body weight, no increase in risk will result due to the stoichiometric nature of the pivalate-carnitine interaction. Premature infants may represent an exception to this due to a lower carnitine biosynthetic capacity and incompletely developed body carnitine stores (Shenai and Borum, 1984). Muscle carnitine stores appear to be near adult levels in full term infants (Shenai and Borum, 1984). A reduction in muscle mass per body weight will not affect total carnitine stores dramatically except in conditions such as morbid obesity, where pivalate-containing prodrugs might be best dosed on a lean body mass basis to yield equivalent pivalate exposures.

Valproate (2-propylpentanoic acid) is a short-chain fatty acid used therapeutically as an anticonvulsant, primarily in pediatric populations (Wallace, 1988). Like pivalate, valproate can be activated to the corresponding acyl-CoA (valproyl-CoA). Unlike pivalate, valproyl-CoA can be metabolized by mammalian tissues to a variety of metabolites (Bjorge and Baillie, 1991; Li et al., 1991; Sugimoto et al., 1996). Although valproylcarnitine can be generated from valproyl-CoA, it is a quantitatively minor pathway of valproate elimination (Millington et al., 1985; Melegh et al., 1990) and thus, valproate-induced absolute secondary carnitine deficiency is not a major consideration. Most studies (Ohtani et al., 1982; van Wouwe, 1995; Castro-Cago et al., 1998) but not all (Hirose et al., 1998) demonstrate that valproate therapy is associated with a mild decrease in plasma carnitine concentrations (carnitine concentrations approximately 20 μM versus a normal range of 30 to 50 μM). Data on tissue carnitine contents during valproate therapy are not available. Lower plasma carnitine concentrations may be more common in valproate-treated individuals with underlying disease (Ohtani et al., 1982; Beghi et al., 1990), but the differential effects of the disease compared with valproate are difficult to define.

Valproate therapy is associated with a rare, severe hepatoxicity that is similar to Reye's syndrome (Bryant and Dreifuss, 1996). This hepatotoxicity has focused attention on the metabolic effects of valproate. Although pivaloyl-CoA appears to be relatively metabolically inert except for coenzyme A sequestration (Ruff and Brass, 1991), valproyl-CoA and its metabolites have profound effects on hepatic intermediary metabolism (Turnbull et al., 1983; Ito et al., 1990; Ponchaut et al., 1992). Although carnitine supplementation can "normalize" the plasma carnitine pool during valproate therapy (Kossack et al., 1993), there are no data demonstrating that carnitine supplementation changes the incidence or severity of the Reye's-like toxicity associated with valproate. Similarly, no data are available that demonstrate an association between the status of the carnitine pool and the development of this rare hepatic toxicity. Studies that report carnitine abnormalities in patients with valproate-associated hepatotoxicity (Krahenbuhl et al., 1995) are difficult to interpret due to the expected changes resulting from the metabolic derangements of the acute hepatic failure. Thus, the data do not support a theoretical concern that coadministration of pivalate-prodrugs with valproate increases the risk of severe hepatotoxicity, and non-carnitine related effects of valproate metabolites are likely to play an etiological role in this syndrome. As the host factors responsible for the idiosyncratic development of hepatotoxicity are not

known, there is no basis to link any aspect of pivalate metabolism with this syndrome.

IV. Regulatory Labeling of Pivalate Prodrugs

Cefditoren pivoxil was recently approved for marketing in the United States and is the only pivalate-containing prodrugs currently marketed in the United States. The U.S. cefditoren pivoxil label notes that the drug is contraindicated in patients with carnitine deficiency or metabolic defects associated with clinically significant carnitine deficiency. Cefditoren pivoxil is intended for short-term therapy, and thus consistent with the concepts discussed above, the label indicates that neither measurement of carnitine concentrations nor carnitine supplementation are recommended with its use.

The labeling of pivalate prodrugs marketed in other countries reflects varied responses to the potential toxicity by manufacturers and regulatory agencies. In Japan, the package inserts for cefteram pivoxil, cefetamet pivoxil, and cefcapen pivoxil all contain a statement that use may reduce serum carnitine. The label for cefetamet pivoxil in Germany contains a contraindication statement for patients with primary carnitine deficiency, as well as for patients on hemodialysis or those with diabetes mellitus. The label also warns against use with valproate. As discussed above, these contraindications without respect to duration of therapy in hemodialysis, diabetes and with valproate are theoretical at best and may not be supported by a careful review of carnitine homeostasis during pivalate exposure. Similar warnings appear on the pivampicillin label in Sweden, with specific mention of unstable diabetes, premature children, patients on valproate, and patients with very small muscle mass. In contrast, the pivampicillin labeling in Canada emphasizes the absence of clinical adverse events with short-term use despite the lowering of serum carnitine concentrations and concludes that neither the monitoring of serum carnitine concentrations or the administration of carnitine supplementation are indicated during pivampicillin use. The United Kingdom labeling for this product cautions against use in patients with carnitine deficiency or those using valproate.

V. Conclusions and Clinical Implications

Animal and clinical studies support a model in which pivalate prodrug administration results in pivaloyl-CoA production that does not adversely affect cellular function. The pivaloyl-CoA accumulation in turn will result in pivaloylcarnitine production, a shift in the distribution of the carnitine pool toward acylcarnitines, and a net increase in total carnitine urinary losses. The impact of these losses on the extracellular carnitine pool may be dramatic, but without clinical sequelae. Tissue total carnitine content will only be impacted as the urinary losses are sustained for a period of time. It is likely that the liver will be the first organ affected, but despite loss of carnitine and a shift in distribution from carnitine toward pivaloylcarnitine, hepatic function is not adversely affected by short-term exposure to pivalate. Continued pivalate exposure for months may more dramatically impact the liver carnitine pool and lead to depletion of the large muscle carnitine stores. Even under these conditions, residual carnitine stores appear sufficient to maintain critical tissue functions, and symptoms appear only under the most extreme conditions. Thus pivalate prodrugs intended for chronic use, such as the antiretroviral adefovir dipivoxil, incorporate carnitine supplementation as part of the dosing regimen (Kahn et al., 1999).

The above model is expected to be only minimally impacted by a variety of underlying disease conditions. Conditions of carnitine deficiency, either primary or secondary to a metabolic defect, might dramatically diminish carnitine stores and/or increase carnitine requirements. However, these patients are rare, should be under the care of a physician, and are often on carnitine supplementation that will mitigate the pivalate effects. Other chronic diseases appear to be associated with relative preservation of carnitine stores.

Clinical experience with pivalate prodrugs appears to support this clinical safety profile with short-term use. It is estimated that between 1996 and 2001, almost 1 billion treatment days experience was accumulated world wide with pivalate prodrugs (data from IMS Health, Fairfield, CT). Despite this broad exposure, published reports of clinical toxicity have been limited to patients with long duration treatment. It is likely that a broad spectrum of populations have been exposed to these drugs without any risk groups being identified. Thus, this experience is also consistent with the safety of short-term use of pivalate prodrugs.

The development of pivalate-containing prodrugs may facilitate optimization of key characteristics for novel therapeutic agents. These drugs will result in perturbations in carnitine homeostasis. In the absence of underlying carnitine deficiency, this change in carnitine metabolism is well tolerated unless the cumulative dose of pivalate (in moles) exceeds the scope of the body carnitine stores. Thus, in circumstances where chronic treatment is anticipated, pivalate-based modifications should be avoided during drug development. The changes in carnitine homeostasis should not be viewed as contraindications for the development of pivalate-prodrugs intended for short-term use with small cumulative doses (for example less than 0.5 mmol/kg of body weight) when pivalate provides properties superior to other potential carboxylic acids or other strategies.

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