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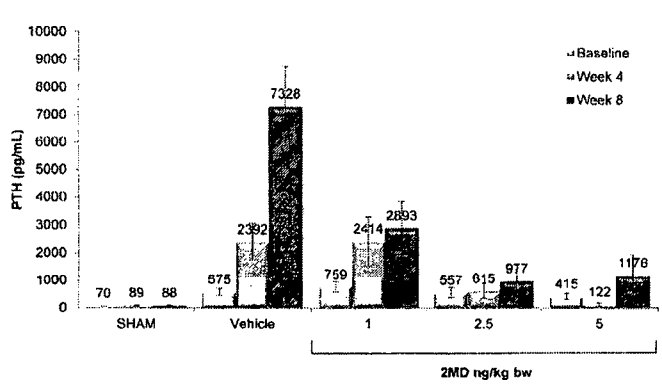
(54) Title: USE OF 2-METHYLENE-19-NOR-(20S)-1α,25-DIHYDROXYVITAMIN D_3 TO TREAT SECONDARY HYPERPARATHYROIDISM

Fig. 5



(57) Abstract: Disclosed is the use of 2-methylene-19-nor-(20S)-1α,25-dihydroxwitamin D_3 to treat and/or prevent secondary hyperparathyroidism and/or its accompanying symptoms in a subject having or at risk for developing secondary hyperparathyroidism without inducing hypercalcemia in the subject.

WO 2014/003849 A1

USE OF 2-METHYLENE-19-NOR-(20S)-1α,25-DIHYDROXYVITAMIN D_3 TO TREAT SECONDARY HYPERPARATHYROIDISM

BACKGROUND

[0001] This invention relates to vitamin D compounds useful in treating and/or preventing secondary hyperparathyroidism and/or the symptoms thereof, and more particularly to the use of the vitamin D compound 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D3 to treat and/or prevent secondary hyperparathyroidism and/or the symptoms thereof.

[0002] Renal disease has become an increasingly important health problem in virtually every country in the world including highly developed countries such as the United States. Presently there are about 250,000 patients on renal dialysis who have lost almost complete use of their kidneys. There are approximately ten times this number of patients who have lost some degree of renal function due to renal disease and are progressing to complete renal failure. Renal failure is evidenced by a decreased glomeruli filtration rate (GFR) from a high value of 110 ml/minute/1.73 m^2 to 30 ml/minute/1.73 m^2 where dialysis is often initiated.

[0003] Many factors contribute to the development of renal disease. High blood pressure is one of the significant contributors, as is having Type I or Type II diabetes. Current treatments for renal failure are limited to hemodialysis, an extremely expensive procedure that currently is supported by federal governments because individuals typically cannot afford this procedure on their own. The annual cost of renal disease in the United States alone is over $42 billion. Accordingly, effective methods for preventing renal disease and treating symptoms thereof would not only provide a major health benefit but would also provide a major economic benefit.

[0004] It is now universally accepted that vitamin D must first be 25-hydroxylated in the liver and subsequently 1α-hydroxylated in the kidney before it can function. (*See* DeLuca, "Vitamin D: The vitamin and the hormone," Fed. Proc. 33, 2211-2219, 1974). These two reactions produce the final active form of vitamin D, namely 1α,25-$(OH)_2D_3$.

(*See* DeLuca & Schnoes, "Vitamin D: Recent advances," Ann. Rev. Biochem. 52, 411-439, 1983). This compound then stimulates a number of physiological processes including: stimulating the intestine to absorb calcium, stimulating the kidney to reabsorb calcium, stimulating the intestine to absorb phosphate, and stimulating bone to mobilize calcium when signaled by high parathyroid hormone (PTH) levels. These actions result in a rise in plasma calcium and phosphorus levels that bring about the healing of bone lesions such as rickets and osteomalacia and prevent the neurological disorder of hypocalcemic tetany.

[0005] Secondary hyperparathyroidism is a universal complication in patients with chronic renal failure. Low levels of 1α,25-$(OH)_2D_3$ and phosphate retention are responsible for the development of secondary hyperparathyroidism. Low levels of circulating 1α,25-$(OH)_2D_3$ are the result of impaired kidney function resulting in the patient's inability to convert 25-hydroxy-vitamin D_3 to 1α,25-dihydroxyvitamin D_3. As a result of low levels of circulating 1α,25-$(OH)_2D_3$, intestinal calcium absorption is minimal which subsequently results in insufficient serum calcium levels. When the parathyroid glands sense a low level of serum calcium, the parathyroid glands secrete PTH which causes calcium to be mobilized from bone to regulate serum calcium. Left unchecked, this abnormal secretion of PTH will lead to the development of renal osteodystrophy. High PTH levels can also lead to: 1) weakening of the bones; 2) calciphylaxis (when calcium forms clumps in the skin and lead to ulcers and potentially death of surrounding tissue); 3) cardiovascular complications; 4) abnormal fat and sugar metabolism; 5) itching (pruritis); and 6) low blood counts (anemia).

[0006] 1α,25-dihydroxyvitamin D_3 has been used as a therapeutic for hyperparathyroidism in patients with renal diseases. In the treatment of secondary hyperparathyroidism of renal osteodystrophy, it is well known that 1α,25-dihydroxyvitamin D_3 binds to the vitamin D receptor (VDR) located in the parathyroid glands to suppress both growth and proliferation of the parathyroid cells and expression of the preproparathyoid gene. (*See* Demay *et al.*, "Sequences in the human parathyroid hormone gene that bind the 1,25-dihydroxyvitamin D_3 receptor and mediate transcriptional repression in response to 1,25-hydroxyvitamin D_3." Proc. Natl. Acad. Sci.

USA 89, 8097-8101,1992; and Darwish & DeLuca, "Identification of a transcription factor that binds to the promoter region of the human parathyroid hormone gene," Arch. Biochem. Biophys. 365, 123-130, 1999). Because of its ability to suppress parathyroid hormone (PTH), 1,25-$(OH)_2D_3$ has been used with success in the treatment of secondary hyperparathyroidism. (*See* Slatopolsky *et al.*, "Marked Suppression of Secondary Hyperparathyroidism by Intravenous Administration of 1,25-dihydroxycholecalciferol in Uremic Patients," J. Clin. Invest. 74:2136-2143, 1984). The use of 1α,25-dihydroxyvitamin D_3 in the treatment of secondary hyperparathyroidism of renal osteodystrophy is often precluded, however, by the development of hypercalcemia resulting from 1α,25-dihydroxyvitamin D_3's potent action on intestinal calcium absorption and bone mineral calcium mobilization.

[0007] As noted previously, secondary hyperparathyroidism typically will occur in patients undergoing renal dialysis. Chronic renal failure is the most common cause of secondary hyperparathyroidism. Failing kidneys do not convert enough vitamin D to its active form and do not adequately excrete phosphate. When this happens, insoluble calcium phosphate forms in the body and removes calcium from circulation. Ultimately, this leads to hypocalcemia and secondary hyperparathyroidism.

[0008] Secondary hyperparathyroidism also can result from gastrointestinal malabsorption syndromes (*e.g.*, chronic pancreatitis, small bowel disease, and malabsorption-dependent bariatric surgery in which the intestines do not absorb vitamins and minerals properly), where these syndromes may result in insufficient absorption of the fat soluble vitamin D. When vitamin D is insufficiently absorbed, hypocalcemia may develop and a subsequent increase in PTH secretion may result where the body attempts to increase serum calcium levels. However, hypocalcemia and secondary hyperparathyroidism also may appear in the early stages of renal disease due to low levels of $1,25(OH)_2D_3$. Other less common causes of secondary hyperparathyroidism are long-term lithium therapy, vitamin D deficiency, malnutrition, vitamin D-resistant rickets, or hypermagnesemia (*i.e.*, abnormally high blood magnesium levels).

[0009] Symptoms of secondary hyperparathyroidism include increased levels of serum PTH, serum phosphorus, and serum creatinine. Less overt symptoms include bone and joint pain, bone deformities, broken bones (fractures), swollen joints, kidney stones, increased urination, muscle weakness and pain, nausea, and loss of appetite. Other less common symptoms include fatigue, upper abdominal pain, and depression.

[00010] Treatment of secondary hyperparathyroidism typically involves addressing the underlying cause of the hypocalcemia. In patients with chronic renal failure, treatment consists of dietary restriction of phosphorus, supplements with an active form of vitamin D such as calcitriol, Hectorol®, or Zemplar®(paricalcitol), and phosphate binders which can be divided into calcium-based binders and non-calcium based binders. A newer class of medication is calcimimetics, one of which is commercially available as Sensipar®(cinacalcet) in the United States and Australia, and as Mimpara® in the European Union. Calcimimetics have achieved positive responses and are FDA approved for use in patients on dialysis, but have not been approved for use in chronic kidney disease pre-dialysis because, among other concerns, they can increase phosphorus levels. Most patients with hyperparathyroidism secondary to chronic kidney disease will improve after renal transplant, but many will continue to have a degree of residual hyperparathyroidism (*i.e.*, tertiary hyperparathyroidism) post-transplant with associated risk of bone loss.

[00011] Although serum phosphorus is usually normal in patients with early renal insufficiency, phosphate restriction can reduce secondary hyperparathyroidism. Dietary phosphate restriction increases 1,25-$(OH)_2D_3$ levels. (*See* Portale *et al.*, "Effect of Dietary Phosphorus on Circulating Concentrations of 1,25-dihydroxyvitamin D and Immunoreactive Parathyroid Hormone in Children with Moderate Renal Insufficiency," J. Clin. Invest. 73:1580-1589, 1984). This in turn decreases PTH by directly suppressing PTH gene transcription and by increasing intestinal calcium absorption. In later stages of renal failure, the extent of hyperparathyroidism and 1,25-$(OH)_2D_3$ deficiency increases, and phosphate restriction has little effect on 1,25-$(OH)_2D_3$ levels. (*See* Lopez-Hilker *et al.*, "Phosphorus Restriction Reverses Hyperparathyroidism in Uremia Independent of

Changes in Calcium and Calcitriol," Am. J. Physiol. 259:F432-F437, 1990). This is presumably due to the decreased renal mass available for 1,25-$(OH)_2D_3$ synthesis.

[00012] Several vitamin D analogs with low calcemic activity have been found to be nearly as effective as 1,25-$(OH)_2D_3$ in suppressing PTH secretion by cultured bovine parathyroid cells. These include 22-oxacalcitriol (OCT), (Brown *et al.*, "The Non-Calcemic Analog of Vitamin D, 22-oxacalcitriol (OCT) Suppresses Parathyroid Hormone Synthesis and Secretion," J. Clin. Invest. 84:728-732, 1989), as well as 1,25-$(OH)_2$-16-ene-23-yne-D_3, 1,25-$(OH)_2$-24-dihomo-D_3, and 1,25-$(OH)_2$-24-trihomo-22-ene-D_3. 22-oxacalcitriol has been examined in detail for this action *in vivo*. (*See* Brown *et al.*, "Selective Vitamin D Analogs and their Therapeutic Applications," Sem. Nephrol 14:156-174, 1994, reporting that 22-oxacalcitriol, despite its rapid clearance *in vivo*, could suppress PTH mRNA). Low, submaximal doses of calcitriol and OCT exhibited comparable inhibition. OCT also has been shown to suppress serum PTH in uremic rats and dogs.

[00013] Another analog of 1,25-$(OH)_2D_3$ with low calcemic and phosphatemic action is 19-nor-1,25-$(OH)_2D_2$. This analog of calcitriol has the carbon 28 and the double bond at carbon 22 that are characteristic of vitamin D_2 compounds, but it lacks carbon 19 and the exocyclic double bond found in all natural vitamin D compounds. Studies *in vitro* utilizing a primary culture of bovine parathyroid cells demonstrated that 19-nor-1,25-$(OH)_2D_2$ had a similar suppressive effect on PTH as 1,25-$(OH)_2D_3$. A 52% suppression on PTH release was obtained with 19-nor-1,25-$(OH)_2D_2$ at 10^{-7}M. There was no significant difference in the suppressive effect of PTH secretion between the two compounds.

[00014] Thereafter, preliminary studies were performed *in vivo* to determine the calcemic activity of 19-nor-1,25-$(OH)_2D_2$. It was found that 1,25-$(OH)_2D_3$ (10 ng/rat/10 days) increased serum calcium to the same magnitude as 19-nor-1,25-(OH_2) D_2 (100 ng/rat/10 days). Because of this, three different doses of 1,25-$(OH)_2D_3$ (2, 4, and 8 ng) and 19-nor-1,25-$(OH)_2D_2$ (8, 25, and 75 ng) were selected for chronic studies. After two months of renal insufficiency, the animals received the above two compounds at the three

indicated doses, four times, during a period of eight days. As expected, 1, 25-$(OH)_2D_3$ suppressed pre-pro-PTH mRNA and PTH secretion. However, this decrease was statistically significant only with a 8 ng dose, and this dose induced hypercalcemia and hyperphosphatemia. On the other hand, none of the doses of 19-nor-1,25-$(OH)_2D_2$ produced statistically significant changes in serum ionized calcium or serum phosphorus.

[00015] 19-nor-1α,25$(OH)_2D_2$ is also known as Paricalcitol and 19-nor-1α,25-dihydroxy-ergocalciferol. Paricalcitol injection is available commercially as Zemplar® from Abbott Laboratories, Abbott Park, Ill. A paricalcitol (Zemplar®) injection is described in U.S. Pat. No. 6,136,799 and has been approved by the FDA and is marketed for the prevention and treatment of secondary hyperparathyroidism associated with chronic renal failure (CKD Stage 5 or end-stage renal disease (ESRD), GFR <15 mL/min/1.73 m^2). This intravenous formulation contains 2-10 micrograms/milliliter of paricalcitol, 30% (v/v) propylene glycol, 20% (v/v) ethanol and approximately 50% (v/v) water. Studies indicate that paricalcitol injection suppresses elevated levels of PTH with minimal effect on serum calcium and phosphorus levels. Since its approval by the FDA in April of 1998, it is estimated that approximately 200,000 patients have received at least one dose of paricalcitol injection. Clinically, the safety and efficacy of paricalcitol injection to treat secondary hyperparathyroidism are well established.

[00016] Hyperphosphatemia is also a persistent problem in chronic hemodialysis patients and can be further aggravated by therapeutic doses of 1,25-$(OH)_2D_3$. (*See* Delmez *et al.*, "Hyperphosphatemia: Its Consequences and Treatment in Patients with Chronic Renal Disease," Am. J. Kidney Dis. 19:303-317, 1992; and Quarles *et al.*, "Prospective trial of Pulse Oral versus Intravenous Calcitriol Treatment of Hyperparathyroidism in ESRD," Kidney Int. 45:1710-1721, 1994). In addition, the control of phosphate absorption with large doses of calcium carbonate only increases the risk of hypercalcemia from 1,25-$(OH)_2D_3$ therapy. (*See* Meyrier *et al.*, "The Influence of a High Calcium Carbonate Intake on Bone Disease in Patients undergoing Hemodialysis," Kidney Int. 4:146-153, 1973; Moriniere *et al.*, "Substitution of Aluminum Hydroxide by High Doses of Calcium Carbonate in Patients on Chronic Hemodialysis: Disappearance of Hyperaluminaemia and Equal Control of

Hyperparathyroidism," Proc. Eur. Dial Transplant Assoc. 19:784-787, 1983; and Slatopolsky *et al.*, "Calcium Carbonate as a Phosphate Binder in Patients with Chronic Renal Failure Undergoing Dialysis," New Engl. J. Med. 315:157-161, 1986). Thus, an analog of 1,25-$(OH)_2D_3$ that can suppress PTH with minor effects on calcium and phosphate metabolism would be an ideal tool for the control and treatment of secondary hyperparathyroidism.

[00017] Another vitamin D analog, namely, 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 (referred to in the literature as "2MD") is also known to suppress PTH production. (*See* U.S. Published Application No. 2011/0034426A1). Although it would therefore appear to be a candidate for treating secondary hyperparathyroidism, it is also well known from U.S. Patent No. 5,843,928 that 2MD has very potent calcemic activity. 2MD significantly increases bone calcium mobilization activity to a level likely to be 10-100 times that of 1α,25-$(OH)_2D_3$ while also exhibiting a modest increase in intestinal calcium transport activity. Due to this highly selective activity for the mobilization of calcium from bone, the compound 2MD was never seriously considered as a pharmaceutical agent for treating secondary hyperparathyroidism, until now.

SUMMARY

[00018] It has now been discovered that the vitamin D analog 2MD has the ability to treat secondary hyperparathyroidism as well as symptoms of secondary hyperparathyroidism when administered under well-controlled conditions to a subject in need thereof. It also now been discovered that the vitamin D analog 2MD has the ability to prevent secondary hyperparathyroidism as well as symptoms of secondary hyperparathyroidism when administered under well-controlled conditions to a subject in need thereof.

[00019] In one embodiment, the present invention provides a novel method of treating secondary hyperparathyroidism by administering a therapeutically effective amount of a composition comprising 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 (2MD) or pharmaceutically acceptable salts thereof as the active agent to a subject

exhibiting symptoms of secondary hyperparathyroidism, without inducing hypercalcemia in the subject.

[00020] In another embodiment, the present invention provides a novel method of treating symptoms of secondary hyperparathyroidism by administering a therapeutically effective amount of a composition comprising 2-methylene-19-nor-(20S)-1α,25-dihyroxyvitamin D_3 (2MD) or pharmaceutically acceptable salts thereof as the active agent to a subject exhibiting symptoms of secondary hyperparathyroidism, without inducing hypercalcemia in the subject.

[00021] In yet another embodiment, the present invention provides a novel method of preventing secondary hyperparathyroidism by administering a therapeutically effective amount of a composition comprising 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 (2MD) or pharmaceutically acceptable salts thereof as the active agent to a subject at risk of developing secondary hyperparathyroidism, without inducing hypercalcemia in the subject.

[00022] In still another embodiment, the present invention provides a novel method of preventing symptoms of secondary hyperparathyroidism by administering a therapeutically effective amount of a composition comprising of 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 (2MD) or pharmaceutically acceptable salts thereof as the active agent to a subject at risk of developing secondary hyperparathyroidism, without inducing hypercalcemia in the subject.

[00023] In one embodiment, the 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 is formulated in an oral, topical, transdermal, parenteral, injectable or infusable form to be administered in amounts ranging from 10 ng/day to about 1 μg/day. Preferably, for the treatment of or prevention of secondary hyperparathyroidism, or for the treatment or prevention of the symptoms of secondary hyperparathyroidism, the compound 2MD is administered either orally or parenterally (i.v.). The dose may be properly selected in accordance with the specific route of administration. Suitable doses may include doses within the range of about 10 ng to about 1 ug per day. Preferably a

dose is administered three times per week either intravenously or orally to subjects receiving hemodialysis treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

[00024] Figure 1 schematically illustrates the intraperitoneal treatment protocol with 2MD contemplated herein.

[00025] Figure 2 is a graph illustrating the effect of intraperitoneal administration of 2MD at various doses on serum PTH in a uremic rat model.

[00026] Figure 3 is a graph illustrating the effect of intraperitoneal administration of 2MD at various doses on serum calcium in a uremic rat model.

[00027] Figure 4 schematically illustrates the oral treatment protocol with 2MD contemplated herein.

[00028] Figure 5 is a graph illustrating the effect of oral administration of 2MD at various doses on serum PTH in a uremic rat model.

[00029] Figure 6 is a graph illustrating the effect of oral administration of 19-nor-1α,25-dihdroxyvitamin D_2 (marketed under the tradename Zemplar®) at various doses on serum PTH in a uremic rat model.

[00030] Figure 7 is a graph illustrating the effect of oral administration of 2MD at various doses on serum calcium in a uremic rat model.

[00031] Figure 8 is a graph illustrating the effect of oral administration of 19-nor-1α,25-dihydroxyvimtin D_2 (marketed under the tradename Zemplar®) at various doses on serum PTH in a uremic rat model.

[00032] Figure 9 is a graph illustrating the effect of oral administration of 2MD at various doses on serum phosphorus in a uremic rat model.

[00033] Figure 10 is a graph illustrating the effect of oral administration of 19-nor-1α,25-dihydroxyvimtin D_2 (marketed under the tradename Zemplar®) at various doses on serum phosphorus in a uremic rat model.

[00034] Figure 11 is a graph illustrating the effect of oral administration of 2MD at various doses on serum creatinine in a uremic rat model.

[00035] Figure 12 is a graph illustrating the effect of oral administration of 19-nor-1α,25-dihydroxyvimtin D_2 (marketed under the tradename Zemplar®) at various doses on serum creatinine in a uremic rat model.

[00036] Figure 13 is a graph illustrating the effect of oral administration of 2MD at various doses on serum PTH in a Phase 1B human trial of postmenopausal women.

DETAILED DESCRIPTION

[00037] Disclosed are methods of treating and/or preventing secondary hyperparathyroidism or the symptoms thereof. The disclosed methods further may described as follows based on the following definitions.

[00038] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications and patents specifically mentioned herein are incorporated by reference in their entirety for all purposes including describing and disclosing the chemicals, instruments, statistical analyses and methodologies which are reported in the publications which might be used in connection with the invention. All references cited in this specification are to be taken as indicative of the level of skill in the art. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue or prior invention.

[00039] In the specification and in the claims, the terms "including" and "comprising" are open-ended terms and should be interpreted to mean "including, but not limited to." These terms encompass the more restrictive terms "consisting essentially of"

and "consisting of." It is also to be noted that the terms "comprising," "including," "characterized by" and "having" can be used interchangeably.

[00040] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. As well, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein.

[00041] Where a range of values is provided, it is understood that each intervening value, and any combination or subcombination of intervening values, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the range of values recited.

[00042] Certain ranges are presented herein with numerical values being preceded by the term "about." The term "about" is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number, and thus will typically refer to a number or value that is 10% below or above the specifically recited number or value.

[00043] The disclosed methods may be utilized to treat and/or prevent secondary hyperthyroidism in a patient in need thereof. A patient in need thereof may include, but is not limited to, a patient having or at risk for developing secondary hyperthyroidism subsequent to a renal disease or disorder. A patient in need thereof may include, but is not limited to, a patient having or at risk for developing secondary hyperthyroidism subsequent to renal osteodystrophy, for example, due to renal failure. A patient in need thereof may include a patient undergoing renal dialysis. A patient in need thereof may include, but is not limited to, a patient having or at risk for developing secondary hyperthyroidism as a result of a gastrointestinal malabsorption syndromes (*e.g.*, chronic pancreatitis, small bowel disease, and malabsorption-dependent bariatric surgery in which the intestines do not absorb vitamins and minerals properly). A patient in need

thereof may include, but is not limited to, a patient having or at risk for developing secondary hyperthyroidism as a result of a long-term lithium therapy, vitamin D deficiency, malnutrition, vitamin D-resistant rickets, or hypermagnesemia (*i.e.*, abnormally high blood magnesium levels).

[00044] The disclosed methods may be utilized to treat and/or prevent the symptoms of secondary hyperthyroidism in a patient in need thereof. Symptoms of secondary hyperthyroidism treated and/or prevented by the disclosed methods may include, but are not limited to: weakening of the bones; calciphylaxis (when calcium forms clumps in the skin and lead to ulcers and potentially death of surrounding tissue); cardiovascular complications; abnormal fat and sugar metabolism; itching (pruritis); and low blood counts (anemia). Other symptoms of secondary hyperthyroidism treated and/or prevented by the disclosed methods may include: increased levels of serum PTH, serum phosphorus, and serum creatinine. Further symptoms of secondary hyperthyroidism treated and/or prevented by the disclosed methods may include: bone and joint pain, bone deformities, broken bones (fractures), swollen joints, kidney stones, increased urination, muscle weakness and pain, nausea, and loss of appetite. Even further symptoms of secondary hyperthyroidism treated and/or prevented by the disclosed methods may include: fatigue, upper abdominal pain, and depression.

[00045] Previously, it has been demonstrated that 300 ng per day of 1α,25-dihydroxyvitamin D_3 ($1,25(OH_2D_3$) administered through the diet can effectively prevent renal disease and renal failure by reducing the symptoms of renal disease. (*See* James Wonkee Kim. Effects of 1α,25-dihydroxyvitamin D_3 on the MRL/MpJ-fas/lpr model of systemic lupus erythematosus (Ph.D. Thesis, University of Wisconsin-Madison (2009)). For instance, it has been previously shown that administering 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) completely prevents proteinuria in the MRL/MpJ-FAS^{lpr} (MRL/lpr) mouse model of systemic lupus erythematosus (SLE). (*See id.*). However, severe hypercalcemia always accompanied this treatment. Hypercalcemia (*i.e.*, increased levels of calcium in the blood) can result in serious physical problems, including death. Specifically, an increase in calcium of approximately 2 mg/100 ml is considered mild hypercalcemia and is not considered a problem. However, an increase in calcium levels

of more than 2 mg/100 ml is considered severe hypercalcemia and can cause calcification of the kidney, heart, and aorta. Clearly, the use of this compound is not optimal to treat or prevent secondary hyperparathyroidism, or the symptoms thereof, because of the resultant hypercalcemia.

[00046] 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 (2MD) is an analog of $1,25(OH)_2D_3$ which has been shown to have increased *in vivo* potency toward bone but not on intestinal calcium absorption. The overall synthesis of 2MD is illustrated and described more completely in U.S. Pat. No. 5,843,928, issued Dec. 1, 1998, and entitled "2-Alkylidene-19-Nor-Vitamin D Compounds" the specification of which is specifically incorporated herein by reference. The biological activity of 2MD is also reported in U.S. Patent No. 5,843,928 and in Shevde *et al.*, "A Potent Analog of 1α,25-dihydroxyvitamin D_3 Selectively Induces Bone Formation" PNAS, Vol. 99, No. 21 pp 13487-13491 (2002), both of which are specifically incorporated herein by reference.

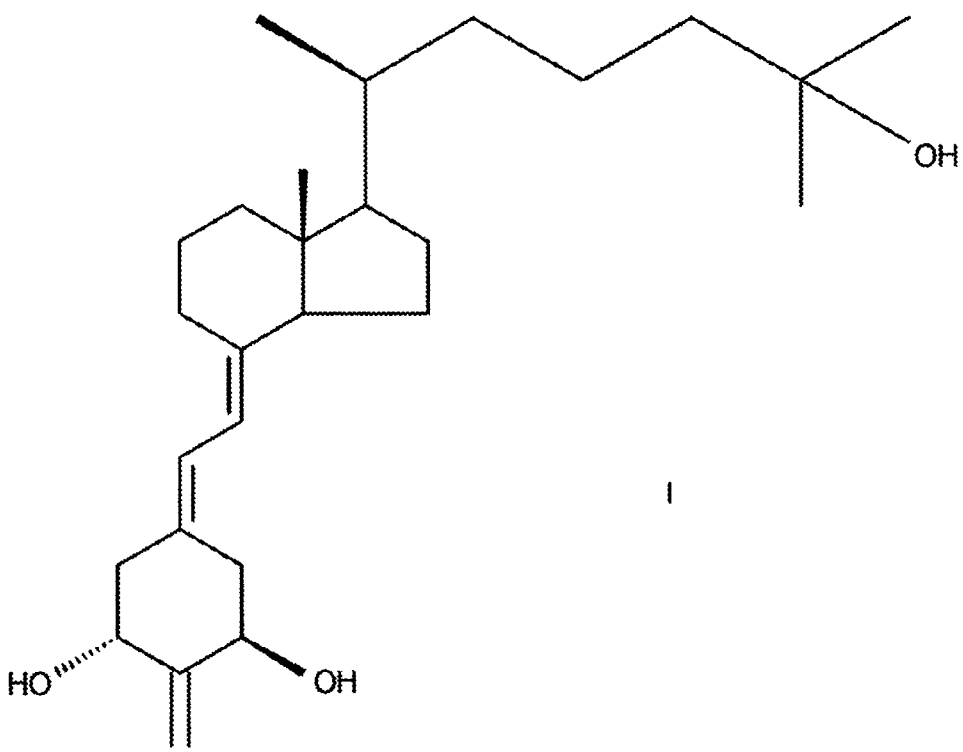
[00047] In the methods disclosed herein, 2MD can be administered to treat and/or prevent secondary hyperparathyroidism and/or its accompanying symptoms without causing severe hypercalcemia, while also resulting in reduced levels of phosphorus and creatinine in blood as well as decreased PTH levels in the blood.

[00048] Also in the methods disclosed herein, 2MD can be used to treat and reduce the severity of secondary hyperparathyroidism of renal disease and its accompanying symptoms, without causing severe hypercalcemia, by reducing phosphorus, creatinine and PTH levels in blood.

[00049] As used herein, "hypercalcemia" means elevated calcium levels in the blood of more than 2 mg/100ml. In a normal subject, calcium levels are approximately 9-10.5 mg/dL or 2.2-2.6 mmol/L. In cases of severe hypercalcemia (*i.e.*, calcium levels above 15-16 mg/dL or 3.75-4 mmol/L) coma and cardiac arrest can develop.

[00050] The present invention therefore provides novel methods of treating and/or preventing secondary hyperparathyroidism and/or its accompanying symptoms in a subject at risk of developing secondary hyperparathyroidism, and of treating and/or

preventing secondary hyperparathyroidism and/or its accompanying symptoms in a subject exhibiting symptoms of secondary hyperparathyroidism, by administering to the subject a therapeutically effective amount of 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 (2MD) or pharmaceutically acceptable salts thereof without inducing hypercalcemia in the subject, where 2MD has the structure (I):



I

[00051] As used herein, "preventing" means forestalling of a clinical symptom indicative of secondary hyperparathyroidism. Such forestalling includes, for example, the maintenance of normal kidney functions in a subject at risk of developing secondary hyperparathyroidism prior to the development of overt symptoms of secondary hyperparathyroidism including, but not limited to, increased levels of serum PTH, phosphorus and creatinine. Therefore, the term "preventing" includes the prophylactic treatment of subjects to guard them from the occurrence of secondary hyperparathyroidism. Preventing secondary hyperparathyroidism in a subject is also intended to include inhibiting or arresting the development of secondary

hyperparathyroidism. Inhibiting or arresting the development of secondary hyperparathyroidism includes, for example, inhibiting or arresting the occurrence of increased levels of serum PTH, phosphorus and creatinine.

[00052] As used herein, a "renal disease" or a "renal disorder" means a condition exhibiting impaired kidney function in a subject who is not on dialysis or a patient with chronic kidney disease (CKD) at stages 2 or 3, such as, for instance, acute kidney failure, acute nephritic syndrome, analgesic nephropathy, atheroembloic renal disease, chronic kidney failure, chronic nephritis, congenital nephrotic syndrome, goodpasture syndrome, interstitial nephritis, kidney cancer, kidney damage, kidney infection, kidney injury, kidney stones, membranoproliferative GNI, membranoproliferative GNII, membranous nephropathy, minimal change disease, necrotizing glomerulonephritis, nephroblastoma, nephrocalcinosis, nephrogenic diabetes insipidus, nephropathy-IgA, nephrosis nephrotic syndrome, polycystic kidney disease, post-strepococcal GN, reflux nephropathy, renal artery embolism, renal artery stenosis, renal disorders, renal papillary necrosis, renal tubular acidosis type I, renal tubular acidosis type II, renal underperfusion, renal vein thrombosis.

[00053] "Renal disease" is also meant to include patients with established kidney failure (*e.g.*, a glomerular filtration rate (GFR) of less than 15 mL/min/1.73 m^2 or permanent renal replacement therapy (RRT)). A subject having "renal disease" is meant to include a subject who has had kidney damage for more than 3 months, as defined by structural or functional abnormalities of the kidney, with or without decreased GFR, manifested by either pathological abnormalities or markers of kidney damage, including abnormalities in the composition of the blood or urine, or abnormalities in imaging tests. Markers of kidney damage include proteinuria of greater than 300 μg/day as measured by 24-HR excretion method. (*See* Table 15, Am. J. of Kidney Diseases, v.39, no. 2, Suppl. 1 (Feb. 2002), pp. 546-575, incorporated herein by reference). This definition may include patients on dialysis.

[00054] As used herein, a patient having "stage 2 chronic kidney disease (CKD)" means a patient exhibiting a mild reduction in GFR (60-89 mL/min/1.73 m^2). Kidney

damage is defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine test or imaging studies. A patient having "stage 3 chronic kidney disease (CKD)" means a patient exhibiting a moderate reduction in GFR (30-59 mL/min/1.73 m^2). Guidelines for characterizing kidney disease may distinguish between stage 3A (GFR 45-59) and stage 3B (GFR 30-44) for purposes of screening and referral. For more information about stages of kidney disease, see Am. J. of Kidney Disease, V. 39, No. 2, Suppl. 1, February 2002, incorporated herein by reference.

[00055] As used herein, a "subject" includes mammals and non-mammals. "Mammals" means any member of the class Mammalia including, but not limited to, humans, non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, and swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice, and guinea pigs; and the like. Examples of non-mammals include, but are not limited to, birds, and the like. The term "subject" does not denote a particular age or sex. The primary subjects to which the present invention is directed are the class of humans being treated with, or receiving, hemodialysis. The term "subject" may be utilized herein interchangeably with the terms "patient" or "individual."

[00056] As used herein, "administering" mean introducing a compound into the body, preferably into the systemic circulation, as described in more detail below. Examples include but are not limited to oral, topical, buccal, sublingual, pulmonary, transdermal, transmucosal, as well as subcutaneous, intraperitoneal, intravenous, and intramuscular injection or in the form of liquid or solid doses via the alimentary canal.

[00057] As used herein, "therapeutically effect" means an amount of a compound that, when administered to a subject for treating or preventing a disease, is sufficient to effect such treatment of prevention of the disease. A "therapeutically effective amount" will vary depending on the compound, the disease state being treated, the severity or the disease treated, the age and relative health of the subject, the route and form of administration, the judgment of the attending medical or veterinary practitioner, and other factors. As disclosed herein, male weanling Harlan-Sprague Dawley rats were

administered several dose levels of 2MD that would not cause significant hypercalcemia. We found that 2 1/2 nanograms/kilogram body weight (ng/kg bw) of 2MD per rat per day is sufficient to prevent and treat secondary hyperparathyroidism, or to prevent or treat symptoms of secondary hyperparathyroidism, without increasing serum calcium levels. Furthermore, 400 ng per day of 2MD in postmenopausal women showed over a 60% reduction in serum PTH levels while maintaining serum calcium levels within the physiologically normal range (Figure 13).

[00058] In one embodiment, the therapeutically effective amount ranges from between about 10 ng/day to about 1 μg/day, and preferably from between about 20 ng/day to about 1 μg/day. In a more preferred embodiment, the therapeutically effective amount ranges from between about 40 ng/day to about 600 ng/day, or between about 50 ng/day to about 600ng/day. In the most preferred embodiment, the therapeutically effective amount ranges from between about 100 ng/day to about 400 ng/day.

[00059] As used herein, "treat," "treating" or "treatment" means amelioration, alleviation or abation of a clinical symptom indicative of secondary hyperparathyroidism. Amelioration, alleviation or abation of a clinical symptom includes, for example, arresting, reducing the severity of or slowing the progression of or causing the regression of a symptom of secondary hyperparathyroidism. For instance, lowering the amount of serum PTH, serum phosphorus or serum creatinine levels in response to treatment with 2MD. Specifically, treating may include reducing the amount of serum PTH, serum phosphorus or serum creatinine by at least about 20%. In one embodiment, the amount of serum PTH, serum phosphorus or serum creatinine in the subject's blood is reduced by about 20-40% or about 35-50%. Other pathological conditions, chronic complications or phenotypic manifestations of secondary hyperparathyroidism are known to those skilled in the art and can similarly be used as a measure of treating secondary hyperparathyroidism so long as there is a reduction in the severity of the condition, complication or manifestation associated with the disease.

[00060] Effective compound formulations are described in U.S. Pat. No. 5,843,928 and include pharmaceutical applications as a solution in innocuous solvents, or as an

emulsion, suspension or dispersion in suitable solvents or carriers, or as pills, tablets, capsules combined with solid carriers. Other formulations may also include other pharmaceutically acceptable and nontoxic excipients such as stabilizers, anti-oxidants, binders, coloring agents or emulsifying or taste-modifying agents and extended release formulations.

[00061] In one embodiment, the 2MD compound is the active pharmaceutical ingredient (API) administered in the disclosed methods. The API may be formulated in an oral pharmaceutical dosage form as a solution in innocuous solvents, emulsion, suspension or dispersion in suitable solvents or carriers. The API may also be formulated in various oral dosage forms, such as pills, tablets or capsules using suitable pharmaceutical solid carriers. Such pharmaceutical formulations may also contain other pharmaceutically suitable USP-approved inactive ingredients, excipients, such as stabilizers, anti-oxidants, binders, coloring agents, emulsifiers, and/or taste-modifying agents, which are referred to as USP approved inactive pharmaceutical ingredients.

[00062] The API may be administered orally, topically, parenterally or transdermally or by inhalation. The compound may be administered by injection or intravenous infusion using suitable sterile solutions. Topical dosage forms may be creams, ointments, patches, or similar vehicles suitable for transdermal and topical dosage forms.

[00063] In some embodiments, the API may be formulated in doses for delivering a dose ranging from between about 10ng/day to about 1 μg/day, preferable from between about 20 ng/day to about 1μg/day, and more preferably from between about 40 ng/day to about 600 ng/day, or from between about 50 ng to about 600 ng per day and most preferably from between about 100 ng/day to about 400 ng/day. The API preferably is formulated in a dose that may be used for the prevention or treatment of secondary hyperparathyroidism, or for the prevention or treatment of symptoms of secondary hyperparathyroidism. Typically, the positive effects of 2MD are observed at dose levels that do not significantly raise serum calcium. Such dose and dosing regimens may be

adjusted to accommodate disease severity or progression, patient predisposition/at-risk/susceptible-to and other known criteria.

[00064] The pharmaceutically suitable oral carrier systems (also referred to as drug delivery systems, which are modern technology, distributed with or as a part of a drug product that allows for the uniform release or targeting or drugs to the body) preferably include FDA-approved and/or USP-approved inactive ingredients. Under 21 CFR 210.3(b)(8), an inactive ingredient is any component of a drug product intended to furnish pharmaceutical activity or other direct effect in the diagnosis, or to affect the structure or any function of the body of humans or other animal. Active ingredients include those components of the product that may undergo chemical change during the manufacture of the drug product and be present in the drug product in a modified form intended to furnish the specified activity or effect. As use herein, a kit (also referred to as a dosage form) is a packaged collection of related material.

[00065] As used herein, "oral dosage" forms may include capsules (*i.e.*, a solid oral dosage form consisting of a shell and a filling), whereby the shell is composed of a single sealed enclosure, or two halves that fit together and which are sometimes sealed with a band, and whereby capsule shells may be made from gelatin, starch, or cellulose, or other suitable materials, may be soft or hard, and are filled with a solid or liquid ingredients that can be poured or squeezed. The oral dosage form may also be a capsule or coated pellets, in which the drug is enclosed within either a hard or soft soluble container or "shell" made from a suitable form of gelatin. The drug itself may be in the form of granules to which varying amount of coating have been applied or in a capsule coated extended release, in which the drug is enclosed within either a hard or soft soluble container or "shell" made from a suitable form of gelatin. Additionally, the capsule may be covered in a designated coating which releases a drug or drugs in such a manner to allow at least a reduction in dosing frequency as compared to that drug or drugs presented as a conventional dosage form.

[00066] The oral dosage form may further be a capsule delayed release, in which the drug is enclosed within either a hard or soft soluble container made from a suitable

form of gelatin, and which releases a drug (or drugs) at a time other than promptly after administration, whereby enteric-coated articles are delayed release dosage forms. Capsule delayed release pellets, in which the drug is enclosed within either a hard or soft container or "shell" are also useful. In these cases, the drug itself is in the form of granules to which enteric coating has been applied, thus delaying release of the drug until its passing into the intestine. Capsule extended release and capsule film-coated extended release are also useful.

[00067] Additionally, the capsule is covered in a designated film coating, and which releases a drug or drugs in such a manner to allow at least a reduction in dosing frequency as compared to that drug or drugs presented as a conventional dosage form), capsule gelatin coated (a solid dosage form in which the drug is enclosed within either a hard or soft soluble container made from a suitable form of gelatin; through a banding process, the capsule is coated with additional layers of gelatin so as to form a complete seal), capsule liquid filled (a solid dosage form in which the drug is enclosed within a soluble, gelatin shell which is plasticized by the addition of a polyol, such as sorbitol or glycerin, and is therefore of a somewhat thicker consistency than that of a hard shell capsule).

[00068] Typically, the active ingredients may be dissolved or suspended in a liquid vehicle, a granule (a small particle or grain), a pellet (a small sterile solid mass consisting of a highly purified drug, with or without excipients, made by the formation of granules, or by compression and molding), or a pellet coated extended release (a solid dosage form in which the drug itself is in the form of granules to which varying amounts of coating have been applied, and which releases a drug or drugs in such a manner to allow a reduction in dosing frequency as compared to that drug or drugs presented as a conventional dosage form).

[00069] Other forms include pills (a small, round solid dosage form containing a medicinal agent intended for oral administration), powder (an intimate mixture of dry, finely divided drugs and/or chemicals that may be intended for internal or external use), elixir (a clear, pleasantly flavored, sweetened hydroalcoholic liquid containing dissolved

medicinal agents; it is intended for oral use), chewing gum (a sweetened and flavored insoluble plastic material of various shapes which when chewed, releases a drug substance into the oral cavity), syrup (an oral solution containing high concentrations of sucrose or other sugars; the term has also been used to include any other liquid dosage form prepared in a sweet and viscid vehicle, including oral suspensions), tablet (a solid dosage form containing medicinal substances with or without suitable diluents), tablet chewable (a solid dosage form containing medicinal substances with or without suitable diluents that is intended to be chewed, producing a pleasant tasting residue in the oral cavity that is easily swallowed and does not leave a bitter or unpleasant after-taste), tablet coated or tablet delayed release, tablet dispersible, tablet effervescent, tablet extended release, tablet film coated, or tablet film coated extended release where the tablet is formulated in such manner as to make the contained medicament available over an extended period of time following ingestion.

[00070] In other forms, a tablet for solution, tablet for suspension, tablet multilayer, tablet multilayer extended release may be provided, where the tablet is formulated in such manner as to allow at least a reduction in dosing frequency as compared to that drug presented as a conventional dosage form. A tablet orally disintegrating, tablet orally disintegrating delayed release, tablet soluble, tablet sugar coated, osmotic, and the like are also suitable.

[00071] The oral dosage form composition may contain an active pharmaceutical ingredient and one or more inactive pharmaceutical ingredients such as diluents, solubilizers, alcohols, binders, controlled release polymers, enteric polymers, disintegrants, excipients, colorants, flavorants, sweeteners, antioxidants, preservatives, pigments, additives, fillers, suspension agents, surfactants (e.g., anionic, cationic, amphoteric and nonionic), and the like. Various FDA-approved topical inactive ingredients are found at the FDA's "The Inactive Ingredients Database" that contains inactive ingredients specifically intended as such by the manufacturer, whereby inactive ingredients can also be considered active ingredients under certain circumstances, according to the definition of an active ingredient given in 21 CFR 210.3(b)(7). Alcohol

is a good example of an ingredient that may be considered either active or inactive depending on the product formulation.

[00072] As used herein, the injectable and infusion dosage forms include, but are not limited to, a liposomal injectable, which either consists of or forms liposomes (a lipid bilayer vesicle usually composed of phospholipids which is used to encapsulate an active drug substance). An injection, which includes a sterile preparation intended for parenteral use; five distinct classes of injections exist as defined by the USP, is also suitable. An emulsion injection, which includes an emulsion consisting of a sterile, pyrogen-free preparation intended to be administered parenterally or a lipid complex injection are also suitable.

[00073] Other forms include a powder for solution injection, which is a sterile preparation intended for reconstitution to form a solution for parenteral use; a powder for suspension injection that is a sterile preparation intended for reconstitution to form a suspension for parenteral use; a powder lyophilized for liposomal suspension injection, which is a sterile freeze dried preparation intended for reconstitution for parenteral use which has been formulated in a manner that would allow liposomes (a lipid bilayer vesicle usually composed of phospholipids which is used to encapsulate an active drug substance, either within a lipid bilayer or in an aqueous space) to be formed upon reconstitution; a powder lyophilized for solution injection, which is a dosage form intended for the solution prepared by lyophilization ("freeze drying"), a process which involves the removal of water from products in the frozen state at extremely low pressures.

[00074] This is intended for subsequent addition of liquid to create a solution that conforms in all respects to the requirements for injections; a powder lyophilized for suspension injection being a liquid preparation, intended for parenteral use that contains solids suspended in a suitable fluid medium and conforms in all respects to the requirements for Sterile Suspensions; the medicinal agents intended for the suspension are prepared by lyophilization ("freeze drying"), a process which involves the removal of water from products in the frozen state at extremely low pressures; a solution injection

being a liquid preparation containing one or more drug substances dissolved in a suitable solvent or mixture of mutually miscible solvents that is suitable for injection; a solution concentrate injection being a sterile preparation for parenteral use which, upon the addition of suitable solvents, yields a solution conforming in all respects to the requirements for injections.

[00075] A suspension injection comprises a liquid preparation, suitable for injection, which consists of solid particles dispersed throughout a liquid phase in which the particles are not soluble that can also consist of an oil phase dispersed throughout an aqueous phase, or vice-versa. A suspension liposomal injection comprises a liquid preparation, suitable for injection, which consists of an oil phase dispersed throughout an aqueous phase in such a manner that liposomes (a lipid bilayer vesicle usually composed of phospholipids which is used to encapsulate an active drug substance, either within a lipid bilayer or in an aqueous space) are formed. A suspension sonicated injection comprises a liquid preparation, suitable for injection, which consists of solid particles dispersed throughout a liquid phase in which the particles are not soluble. In addition, the product is sonicated while a gas is bubbled through the suspension, and this results in the formation of microspheres by the solid particles.

[00076] The parenteral carrier system includes one or more pharmaceutically suitable excipients, such as solvents and co-solvents, solubilizing agents, wetting agents, suspending agents, thickening agents, emulsifying agents, chelating agents, buffers, pH adjusters, antioxidants, reducing agents, antimicrobial preservatives, bulking agents, protectants, tonicity adjusters, and special additives. Formulations suitable for parenteral administration conveniently comprise a sterile oily or aqueous preparation of the active ingredient which is preferably isotonic with the blood of the recipient.

[00077] As used herein, inhalation dosage forms include, but are not limited to, aerosol being a product that is packaged under pressure and contains therapeutically active ingredients that are released upon activation of an appropriate valve system intended for topical application to the skin as well as local application into the nose (nasal aerosols), mouth (lingual and sublingual aerosols), or lungs (inhalation aerosols); foam

aerosol being a dosage form containing one or more active ingredients, surfactants, aqueous or nonaqueous liquids, and the propellants, whereby if the propellant is in the internal (discontinuous) phase (i.e., of the oil-in-water type), a stable foam is discharged, and if the propellant is in the external (continuous) phase (i.e., of the water-in-oil type), a spray or a quick-breaking foam is discharged; metered aerosol being a pressurized dosage form consisting of metered dose valves which allow for the delivery of a uniform quantity of spray upon each activation; powder aerosol being a product that is packaged under pressure and contains therapeutically active ingredients, in the form of a powder, that are released upon activation of an appropriate valve system; and, aerosol spray being an aerosol product which utilizes a compressed gas as the propellant to provide the force necessary to expel the product as a wet spray and being applicable to solutions of medicinal agents in aqueous solvents.

[00078] As used herein, transdermal dosage form includes, but is not limited to, a patch being a drug delivery system that often contains an adhesive backing that is usually applied to an external site on the body, whereby the ingredients either passively diffuse from, or are actively transported from, some portion of the patch, and whereby depending upon the patch, the ingredients are either delivered to the outer surface of the body or into the body; and, other various types of transdermal patches such as matrix, reservoir and others known in the art.

[00079] As used herein, the topical dosage form includes various dosage forms known in the art such as lotions (an emulsion, liquid dosage form, whereby this dosage form is generally for external application to the skin), lotion augmented (a lotion dosage form that enhances drug delivery, whereby augmentation does not refer to the strength of the drug in the dosage form), gels (a semisolid dosage form that contains a gelling agent to provide stiffness to a solution or a colloidal dispersion, whereby the gel may contain suspended particles) and ointments (a semisolid dosage form, usually containing less than 20% water and volatiles and greater than 50% hydrocarbons, waxes, or polyols as the vehicle, whereby this dosage form is generally for external application to the skin or mucous membranes).

[00080] Ointment augmented (an ointment dosage form that enhances drug delivery, whereby augmentation does not refer to the strength of the drug in the dosage form), creams (an emulsion, semisolid dosage form, usually containing greater than 20% water and volatiles and/or less than 50% hydrocarbons, waxes, or polyols may also be used as the vehicle, whereby this dosage form is generally for external application to the skin or mucous membranes. Cream augmented (a cream dosage form that enhances drug delivery, whereby augmentation does not refer to the strength of the drug in the dosage form), emulsions (a dosage form consisting of a two-phase system comprised of at least two immiscible liquids, one of which is dispersed as droplets, internal or dispersed phase, within the other liquid, external or continuous phase, generally stabilized with one or more emulsifying agents, whereby emulsion is used as a dosage form term unless a more specific term is applicable, e.g. cream, lotion, ointment), suspensions (a liquid dosage form that contains solid particles dispersed in a liquid vehicle), suspension extended release, pastes (a semisolid dosage form, containing a large proportion, 20-50%, of solids finely dispersed in a fatty vehicle, whereby this dosage form is generally for external application to the skin or mucous membranes), solutions (a clear, homogeneous liquid dosage form that contains one or more chemical substances dissolved in a solvent or mixture of mutually miscible solvents), and powders are also suitable.

[00081] Shampoos (a lotion dosage form which has a soap or detergent that is usually used to clean the hair and scalp) are often used as a vehicle for dermatologic agents. For instance, shampoo suspensions (a liquid soap or detergent containing one or more solid, insoluble substances dispersed in a liquid vehicle that is used to clean the hair and scalp and is often used as a vehicle for dermatologic agents) are often used. Aerosol foams (i.e., a dosage form containing one or more active ingredients, surfactants, aqueous or nonaqueous liquids, and the propellants; if the propellant is in the internal discontinuous phase, i.e., of the oil-in-water type, a stable foam is discharged, and if the propellant is in the external continuous phase, i.e., of the water-in-oil type, a spray or a quick-breaking foam is discharged), sprays (a liquid minutely divided as by a jet of air or steam), metered spray (a non-pressurized dosage form consisting of valves which allow the dispensing of a specified quantity of spray upon each activation), and suspension spray (a liquid preparation containing solid particles dispersed in a liquid vehicle and in

the form of coarse droplets or as finely divided solids to be applied locally, most usually to the nasal-pharyngeal tract, or topically to the skin) are also suitable.

[00082] Jellies (a class of gels, which are semisolid systems that consist of suspensions made up of either small inorganic particles or large organic molecules interpenetrated by a liquid--in which the structural coherent matrix contains a high portion of liquid, usually water) and films (a thin layer or coating), including film extended release (a drug delivery system in the form of a film that releases the drug over an extended period in such a way as to maintain constant drug levels in the blood or target tissue) and film soluble (a thin layer or coating which is susceptible to being dissolved when in contact with a liquid) are also suitable.

[00083] Sponges (a porous, interlacing, absorbent material that contains a drug, whereby it is typically used for applying or introducing medication, or for cleansing, and whereby a sponge usually retains its shape), swabs (a small piece of relatively flat absorbent material that contains a drug, whereby a swab may also be attached to one end of a small stick, and whereby a swab is typically used for applying medication or for cleansing).

[00084] Patches (a drug delivery system that often contains an adhesive backing that is usually applied to an external site on the body, whereby its ingredients either passively diffuse from, or are actively transported from, some portion of the patch, whereby depending upon the patch, the ingredients are either delivered to the outer surface of the body or into the body, and whereby a patch is sometimes synonymous with the terms 'extended release film' and 'system'), patch extended release (a drug delivery system in the form of a patch that releases the drug in such a manner that a reduction in dosing frequency compared to that drug presented as a conventional dosage form, e.g., a solution or a prompt drug-releasing, conventional solid dosage form), patch extended release electronically controlled (a drug delivery system in the form of a patch which is controlled by an electric current that releases the drug in such a manner that a reduction in dosing frequency compared to that drug presented as a conventional dosage form, e.g., a solution or a prompt drug-releasing, conventional solid dosage form), and the like. The

various topical dosage forms may also be formulated as immediate release, controlled release, sustained release, or the like.

[00085] The topical dosage form composition contains an active pharmaceutical ingredient and one or more inactive pharmaceutical ingredients such as excipients, colorants, pigments, additives, fillers, emollients, surfactants (e.g., anionic, cationic, amphoteric and nonionic), penetration enhancers (e.g., alcohols, fatty alcohols, fatty acids, fatty acid esters and polyols), and the like. Various FDA-approved topical inactive ingredients are found at the FDA's "The Inactive Ingredients Database" that contains inactive ingredients specifically intended as such by the manufacturer, whereby inactive ingredients can also be considered active ingredients under certain circumstances, according to the definition of an active ingredient given in 21 CFR 210.3(b)(7). Alcohol is a good example of an ingredient that may be considered either active or inactive depending on the product formulation.

EXAMPLES

[00086] The following examples are presented for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. The examples illustrate that 2MD, an analog of $1,25(OH)_2D_3$ originally thought to be important in prevention and treatment of osteoporosis, is also important in preventing and treating secondary hyperparathyroidism and its accompanying symptoms. A study conducted in rats in which their kidneys were surgically removed, showed that daily oral and intraperitoneal (ip) 2MD administration results in lower levels of serum PTH, phosphorus, and creatinine, all indicators of kidney failure, as compared to vehicle control animals. Furthermore, 2MD administration results in lower PTH, phosphorus and creatinine levels at dose levels that do not raise serum calcium.

[00087] Example 1

[00088] Materials and Methods

[00089] Nephrectomy Rat Model. Disease Induction. Weanling, male Sprague-Dawley rats were obtained from Harlan (Madison, Wis.). Following a 10-13 day

acclimation period, the animals had two-thirds of one kidney removed. After a week, the other entire kidney was removed. The animals were then switched from a chow diet to a purified rodent diet (Suda *et al.*, Purified Rodent Diet-Diet 11) containing 0.6% Ca and 0.9% phosphorus and fat soluble vitamins A, D, E and K. Water and diet were provided ad libitum.

[00090] Animal Husbandry. Animals were housed in suspended, plastic shoe-box style cages with corn cob bedding (prior to surgery) or in stainless steel, wire-bottom cages (approximately one week after surgery). The animal rooms were maintained at a temperature of 68 to 72 °F. and a relative humidity of 25 to 75%. The holding rooms were set to provide 12 hours of light per day.

[00091] Treatment Groups. Approximately four weeks after the second surgery, animals were assigned to treatment groups (14-15 animals/group) so that each group had the same average PTH level.

[00092] Dose Preparation (Vehicle Formulation). The negative control material was prepared by volumetrically measuring ethanol (5%) and Neobee oil, mixing and then placing in storage at 2 to 8 °C.

[00093] Dose Preparation (2MD Formulation). 2MD formulations (DP001, Sigma Aldrich Fine Chemicals, Madison, Wis.) were prepared by first determining the concentration of an ethanol stock solution using UV spectrophotometry (extinction coefficient=42,000; λ_{max}=252 nm). The solutions were then volumetrically added to Neobee oil so that there was no more than 5% ethanol in the final solution. If necessary, additional ethanol was added to bring the final ethanol amount to 5%. The solution was mixed and stored at 2 to 8 °C.

[00094] Dose Administration Method. Both vehicle and 2MD were administered orally to the back of the tongue at 0.5 ml/kg body weight once daily for 8 weeks, or intraperitoneally three times per week for 4 weeks.

[00095] Serum Parathyroid Hormone (PTH) Levels. By "serum PTH levels" we mean the amount of PTH released by the parathyroid gland. PTH is the most important

regulator of the body's calcium and phosphorus levels, and is controlled by the level of calcium in the blood. Low blood calcium levels cause increased PTH to be released, while high blood calcium levels inhibit PTH release. Normal values are 10-55 picograms per milliliter (pg/mL). Four weeks after surgery and 4 and 8 weeks after treatment initiation, blood was collected from the tail artery and the concentration of bioactive serum PTH was measured using the rat BioActive Intact PTH ELISA Kit from Immutopics, Inc. (San Clemente, Calif.).

[00096] Serum Calcium Analysis. Four weeks following surgery and 4 and 8 weeks after treatment started, blood was collected from the tail artery of each experimental animal. The blood was allowed to coagulate at room temperature and then centrifuged at 3000xg for 15 minutes. The serum was transferred to a polypropylene tube and stored frozen at -20 °C. The level of calcium was determined by diluting the serum into 0.1% lanthum chloride and measuring the absorbance on an atomic absorption spectrophotometer (Perkin Elmer Model 3110, Shelton, Conn.).

[00097] Phosphorus Assay. Four weeks after surgery and 8 weeks after treatment started, blood was collected from the tail artery of each experimental animal. The blood was allowed to coagulate at room temperature and then centrifuged at 3000xg for 15 minutes. The serum was transferred to a polypropylene tube and stored frozen at -20 °C. The level of phosphorus was determined using a clinical analyzer (Pentra 400, Horiba ABX Diagnostics--France; UV method using phosphomolybdate).

[00098] Creatinine Assay. Measuring serum creatinine levels is a useful and inexpensive method of evaluating renal dysfunction. Creatinine is a non-protein waste product of phosphocreatinine metabolism by skeletal muscle tissue. Creatinine production is continuous and is proportional to muscle mass. Creatinine is freely filtered and therefore the serum creatinine level depends on the Glomerular Filtration Rate (GFR). Renal dysfunction diminishes the ability to filter creatinine and the serum creatinine rises. If the serum creatinine level doubles, the GFR is considered to have been halved. A threefold increase is considered to reflect a 75% loss of kidney function.

[00099] In the following examples, serum creatinine levels were evaluated four weeks after surgery and 8 weeks after treatment started. Blood was collected from the tail artery of each experimental animal. The blood was allowed to coagulate at room temperature and then centrifuged at 3000xg for 15 minutes. The serum was transferred to a polypropylene tube and stored frozen at -20 °C. The level of creatinine was determined using a clinical analyzer (Pentra 400, Horiba ABX Diagnostics--France; Jaffe reaction) and is indicative of impaired renal function and chronic nephritis. In one embodiment of the invention, a minimum decrease in serum creatinine levels of approximately 30% is expected after treatment according to the method of the present invention.

[000100] Example 2

[000101] Uremic Rat model – Intraperitoneal (ip) Adminstratoin of 2MD

[000102] Figure 1 schematically illustrates the ip treatment protocol with 2MD. As shown in Figure 2, ip administration of 2MD at 5 ng/kg bw three times per week prevented increases in serum PTH, and suppresses circulating PTH levels at 10 ng/kg bw. As shown in Figure 3, ip administration of 2MD did not raise serum calcium levels until a dose of 10 ng/kg bw was administered.

[000103] Example 3

[000104] Uremic Rat Model – Oral Administration of 2MD compared to Zemplar®

[000105] Figure 4 schematically illustrates the oral treatment protocol with 2MD. As shown in Figure 5, oral administration of 2MD at daily doses of 1-5 ng/kg bw prevented an increase or effected a reduction in serum PTH levels. The observed effect lasted for eight weeks of therapy. As shown in Figure 6, oral administration of Zemplar® at daily doses of 30-300 ng/kg bw prevented an increase in serum PTH levels, but the therapeutic effect was lost as the disease progressed. Figure 7 illustrates that when orally administered clinically significant serum calcium increases are observed at 2MD doses of 5 ng/kg bw. Figure 8 illustrates that when orally administered clinically significant serum calcium increases are observed at Zemplar® doses of 100 and 300 ng/kg bw.

[000106] As shown in Figure 9, oral administration of 2MD at daily doses of 1-5 ng/kg bw reduced serum phosphorus levels in nephrectomized rats. In contrast, oral administration of Zemplar® did not reduce serum phosphorus levels in nephrectomized rats.

[000107] As shown in Figure 11, oral administration of 2MD at daily doses of 1-5 ng/kg bw resulted in lower serum creatinine levels compared to Vehicle control animals. In contrast, oral administration of Zemplar® lowered serum creatinine levels compared to Vehicle control animals, however, only at dose levels that significantly increased serum calcium.

[000108] Example 4

[000109] Phase 1B Trial – Oral Administration of 2MD to Postmenopausal Women

[000110] Figure 13 illustrates the oral administration of 2MD once daily for 28 days to postmenopausal women at a dose of 110 nanograms (ng) reduced serum PTH levels by 21%, and a dose of 440 ng reduced serum PTH levels by 67%.

[000111] Interpretation of Data

[000112] 2MD or 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 effectively reduces secondary hyperparathyroidism in a rat model of renal failure. Rats that have all but one sixth of their kidney mass surgically removed, and are placed on a high phosphorus diet will develop elevated PTH levels in the blood. Oral or intraperitoneal administration of 2MD on a daily or 3 times per week regimen will reduce the circulating levels of PTH. In addition, 2MD has the added benefit of preventing further increases or possibly reducing the levels of both phosphorus and creatinine in the blood. Furthermore, 2MD exhibits long-lasting effects in that rats treated orally for 8 weeks still show reduced PTH levels; whereas, other vitamin D compounds lose their effectiveness after 4 weeks of treatment in this animal model.

CLAIMS

We claim:

1. Use of 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 or pharmaceutically acceptable salts thereof for treating or preventing secondary hyperparathyroidism or the symptoms thereof in a subject having or at risk for developing secondary hyperparathyroidism or the symptoms thereof without inducing hypercalcemia in the subject.

2. The use of claim 1, wherein the 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 is formulated in an oral, topical, transdermal, parenteral, injection or infusion dosage form.

3. The use of claim 1, wherein the 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 is formulated in a dose for administering about 20 ng/day to about 1 ug/day to the subject.

4. The use of claim 1, wherein the 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 is formulated in a dose for administering about 40 ng/day to about 600 ng/day to the subject.

5. The use of claim 1, wherein the 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 is formulated in a dose for administering about 600 ng/day to the subject.

6. The use of claim 1, wherein the 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 is formulated in a dose for administering about 400 ng/day to the subject.

7. The use of claim 1, wherein the subject is receiving hemodialysis treatment.

8. The use of claim 7, wherein the 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 is formulated in a dosage form for administering about three times per week to the subject.

9. The use of claim 1, wherein the symptoms of secondary hyperparathyroidism are selected from the group consisting of serum PTH, serum phosphorus and serum creatinine.

Fig. 1

Uremic Rat Model – ip

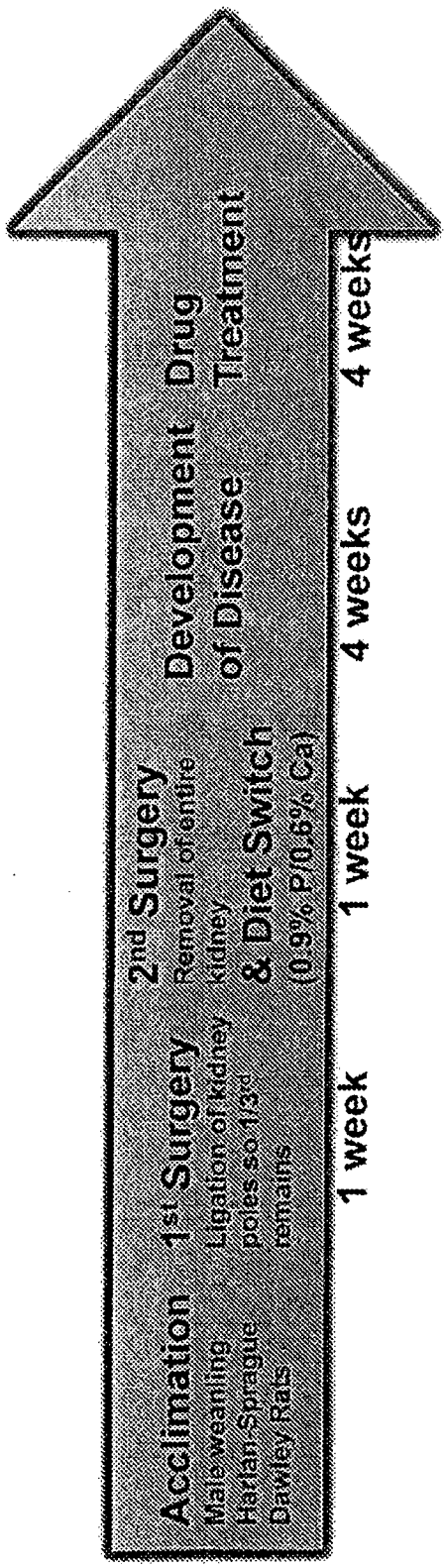


Fig. 2

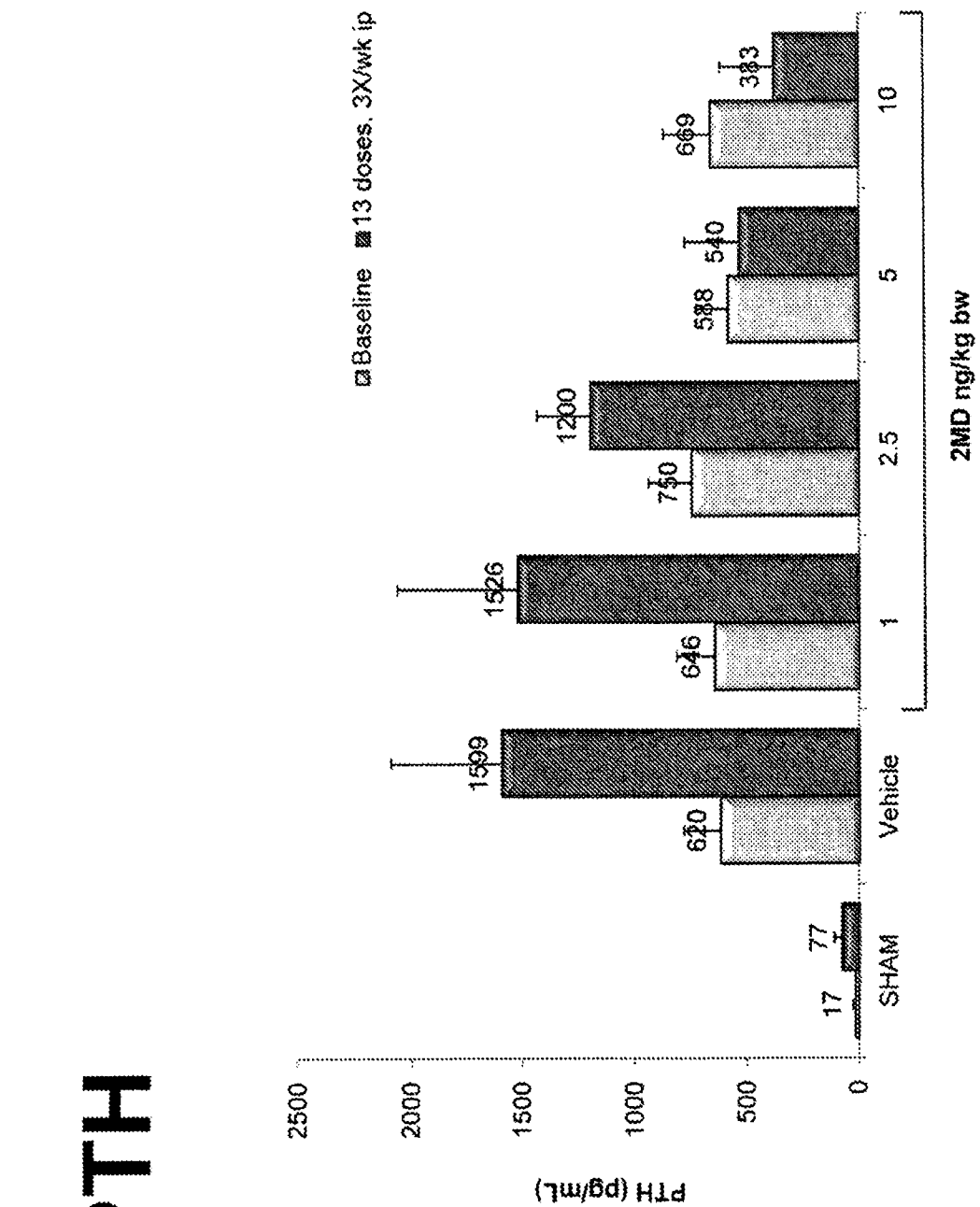

PTH
Baseline
13 doses, 3X/wk ip
PTH (pg/mL)
2500
2000
1500
1000
500
0
17
77
SHAM
620
1599
Vehicle
646
1526
1
750
1200
2.5
588
540
5
669
383
10
2MD ng/kg bw

Fig. 3

Serum Calcium

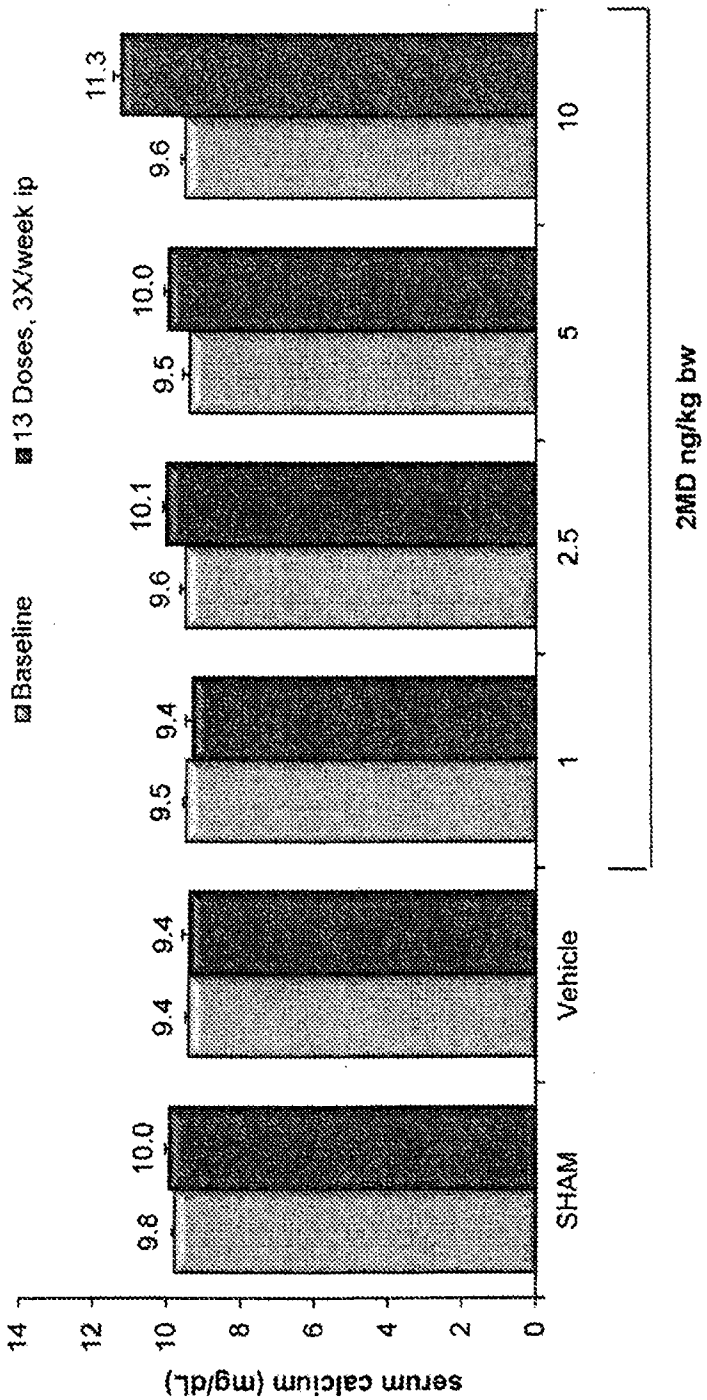
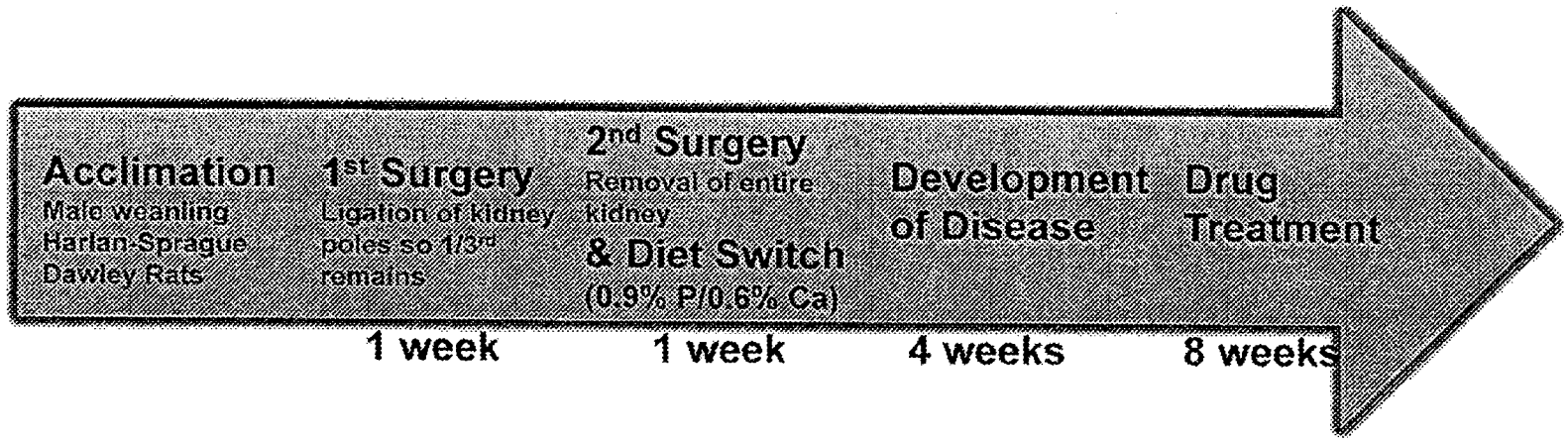


Fig. 4

Uremic Rat Model - Oral



Endpoints: Blood collected at baseline and termination for serum PTH (Intact PTH ELISA from Immutopics) and calcium (atomic absorption) measurements.

Fig. 5

PTH

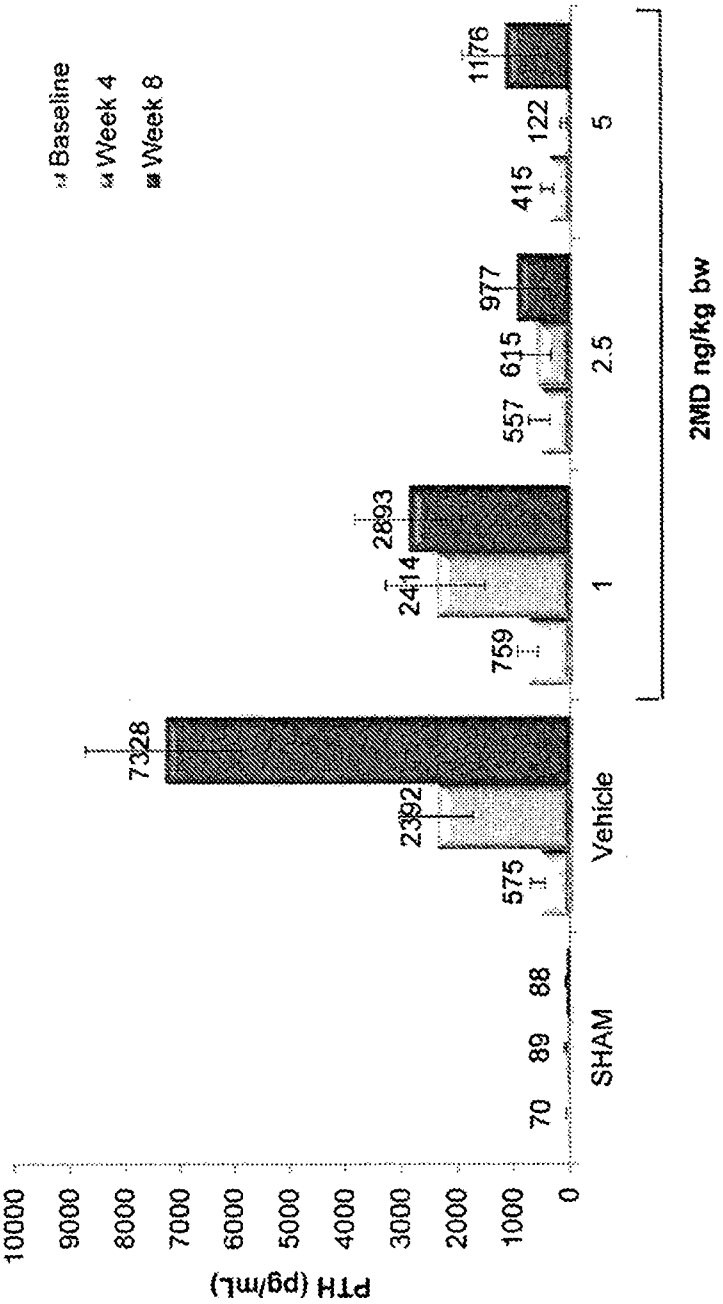

Baseline
Week 4
Week 8
PTH (pg/mL)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
70
89
88
575
2392
7328
759
2414
2893
557
615
977
415
122
1176
SHAM
Vehicle
1
2.5
5
2MD ng/kg bw

Fig. 6

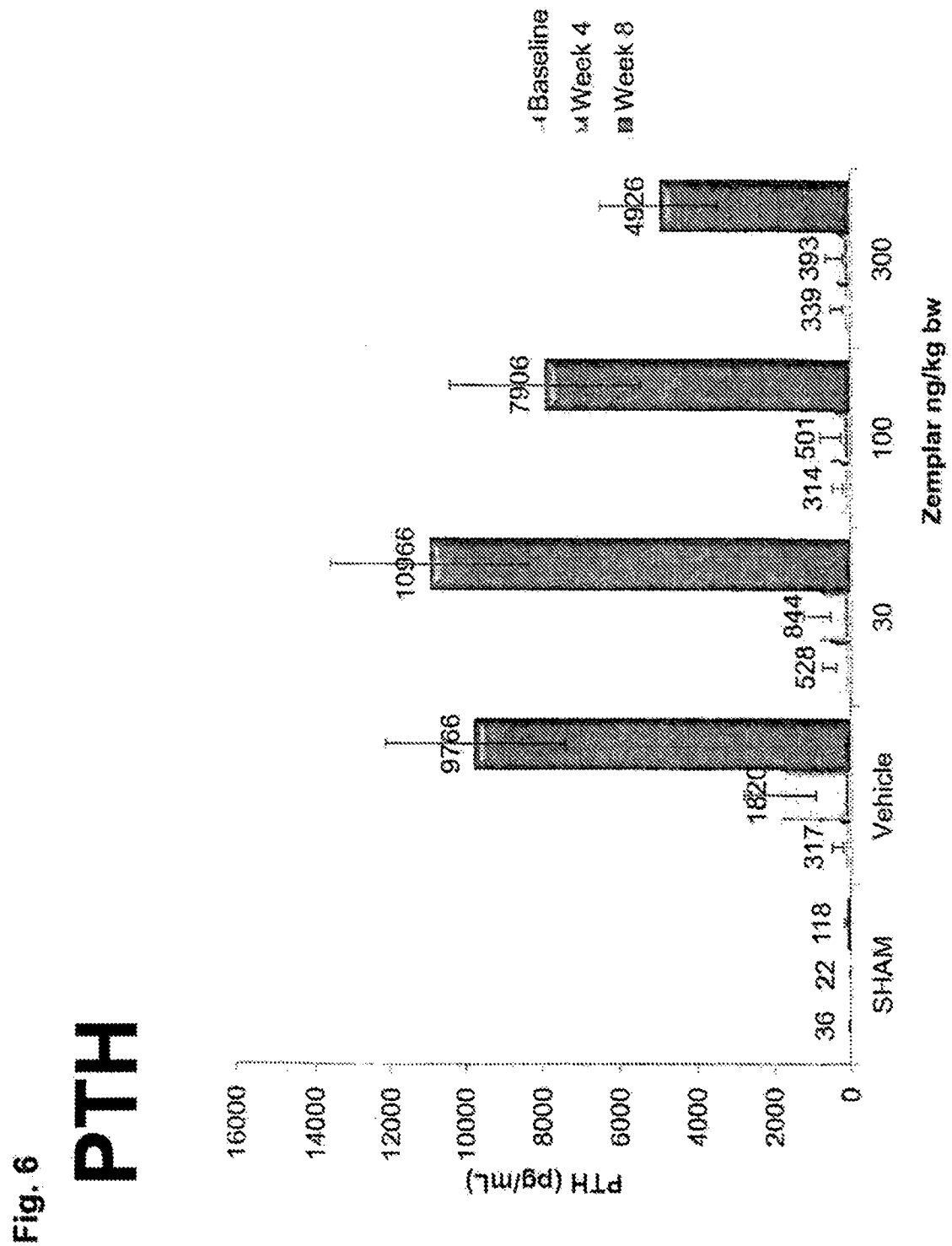

PTH
PTH (pg/mL)
16000
14000
12000
10000
8000
6000
4000
2000
0
Baseline
Week 4
Week 8
36
22
118
1820
317
9766
528
844
10966
314
501
7906
339
393
4926
SHAM
Vehicle
30
100
300
Zemplar ng/kg bw

Fig. 7

Serum Calcium

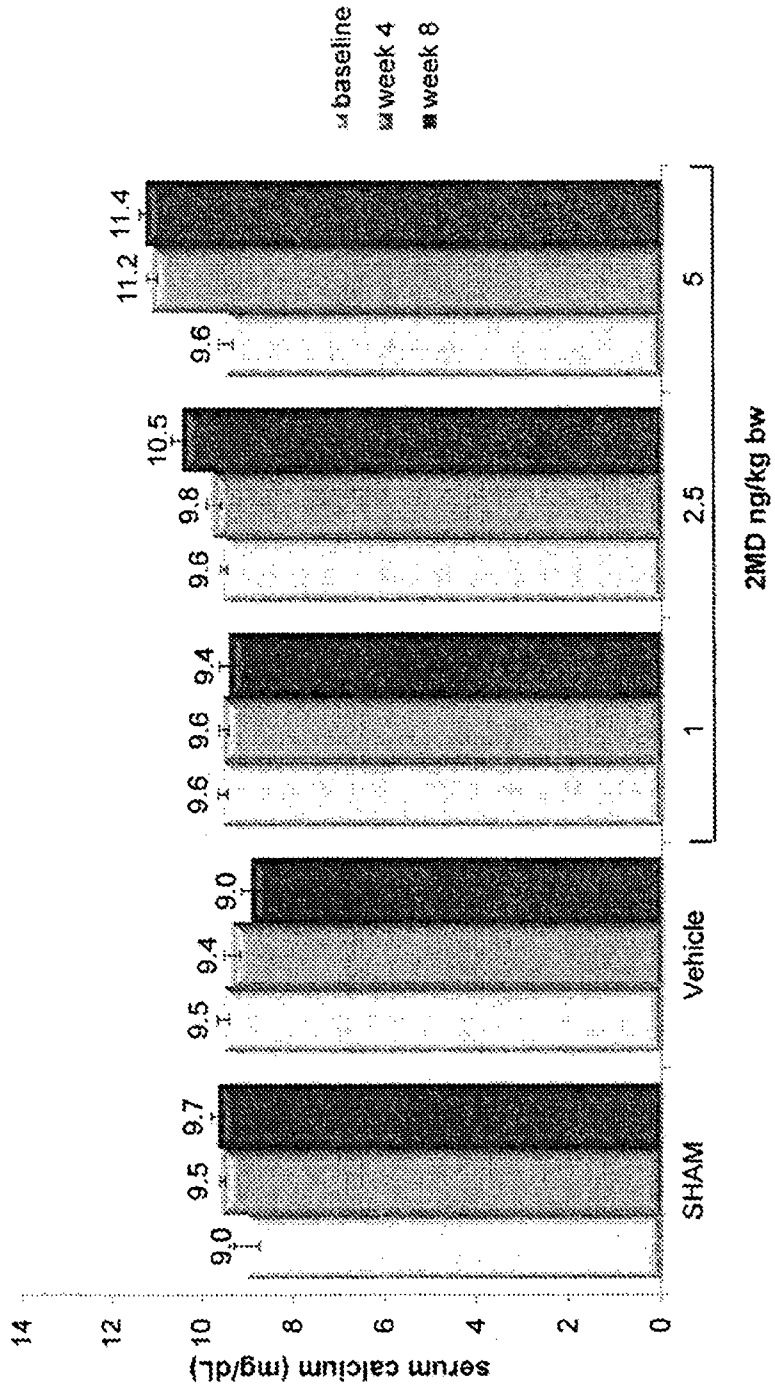


Fig. 8

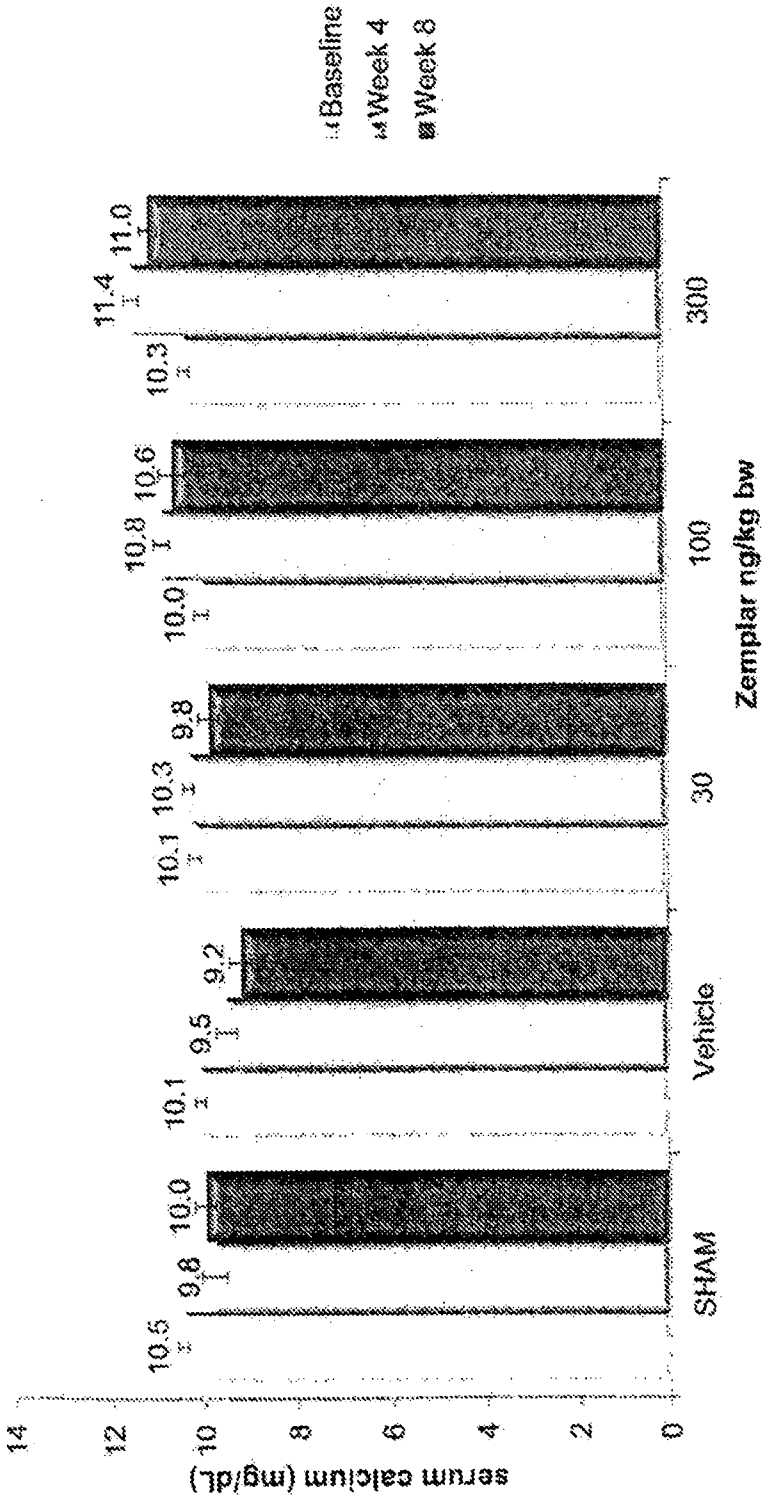

Serum Calcium
Baseline
Week 4
Week 8
serum calcium (mg/dL)
0
2
4
6
8
10
12
14
10.5
9.8
10.0
10.1
9.5
9.2
10.1
10.3
9.8
10.8
10.0
10.6
11.4
10.3
11.0
SHAM
Vehicle
30
100
300
Zemplar ng/kg bw

Fig. 9

Serum Phosphorus

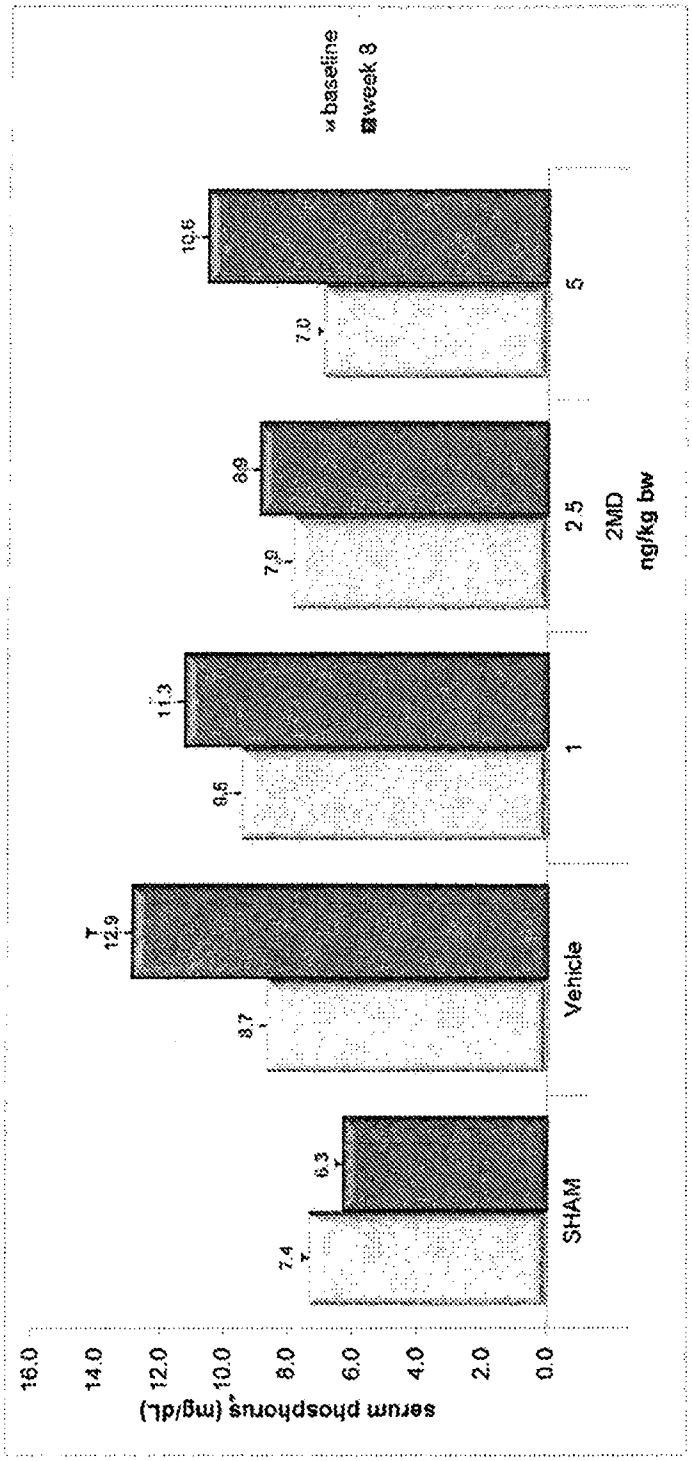


Fig. 10

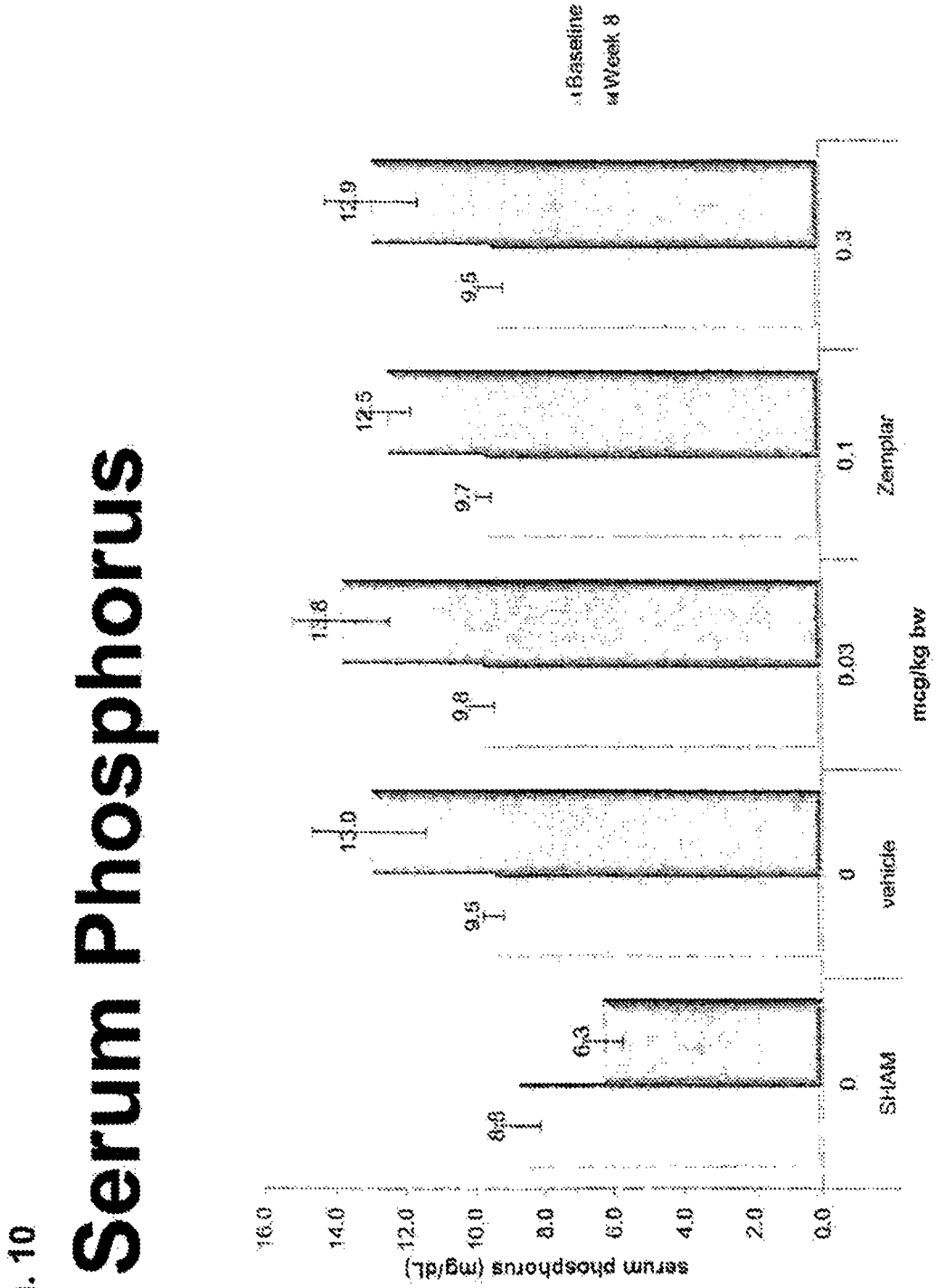

Serum Phosphorus
Baseline
Week 8
serum phosphorus (mg/dL)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
8.8
6.3
9.5
13.0
9.8
11.6
9.7
12.5
9.5
13.9
SHAM
vehicle
Zemplar
0
0
0.03
0.1
0.3
mcg/kg bw

Fig. 11

Serum Creatinine

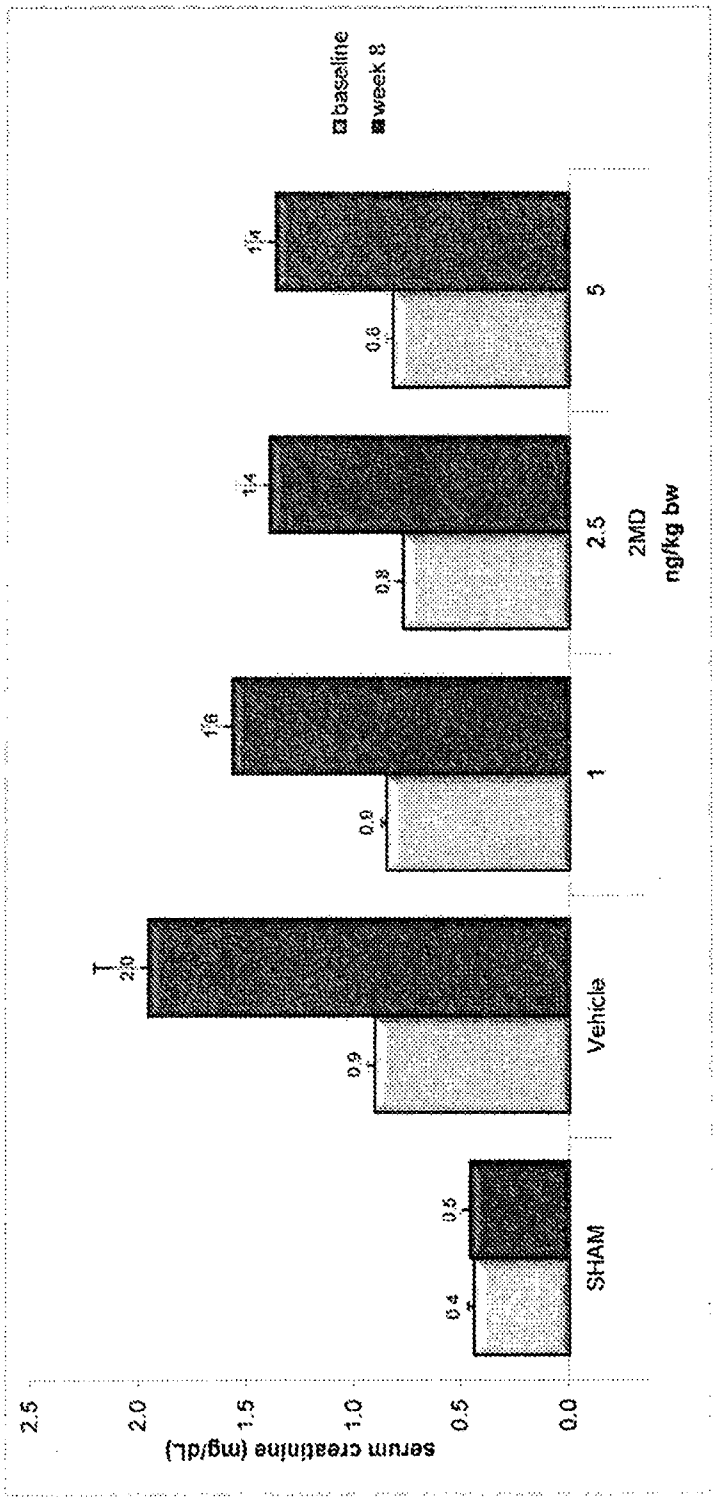


Fig. 12

Serum Creatinine

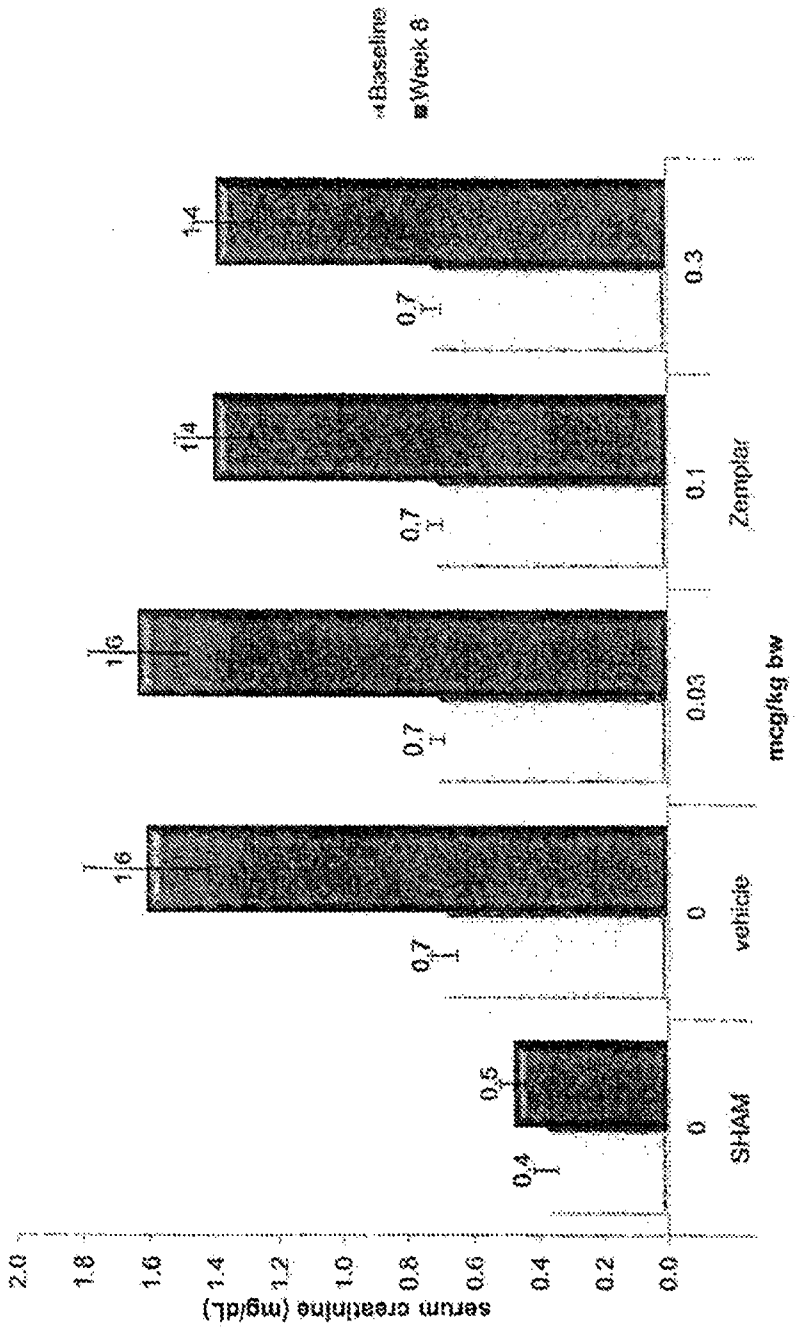
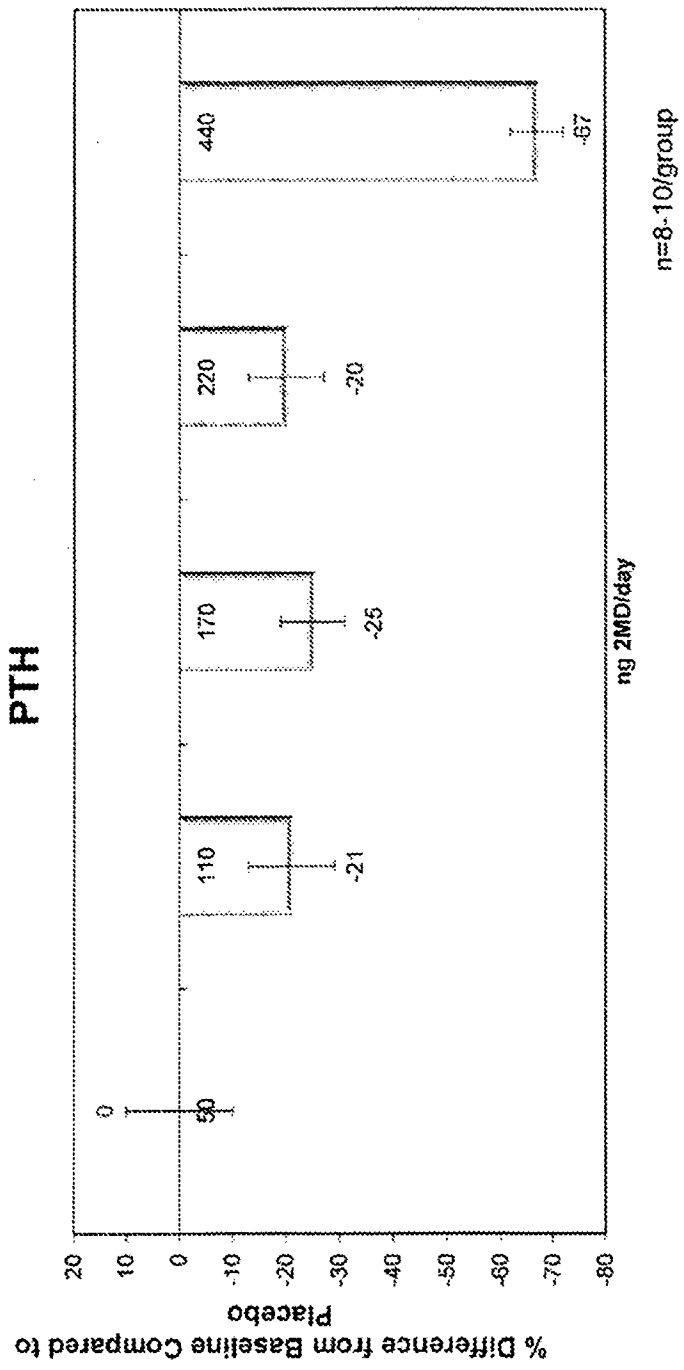


Fig. 13

Phase 1B Trial



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/031574

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/593 A61P3/14
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2011/034426 A1 (DELUCA HECTOR F [US] ET AL) 10 February 2011 (2011-02-10) paragraphs [0006], [0015], [0045]; claims 12,13 -----	1-4,9 1-9
Y	WO 02/05823 A2 (WISCONSIN ALUMNI RES FOUND [US]) 24 January 2002 (2002-01-24) page 11 -----	1-9
Y	BROWN A J ET AL: "Selective vitamin D analogs and their therapeutic applications", SEMINARS IN NEPHROLOGY 1994 US, vol. 14, no. 2, 1994, pages 156-174, XP009168757, ISSN: 0270-9295 page 163, right-hand column - page 165, paragraph 1 -----	1-9

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 April 2013	25/04/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Loher, Florian

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/031574

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 2461815 A1	13-06-2012
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		EP 1301189 A2	16-04-2003
		HK 1060304 A1	21-04-2006
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		US 2006135492 A1	22-06-2006
		US 2006135493 A1	22-06-2006
		WO 0205823 A2	24-01-2002

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(51)Int.Cl.
A61K 31/593(2006.01)
A61P 3/14(2006.01)

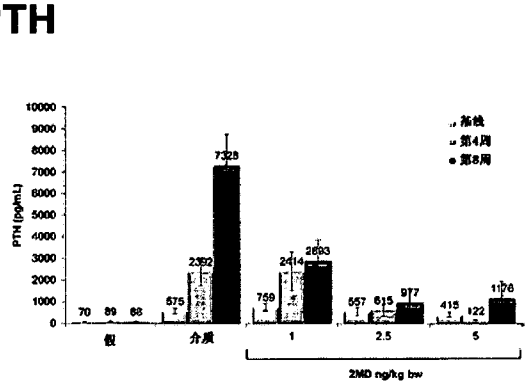
权利要求书1页 说明书16页 附图13页

(54)发明名称

2-亚甲基-19-去甲-(20S)-1α,25-二羟基维生素D_3治疗继发性甲状旁腺功能亢进的用途

(57)摘要

本发明公开了2-亚甲基-19-去甲-(20S)-1α,25-二羟基维生素D_3的用途,其用于在患有或有风险发生继发性甲状旁腺功能亢进的受试者中治疗和/或预防继发性甲状旁腺功能亢进和/或其伴随症状,而不在所述受试者中诱导高钙血症。



CN 104394871 A

1. 2- 亚甲基 -19- 去甲 -(20S)-1α, 25- 二羟基维生素 D_3 或其可药用盐的用途，其用于在患有或有风险发生继发性甲状旁腺功能亢进或其症状的受试者中治疗或预防继发性甲状旁腺功能亢进或其症状，而不在所述受试者中诱导高钙血症。

2. 权利要求 1 的用途，其中所述 2- 亚甲基 -19- 去甲 -(20S)-1α, 25- 二羟基维生素 D_3 被配制在口服、表面、透皮、肠胃外、注射或输注剂型中。

3. 权利要求 1 的用途，其中所述 2- 亚甲基 -19- 去甲 -(20S)-1α, 25- 二羟基维生素 D_3 以向所述受试者给药约 20ng/ 天至约 1ug/ 天的剂量被配制。

4. 权利要求 1 的用途，其中所述 2- 亚甲基 -19- 去甲 -(20S)-1α, 25- 二羟基维生素 D_3 以向所述受试者给药约 40ng/ 天至约 600ng/ 天的剂量被配制。

5. 权利要求 1 的用途，其中所述 2- 亚甲基 -19- 去甲 -(20S)-1α, 25- 二羟基维生素 D_3 以向所述受试者给药约 600ng/ 天的剂量被配制。

6. 权利要求 1 的用途，其中所述 2- 亚甲基 -19- 去甲 -(20S)-1α, 25- 二羟基维生素 D_3 以向所述受试者给药约 400ng/ 天的剂量被配制。

7. 权利要求 1 的用途，其中所述受试者正接受血液透析治疗。

8. 权利要求 7 的用途，其中所述 2- 亚甲基 -19- 去甲 -(20S)-1α, 25- 二羟基维生素 D_3 被配制在用于每周约三次向所述受试者给药的剂型中。

9. 权利要求 1 的用途，其中所述继发性甲状旁腺功能亢进的症状选自血清 PTH、血清磷和血清肌酸酐。

2-亚甲基-19-去甲-(20S)-1α,25-二羟基维生素D_3治疗继发性甲状旁腺功能亢进的用途

技术领域

[0001] 本发明涉及在治疗和/或预防继发性甲状旁腺功能亢进和/或其症状中有用的维生素D化合物,更具体来说涉及维生素D化合物2-亚甲基-19-去甲-(20S)-1α,25-二羟基维生素D3治疗和/或预防继发性甲状旁腺功能亢进和/或其症状的用途。

背景技术

[0002] 在事实上世界上的每个国家、包括高度发达国家例如美国中,肾病已变成越来越重要的健康问题。目前存在约250,000位已几乎完全失去他们的肾脏用途的进行肾透析的患者。有约10倍于该数量的患者已由于肾病而失去一定程度的肾功能并正向完全肾衰竭发展。肾衰竭的证据是肾小球滤过率(GFR)从110ml/分钟/1.73m^2的高值降低到30ml/分钟/1.73m^2,此时通常开始透析。

[0003] 许多因素造成肾病的发生。高血压是重要的贡献因素之一,患有I型或II型糖尿病也是如此。目前肾衰竭的治疗限于血液透析,这是一种极为昂贵的程序,目前由联邦政府支持,因为个人自身通常不能负担得起这一程序。单单在美国,肾病的每年花费就超过420亿美元。因此,用于预防肾病和治疗其症状的有效方法,不仅提供了重要的健康益处,而且也将提供重要的经济益处。

[0004] 现在普遍接受,维生素D在能够发挥作用之前,必须首先在肝脏中被25-羟基化,然后在肾脏中被1α-羟基化。(参见DeLuca,"维生素D:维生素和激素"(Vitamin D:The vitamin and the hormone),Fed. Proc. 33,2211-2219,1974)。这两个反应产生最终有活性形式的维生素D,即1α,25-$(OH)_2D_3$。(参见DeLuca&Schnoes,"维生素D:最新进展"(Vitamin D:Recent advances),Ann. Rev. Biochem. 52,411-439,1983)。这种化合物随后刺激大量生理过程,包括:刺激肠吸收钙,刺激肾脏重吸收钙,刺激肠吸收磷酸盐,以及在存在高甲状旁腺激素(PTH)水平信号时刺激骨骼动员钙。这些作用引起血浆钙和磷水平升高,引起骨损伤例如软化病和软骨病的愈合,并预防低血钙性抽搐的神经性障碍。

[0005] 继发性甲状旁腺功能亢进在患有慢性肾衰竭的患者中是一种普遍并发症。低水平的1α,25-$(OH)_2D_3$和磷酸盐保留造成继发性甲状旁腺功能亢进的发生。低水平的循环1α,25-$(OH)_2D_3$是肾功能受损,导致患者不能将25-羟基-维生素D_3转变成1α,25-二羟基维生素D_3的结果。作为低水平的循环1α,25-$(OH)_2D_3$的结果,肠的钙吸收降至最低,其随后引起血清钙水平不足。当甲状旁腺感应到低水平的血清钙时,甲状旁腺分泌PTH,其引起从骨骼动员钙以调控血清钙。如果任其发展,这种异常的PTH分泌将引起肾性骨营养不良的发生。高的PTH水平也可以引起:1)骨骼弱化;2)钙化防御(此时钙在皮肤中形成结块并引起周围组织的溃疡和潜在的死亡);3)心血管并发症;4)异常的脂肪和糖代谢;5)瘙痒(瘙痒症);和6)低血液计数(贫血症)。

[0006] 1α,25-二羟基维生素D_3已在患有肾病的患者中被用作甲状旁腺功能亢进的治疗剂。在肾性骨营养不良的继发性甲状旁腺功能亢进的治疗中,众所周知,

1α,25-二羟基维生素D_3结合到位于甲状旁腺中的维生素D受体(VDR),以抑制甲状旁腺细胞的生长和增殖两者以及前甲状旁腺素原基因的表达。(参见Demay等,"结合1,25-二羟基维生素D_3受体并对1,25-羟基维生素D_3作出响应介导转录阻遏的人类甲状旁腺激素基因的序列"(Sequences in the human parathyroid hormone gene that bind the 1,25-dihydroxyvitamin D_3receptor and mediate transcriptional repression in response to 1,25-hydroxyvitamin D_3),Proc.Natl.Acad.Sci.USA 89,8097-8101,1992;和Darwish&DeLuca,"结合于人类甲状旁腺激素基因的启动子区域的转录因子的鉴定"(Identification of a transcription factor that binds to the promoter region of the human parathyroid hormone gene),Arch.Biochem.Biophys.365,123-130,1999)。由于其抑制甲状旁腺激素(PTH)的能力,$1,25-(OH)_2D_3$已被成功地用于继发性甲状旁腺功能亢进的治疗。(参见Slatopolsky等,"在尿毒症患者中通过1,25-二羟基胆钙化醇的静脉内给药显著抑制继发性甲状旁腺功能亢进"(Marked Suppression of Secondary Hyperparathyroidism by Intravenous Administration of1,25-dihydroxycholecalciferol in Uremic Patients),J.Clin.Invest.74:2136-2143,1984)。然而,1α,25-二羟基维生素D_3在肾性骨营养不良的继发性甲状旁腺功能亢进的治疗中的使用,通常被1α,25-二羟基维生素D_3对肠钙吸收和骨骼矿物钙动员的强力作用导致的高钙血症的发生而被排除。

[0007] 正如以前提到的,继发性甲状旁腺功能亢进通常发生在经历肾透析的患者中。慢性肾衰竭是继发性甲状旁腺功能亢进的最常见的病因。衰竭的肾脏无法将足够的维生素D转变成其活性形式,并且无法充分排泄磷酸盐。当这种情况发生时,不溶性的磷酸钙在体内形成并将钙从循环移除。最终,这引起低钙血症和继发性甲状旁腺功能亢进。

[0008] 继发性甲状旁腺功能亢进也可以由胃肠道吸收不良综合征(例如慢性胰腺炎、小肠病和吸收不良依赖性减肥手术,其中肠不适当地吸收维生素和矿物质)引起,其中这些综合征可能导致脂溶性维生素D的吸收不足。当维生素D吸收不足时,可能发生低钙血症,并且随后可能引起PTH分泌的增加,此时身体试图提高血清钙水平。然而,由于$1,25(OH)_2D_3$的低水平,低钙血症和继发性甲状旁腺功能亢进也可能出现在肾病的早期阶段中。继发性甲状旁腺功能亢进的其他较不常见的病因是长期锂治疗、维生素D缺乏、营养不良、维生素D抗性佝偻病或高镁血症(即异常高的血液镁水平)。

[0009] 继发性甲状旁腺功能亢进的症状包括血清PTH、血清磷和血清肌酸酐水平增高。较不明显的症状包括骨和关节痛、骨骼畸形、骨骼断裂(骨折)、关节肿胀、肾结石、排尿增加、肌肉虚弱和疼痛、恶心和食欲不振。其他较不常见的症状包括疲劳、上腹疼痛和抑郁。

[0010] 继发性甲状旁腺功能亢进的治疗通常涉及解决低钙血症的隐伏的病因。在患有慢性肾衰竭的患者中,治疗由磷的饮食限制、增补维生素D的活性形式例如骨化三醇、Hectorol®或Zemplar®(帕立骨化醇)以及磷酸盐结合剂构成,所述磷酸盐结合剂可以分成基于钙的结合剂和不基于钙的结合剂。一类更新的药物是钙模拟物,其中一种可以在美国和澳大利亚作为Sensipar®(西那卡塞)并在欧盟作为Mimpara®商购。钙模拟物已获得正面响应,并且被FDA批准用于透析患者,但是尚未被批准用于透析前的慢性肾病,这是由于它们能够提高磷水平等顾虑。大多数患有慢性肾病继发的甲状旁腺功能亢进的患者在肾移植后改善,但是许多患者继续具有一定程度的移植后残留甲状旁腺功能亢进(即三发

性甲状旁腺功能亢进）以及相伴的骨丧失风险。

[0011] 尽管在患有早期肾功能不全的患者中血清磷通常是正常的，但磷限制可以减轻继发性甲状旁腺功能亢进。饮食磷限制提高了 1,25-$(OH)_2D_3$ 水平。（参见 Portale 等，“在患有中度肾功能不全的儿童中饮食磷对 1,25- 二羟基维生素 D 和免疫反应性甲状旁腺激素的循环浓度的影响”(Effect of Dietary Phosphorus on Circulating Concentrations of1,25-dihydroxyvitamin D and Immunoreactive Parathyroid Hormone in Children with Moderate Renal Insufficiency)，J. Clin. Invest. 73:1580-1589, 1984)。这进而通过直接抑制 PTH 基因转录并通过增加肠的钙吸收来降低 PTH。在肾衰竭的较晚阶段，甲状旁腺功能亢进和 1,25-$(OH)_2D_3$ 缺陷的程度增加，并且磷酸盐限制对 1,25-$(OH)_2D_3$ 水平几乎没有影响。（参见 Lopez-Hilker 等，“在尿毒症中磷限制独立于钙和骨化三醇的变化逆转甲状旁腺功能亢进”(Phosphorus Restriction Reverses Hyperparathyroidism in Uremia Independent of Changes in Calcium and Calcitriol)，Am. J. Physiol. 259:F432-F437, 1990)。这推测是由可用于 1,25-$(OH)_2D_3$ 合成的肾质的减少造成的。

[0012] 已发现，几种具有低钙血活性的维生素 D 类似物在抑制培养的牛甲状旁腺细胞的 PTH 分泌中与 1,25-$(OH)_2D_3$ 几乎同样有效。它们包括 22- 奥沙骨化三醇 (OCT)(Brown 等，“维生素 D 的非钙血型类似物 22- 奥沙骨化三醇 (OCT) 抑制甲状旁腺激素合成和分泌”(The Non-Calcemic Analog of Vitamin D, 22-oxacalcitriol(OCT) Suppresses Parathyroid Hormone Synthesis and Secretion)，J. Clin. Invest. 84:728-732, 1989) 以及 1,25-$(OH)_2$-16- 烯 -23- 炔 -D_3、1,25-$(OH)_2$-24- 二同型 -D_3 和 1,25-$(OH)_2$-24- 三同型 -22- 烯 -D_3。已在体内详细研究了 22- 奥沙骨化三醇的这种作用（参见 Brown 等，“选择性维生素 D 类似物及其治疗应用”(Selective Vitamin D Analogs and their Therapeutic Applications)，Sem. Nephrol 14:156-174, 1994，其报道了 22- 奥沙骨化三醇尽管在体内快速清除，但能够抑制 PTH mRNA)。低的、亚最大剂量的骨化三醇和 OCT 表现出可比的抑制。也已显示，OCT 在尿毒症大鼠和狗中抑制血清 PTH。

[0013] 1,25-$(OH)_2D_3$ 的具有低钙血和磷酸盐血作用的另一种类似物是 19- 去甲 -1,25-$(OH)_2D_2$。骨化三醇的这种类似物具有维生素 D_2 化合物特征性的碳 28 和碳 22 处的双键，但是它缺少在所有天然维生素 D 化合物中存在的碳 19 和环外双键。利用牛甲状旁腺细胞的原代培养物的体外研究证实，19- 去甲 -1,25-$(OH)_2D_2$ 具有与 1,25-$(OH)_2D_3$ 相似的对 PTH 的抑制效应。使用 10^{-7}M 的 19- 去甲 -1,25-$(OH)_2D_2$ 获得对 PTH 释放的 52％的抑制。在这两种化合物之间，在对 PTH 分泌的抑制性效应中没有明显差异。

[0014] 随后，在体内进行了初步研究以确定 19- 去甲 -1,25-$(OH)_2D_2$ 的钙血活性。已发现，1,25-$(OH)_2D_3$(10ng/ 大鼠 /10 天）与 19- 去甲 -1,25-$(OH_2)D_2$(100ng/ 大鼠 /10 天）以相同幅度增加血清钙。因此，选择了三种不同剂量的 1,25-$(OH)_2D_3$(2、4 和 8ng) 和 19- 去甲 -1,25-$(OH)_2D_2$(8、25 和 75ng) 用于长期研究。在肾功能不全两个月后，动物在 8 天的时间段中以三种所指示的剂量四次接受上述两种化合物。正如预期，1,25-$(OH)_2D_3$ 抑制 pre-pro-PTH mRNA 和 PTH 分泌。然而，这种降低仅仅在使用 8ng 剂量时是统计上显著的，并且这种剂量诱导高钙血症和高磷酸盐血症。另一方面，所有剂量的 19- 去甲 -1,25-$(OH)_2D_2$ 都不产生血清离子化钙或血清磷的统计上显著的变化。

[0015] 19- 去甲 -1α,25$(OH)_2D_2$ 也被称为帕立骨化醇和 19- 去甲 -1α,25- 二羟基 - 麦

角钙化醇。一种帕立骨化醇注射剂可以以Zemplar®从Abbott Laboratories, Abbott Park, Ill 商购。帕立骨化醇(Zemplar®)注射剂描述在美国专利号6,136,799中,并且已被FDA批准并销售,用于伴有慢性肾衰竭(CKD阶段5或晚期肾病(ESRD),GFR<15mL/min/1.73m^2)的继发性甲状旁腺功能亢进的预防和治疗。这种静脉内配方含有2-10微克/毫升的帕立骨化醇、30%(v/v)的丙二醇、20%(v/v)的乙醇和约50%(v/v)的水。研究表明,帕立骨化醇注射剂抑制高水平的PTH,并对血清钙和磷水平具有极小影响。由于它在1998年4月被FDA批准,因此估计已有约200,000位患者接受至少一剂帕立骨化醇注射剂。在临床上,帕立骨化醇注射剂治疗继发性甲状旁腺功能亢进的安全性和效能已被很好地确立。

[0016] 高磷酸盐血症也是长期血液透析患者中的长期存在的问题,并且可以被治疗剂量的1,25-$(OH)_2D_3$进一步加剧。(参见Delmez等,"高磷酸盐血症:其在慢性肾病患者中的结果和治疗"(Hyperphosphatemia:Its Consequences and Treatment in Patients with Chronic Renal Disease),Am. J. Kidney Dis. 19:303-317,1992;以及Quarles等,"ESRD中的甲状旁腺功能亢进的脉冲口服与静脉内骨化三醇治疗的比较的前瞻性试验"(Prospective trial of Pulse Oral versus Intravenous Calcitriol Treatment of Hyperparathyroidism in ESRD),Kidney Int. 45:1710-1721,1994)。此外,使用大剂量碳酸钙控制磷酸盐吸收仅仅提高了来自于1,25-$(OH)_2D_3$疗法的高钙血症的风险。(参见Meyrier等,"在经历血液透析的患者中高碳酸钙摄入对骨病的影响"(The Influence of a High Calcium Carbonate Intake on Bone Disease in Patients undergoing Hemodialysis),Kidney Int. 4:146-153,1973;Moriniere等,"在长期血液透析患者中用高剂量碳酸钙替代氢氧化铝:高氨血症的消失和甲状旁腺功能亢进的同等控制"(Substitution of Aluminum Hydroxide by High Doses of Calcium Carbonate in Patients on Chronic Hemodialysis:Disappearance of Hyperaluminaemia and Equal Control of Hyperparathyroidism),Proc. Eur. Dial Transplant Assoc. 19:784-787,1983;以及Slatopolsky等,"在患有慢性肾衰竭的经历透析的患者中碳酸钙作为磷酸盐结合剂"(Calcium Carbonate as a Phosphate Binder in Patients with Chronic Renal Failure Undergoing Dialysis),New Engl. J. Med. 315:157-161,1986)。因此,1,25-$(OH)_2D_3$的可以抑制PTH并对钙和磷酸盐代谢具有次要影响的类似物,将是用于控制和治疗继发性甲状旁腺功能亢进的理想工具。

[0017] 另一种维生素D类似物即2-亚甲基-19-去甲-(20S)-1α,25-二羟基维生素D_3(在文献中被称为"2MD"),也已知抑制PTH产生。(参见美国公开申请号2011/0034426A1)。尽管因此它似乎是用于治疗继发性甲状旁腺功能亢进的候选物,但是从美国专利号5,843,928也已公知,2MD具有非常强的钙血活性。2MD将骨骼钙动员活性显著提高到可能为1α,25-$(OH)_2D_3$的10-100倍的水平,同时也表现出肠钙运输活性的中度增加。由于对钙从骨骼的动员的这种高度选择性活性,到目前为止,化合物2MD从未被严肃地当作用于治疗继发性甲状旁腺功能亢进的药剂。

[0018] 发明概述

[0019] 现在已发现,维生素D类似物2MD当在良好控制的条件下给药于需要的受试者时,具有治疗继发性甲状旁腺功能亢进以及继发性甲状旁腺功能亢进的症状的能力。现在还已

发现，维生素 D 类似物 2MD 当在良好控制的条件下给药于需要的受试者时，具有预防继发性甲状旁腺功能亢进以及继发性甲状旁腺功能亢进的症状的能力。

[0020] 在一种实施方式中，本发明提供了一种通过向表现出继发性甲状旁腺功能亢进的症状的受试者给药治疗有效量的包含 2- 亚甲基 -19- 去甲 -(20S)-1α,25- 二羟基维生素 D_3(2MD) 或其可药用盐作为活性药剂的组合物，来治疗继发性甲状旁腺功能亢进而不在所述受试者中诱导高钙血症的新方法。

[0021] 在另一种实施方式中，本发明提供了一种通过向表现出继发性甲状旁腺功能亢进的症状的受试者给药治疗有效量的包含 2- 亚甲基 -19- 去甲 -(20S)-1α,25- 二羟基维生素 D_3(2MD) 或其可药用盐作为活性药剂的组合物，来治疗继发性甲状旁腺功能亢进的症状而不在所述受试者中诱导高钙血症的新方法。

[0022] 在又一种实施方式中，本发明提供了一种通过向处于发生继发性甲状旁腺功能亢进的风险中的受试者给药治疗有效量的包含 2- 亚甲基 -19- 去甲 -(20S)-1α,25- 二羟基维生素 D_3(2MD) 或其可药用盐作为活性药剂的组合物，来预防继发性甲状旁腺功能亢进而不在所述受试者中诱导高钙血症的新方法。

[0023] 在又一种实施方式中，本发明提供了一种通过向处于发生继发性甲状旁腺功能亢进的风险中的受试者给药治疗有效量的包含 2- 亚甲基 -19- 去甲 -(20S)-1α,25- 二羟基维生素 D_3(2MD) 或其可药用盐作为活性药剂的组合物，来预防继发性甲状旁腺功能亢进的症状而不在所述受试者中诱导高钙血症的新方法。

[0024] 在一种实施方式中，所述 2- 亚甲基 -19- 去甲 -(20S)-1α,25- 二羟基维生素 D_3 被配制成口服、表面、透皮、肠胃外、可注射或可输注形式，以 10ng/ 天至约 1μg/ 天范围内的量给药。优选地，对于继发性甲状旁腺功能亢进的治疗或预防来说，或者对于继发性甲状旁腺功能亢进的症状的治疗或预防来说，将化合物 2MD 口服或肠胃外 (i.v.) 给药。剂量可以根据具体的给药途径适合地选择。适合的剂量可以包括每天约 10ng 至约 1ug 范围内的剂量。优选地，将药剂每周三次静脉内或口服给药于接受血液透析治疗的受试者。

附图说明

[0025] 图 1 示意说明了使用本文中设想的 2MD 的腹膜内治疗方案。

[0026] 图 2 的图示出了在尿毒症大鼠模型中各种不同剂量的 2MD 的腹膜内给药对血清 PTH 的影响。

[0027] 图 3 的图示出了在尿毒症大鼠模型中各种不同剂量的 2MD 的腹膜内给药对血清钙的影响。

[0028] 图 4 示意说明了使用本文中设想的 2MD 的口服治疗方案。

[0029] 图 5 的图示出了在尿毒症大鼠模型中各种不同剂量的 2MD 的口服给药对血清 PTH 的影响。

[0030] 图 6 的图示出了在尿毒症大鼠模型中各种不同剂量的 19- 去甲 -1α,25- 二羟基维生素 D_2(在商品名 Zemplar® 下销售) 的口服给药对血清 PTH 的影响。

[0031] 图 7 的图示出了在尿毒症大鼠模型中各种不同剂量的 2MD 的口服给药对血清钙的影响。

[0032] 图 8 的图示出了在尿毒症大鼠模型中各种不同剂量的 19- 去甲 -1α,25- 二羟基

维生素 D_2（在商品名 Zemplar® 下销售）的口服给药对血清 PTH 的影响。

[0033] 图 9 的图示出了在尿毒症大鼠模型中各种不同剂量的 2MD 的口服给药对血清磷的影响。

[0034] 图 10 的图示出了在尿毒症大鼠模型中各种不同剂量的 19- 去甲 -1α,25- 二羟基维生素 D_2（在商品名 Zemplar® 下销售）的口服给药对血清磷的影响。

[0035] 图 11 的图示出了在尿毒症大鼠模型中各种不同剂量的 2MD 的口服给药对血清肌酸酐的影响。

[0036] 图 12 的图示出了在尿毒症大鼠模型中各种不同剂量的 19- 去甲 -1α,25- 二羟基维生素 D_2（在商品名 Zemplar® 下销售）的口服给药对血清肌酸酐的影响。

[0037] 图 13 的图示出了在绝经后妇女的 1B 期人类试验中各种不同剂量的 2MD 的口服给药对血清 PTH 的影响。

[0038] 详细描述

[0039] 公开了治疗和 / 或预防继发性甲状旁腺功能亢进或其症状的方法。所公开的方法可以基于下面的定义进行如下的进一步描述。

[0040] 除非另有定义，否则在本文中使用的所有技术和科学术语具有与本发明所属领域的普通技术人员所通常理解的相同的意义。在本文中具体提到的所有出版物和专利，为包括描述和公开在出版物中报道的可能与本发明相结合使用的化学物质、仪器、统计分析和方法在内的所有目的，以其全部内容通过参考并入本文。在本说明书中引用的所有参考文献应该被视为指示了本领域的技术水平。本文中的任何内容不应被解释为承认本发明没有权利早于通过在先发明的这些公开。

[0041] 在说明书和权利要求书中，术语“包括”和“包含”是开放性术语，并且应该被解释为意味着“包括但不限于”。这些术语涵盖了更加限制性的术语“基本上由……构成”和“由……构成”。还应该指出，术语“包含”、“包括”、“特征在于”和“具有”可以互换使用。

[0042] 当在本文中和权利要求书中使用时，没有具体数量的指称包括其复数指称物，除非上下文明确叙述不是如此。此外，无具体数量的指称、术语“一个或多个”和“至少一个”可以互换使用。

[0043] 当提供值的范围时，应该理解，在所述范围的上下限之间的每个居间值和居间值的任何组合或子组合，以及所述陈述的范围内的任何其他陈述的或居间的值，都被涵盖在所叙述的值的范围之内。

[0044] 某些范围在本文中用前面带有术语“约”的数值描述。术语“约”在本文中被用于为它之后的准确数字以及接近或近似于该术语之后的数字的数字提供文字上的支持。在确定数字是否接近或近似于具体叙述的数字时，接近或近似的未叙述的数字可以是在提出它的上下文中提供了具体叙述的数字的基本等同性的数字，因此一般是指比具体叙述的数字或值低或高 10％的数字或值。

[0045] 所公开的方法可用于在需要的患者中治疗和 / 或预防继发性甲状腺机能亢进。需要的患者可以包括但不限于患有或有风险发生肾脏疾病或障碍后的继发性甲状腺机能亢进的患者。需要的患者可以包括但不限于患有或有风险发生例如由肾衰竭造成的肾性骨营养不良后的继发性甲状腺机能亢进的患者。需要的患者可以包括经历肾透析的患者。需要

的患者可以包括但不限于作为胃肠道吸收不良综合征（例如慢性胰腺炎、小肠病和吸收不良依赖性减肥手术，其中肠不适合地吸收维生素和矿物质）的结果而患有或有风险发生继发性甲状腺机能亢进的患者。需要的患者可以包括但不限于作为长期锂疗法、维生素 D 缺乏、营养不良、维生素 D 抗性佝偻病或高镁血症（即异常高的血镁水平）的结果而患有或有风险发生继发性甲状腺机能亢进的患者。

[0046] 所公开的方法可用于在需要的患者中治疗和 / 或预防继发性甲状腺机能亢进的症状。通过本公开的方法治疗和 / 或预防的继发性甲状腺机能亢进的症状可以包括但不限于 :骨骼弱化 ;钙化防御（此时钙在皮肤中形成结块并引起周围组织的溃疡和潜在的死亡）;心血管并发症 ;异常的脂肪和糖代谢 ;瘙痒（瘙痒症）;和低血液计数（贫血症）。通过本公开的方法治疗和 / 或预防的继发性甲状腺机能亢进的其他症状可以包括 :血清 PTH、血清磷和血清肌酸酐水平的提高。通过本公开的方法治疗和 / 或预防的继发性甲状腺机能亢进的其他症状可以包括 :骨骼和关节痛，骨骼畸形，骨骼断裂（骨折），关节肿胀，肾结石，排尿增加，肌肉虚弱和疼痛，恶心和食欲不振。通过本公开的方法治疗和 / 或预防的继发性甲状腺机能亢进的另外症状可以包括 :疲劳，上腹疼痛和抑郁。

[0047] 以前已证实，每天通过饮食给药 300ng 1α,25- 二羟基维生素 D_3(1,25(OH)$_2D_3$)，可以通过减轻肾病的症状而有效地预防肾病和肾衰竭。（参见 James Wonkee Kim，“1α,25- 二羟基维生素 D_3 对系统性红斑狼疮的 MRL/MpJ-fas/lpr 模型的影响”(Effects of 1α,25-dihydroxyvitamin D_3on the MRL/MpJ-fas/lpr model of systemic lupus erythematosus)，(Ph.D. 论文，University of Wisconsin-Madison(2009))。例如，以前已经显示，在系统性红斑狼疮 (SLE) 的 MRL/MpJ-FAS^{lpr}(MRL/lpr) 小鼠模型中，给药 1α,25- 二羟基维生素 D_3(1,25(OH)$_2D_3$) 完全阻止了蛋白尿症。（参见同上）。然而，这种治疗总是伴有严重的高钙血症。高钙血症（即血液中钙水平提高）可以引起严重的身体问题，包括死亡。具体来说，钙增加约 2mg/100ml 被认为是轻度高钙血症，并且被认为没有问题。然而，钙水平提高超过 2mg/100ml 被认为是严重高钙血症，并且可以引起肾脏、心脏和主动脉的钙化。显然，由于引起的高钙血症，这种化合物的使用对于治疗或预防继发性甲状旁腺功能亢进或其症状来说不是最佳的。

[0048] 2- 亚甲基 -19- 去甲 -(20S)-1α,25- 二羟基维生素 D_3(2MD) 是 1,25(OH)$_2D_3$ 的一种类似物，其已被显示对骨骼具有提高的体内效力，但是对肠的钙吸收没有变化。2MD 的整体合成在 1998 年 12 月 1 日出版的题为“2- 亚烷基 -19- 去甲 - 维生素 D 化合物”(2-Alkylidene-19-Nor-Vitamin D Compounds) 的美国专利号 5,843,928 中更完整地说明和描述，所述专利的说明书具体地通过参考并入本文。2MD 的生物学活性也被报道在美国专利号 5,843,928 和 Shevde 等，“1α,25- 二羟基维生素 D_3 的强力类似物选择性地诱导骨形成”(A Potent Analog of 1α,25-dihydroxyvitamin D_3Selectively Induces Bone Formation)，PNAS,Vol.99,No.21pp 13487-13491(2002) 中，二者具体地通过参考并入本文。

[0049] 在本文中公开的方法中，2MD 可以被给药以治疗和 / 或预防继发性甲状旁腺功能亢进和 / 或其伴随的症状而不引起严重的高钙血症，同时还导致血液中磷和肌酸酐水平降低以及血液中 PTH 水平降低。

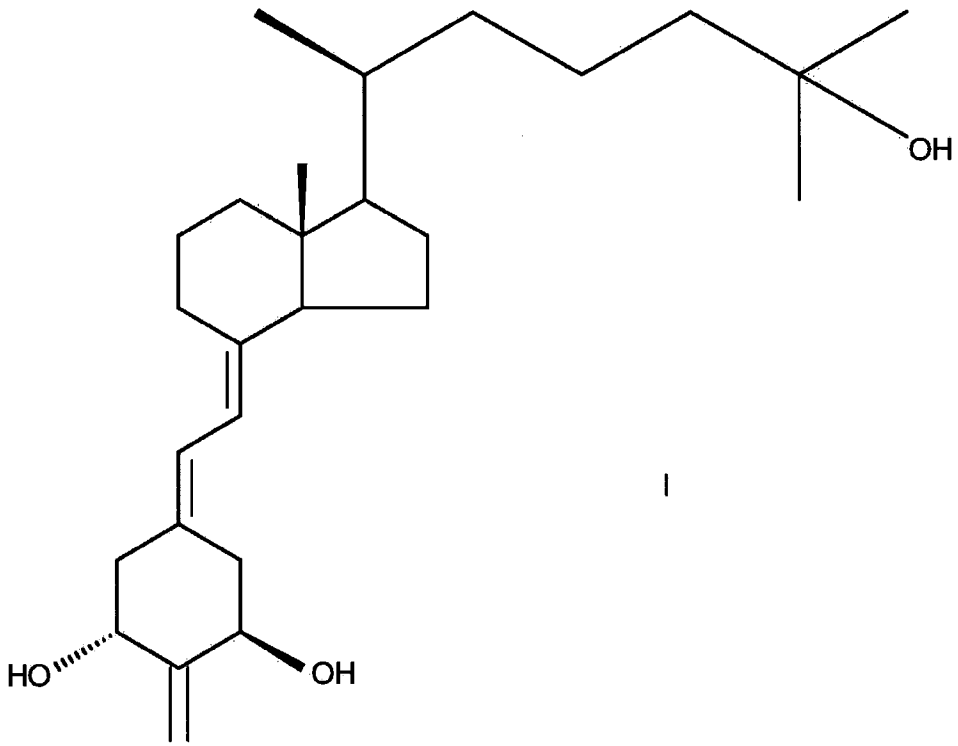
[0050] 此外，在本文中公开的方法中，通过降低血液中磷、肌酸酐和 PTH 的水平，2MD 可用

于治疗和减轻肾病的继发性甲状旁腺功能亢进的严重性及其伴随的症状，而不引起严重的高钙血症。

[0051] 当在本文中使用时，“高钙血症”是指血液中高于 2mg/100ml 的升高的钙水平。在正常受试者中，钙水平约为 9-10.5mg/dL 或 2.2-2.6mmol/L。在严重高钙血症（即钙水平高于 15-16mg/dL 或 3.75-4mmol/L）的情形中，可能发生昏迷和心脏停搏。

[0052] 因此，本发明提供了通过向受试者给药治疗有效量的 2- 亚甲基 -19- 去甲 -(20S)-1α,25- 二羟基维生素 D_3(2MD) 或其可药用盐，在有风险发生继发性甲状旁腺功能亢进的受试者中治疗和 / 或预防继发性甲状旁腺功能亢进和 / 或其伴随的症状，和在表现出继发性甲状旁腺功能亢进的症状的受试者中治疗和 / 或预防继发性甲状旁腺功能亢进和 / 或其伴随的症状，而不在所述受试者中诱导高钙血症的新方法，其中 2MD 具有结构 (I)：

[0053]



I

[0054] 当在本文中使用时，“预防”意味着预先阻止表明继发性甲状旁腺功能亢进的临床症状。这样的预先阻止包括例如在有风险发生继发性甲状旁腺功能亢进的受试者中，在继发性甲状旁腺功能亢进的明显症状包括但不限于血清 PTH、磷和肌酸酐水平升高发生之前，维持正常的肾功能。因此，术语“预防”包括受试者的预防性治疗以保护他们免于继发性甲状旁腺功能亢进的发生。在受试者中预防继发性甲状旁腺功能亢进还打算包括抑制或中止继发性甲状旁腺功能亢进的发生。抑制或中止继发性甲状旁腺功能亢进的发生包括例如抑制或中止血清 PTH、磷和肌酸酐水平升高的发生。

[0055] 当在本文中使用时，“肾病”或“肾障碍”意味着在没有进行透析的受试者或患有阶段 2 或 3 的慢性肾病 (CKD) 的患者中表现为肾功能受损的病症，例如急性肾衰竭、急性肾炎综合征、镇痛剂肾病、动脉栓塞性肾病、慢性肾衰竭、慢性肾炎、先天性肾病综合征、肺出血肾炎综合征、间质性肾炎、肾癌、肾损害、肾感染、肾损伤、肾结石、膜增生性 GNI、膜增生性

GNII、膜性肾病、微小病变性肾病、坏死性肾小球肾炎、肾母细胞瘤、肾钙质沉着症、肾源性尿崩症、肾病 -IgA、肾病综合症、多囊性肾病、链球菌后 GN、反流性肾病、肾动脉栓塞、肾动脉狭窄、肾脏障碍、肾乳头坏死、I 型肾小管性酸中毒、II 型肾小管性酸中毒、肾灌注不足、肾静脉血栓形成。

[0056] “肾病”还打算包括患有已确定的肾衰竭（例如肾小球滤过率 (GFR) 小于 15mL/min/1.73m^2 或永久性肾替代疗法 (RRT)）的患者。具有“肾病”的受试者打算包括已具有肾损害超过 3 个月的受试者，所述肾损害由伴有或不伴有 GFR 降低的肾脏的结构或功能异常所定义，所述异常由肾损害的病理学异常或标志物，包括血液或尿液的组成异常或成像试验的异常所证明。肾损害的标志物包括通过 24-HR 排泄方法测量时大于 300μg/ 天的蛋白尿。（参见表 15，Am. J. of Kidney Diseases, v. 39, no. 2, Suppl. 1 (Feb. 2002), pp. 546-575，通过参考并入本文）。这一定义可以包括正在透析的患者。

[0057] 当在本文中使用时，患有“阶段 2 慢性肾病 (CKD)”的患者是指表现出 GFR 轻度降低 (60-89mL/min/1.73m^2) 的患者。肾损害被定义为病理学异常或损害的标志物，包括血液或尿液试验或成像研究的异常。患有“阶段 3 慢性肾病 (CKD)”的患者是指表现出 GFR 中度降低 (30-59mL/min/1.73m^2) 的患者。表征肾病的指导方针可以在阶段 3A (GFR 45-59) 与阶段 3B (GFR 30-44) 之间作出区分，用于筛选和转诊的目的。对关于肾病阶段的更多信息，参见 Am. J. of Kidney Disease, V. 39, No. 2, Suppl. 1, February 2002，通过参考并入本文。

[0058] 当在本文中使用时，“受试者”包括哺乳动物和非哺乳动物。“哺乳动物”是指哺乳纲的任何成员，包括但不限于人类、非人类灵长动物例如黑猩猩和其他猿类和猴物种；农场动物例如牛、马、绵羊、山羊和猪，家畜例如兔、狗和猫；实验室动物包括啮齿动物例如大鼠、小鼠和豚鼠等。非哺乳动物的实例包括但不限于鸟类等。术语“受试者”不指示特定年龄或性别。本发明所针对的主要受试者是用血液透析治疗或接受血液透析的人类。术语“受试者”在本文中可以与术语“患者”或“个体”互换使用。

[0059] 当在本文中使用时，“给药”是指将化合物导入到身体中，优选到系统循环中，正如在下面更详细描述的。实例包括但不限于口服、表面、颊、舌下、肺、透皮、透粘膜以及皮下、腹膜内、静脉内和肌肉内注射，或采取通过消化道的液体或固体药剂的形式。

[0060] 当在本文中使用时，“治疗效果”是指一定量的化合物，当给药到受试者用于治疗或预防疾病时，足以执行所述疾病的这样的治疗或预防。“治疗有效量”将随着化合物、待治疗的疾病状态、待治疗疾病的严重性、受试者的年龄和相对健康、给药途径和形式、主治医疗或兽医从业人员的判断和其他因素而变。正如本文中所公开的，雄性刚断奶的 Harlan-Sprague Dawley 大鼠被给药几种剂量水平的 2MD，其不引起显著的高钙血症。我们发现，每只大鼠每天 21/2 纳克 / 千克体重 (ng/kg bw) 的 2MD 足以预防和治疗继发性甲状旁腺功能亢进或预防或治疗继发性甲状旁腺功能亢进的症状，而不提高血清钙水平。此外，在绝经后妇女中，每天 400ng 的 2MD 显示出血清 PTH 水平的超过 60％的降低，同时将血清钙水平维持在生理上正常的范围之内（图 13）。

[0061] 在一种实施方式中，治疗有效量在约 10ng/ 天至约 1μg/ 天之间，优选地约 20ng/ 天至约 1μg/ 天之间的范围内。在更优选的实施方式中，治疗有效量在约 40ng/ 天至约 600ng/ 天之间或约 50ng/ 天至约 600ng/ 天之间的范围内。在最优选的实施方式中，治疗有效量在约 100ng/ 天至约 400ng/ 天之间的范围内。

[0062] 当在本文中使用时，“治疗”意味着指示继发性甲状旁腺功能亢进的临床症状的改善、缓解或消除。临床症状的改善、缓解或消除包括例如继发性甲状旁腺功能亢进的症状的阻止、严重性的减轻或进展的减缓或引起所述症状的减退。例如，对使用 2MD 的治疗做出响应降低血清 PTH、血清磷或血清肌酸酐水平的量。具体来说，治疗可以包括将血清 PTH、血清磷或血清肌酸酐的量减少至少约 20％。在一种实施方式中，受试者血液中血清 PTH、血清磷或血清肌酸酐的量被减少约 20-40％或约 35-50％。继发性甲状旁腺功能亢进的其他病理状况、慢性并发症或表型表现对于本领域技术人员来说是已知的，并且可以类似地用作治疗继发性甲状旁腺功能亢进的措施，只要存在与疾病相关的状况、并发症或表现的严重性的降低即可。

[0063] 有效的化合物配方描述在美国专利号 5,843,928 中，并包括作为在无毒溶剂中的溶液，或作为在适合的溶剂或载体中的乳液、悬液或分散系，或作为与固体载体合并的丸剂、片剂、胶囊的制药应用。其他配方也可以包括其他可药用且无毒性的赋形剂，例如稳定剂、抗氧化剂、粘合剂、着色剂或乳化剂或矫味剂和延长释放配方。

[0064] 在一种实施方式中，2MD 化合物是在所公开的方法中给药的活性药物成分 (API)。API 可以作为在无毒溶剂中的溶液、在适合的溶剂或载体中的乳液、悬液或分散系配制在口服药物剂型中。API 也可以使用适合的固体制药载体配制成各种不同口服剂型，例如丸剂、片剂或胶囊。这样的药物配方也可以含有其他适合制药的 USP 批准的无活性成分、赋形剂例如稳定剂、抗氧化剂、粘合剂、着色剂、乳化剂和 / 或矫味剂，其被称为 USP 批准的无活性药物成分。

[0065] API 可以口服、表面、肠胃外或透皮或通过吸入给药。化合物可以使用适合的无菌溶液，通过注射或静脉内输注来给药。表面剂型可以是霜剂、软膏、贴片或适合于透皮和表面剂型的类似介质。

[0066] 在某些实施方式中，可以将 API 配制在药剂中，用于递送约 10ng/ 天至约 1μg/ 天之间、优选地约 20ng/ 天至约 1μg/ 天之间、更优选地约 40ng/ 天至约 600ng/ 天之间或每天约 50ng 至约 600ng 之间、最优选地约 100ng/ 天至约 400ng/ 天之间的范围内的剂量。API 优选被配制在可用于预防或治疗继发性甲状旁腺功能亢进或用于预防或治疗继发性甲状旁腺功能亢进的症状的药剂中。通常，在不显著升高血清钙的剂量水平下观察到 2MD 的正面效应。这样的剂量和给药方案可以被调整，以适应于疾病的严重性或进展、患者的素因 / 风险 / 易感性和其他已知判据。

[0067] 制药上适合的口服载体系统（也称为药物递送系统，其是随着药品或作为药品的一部分分销的现代技术，允许将药物均匀地释放或靶向身体）优选地包括 FDA 批准的和 / 或 USP 批准的无活性药物成分。在 21CFR 210.3(b)(8) 下，无活性成分是药品的旨在提供制药活性或诊断中的其他直接效应，或影响人类或其他动物的身体的结构或任何功能的任何组分。活性成分包括可能在药品制造期间经历化学变化，并以旨在提供特定活性或效果的改性的形式存在于药品中的药品组分。当在本文中使用时，药剂套装（也成为剂型）是相关材料的包装好的集合。

[0068] 当在本文中使用时，“口服剂型”可以包括胶囊（即由外壳和填充物构成的固体口服剂型），其中外壳由单一密封外套或配合在一起并有时用带子密封的两个半外套构成，并且其中胶囊外壳可以由明胶、淀粉或纤维素或其他适合的材料制成，可以是软质或硬质的，

并且填充有可以倾倒或挤压的固体或液体成分。口服剂型也可以是胶囊或包衣球粒，其中将药物包封在由适合形式的明胶制成的硬质或软质的可溶性容器或“外壳”内。药物本身可以采取颗粒的形式，所述颗粒已被施加不同量的包衣，或者采取延迟释放包衣胶囊形式，其中药物被包封在由适合形式的明胶制成的硬质或软质的可溶性容器或“外壳”内。此外，可以将胶囊覆盖在指定的包衣中，其以与作为常规剂型存在的一种或多种药物相比，至少允许降低给药频率的方式释放一种或多种药物。

[0069] 口服剂型还可以是延迟释放胶囊，其中药物被包封在由适合形式的明胶制成的硬质或软质的可溶性容器内，并且其在不是给药后立即的其他时间释放药物（或多种药物），因此肠溶包衣制品是延迟释放剂型。药物被包封在硬质或软质容器或“外壳”内的延迟释放球粒胶囊，也是有用的。在这些情形中，药物本身采取已被施加肠溶包衣的颗粒的形式，因此将药物的释放延迟到它通入到肠中为止。延迟释放胶囊和薄膜包衣延迟释放胶囊，也是有用的。

[0070] 此外，将胶囊覆盖在指定的薄膜包衣中，其以与作为常规剂型存在的一种或多种药物相比，至少允许降低给药频率的方式释放一种或多种药物。明胶包衣的胶囊（其中药物被包封在由适合形式的明胶制成的硬质或软质的可溶性容器内的固体剂型；通过加带过程，将胶囊用另外的明胶层包衣，以便形成完整密封），液体填充的胶囊（一种固体剂型，其中药物被包封在通过添加多元醇例如山梨糖醇或甘油而增塑，并且因此具有比硬壳胶囊略微更稠的稠度的可溶性明胶外壳内）。

[0071] 通常，活性成分可以被溶解或悬浮在液体介质中，可以是颗粒（小粒子或细粒）、球粒（含有或不含赋形剂的由高度纯化的药物构成的小的无菌固体物质，其通过颗粒形成或通过压制和模制来制造）或延迟释放包衣的球粒（一种固体剂型，其中药物本身采取颗粒的形式，所述颗粒已被施加不同量的包衣，并且其以与作为常规剂型存在的一种或多种药物相比，允许降低给药频率的方式释放一种或多种药物）。

[0072] 其他形式包括丸剂（旨在用于口服给药的含有药剂的小的圆形固体剂型）、粉剂（可能旨在内服或外用的干燥的细粉药物和/或化学物质的密切混合物）、酏剂（含有溶解的药剂的透亮、香味宜人的增甜的含水酒精液体；它旨在口服使用）、口香糖（各种不同形状的增甜和调味的不溶性塑性材料，其在被咀嚼时将药物物质释放到口腔中）、糖浆（含有高浓度蔗糖或其他糖类的口服溶液；该术语也被用于包括在甜味和粘性介质中制备的任何其他液体剂型，包括口服悬液）、片剂（含有药物、带有或不带有适合的稀释剂的固体剂型）、咀嚼片剂（用于咀嚼的含有药物、带有或不带有适合的稀释剂的固体剂型，其在口腔中产生口味怡人的残留物，所述残留物易于吞咽并且不留下苦味或令人不快的余味）、包衣片剂或延迟释放片剂、可分散片剂、泡腾片剂、延迟释放片剂、薄膜包衣片剂或薄膜包衣延迟释放片剂，其中片剂被配制成使得所包含的药物可以在摄入后长时间段内可用。

[0073] 在其他形式中，可以提供溶液用片剂、悬液用片剂、多层片剂、延迟释放多层片剂，其中片剂被配制成与作为常规剂型存在的药物相比，至少允许降低给药频率。口服崩解片剂、口服崩解延迟释放片剂、可溶性片剂、糖衣片剂、渗透片剂等，也是适合的。

[0074] 口服剂型组合物可以包含活性药物成分和一种或多种无活性药物成分例如稀释剂、增溶剂、醇类、粘合剂、受控释放聚合物、肠溶聚合物、崩解剂、赋形剂、着色剂、调味剂、甜味剂、抗氧化剂、防腐剂、颜料、添加剂、填充剂、悬浮剂、表面活性剂（例如阴离子型、阳

离子型、两性或非离子型）等。各种 FDA 批准的表面用无活性成分可以在 FDA 的“无活性成分数据库”(The Inactive Ingredients Database) 中找到，该数据库含有制造商旨在用于此目的的无活性成分，其中根据 21CFR 210.3(b)(7) 中给出的活性成分的定义，无活性成分在某些情况下也可以被当作活性成分。酒精是取决于产品配方、可以被当作有活性或无活性的成分的良好实例。

[0075] 当在本文中使用时，可注射和输注的剂型包括但不限于可注射脂质体，其由脂质体构成或形成脂质体（通常由磷脂构成的脂质双层囊泡，其被用于包封活性药物物质）。包含无菌制剂的旨在肠胃外使用的注射液，也是适合的；正如 USP 所定义，存在 5 种不同类型的注射液。包括由无菌、无热原制剂构成的乳液，旨在肠胃外给药的注射乳液或脂质复合物注射液，也是适合的。

[0076] 其他形式包括用于溶液注射的粉剂，其是无菌制剂，旨在重构以形成用于肠胃外使用的溶液；用于悬液注射的粉剂，其是无菌制剂，制造重构以形成用于肠胃外使用的悬液；用于脂质体悬液注射的冷冻干燥的粉剂，其是无菌的冷冻干燥的制剂，旨在重构用于肠胃外使用，其被配制成允许在重构后形成脂质体（通常由磷脂构成的脂质双层囊泡，其被用于将活性药物物质包封在脂质双层内或水性空间中）；用于溶液注射的冷冻干燥的粉剂，其是通过冻干（“冷冻干燥”）制备的旨在用于溶液的剂型，所述冻干是涉及在极低压力下，在冷冻状态下从产品除去水的过程。

[0077] 这旨在用于随后添加液体以产生在所有方面符合注射要求的溶液；用于注射悬液的冻干粉剂，所述注射悬液是旨在肠胃外使用的含有悬浮在适合的流体介质中并在所有方面符合无菌悬液要求的液体制剂；旨在用于悬液的药剂通过冻干（“冷冻干燥”）来制备，所述冻干是涉及在极低压力下，在冷冻状态下从产品除去水的过程；注射溶液，其是含有溶解在适用于注射的适合的溶剂或互溶溶剂的混合物中的一种或多种药物的液体制剂；注射溶液浓缩物，其是用于肠胃外使用的无菌制剂，在添加适合的溶剂后产生在所有方面符合注射要求的溶液。

[0078] 注射悬液包含适用于注射的液体制剂，其由分散在粒子不溶于其中的整个液相中的固体粒子构成，所述液相也可以由分散在整个水性相中的油相或分散在整个油相中的水性相构成。脂质体注射悬液包含适合于注射的液体制剂，其由分散在整个水性相中的油相构成，以便形成脂质体（通常由磷脂构成的脂质双层囊泡，其被用于将活性药物物质包封在脂质双层内或水性空间中）。超声注射悬液包含适合于注射的液体制剂，其由分散在粒子不溶于其中的整个液相中的固体粒子构成。此外，当将气体鼓泡通过悬液时对产品进行超声，这导致由固体粒子形成微球。

[0079] 肠胃外载体系统包括一种或多种制药上适合的赋形剂，例如溶剂和共溶剂、增溶剂、润湿剂、悬浮剂、增稠剂、乳化剂、螯合剂、缓冲剂、pH 调节剂、抗氧化剂、还原剂、抗微生物防腐剂、增量剂、防护剂、渗涨度调节剂和特殊添加剂。适合于肠胃外给药的配方方便地包含活性成分的无菌油性或水性制剂，其优选地与受体的血液等渗。

[0080] 当在本文中使用时，吸入剂型包括但不限于：气溶胶，其是在压力下包装并含有治疗活性成分的产品，所述治疗活性成分在激活适合的阀系统后释放，旨在表面施用到皮肤以及局部施用到鼻（鼻气溶胶）、口（舌和舌下气溶胶）或肺（吸入气溶胶）中；泡沫气溶胶，其是含有一种或多种活性成分、表面活性剂、水性或非水性液体和推进剂的剂型，其中

如果推进剂处于内部（不连续）相中（即是水包油类型），则排出稳定的泡沫，并且如果推进剂处于外部（连续）相中（即是油包水类型），则排出喷雾或快速破裂的泡沫；计量气溶胶，其是由计量药剂阀构成的加压剂型，所述阀在每次激活后允许递送均一量的喷雾；粉末气溶胶，其是在压力下包装并含有采取粉末形式的治疗活性成分的产品，所述治疗活性产品在激活适合的阀系统后释放；以及气溶胶喷剂，其是利用压缩气体作为推进剂以提供将产品作为湿润喷雾排出所必需的力的气溶胶产品，并且适用于药剂在水性溶剂中的溶液。

[0081] 当在本文中使用时，透皮剂型包括但不限于贴片，其是通常含有胶粘衬垫的药物递送系统，通常被施用到身体上的外部位点，由此使成分从贴片的某些部分被动扩散或主动运输，并且取决于贴片，由此将成分递送到身体的外表面或身体内；以及其他各种不同类型的透皮贴片，例如基质、储库和本领域中已知的其他类型的贴片。

[0082] 当在本文中使用时，表面剂型包括本领域中已知的各种不同剂型，例如洗剂（一种乳液液体剂型，其中该剂型一般用于皮肤的外部施用）、增强洗剂（一种增强药物递送的洗剂剂型，其中增强不是指剂型中药物的强度）、凝胶（一种含有胶凝剂以为溶液或胶体分散系提供刚性的半固体剂型，其中凝胶可以含有悬浮的粒子）和软膏（一种半固体剂型，通常含有少于 20％的水和可挥发物和多于 50％的烃类、蜡或多元醇作为介质，其中该剂型一般用于外部施用到皮肤或粘膜）。

[0083] 增强软膏（一种增强药物递送的软膏剂型，其中增强不是指剂型中药物的强度）、霜剂（一种乳液半固体剂型，通常含有超过 20％的水和可挥发物和 / 或少于 50％的烃类、蜡或多元醇）也可以用作介质，其中这种剂型一般用于外部施用到皮肤或粘膜。增强霜剂（增强药物递送的霜剂剂型，其中增强不是指剂型中药物的强度）、乳液（一种由包含至少两种不混溶液体的两相系统构成的剂型，一种液体作为液滴、内部或分散相分散在一般用一种或多种乳化剂稳定化的另一种液体外部或连续相中，其中乳液被用作剂型术语，除非适用更具体的术语例如霜剂、洗剂、软膏）、悬液（一种含有分散在液体介质中的固体粒子的液体剂型）、延迟释放悬液、糊剂（一种含有细小分散在脂肪介质中的 20-50％的大比例固体的半固体剂型，其中所述剂型一般用于外部施用到皮肤或粘膜）、溶液（一种透亮、均匀的液体剂型，其含有溶解在溶剂或可互溶溶剂的混合物中的一种或多种化学物质）和粉剂也是适合的。

[0084] 香波（一种具有皂或去污剂的洗剂剂型，通常用于清洁头发和头皮）通常被用作皮肤科药剂的介质。例如，常常使用香波悬液（含有分散在液体介质中的一种或多种固体不溶物质的液体皂或去污剂，其被用于清洁头发和头皮并通常被用作皮肤科药剂的介质）。气溶胶泡沫（即含有一种或多种活性成分、表面活性剂、水性或非水性液体和推进剂的剂型；如果推进剂处于内部不连续相中、即是水包油类型，则排出稳定的泡沫，并且如果推进剂处于外部连续相中、即是油包水类型，则排出喷雾或快速破裂的泡沫）、喷剂（通过空气或水蒸汽的喷射细分的液体）、计量喷剂（一种由阀构成的非加压剂型，所述阀允许在每次激活后分发规定量的喷雾）和悬浮喷剂（一种含有分散在液体介质中的固体粒子的液体制剂，并采取粗液滴形式或作为细分固体局部施用，最通常施用到鼻咽道或表面施用到皮肤）也是适合的。

[0085] 胶冻（一类凝胶，其是由液体浸透的小无机粒子或大有机分子构成的悬液所组成的半固体系统——其中结构上内聚的基质含有高比例的液体，通常为水）和薄膜（薄的层

或涂层）、包括延迟释放薄膜（一种采取薄膜形式的药物递送系统，其在长时期内释放药物以便在血液或靶组织中维持恒定的药物水平）和可溶性薄膜（当与液体接触时易于被溶解的薄层或涂层），也是适合的。

[0086] 海绵（一种含有药物的多孔、交错的吸收材料，它通常被用于施用或导入药物或用于清洁，其中海绵通常保持其形状），拭子（含有药物的一小块相对扁平的吸收材料，其中拭子也可以附连到小棒的一个末端，并且其中拭子通常被用于施用药物或用于清洁）。

[0087] 贴片（一种通常含有胶粘衬垫的药物递送系统，通常被施用到身体上的外部位点，使其成分从贴片的某些部分被动扩散或主动运输，并且取决于贴片，将成分递送到身体的外表面或身体内，并且其中贴片有时与术语“延迟释放薄膜”和“系统”同义），延迟释放贴片（一种采取贴片形式的药物递送系统，其释放药物的方式使得与作为常规剂型例如溶液或即时释放药物的常规固体剂型存在的药物相比降低给药频率），电子控制的延迟释放贴片（一种采取受电流控制的贴片形式的药物递送系统，其释放药物的方式使得与作为常规剂型例如溶液或即时释放药物的常规固体剂型存在的药物相比降低给药频率）等。各种表面剂型也可以配制成立即释放、受控释放、持续释放等。

[0088] 表面剂型组合物含有活性药物成分和一种或多种无活性药物成分例如赋形剂、着色剂、颜料、添加剂、填充剂、软化剂、表面活性剂（例如阴离子型、阳离子型、两性和非离子型）、穿透增强剂（例如醇类、脂肪醇类、脂肪酸、脂肪酸酯和多元醇）等。各种 FDA 批准的表面用无活性成分可以在 FDA 的“无活性成分数据库”(The Inactive Ingredients Database) 中找到，该数据库含有制造商旨在用于此目的的无活性成分，其中根据 21CFR 210.3(b)(7) 中给出的活性成分的定义，无活性成分在某些情况下也可以被当作活性成分。酒精是取决于产品配方、可以被当作有活性或无活性的成分的良好实例。

实施例

[0089] 提出下面的实施例仅仅是出于说明的目的，而不打算以任何方式限制本发明的范围。所述实施例说明，2MD 这种最初被认为在骨质疏松症的预防和治疗中重要的 $1,25(OH)_2D_3$ 的类似物，在预防和治疗继发性甲状旁腺功能亢进及其相伴症状中也是重要的。在肾脏被手术摘除的大鼠中进行的研究显示，每日口服和腹膜内 (ip)2MD 给药与介质对照动物相比，产生较低的血清 PTH、磷和肌酸酐水平，所述血清 PTH、磷和肌酸酐都是肾衰竭的指示物。此外，2MD 给药在不升高血清钙的剂量水平下产生较低的 PTH、磷和肌酸酐水平。

[0090] 实施例 1

[0091] 材料和方法

[0092] 肾切除术大鼠模型。疾病诱导。刚断奶的雄性 Sprague-Dawley 大鼠从 Harlan(Madison, Wis.) 获得。在 10-13 天的适应期后，将动物的一个肾的三分之二移除。一周后，移除另一个完整的肾。然后将动物从普通饲料切换成含有 0.6% Ca 和 0.9%磷和脂溶性维生素 A、D、E 和 K 的纯化的啮齿动物饮食 (Suda 等，纯化的啮齿动物饮食 (Purified Rodent Diet-Diet)11)。水和饮食被提供以随意取用。

[0093] 动物饲养。动物被饲养在带有玉米芯垫层的悬挂的塑料鞋盒式笼子（在手术之前）或不锈钢金属丝底笼子（手术后约一周）中。将动物房维持在 68 至 72 °F 的温度和 25

至 75%的相对湿度下。保育室被设置成每天提供 12 小时光照。

[0094] 治疗组 :在第二次手术后约四周,将动物指派到治疗组 (14-15 只动物 / 组),以使每个组具有相同的平均 PTH 水平。

[0095] 药剂制备 (介质配方)。阴性对照材料通过按体积计量乙醇 (%) 和 Neobee 油,混合,然后置于 2 至 8℃下储存来制备。

[0096] 药剂制备 (2MD 配方)。2MD 配方 (DP001, Sigma Aldrich Fine Chemicals, Madison, Wis.) 如下制备 :首先使用 UV 分光光度测量法 (消光系数 = 42,000 ; λ_{max} = 252nm) 测定乙醇储用溶液的浓度。然后按体积向 Neobee 油加入溶液,使得在最终溶液中存在不超过 5%乙醇。如果需要,加入另外的乙醇以使最终乙醇含量达到 5%。将溶液混合并储存在 2 至 8℃。

[0097] 药剂给药方法。介质和 2MD 两者以 0.5ml/kg 体重的量每天一次口服给药到舌背共 8 周,或每周三次腹膜内给药共 4 周。

[0098] 血清甲状旁腺激素 (PTH) 水平。使用“血清 PTH 水平”,我们意指由甲状旁腺释放的 PTH 的量。PTH 是身体的钙和磷水平的最重要的调控物,并且受血液中钙水平的控制。低的血钙水平引起被释放的 PTH 增加,而高的血钙水平抑制 PTH 释放。正常值为 10-55 皮克每毫升 (pg/mL)。手术后 4 周和治疗开始后 4 和 8 周,从尾动脉收集血液并使用来自于 Immutopics, Inc. (San Clemente, Calif.) 的大鼠 BioActive Intact PTH ELISA 试剂盒测量生物活性血清 PTH 的浓度。

[0099] 血清钙分析。手术后 4 周和治疗开始后 4 和 8 周,从每只实验动物的尾动脉收集血液。使血液在室温凝结,然后以 3000xg 离心 15 分钟。将血清转移到聚丙烯管,并在 -20℃下冷冻储存。通过将血清在 0.1%氯化镧中稀释,并在原子吸收分光光度计 (Perkin Elmer 3110 型, Shelton, Conn.) 上测量吸收值,来测定钙水平。

[0100] 磷测定法。手术后 4 周和治疗开始后 8 周,从每只实验动物的尾动脉收集血液。使血液在室温凝结,然后以 3000xg 离心 15 分钟。将血清转移到聚丙烯管,并在 -20℃下冷冻储存。使用临床分析仪 (Pentra 400, Horiba ABX Diagnostics--France ;使用磷钼酸盐的 UV 方法) 测定磷水平。

[0101] 肌酸酐测定法。测量血清肌酸酐水平是评估肾机能障碍的有用且廉价的方法。肌酸酐是骨骼肌组织的磷酸肌酸酐代谢的非蛋白质废物。肌酸酐生产是连续的并且与肌肉质量成正比。肌酸酐被自由滤过,因此血清肌酸酐水平取决于肾小球滤过率 (GFR)。肾机能障碍降低滤过肌酸酐的能力,并且血清肌酸酐升高。如果血清肌酸酐水平加倍,GFR 被认为已经减半。三倍的增加被认为反映出肾功能的 75%的丧失。

[0102] 在下面的实施例中,在手术后 4 周和治疗开始后 8 周评估血清肌酸酐水平。从每只实验动物的尾动脉收集血液。使血液在室温凝结,然后以 3000xg 离心 15 分钟。将血清转移到聚丙烯管,并在 -20℃下冷冻储存。肌酸酐水平使用临床分析仪 (Pentra 400, Horiba ABX Diagnostics--France ;Jaffe 反应) 来测定,并且指示了受损的肾功能和慢性肾炎。在本发明的一种实施方式中,预计在按照本发明的方法治疗后,血清肌酸酐水平最少降低约 30%。

[0103] 实施例 2

[0104] 尿毒症大鼠模型 - 2MD 的腹膜内 (ip) 给药

[0105] 图 1 示意示出了使用 2MD 的 ip 治疗方案。如图 2 中所示，每周三次以 5ng/kg bw 的剂量 ip 给药 2MD 阻止了血清 PTH 升高，并且在 10ng/kg bw 剂量下抑制循环 PTH 水平。如图 3 中所示，直至给药 10ng/kg bw 的剂量为止，2MD 的 ip 给药不升高血清钙水平。

[0106] 实施例 3

[0107] 尿毒症大鼠模型 - 2MD 与 Zemplar® 的口服给药的比较

[0108] 图 4 示意示出了使用 2MD 的口服治疗方案。如图 5 中所示，以 1-5ng/kg bw 的每日剂量口服给药 2MD 阻止了血清 PTH 水平升高或使其降低。观察到的效果在治疗的 8 周内持续。如图 6 中所示，以 30-300ng/kg bw 的每日剂量口服给药 Zemplar® 阻止了血清 PTH 水平升高，但是随着疾病的进展而失去治疗效果。图 7 示出了当口服给药时，在 5ng/kg bw 的 2MD 剂量下观察到临床上显著的血清钙升高。图 8 示出了当口服给药时，在 100 和 300ng/kg bw 的 Zemplar® 剂量下观察到临床上显著的血清钙升高。

[0109] 如图 9 中所示，以 1-5ng/kg bw 的每日剂量口服给药 2MD 在肾切除的大鼠中降低血清磷水平。相反，Zemplar® 的口服给药在肾切除的大鼠中不降低血清磷水平。

[0110] 如图 11 中所示，以 1-5ng/kg bw 的每日剂量口服给药 2MD 引起与介质对照动物相比更低的血清肌酸酐水平。相反，Zemplar® 的口服给药与介质对照动物相比降低血清肌酸酐水平，但是只在明显提高血清钙的剂量水平下才行。

[0111] 实施例 4

[0112] 1B 期临床 - 2MD 向绝经后妇女的口服给药

[0113] 图 13 示出了每日一次以 110 纳克 (ng) 的剂量向绝经后妇女口服给药 2MD 共 28 天，使血清 PTH 水平降低 21%，并且 440ng 的剂量使血清 PTH 水平降低 67%。

[0114] 数据的解释

[0115] 在肾衰竭的大鼠模型中，2MD 或 2- 亚甲基 -19- 去甲 -(20S)-1α,25- 二羟基维生素 D_3 有效地减轻继发性甲状旁腺功能亢进。除了六分之一之外的所有肾物质被手术移除并置于高磷饮食下的大鼠，将在血液中产生高的 PTH 水平。以每日或每周三次的方式口服或腹膜内给药 2MD，将降低 PTH 的循环水平。此外，2MD 具有防止血液中磷和肌酸酐两者的水平进一步升高或可能降低两者的水平的额外的益处。此外，2MD 表现出长期持续的效果，因为口服治疗 8 周的大鼠仍显示出降低的 PTH 水平；然而，在这一动物模型中，其他维生素 D 化合物在治疗 4 周后失去它们的有效性。

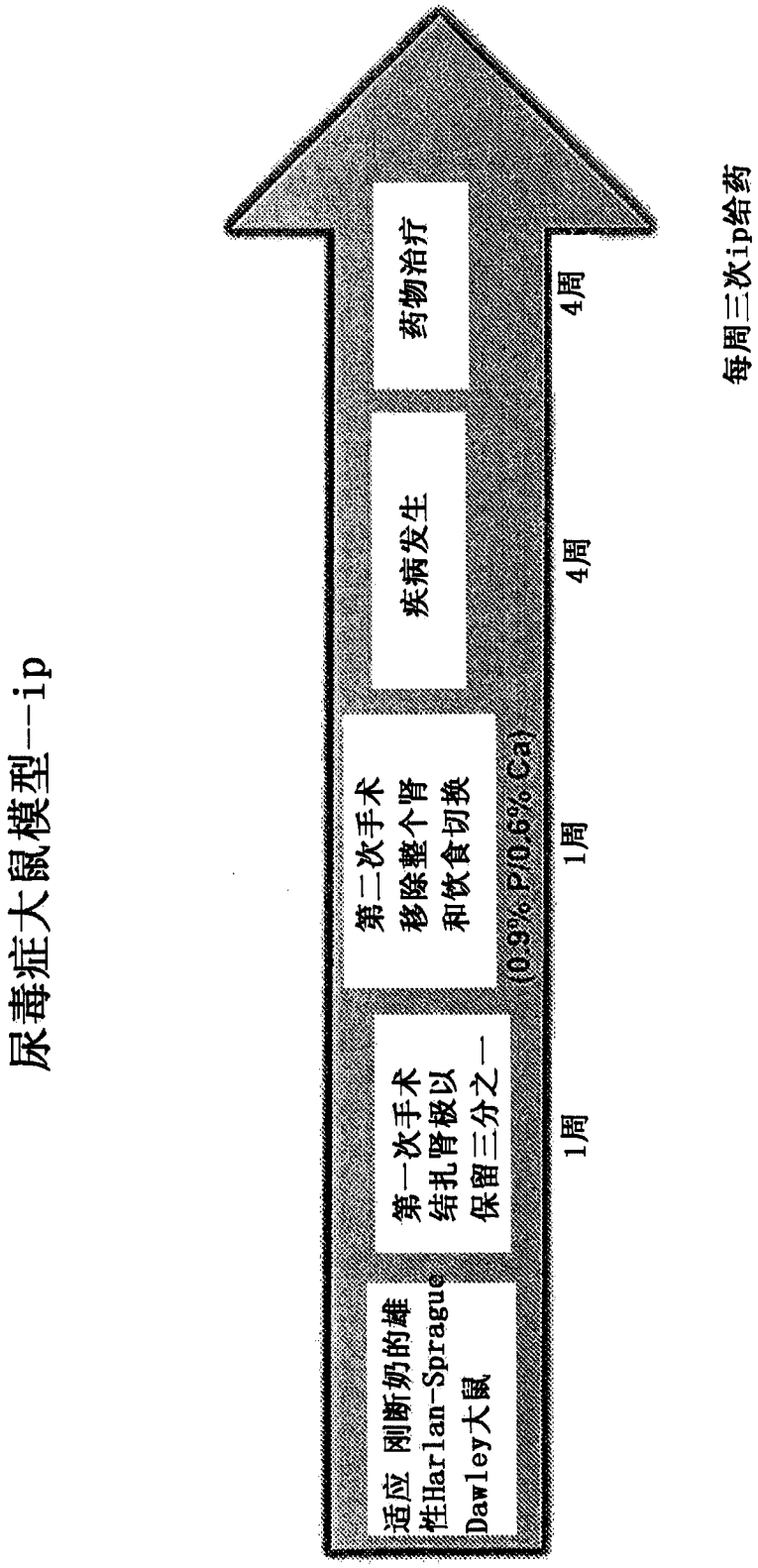

尿毒症大鼠模型--ip
适应 刚断奶的雄性Harlan-Sprague Dawley大鼠
第一次手术 结扎肾极以 保留三分之一
第二次手术 移除整个肾 和饮食切换
{0.9%P/0.6%Ca}
疾病发生
药物治疗
1周
1周
4周
4周
每周三次ip给药

图 1

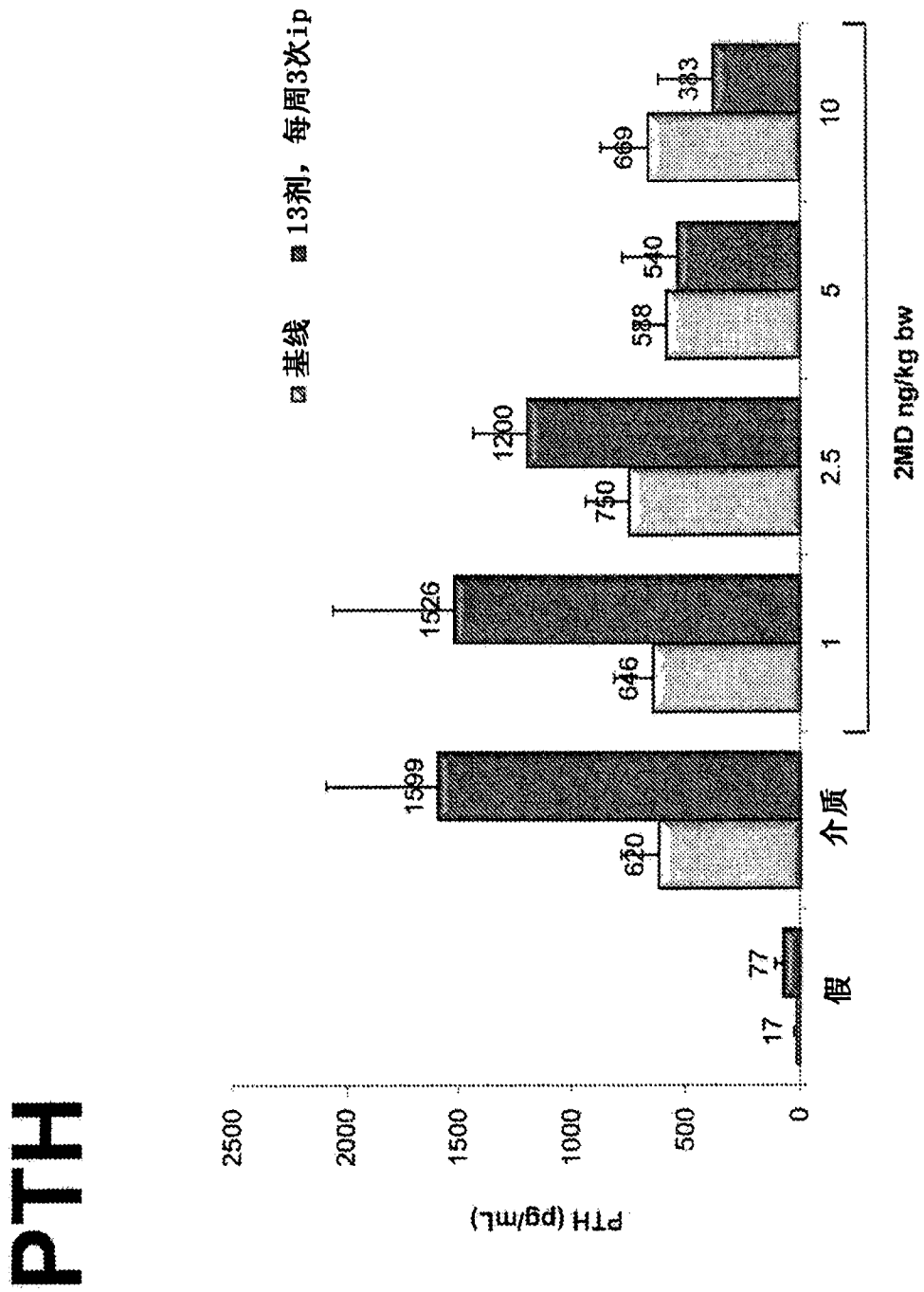

PTH
基线
13剂，每周3次ip
PTH (pg/mL)
2500
2000
1500
1000
500
0
假
介质
1
2.5
5
10
2MD ng/kg bw
17
77
630
1599
646
1526
750
1200
588
540
669
383

图 2

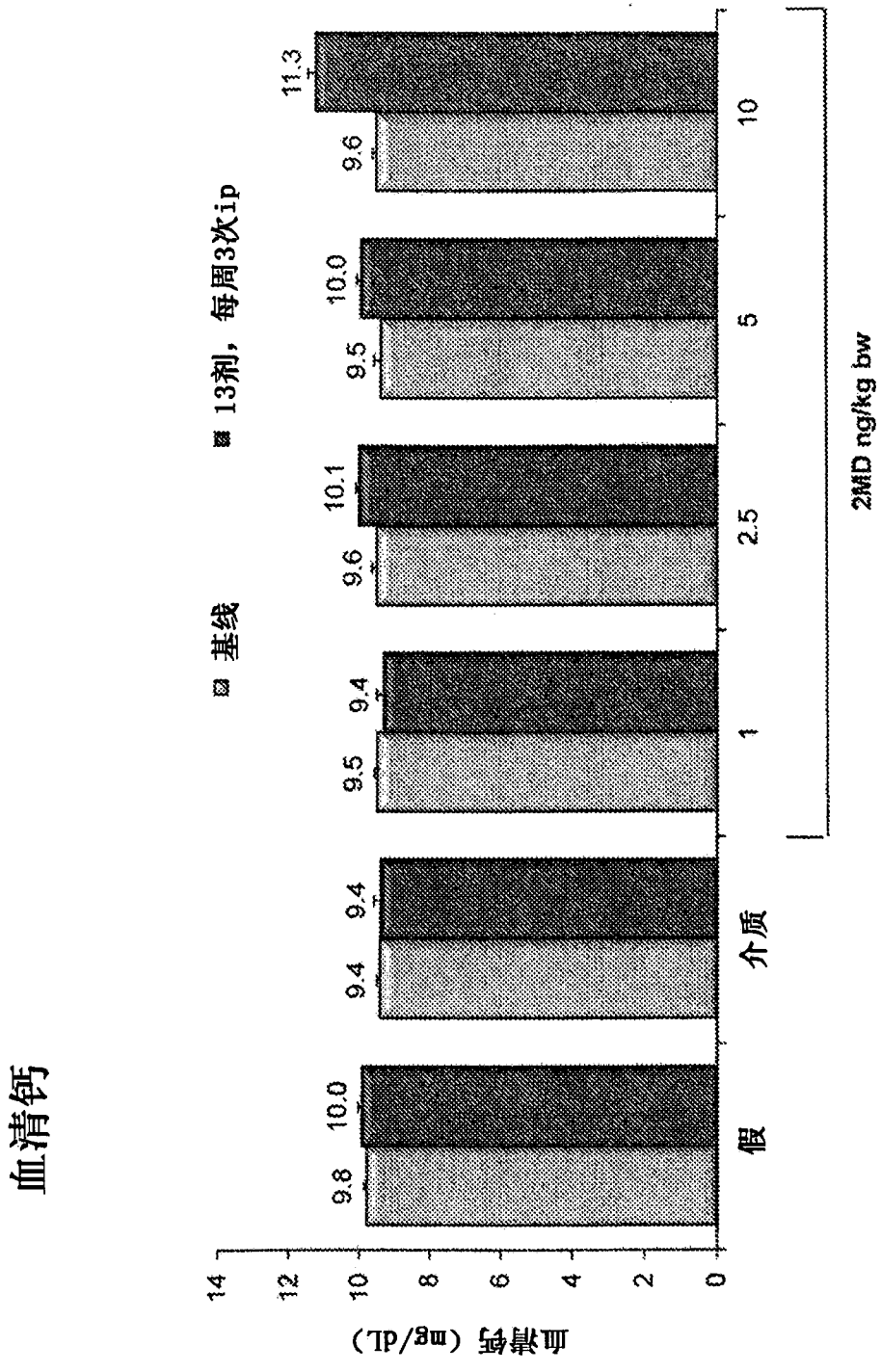

血清钙
基线
13剂，每周3次ip
血清钙 (mg/dL)
0
2
4
6
8
10
12
14
假
介质
1
2.5
5
10
2MD ng/kg bw
9.8
10.0
9.4
9.4
9.5
9.4
9.6
10.1
9.5
10.0
9.6
11.3

图 3

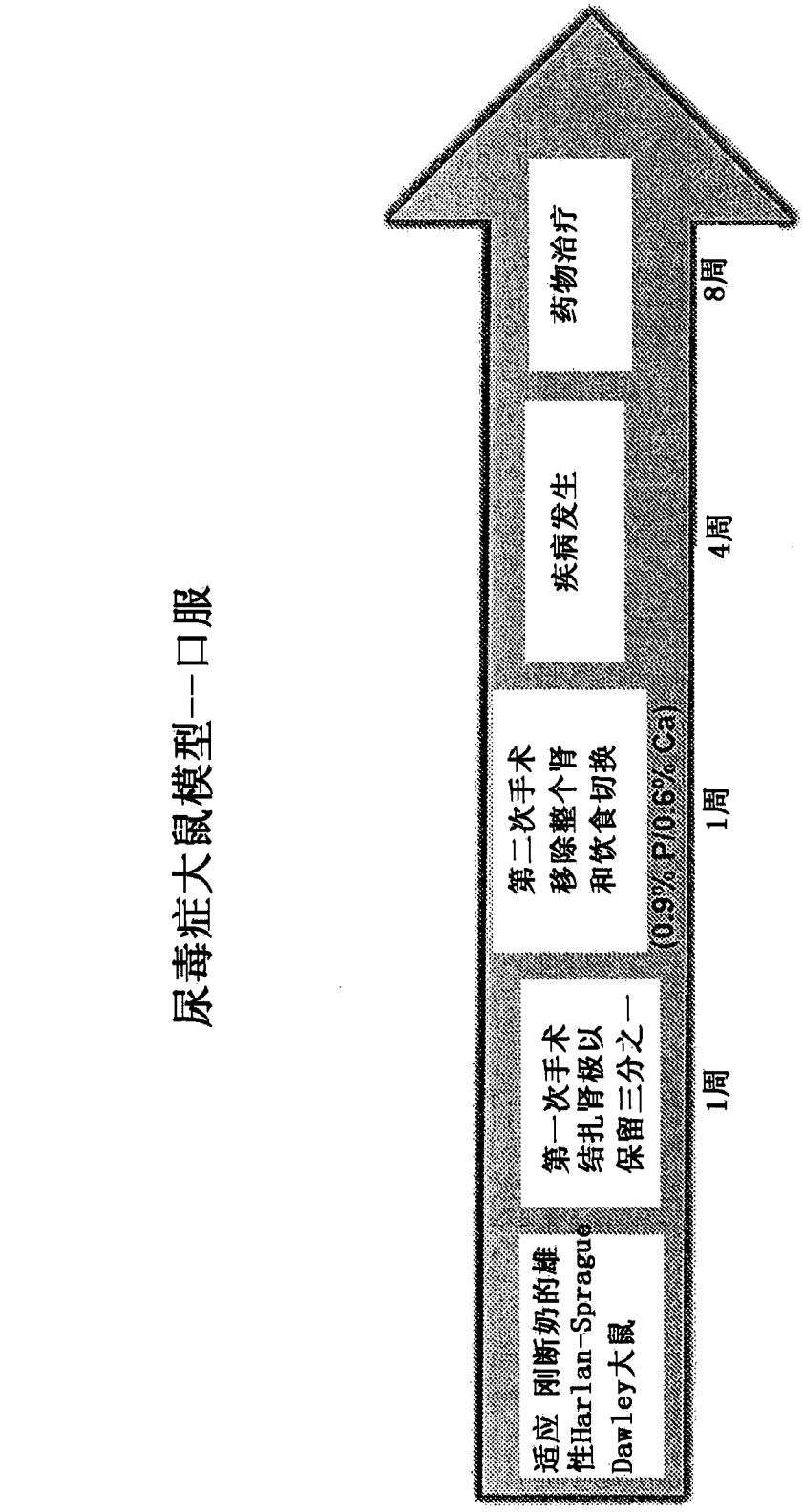

尿毒症大鼠模型--口服
适应 刚断奶的雄性Harlan-Sprague Dawley大鼠
第一次手术结扎肾极以保留三分之一
第二次手术移除整个肾和饮食切换
(0.9%P/0.6%Ca)
疾病发生
药物治疗
1周
1周
4周
8周
每日一次口服给药
终点：在基线和结束时收集血液，用于血清PTH
(来自于Immutopics的完整PTH ELISA)和钙(原子吸收)测量。

图 4

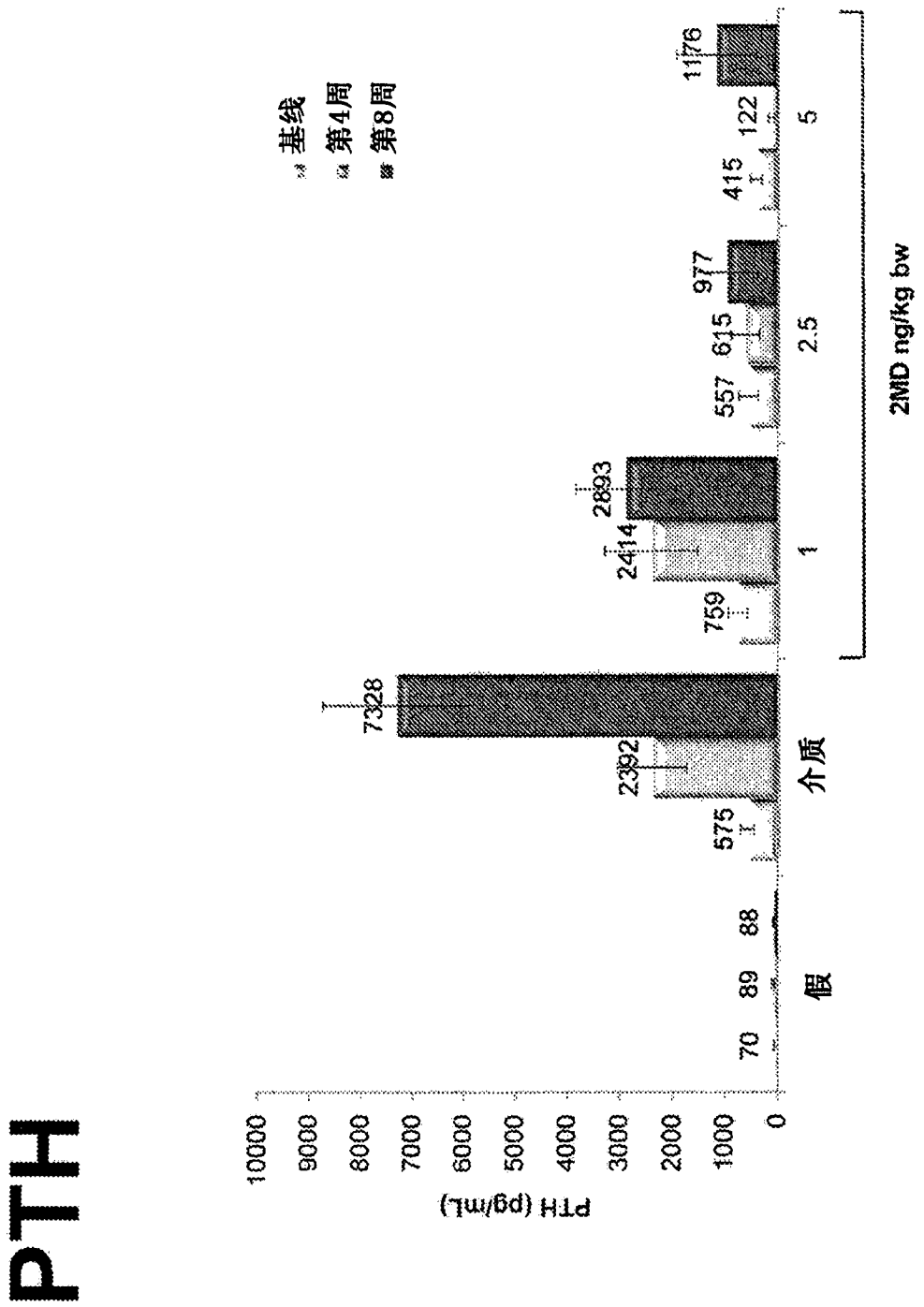

PTH
基线
第4周
第8周
PTH (pg/mL)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
70
89
88
575
2392
7328
759
2414
2893
557
615
977
415
122
1176
假
介质
1
2.5
5
2MD ng/kg bw

图 5

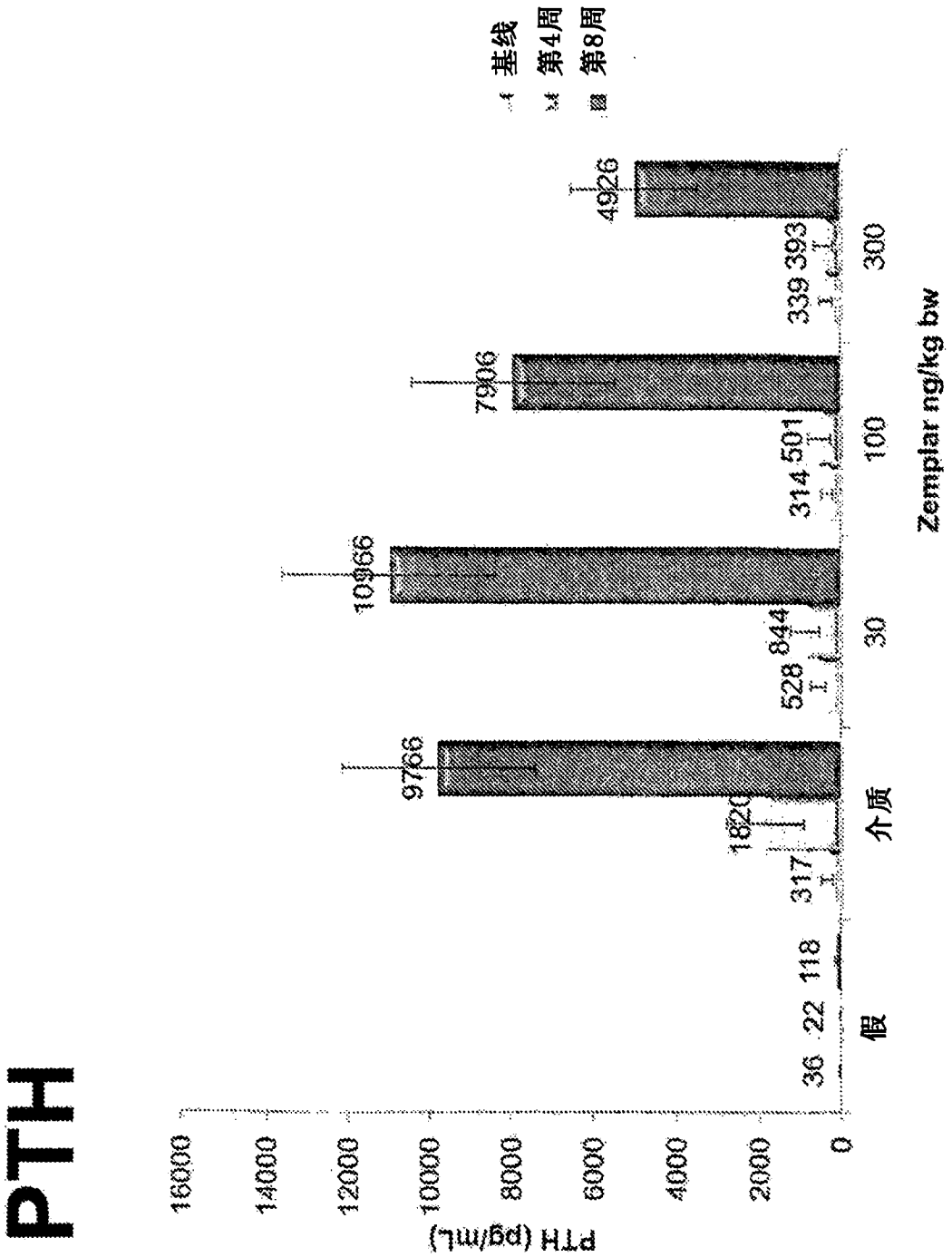

PTH
基线
第4周
第8周
PTH (pg/mL)
16000
14000
12000
10000
8000
6000
4000
2000
0
假
介质
Zemplar ng/kg bw
30
100
300
36
22
118
317
1820
9766
528
844
10966
314
501
7906
339
393
4926

图 6

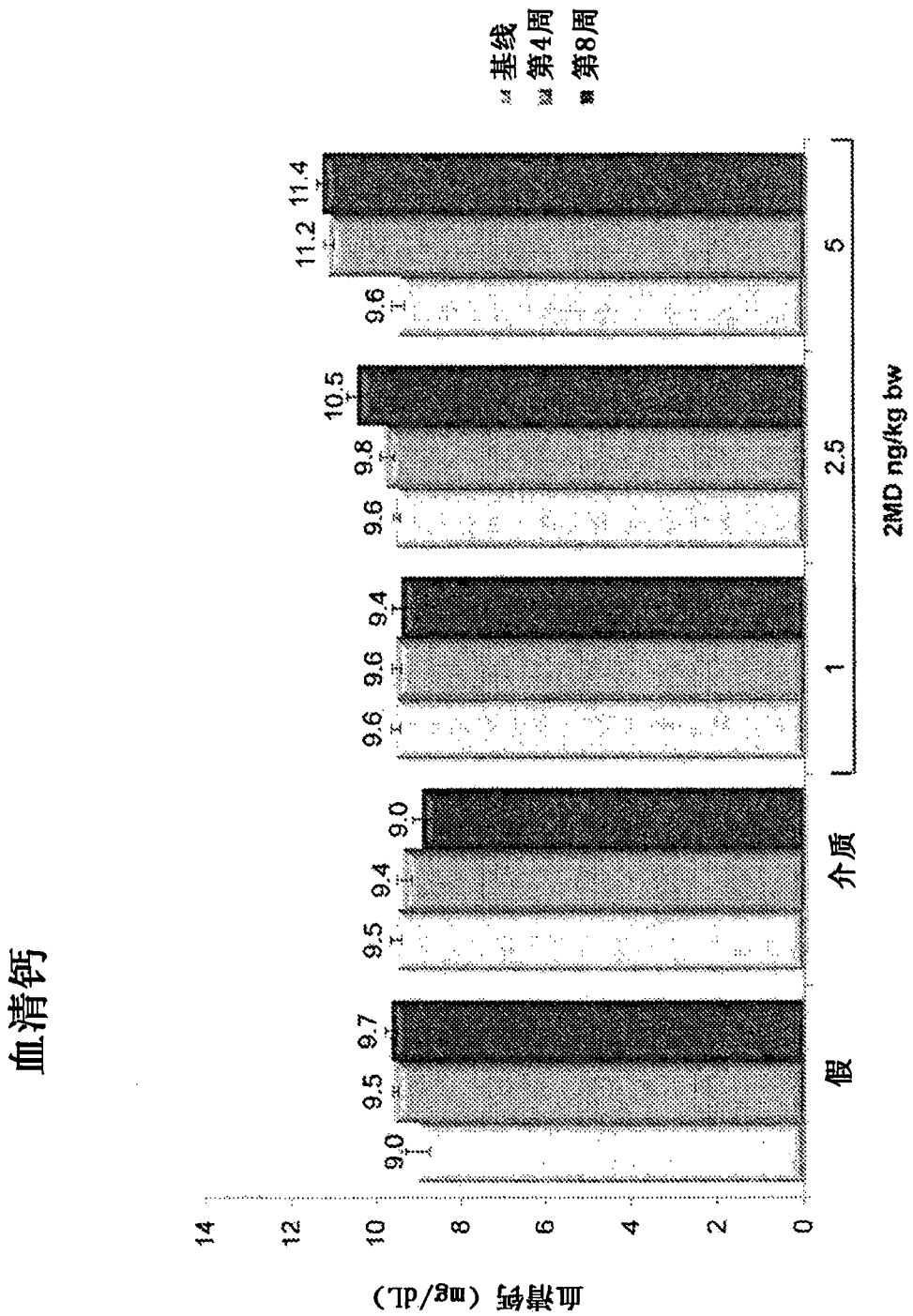

血清钙
基线
第4周
第8周
血清钙 (mg/dL)
0
2
4
6
8
10
12
14
9.0
9.5
9.7
9.5
9.4
9.0
9.6
9.6
9.4
9.6
9.8
10.5
9.6
11.2
11.4
假
介质
1
2.5
5
2MD ng/kg bw

图 7

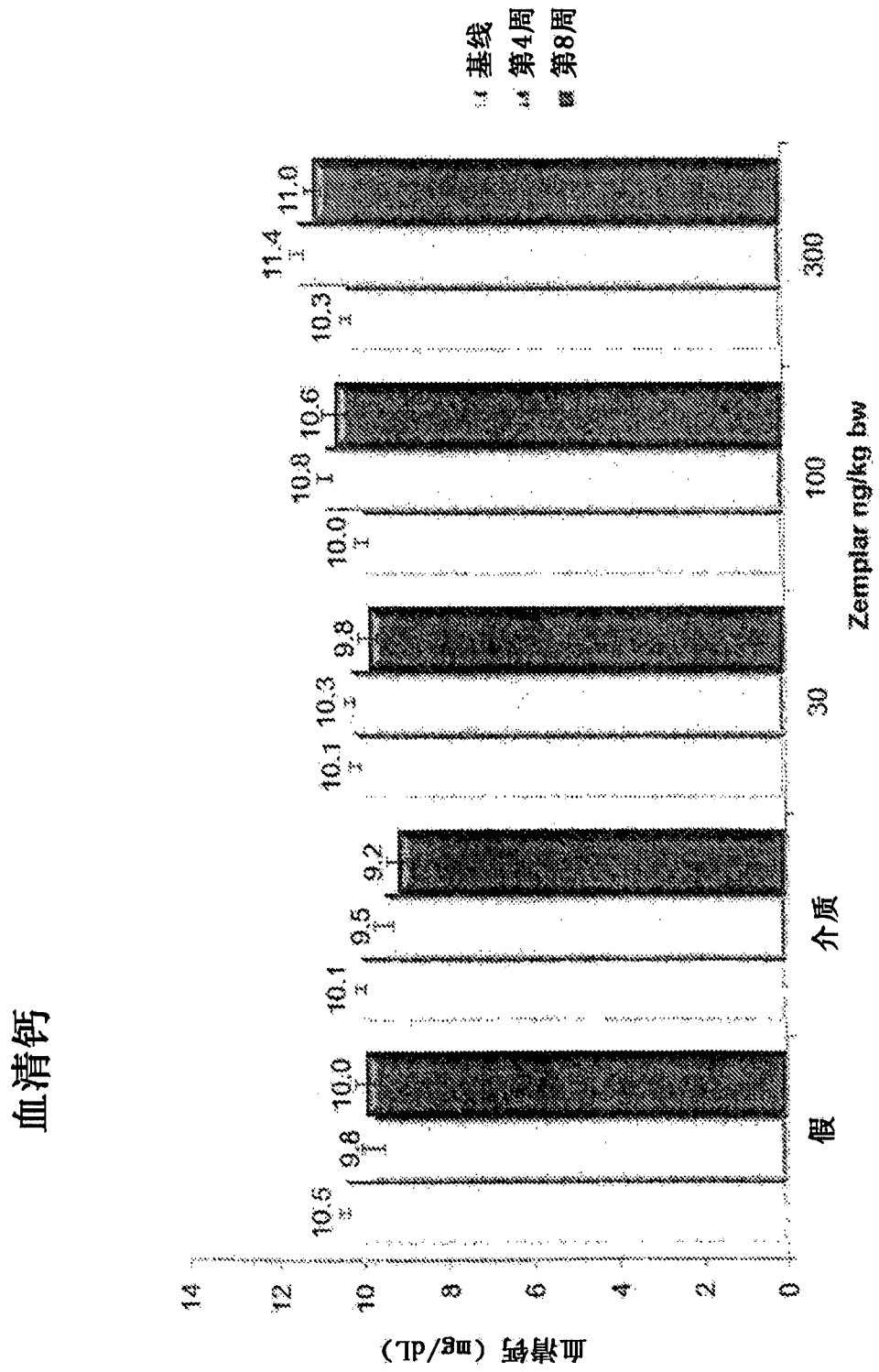
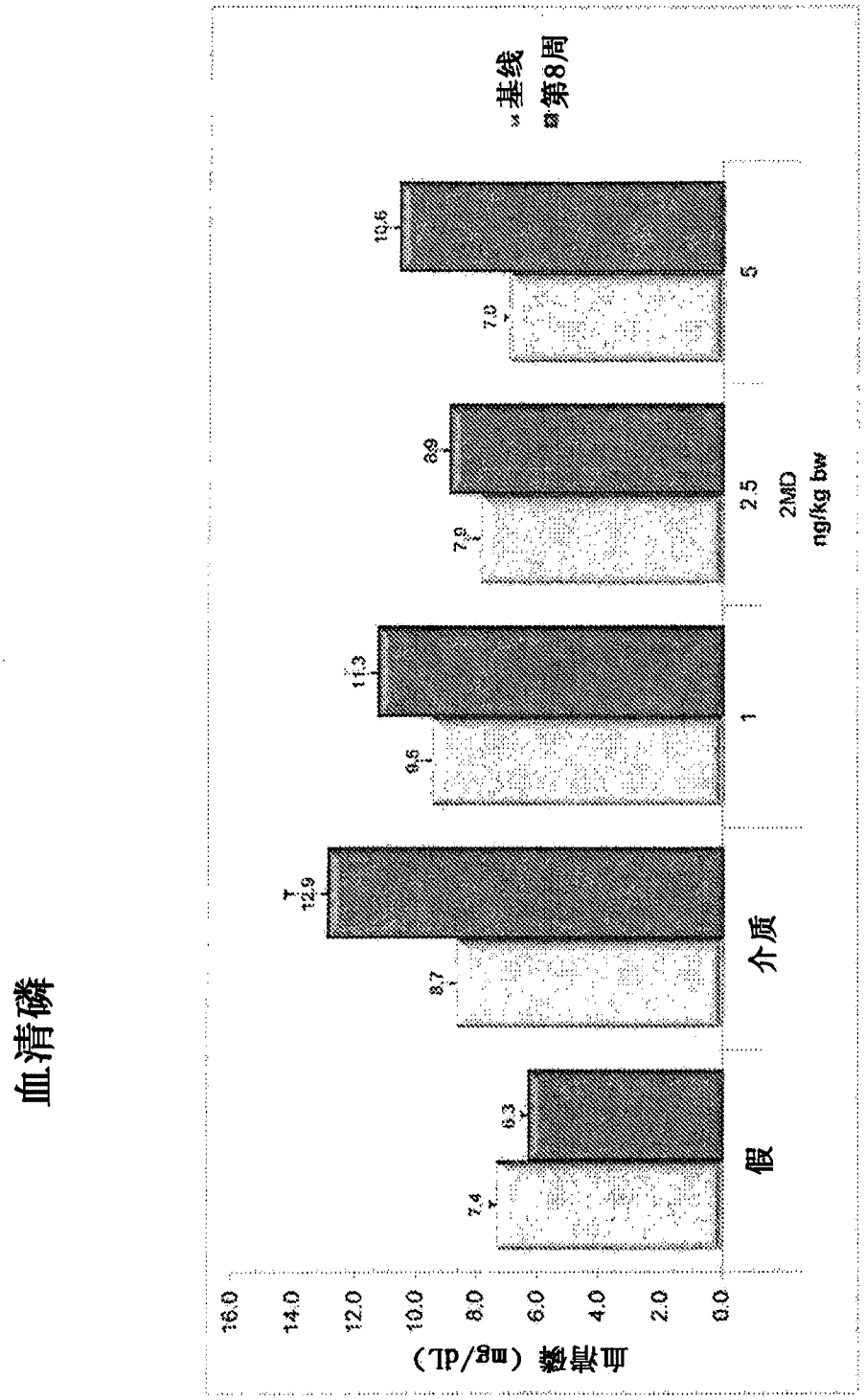

血清钙
基线
第4周
第8周
血清钙 (mg/dL)
0
2
4
6
8
10
12
14
10.5
9.8
10.0
10.1
9.5
9.2
10.1
10.3
9.8
10.8
10.0
10.6
11.4
10.3
11.0
假
介质
30
100
300
Zemplar ng/kg bw

图 8


基线
第8周
血清磷 (mg/dL)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
7.4
8.3
8.7
12.9
9.5
11.3
7.9
8.9
7.0
12.6
假
介质
1
2.5
5
2MD
ng/kg bw

血清磷

图 9

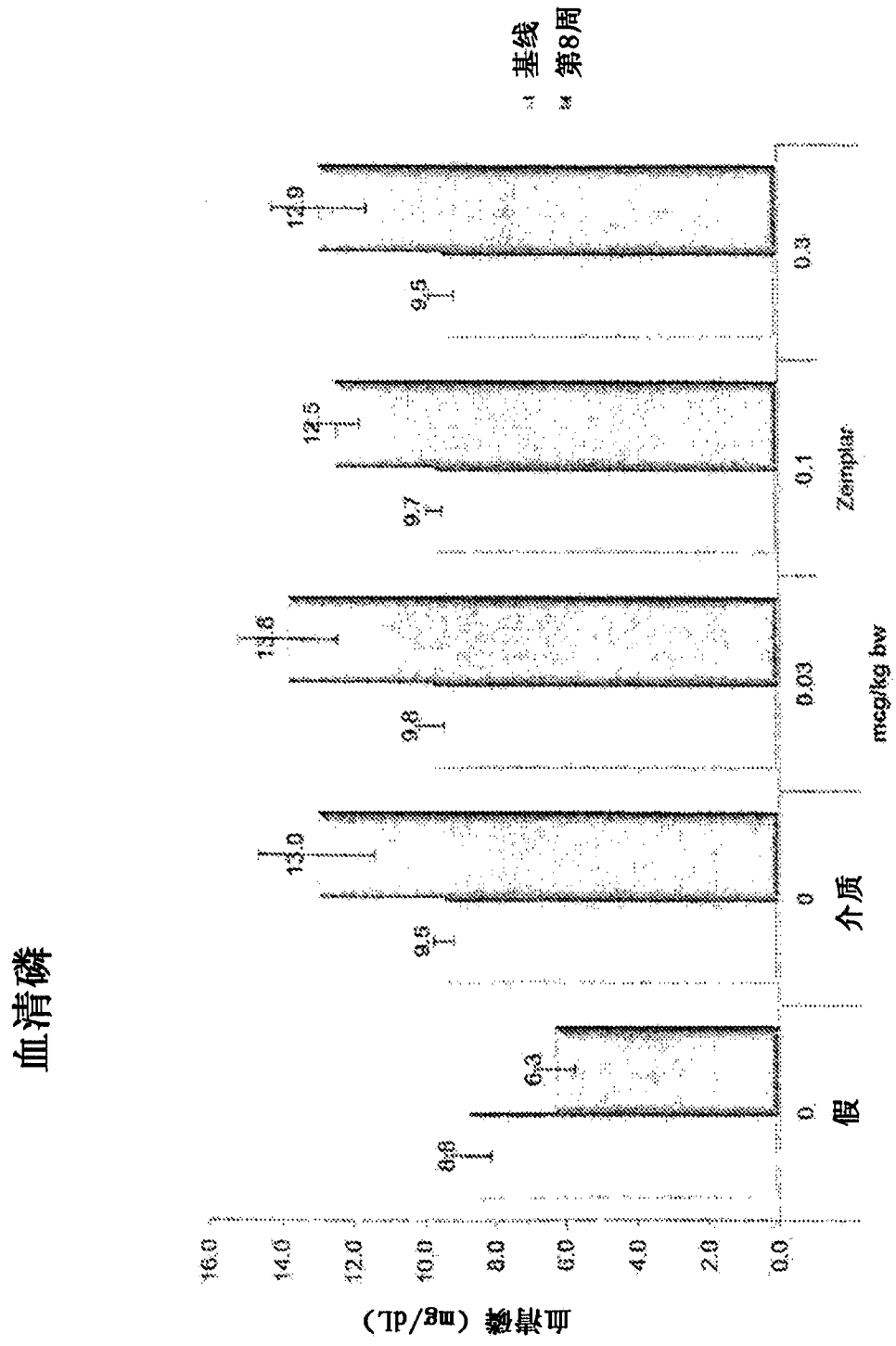

血清磷
基线
第8周
血清磷 (mg/dL)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
8.8
6.3
9.5
13.0
9.8
13.6
9.7
12.5
9.5
12.9
假
0
介质
0
0.03
0.1
0.3
Zemplar
mcg/kg bw

图 10

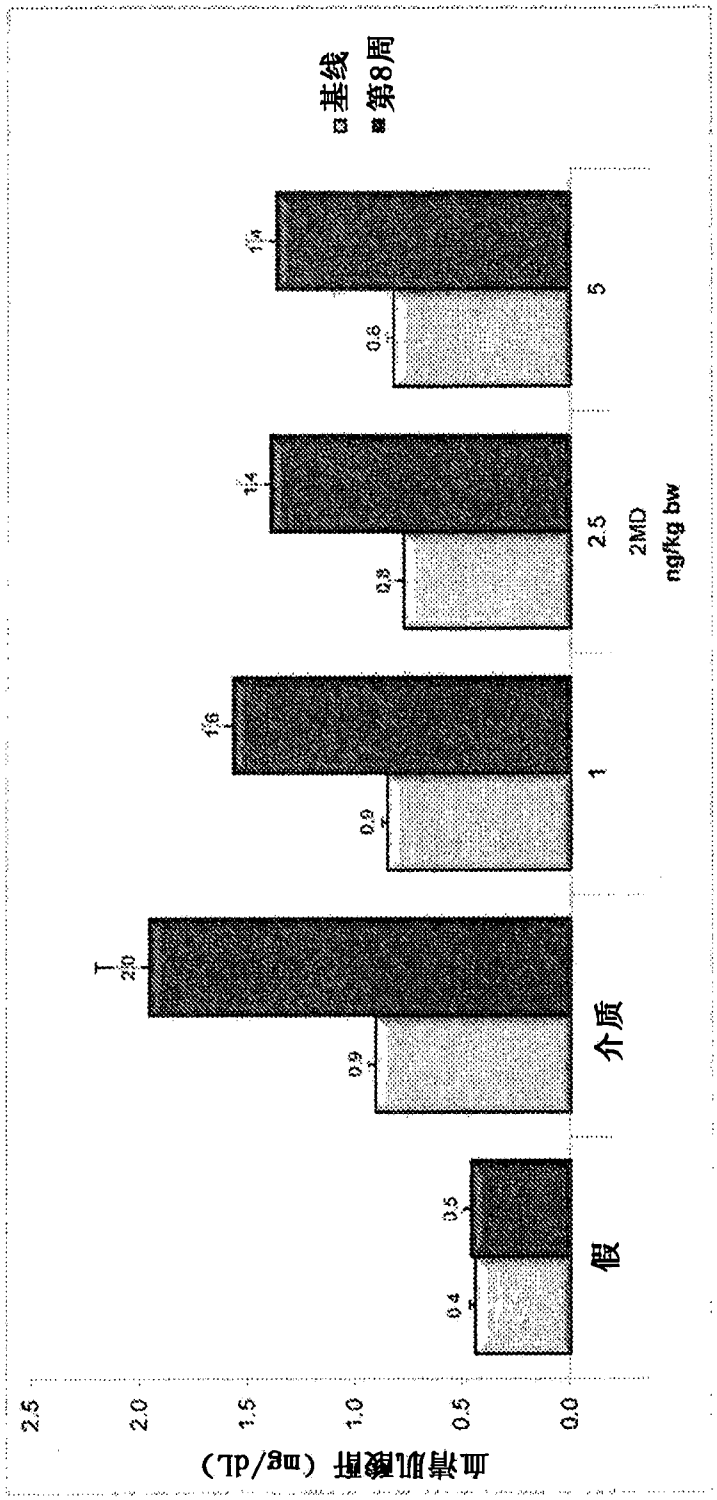

血清肌酸酐
基线
第8周
血清肌酸酐 (mg/dL)
2.5
2.0
1.5
1.0
0.5
0.0
假
介质
1
2.5
5
2MD
ng/kg bw
0.4
0.5
0.9
2.0
0.9
1.6
0.8
1.4
0.8
1.4
0.6
1.4

图 11

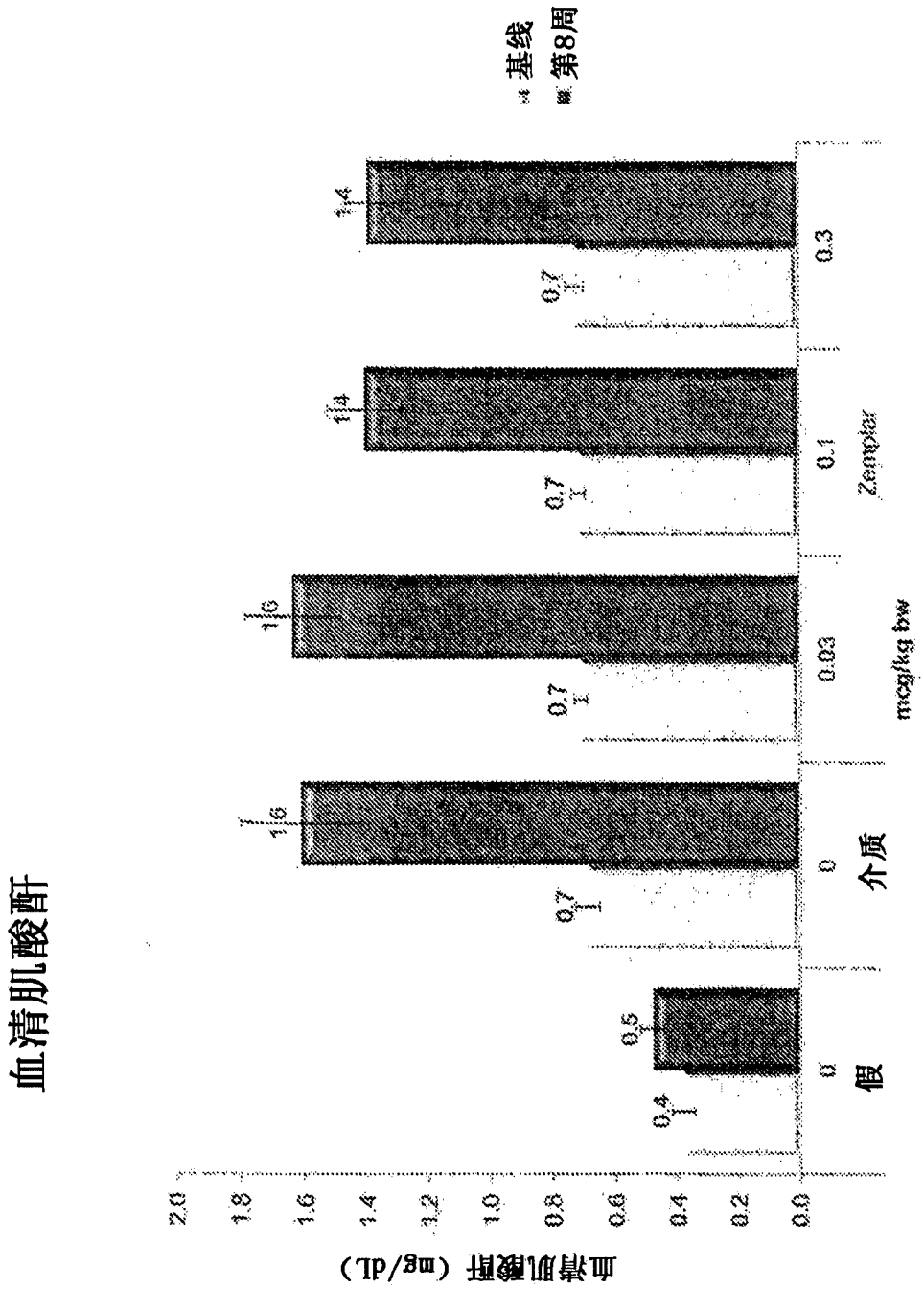

血清肌酸酐
基线
第8周
血清肌酸酐 (μg/dL)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
假
介质
Zenpiar
mcg/kg bw
0
0
0.03
0.1
0.3
0.4
0.5
1.6
1.6
1.4
1.4
0.7
0.7
0.7
0.7

图 12

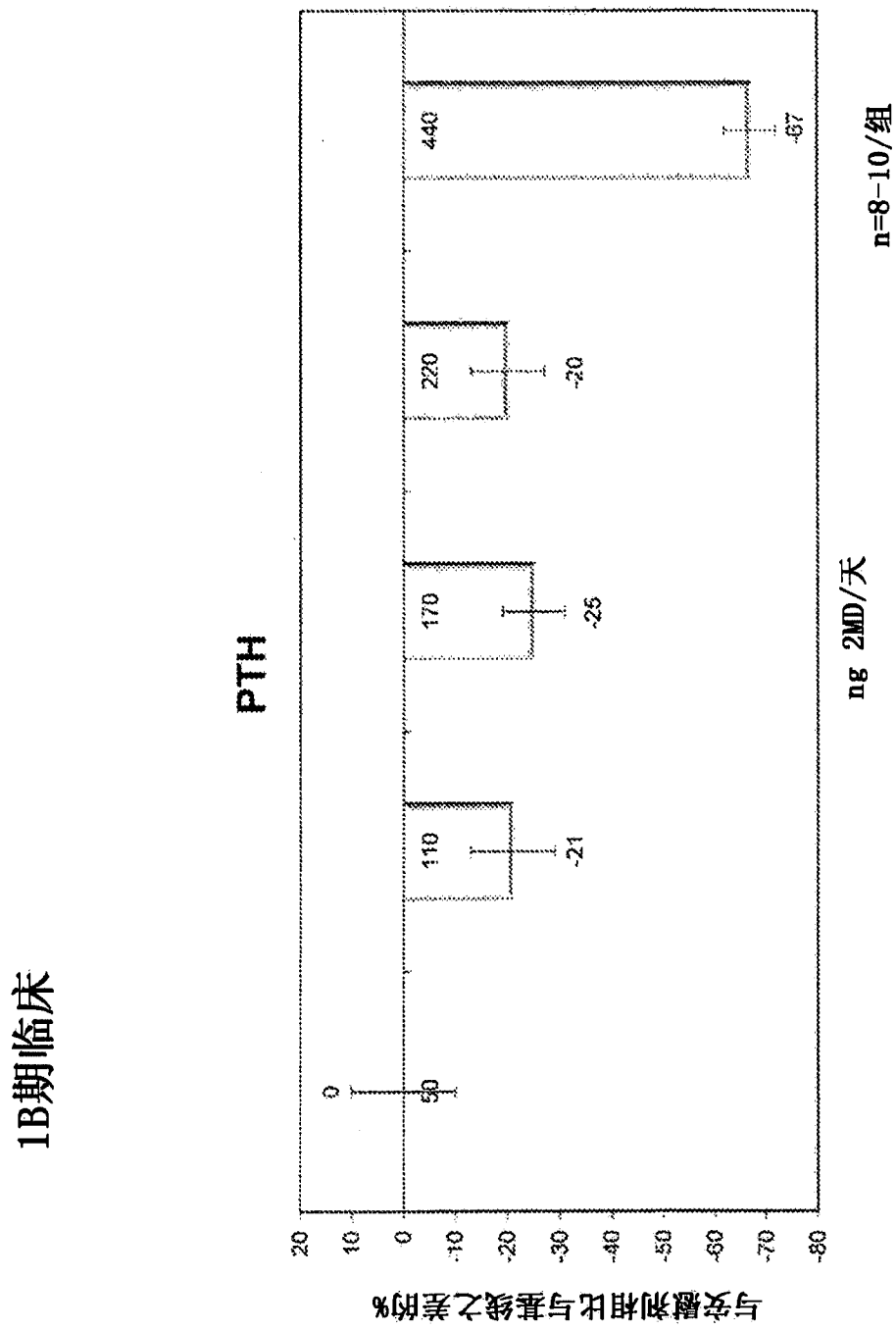

1B期临床
PTH
与安慰剂相比与基线之差的%
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
0
50
110
-21
170
-25
220
-20
440
-67
ng 2MD/天
n=8-10/组

图 13