P decreased slightly, but n.s., in a dd and td manner in MD, HD, XD.
Bilirubin increased significantly (1.5x) in HD @ 2 mo.
ALP decreased dd and td, but n.s., in MD, HD, XD, down to 0.6x in HD @ 2 mo and 0.6x in MD @ 3mo.
ALT decreased in HD @ 2 mo (0.5x, n.s.), and in MD @ 3 mo (0.6x).
TCO_2 increased dd and td in MD, HD, XD, significantly so in HD @ 2 mo (1.2x) and in MD @ 3 mo (1.1x).
Females -
Blood urea nitrogen (BUN) increased dose-dependently (dd) and time-dependently (td) in HD, XD, up to 2x in XD @ 1 mo. Creatinine increased dd and td in HD, XD, up to 1.5x in XD @1 mo. Changes become significant as time progresses and dose increases.
Ca increased dd and td in MD, HD, XD, up to 1.4x in XD @ 1 mo. Changes significant in MD, HD, XD @ 3 mo, 2 mo and 1 mo, respectively.
P decreased slightly, but n.s., in a dd and td manner in HD, XD.
Bilirubin increased n.s. (1.7x) in HD @ 2 mo.
ALP decreased dd and td in MD, HD, XD, significantly so in HD @ 1mo (0.5x) and @ 2 mo (0.2x).
TCO_2 increased dd and significantly in MD, HD, XD at all time points, up to 1.1x in XD @ 1 mo, 1.2x in HD @ 2 mo, and 1.1x in MD @ 3 mo.

Fractional Ca excretion -
Incidence of hypercalciuria (fract. excr.>0.4) at -7 days, 1 mo, 2 mo, 3 mo (n=4-4-4-4/sex) -
Males: controls: 0-0-0-0, LD: 0-1-0-0, MD: 0-2-4-2, HD: 0-4-3-NA, XD: 0-1-NA-NA
Females: controls: 0-0-0-0, LD: 0-0-0-0, MD: 0-3-1-3, HD: 0-4-2-NA, XD: 0-2-NA-NA
Thus, fractional Ca excretion peaked after 1-2 months, and at the HD dose.

Urinalysis - No other changes.

Organ Weights -
Males and females (MD,HD,XD)*:*
Kidney: Slight to moderate increase in relative weight
Males (XD) *and females* (HD, XD):
Brain, Heart, Liver and Adrenal: Slight to moderate increases in relative weight
Spleen: Moderate to large, and slight to moderate decreases in absolute and relative weight, respectively
Thymus: Profound and large decreases in absolute and relative weight, respectively
Testis and Prostate: Moderate decrease in absolute weight

Gross Pathology - *Numbers in parentheses indicate incidence ($N_{examined}$ = 8-8-8-8-8)*
(Control, LD, MD terminated at 3 mo, HD at 2 mo, XD at 3 mo)

Aorta with raised firm foci in sinus (0-0-0-7-6)
Gall bladder abnormal surface (0-0-0-0-2)
Heart discoloration (0-0-0-0-2)

Kidney enlarged or discolored (0-0-2-0-3)
Ovary small (0-0-0-0-1)
Prostate small (0-0-0-2-1)
Small intestine discoloration (0-0-0-5-7)
Testis small (0-0-0-0-1)
Thymus small (0-0-0-6-5)
Vein injection site discoloration (0-0-1-3-4)
Whole animal thin (0-0-3-8-8)

Histopathology - *Numbers in parentheses indicate incidence ($N_{examined}$ = 8-8-8-8-8)* (Control, LD and MD terminated at 3 mo, HD at 2 mo, XD at 3 mo)
Aorta mineralization (0-0-0-5-7) and fibroplasia (0-0-0-1-4)
Bone marrow hypocellularity (0-0-0-2-1)
Epididymis abnormal spermatozoa (0-0-0-2-0)
Heart mineralization (0-0-0-0-1) and myofiber degeneration (0-0-0-0-1)
Kidney degeneration (0-0-3-8-8), mineralization (0-0-2-8-7), fibroplasia ((0-0-4-4-4), inflammation (1-1-7-8-7), tubular regeneration (1-0-7-8-8) and dilatation (0-0-7-8-8)
Lymph node cervical hemosiderin pigment (0-0-0-0-2)
Parathyroid gland atrophy (0-0-7-8-8)
Prostate atrophy or hypoplasia (0-0-0-3-2)
Ovary mineralization (0-0-0-1-0)
Salivary gland mineralization (0-0-0-1-1/1)
Skeletal muscle mineralization and fiber degeneration (0-0-0-0-1)
Stomach mineralization (0-0-0-0-2)
Testis aspermia or hypospermia (0-0-0-2-1), degeneration (1-0-0-1-3), sperm giant cells (0-0-0-3-2)
Thymus atrophy (0-0-2-7-7)
Thyroid gland mineralization (0-0-0-1-1)
Vein injection site hemorrhage (0-1-0-1-3), fibroplasia (0-2-3-2-0), hyperplasia (0-0-1-1-0)\

Comments:
(1) The 1-month serum Ca value of XD animals were, as predicted by Sponsor, increased slightly (but n.s.) more than those of HD animals. However, the 1-month PTH levels were also decreased slightly more in XD than in HD (n.s.). This neither confirms nor rejects the sponsor's hypothesis that vitD$_2$ analog will lead to less hypercalcemia than calcitriol.
(2) Hypercalcemia/hypercalciuria may be partly due to increased intestinal Ca uptake, partly to increased bone resorption (extent of both parts uncertain).

THREE-MONTH I.V. TOXICITY STUDY IN RATS (Vol. 5.1)
Abbott Study Nr. TA94-344 (Abbott Laboratories, IL). Study Period January - April 1995.
Lot Nr. #94-0584 (19-nor-vitD$_2$) and Lot Nrs. 95-0053 and 95-109-DK (calcitriol).

PURPOSE - To determine toxicity of 19-nor-vitD2 analog in three-month chronic rat study

PROCEDURES - Crl:CDR(SD)BR rats (10/sex/dose group), age ___ weeks, weight _____ g, were dosed intravenously, three times a week, 2 or 3 days apart, with **0, 0.1, 0.5, 3.0 µg/kg/day** (control, LD, MD, HD) of the 19-nor-vitD2 analog in 30% (v/v) propylene glycol and 20% (v/v) ethanol in water (1ml/kg), or with **3.0 µg/kg/day** (XD) of calcitriol (Calcijex®) (1.5 ml/kg) for 13 weeks. Administration was via lateral tail vein (0.5-1 ml/min; _____ _____ From 2 weeks before dosing, rats were fed a diet containing ca. 0.5% Ca, 0.4% P, and 1.0 IU/g vitamin D. Five satellite rats/sex/dose group of 19-nor-vitD2 were used for plasma drug concentration measurements.

RESULTS -

Mortality - Four controls (f), 2 LD (f), 2 MD (m) and 2HD (m) died during blood collection. 1MD (m) and 1 XD (f) were euthanized because of bad tail condition. 1MD (m) found dead.

Clinical signs - Dry tail, redness of tail, or swelling of tail increased in MD, HD. Decreased activity, dehydration, emaciation and hunched posture in 3-5/20 of XD. Rough coat in 9/20 XD.

Body Weight - Cessation of BW gain in XD males from 1 month (mo). BW slightly to moderately decreased in XD males after 3 mo.

Food Consumption - FC slightly decreased in XD males after 1 mo.

Ophthalmoscopy - After 3 months, superficial, punctate keratitis in 6/10 XD m, and 3/9 XD f.

Hematology - *(significant changes)* -
WBC count increased moderately and dose-dependently (dd) in LD f, MD f, HD f, and in XD f. Reticulocyte count decreased moderately in XD m at 3 mo, and XD f at 1 mo. Platelet count slightly decreased in XD m.

Coagulation - *(significant changes)* -
Prothrombin time slightly decreased in XD m,f. Activated partial thromboplastin time (APTT) decreased at 3 mo, not at 1 mo, slightly and similarly in LD m, MD m, HD m, XD m.

Clinical Chemistry - Blood urea nitrogen (BUN) slightly increased in HD m. K slightly but non-significantly (n.s.) increased in HD. Ca increased in MD m, and in HD and XD m and f. Ca levels at 3 mo were, in males: 9.28, 9.99, 10.63, 11.03, 12.8, and in females: 10.68, 10.78, 10.71, 11.56, 12.6 mg/dl. P increased dd in all treated, by 55% in HD, by 30% in XD. ALKPH increased ca. 2x in XD. AST slightly decreased in XD. Cholesterol slightly increased in XD. Triglycerides moderately increased in XD f.

Serum PTH - PTH levels decreased in all treated, significantly so in MD, HD, and XD m, and in HD and XD f. PTH levels after 3 mo in males were: 165, 105, 47, 4.66, 3.46 pg/ml, in females: 205, 185, 97, 54, 3.96 pg/ml. In HD and XD, 3-mo values appeared lower than 1-

mo values.

Urinalysis - Values similar after 1.5 and 3 mo. Urine Ca increased dd in all treated, significantly so in MD, HD, XD, and 15x in XD after 3 mo. High incidence of aciduria in HD, XD. Some proteinuria in all 19-nor-treated male groups. Proteinuria marked in some XD males, mild in some XD females.

Organ Weights -
Brain - slight increase in relative weigth in XD m
Heart and liver- slight decreases in absolute weight in XD m
Spleen - moderate increase in relative weight in XD m
Adrenal - dose-dependent increase in absolute weight in LD-HD and XD m, and in relative weight in HD, XD m
Testis - slight increase in relative weight in XD m.
Kidney - slight increases in absolute and relative weight in XD f, and in relative weight in XD m.
Ovary - slight increase in absolute weight in HD f.

Gross Pathology - *Numbers in parentheses indicate incidence ($N_{examined}$ = 20-20-20-20-20)*
Aorta - Abnormal consistency or surface (0-0-0-0-3)
Heart - discoloration (0-0-1-0-1)
Vein injection site - discoloration (0-2-1-2-1)
Uterus - fluid-filled (0-0-1-1-4)

Histopathology - *Numbers in parentheses indicate incidence [$N_{examined}$= (m 10-0-3-10-10) (f 10-2-0-10-10)]*
Aorta - mineralization (m 0-?-0/3-0-10) (f 0-0/2-?-0-4) (? = not examined)
Femur - hyperostosis (m 0-?-0/3-0-7) (f 0-0/2-?-0-1), mineralization (m 0-?-0/3-0-1) (f 0-0/2- ?-0-0), coagulative necrosis (m 0-?-0/3-1-0) (f 0-0/2-?-1-0)
Colon - mineralization (m 0-?-0/3-0-1)
Epididymis - Mononuclear infiltrate (m 5-?-2/3-8-4)
Eye - mineralization (m 0-?-0/3-0-6)
Heart - mineralization (m 0-?-0/3-0-10) (f 0-0/2-?-0-6)
Kidney - degeneration (m 0-?-0/3-1-10) (f 1-0/2-?-0-3), fibrosis (m 0-?-0/3-0-2), chronic inflammation (m 0-?-0/3-2-7) (f 1-0/2-?-0-2), mineralization (m 0-?-0/3-9-10) (f 10-2/2-?-6-10), microlith (m 0-?-0/3-1-3) (f 0-0/2-?-3-2), tubular regeneration (m 0-?-0/3-0-6) (f 0-0/2-?-0-1) and dilatation (m 0-?-0/3-0-7) (f 0-0/2-?-0-1)
Liver - single cell necrosis (m 0-?-1/3-2-1), cytoplasmic vacuolization (f 0-0/2-?-2-3)
Lung - granuloma(s) (m 3-?-1/3-2-7) (f 1-0/2-?-8-6), osseous metaplasia (m 0-?-0-1-0) (f 0-0/2-?-0-1), mineralization (m 1-?-0/3-0-2) (f 0-0/2-?-1-0)
Lymph node (thoracic) - mineralization (m 0-?-0/3-0-1/8), pigmentation (m 0-?-0/3-2/9-0)
Lymph node (cervical) - infiltrate (m 0-?-0/3-2-0), mineralization (m 0-?-0/3-0-1)
Lymph node (mesenteric) - infiltrate (m 1-?-0/3-0-2), mineralization (m 0-?-0/3-0-5) (f 0-0/2-?-0-4)

Pancreas - mineralization (m 0-?-0/3-0-5) (f 0-0/2-?-0-1), mononuclear infiltrate (f 1-0/2-?-0-2)
Prostate - mineralization (m 0-?-0/3-0-1), mononuclear infiltrate (m 0-?-0/3-1-2), inflammation (m 0-?-0/3-4-0), concretion (m 1-0-0-5-5)
Salivary gland - mineralization (m 0-?-0/3-0-5) (f 0-0/2-?-0-1)
Skeletal muscle - mineralization (m 0-?-0/3-0-4)
Skin - mineralization (m 0-?-0/3-0-4)
Ileum - mineralization (m 0-?-0/3-0-1)
Spinal cord (cervical) - mineralization (m 0-?-0/3-0-1)
Spleen - extramedullary hematopoiesis (m 0-?-0/3-0-2) (f 0-0/2-?-0-1)
Stomach - degeneration or gland dilatation (m 0-?-0/3-0-2), mineralization (m 0-?-0/3-0-7)
Thymus - mineralization (m 0-?-0/3-0-1/9)
Thyroid - mineralization (m 0-?-0/3-0-1), mononuclear infiltrate (f 0-0/2-?-1-1)
Trachea - osseous metaplasia (m 0-?-0/3-0-1), mineralization (m 0-?-0/3-0-1)
Urinary bladder - microlith (m 0-?-0/3-0-1), mononuclear infiltrate (f 0-0/2-?-1-2)
Uterus - dilatation (f 1-0/2-?-2-3)
Vein injection site - granuloma(s) (m 1-?-0/3-1-2) (f 0-0/2-?-3-2), thrombus (m 0-?-1/1-2/7-0) (f 0-0/2-?-2-0)

THREE-MONTH S.C. MAXIMUM-TOLERATED DOSAGE STUDY IN RATS (Vol. 3.2)
Abbott Study Nr. TA94-370 (Abbott Laboratories, IL). Study period March - June 1995.
Lot Nr. 95-0135.

PURPOSE - Determine a MTD for an anticipated _____

PROCEDURES - Crl:CDR(SD)BR rats (10/sex/dose group), age_____, weight _____ were dosed subcutaneously, three times a week, 2 or 3 days apart, with 0, 0.1, 0.5, 3.0 µg/kg/day (control, LD, MD, HD) of the 19-nor-vitD2 analog in 30% (v/v) propylene glycol and 20% (v/v) ethanol in water (1ml/kg) for 13 weeks. From 2 weeks before dosing, rats were fed a diet containing ca. 0.5% Ca, 0.4% P, and 1.0 IU/g vitamin D. In addition, five satellite rats/sex/dose group of 19-nor-vitD2 were used for measurement of plasma drug concentrations on Day 0 and Day 77.

RESULTS -

Mortality - Of the satellite rats, 6 died during blood collection, 1 HD died on Day 72 with cause unknown.

Clinical signs - No treatment-related effects

Body Weight - From Day 40, decrease in BW in HD males (m) as compared to controls, up to 10% at end of study. Decrease in BW gain in HD m ca. 17%. Also, trend to small decrease of BW in LD and MD m.

Food Consumption - No treatment-related effects.

Vital Signs - Not examined

Ophthalmoscopy - Ocular opacity in 3 HD m at time of necropsy.

Hematology - No treatment-related effects.

Coagulation - Slight, dose-dependent (dd) reduction of APTT in male dose groups, significant in MD and HD m.

Clinical Chemistry - Creatinine minimally decreased in LD, MD, HD m and f. Ca and P increased dd in all m and f dose groups.

Serum PTH - PTH levels decreased significantly in all treated In LD and MD, values after 3 mo were lower than after 1.5 mo.

Urinalysis - Urinary Ca values dd and sharply increased in all treated, from in HD m, and from . HD f. Urinary pH decreased dd from controls to 5 in HD m and f. In HD m, there was an occurrence of single animals with multiple Ca or P crystals in urine.

Organ Weights -
Heart, Liver, Kidney, Spleen - Slight dd increase in relative weight of all organs in males, and slight increases of absolute and relative kidney and spleen weight in females. Changes were significant only in HD groups.
Adrenal - Slight to moderate dd increases of absolute and relative weight in males, significant in MD, HD.
Prostate - Slight decrease of absolute weight in HD.

Gross Pathology - *Numbers in parentheses indicate incidence ($N_{examined}$ = 20-20-20-20)*
Stomach - discoloration (0-0-0-1)
Eye - opacity (0-0-0-3), small (0-0-1-1)
Kidney - abnormal surface (0-0-0-1)
Uterus - fluid-filled (1-2-0-1)

Histopathology - *Numbers in parentheses indicate incidence [$N_{examined}$ = (m 10 - either 0 or 10 - either 0 or 10 - 10) (f 10 - either 0 or 10 - either 0 or 10 - 10)]*
Adrenal - extramedullary hematopoiesis (m 0-?-?-1) (? = 0 animals examined)
Aorta - mineralization (m 0-?-0-6)
Femur - hyperostosis (m 0-?-0-6/9) (f 0-?-0-1)
Bronchus - mineralization (m 0-?-?-1/1)
Eye - mineralization (m 0-?-?-1)

Heart - mineralization (m 0-?-0-3)
Kidney - atrophy (m 0-?-0-3), fibrosis (m 0-?-0-1), mineralization (m 0-1-3-10) (f 2-2-9-9), necrosis (m 0-0-0-1), cast (m 0-1-1-2), tubular dilatation (m 0-0-2-7) (f 0-0-2-1)
Lung - osseous metaplasia (m 2-?-0-0) (f 0-?-0-1), mineralization (m 1-?-1-1) (f 1-?-4-1)
Mesentery - mineralization (m 0-?-0-2) (f 0-?-1-0)
Pancreas - mineralization (m 0-?-?-2)
Prostate - inflammation (m 1-?-?-3)
Salivary gland - mineralization (m 0-?-0-1)
Skeletal muscle - mineralization (m 0-?-0-1)
Stomach - mineralization (m 0-?-0-3) (f 0-?-0-1)
Trachea - mineralization (m 0-?-0-4)
Vessels - mineralization (m 1-0-2-6) (f 1-0-0-4)

Note: Drug-related changes were mostly seen in males. A few animals of the HD m group were found with changes in many organs, while in most HD m and f only a few organs were affected.

THREE-MONTH S.C. MAXIMUM-TOLERATED DOSAGE STUDY IN MICE (Vol. 5.2)
Abbott Study Nr. TD94-381 (Abbott Laboratories, IL). Study period March - June 1995. Lot Nr. 95-0135.

PURPOSE - Determine a MTD for an anticipated chronic bioassay.

PROCEDURES - Crl:CD^{R}-1(ICR)BR mice (10/sex/dose group), age_____, weight_____ were dosed subcutaneously, three times a week, 2 or 3 days apart, with 0, 0.1, 0.5, 3.0, 10.0 µg/kg/day (control, LD, MLD, MHD, HD) of the 19-nor-vitD2 analog in 30% (v/v) propylene glycol and 20% (v/v) ethanol in water (2ml/kg) for 13 weeks. From 2 weeks before dosing, rats were fed a diet containing ca. 0.5% Ca, 0.4% P, and 1.0 IU/g vitamin D. In addition, 5 (control) or 25 (drug dose groups) satellite mice/sex/dose group of 19-nor-vitD2 were used for measurement of plasma drug levels at the end of the treatment period.

RESULTS -
Mortality - 1 MLD f died, probably related to ovarian teratoma. Scabs on skin seen in all groups including control, probably due to repeated s.c. dosing.

Clinical signs - No effects.

Body Weight - No effects.

Food Consumption - No effects.

Ophthalmoscopy - No abnormalities.

Hematology - Moderate, dose-dependent (dd) decrease of WBC count in MHD and HD m, and in all f dose groups.

Coagulation - No changes.

Clinical Chemistry - Serum Ca increased dd in all m and f dose groups. Increase was somewhat more pronounced in m than in f. Serum P increased non-dd in all m and f dose groups. Serum ALP mildly increased in MHD and HD f.

Serum PTH - After 92 days, PTH levels decreased significantly in all treated, from 12.8 pg/ml in control m to 1.0 pg/ml in HD m, and from 7.8 pg/ml in control f to 2.0 pg/ml in HD f.

Urinalysis - No data.

Organ Weights - No changes.

Gross Pathology - *Numbers in parentheses indicate incidence ($N_{examined}$ = 20-20-20-20-20)*
Skin - discoloration (2-6-6-6-5), scab (4-3-6-4-2)

Histopathology - *Numbers in parentheses indicate incidence ($N_{examined}$ = 10-10-10-10-10)*
Kidney - Inflammation (m 0-1-1-5-5) (f 0-1-1-0-1), mineralization (m 0-1-1-5-8) (f 0-2-0-1-1).
Note: Kidney changes localized in renal medulla, especially outer medullary stripe (thin loops of Henle).

E. GENETIC TOXICOLOGY

19-Nor-1α,25-dihydroxyvitamin D2 Test of Chemical Induction of Chromosome Aberration in Cultured Human Peripheral Blood Lymphocytes (Vol. 2.1)
Study Nr. 94-309 Study Period March 1995-October 1995. Lot No. 96-016-JE.

Doses tested: Without metabolic activation: **0** *(untreated control)*, **0** *(solvent control)*, **0.1, 1.0, 10 μg/ml**, with metabolic activation: **0, 0, 30, 60, 100 μg/ml.**
Metabolic activation system: S-9 mixture prepared from the livers of Aroclor 1254-induced male SD rats.
Positive controls: Mitomycin C (MMC) (0.2 μg/ml) in absence, and cyclophosphamide (CP) (20 μg/ml) in presence of metabolic activation. MMC and CP were dissolved in water.
Solvent control: 1% DMSO.
Methods: Human peripheral blood lymphocytes (HPBL) were derived from a healthy female donor. Blood cells were cultured in RPMI-1640 medium containing 15% fetal bovine serum

and 1% PHA for approximately 48 h before exposure to test article. For each dose level duplicate cultures were maintained. In the non-activated system, cells were treated for 22 h with test article in a medium containing 15% bovine serum, and for 2 h without test article before harvesting. In the activated system, cells were treated in serum-free medium containing the S-9 mixture with test article for 4 h, and incubated for another 20 h before harvesting. Final concentration of DMSO in treated cells was 1%. Exposure to colcemid (0.1 μg/ml) was for 2 h before harvesting. From each culture ≥2 slides were prepared. From each of the duplicate cultures 100 metaphases were scored. 1000 cells were scored for each dose to determine MI. Simple and complex structural chromatid and chromosome aberrations, and numerical aberrations were identified. Percentages polyploid and endoreduplicated cells were determined separately in 100 metaphases from each culture.
Statistical tests: Fisher's Exact Test (for each dose level), Cochran-Armitage Test (for evidence of dose-related response).
Results: From the results of a parallel toxicity test with doses spaced on log-base between 0.1 and 100 μg/ml, the highest doses selected were 10 μg/ml in non-activated assay (50% reduction in relative MI), and 100 μg/ml in activated assay (42% reduction in relative MI). Reductions in relative MI for MMC and CP were 18% and 94% respectively. MI was not scored in mutagenicity test itself. Results on aberrations are shown in Tables 1 and 2.

Table 1. Chromosome aberrations in lymphocyte assay without metabolic activation

Treatment/ Dose	#cells scored	chroma-tid gaps	chromo-some gaps	%endo re-dupl. cells	% polyploid cells	% cells with simple or complex, chromatid or chromosome aberrations	No. of aberrations per cell
untreated	200	1	0	0	0	1.5	0.015
solvent control	200	0	0	0	0	0	0
0.1 µg/ml	200	0	1	0	1	0.5	0.005
1.0 µgml	200	0	0	0	0	0.5	0.005
10 µg/ml	200	1	0	0	0	0	0
MMC 0.2 µg/ml	200	2	1	0	0	23	0.28

Table 2. Chromosome aberrations in lymphocyte assay with metabolic activation

Treatment/ Dose	#cells scored	chroma-tid gaps	chromo-some gaps	%endo re-dupl. cells	% polyploid cells	% cells with simple or complex, chromatid or chromosome aberrations	No. of aberrations per cell
untreated	200	1	0	0	0	0.5	0.005
solvent control	200	1	0	0	0	0	0
30 µg/ml	200	1	1	0	0	0.5	0.005
60 µgml	200	1	1	0	0	0.5	0.005
100 µg/ml	200	2	0	0	0	0	0
CP 20 µg/ml	200	21	13	0	0	37	0.49

Since solvent control produced no aberrant cells, test substance was compared to historical solvent control data (0.5% in both systems). Statistical test showed that test substance did not induce a significant increase in the % cells with aberrations over historical solvent data at any of the doses tested. There was also no indication for a positive dose-response trend.

Comment: Although the doses selected were rather minimal (50% reduction in MI is recommended minimum), this result indicates clearly negative response.

Conclusion: 19-nor-1α,25-dihydroxyvitamin D_2 was non-mutagenic in the Human Peripheral Blood Lymphocyte chromosome aberration assay.

F. SUMMARY AND EVALUATION

PHARMACOKINETICS

- In the rat, the 19-nor-vitaminD2 analog is eliminated mainly in feces, through biliary excretion (fecal excretion > 90% at 72h after a single dose of 1.7 μg/kg i.v.).
- Major excretory component in bile, feces and urine is a metabolite (M3).
- After a single i.v. dose of 1.7 μg/kg, plasma $T_{½}$ is 7.5h and $AUC_{(0\text{-}12h)}$ is 25 ng.h/ml.
- In mouse, rat, dog, monkey and human plasma, the compound 19-nor-vitD2 is extensively bound to proteins.
- 19-nor-vitD2 does not bind to red blood cells.

TOXICOLOGY

Synopsis

A. Dog (3-month i.v. study; doses 0, 0.02, 0.1, 0.3, 0.3 (calcitriol) μkd)

- Mortality: High incidence in 19-nor high dose and calcitriol groups.
- Body weight and food consumption ↓ in 19-nor high dose and calcitriol groups.
- Blood: Neutropenia in 19-nor high dose group, aPTT ↑ in 19-nor high dose and calcitriol groups.
- Serum: PTH ↓↓ in all dose groups. Hypercalcemia in all dose groups. BUN and creatinine ↑ in mid and high dose 19-nor and calcitriol groups. ALP ↓ in 19-nor mid and high dose and in calcitriol groups. ALT ↓ in male 19-nor mid and high dose groups. Bilirubin ↑ in 19-nor high dose groups.
- Urine: Hypercalciuria in almost (or) all animals of 19-nor mid and high dose and calcitriol groups.
- Pathology:

Ectopic mineralization of aorta, heart, salivary gland, skeletal muscle, stomach and thyroid gland in calcitriol-treated. Mineralization of aorta, ovary, salivary gland, and thyroid gland in 19-nor high dose group.
Kidney degeneration, mineralization, fibroplasia and inflammation in 19-nor mid/high dose and calcitriol groups. Tubule regeneration and dilatation in 19-nor high dose and calcitriol groups.
Parathyroid gland atrophy in 19-nor high dose and calcitriol groups.
Small intestine discoloration in both 19-nor high dose and calcitriol groups.
Thymus atrophy in 19-nor mid and high dose and in calcitriol groups.
Prostate atrophy in 19-nor high dose and calcitriol groups.
Testis hypospermia and/or sperm giant cells in 19-nor high dose and calcitriol groups, and testis degeneration in calcitriol group. Epididymis with abnormal spermatozoa in 19-nor high dose group.

B. Rat (3-mo i.v. study: 0.1, 0.5, 3, 3 (calcitriol) μkd; 3-month s.c. study: 0.1, 0.5, 3 μkd)

- Mortality: Very low incidence in mid- and high dose groups (iv and sc).

- Signs: (In iv study:) Injection site red/dry/swollen in mid/high dose groups, and emaciation and dehydration in calcitriol group.
- Body weight: Small ↓ in iv calcitriol group and sc 19-nor high dose group.
- Ophtalmoscopy: Keratitis in iv calcitriol group, and ocular opacity in sc 19-nor high dose group.
- Hematology: (In iv study:) WBC count ↑ in all female 19-nor and calcitriol dose groups, reticulocyte count ↓ and platelet count ↓ in calcitriol group.
- Serum: PTH: ↓↓ in all iv and sc dose groups. Ca ↑ and P ↑ in mid and high dose iv-treated, and in all sc-treated.
- Urine: Urine Ca ↑ in all iv and sc dose groups. Aciduria in all sc dose groups, and in iv 19-nor high dose and calcitriol groups. Proteinuria in all iv dose groups.
- Pathology:
  Ectopic mineralization:
  Small-moderate incidence in 19-nor high dose and calcitriol groups. Affected organs: aorta, bronchus, colon, eye, femur, heart, ileum, lung, lymph nodes, mesentery, pancreas, prostate, salivary gland, skeletal muscle, skin, spinal cord, stomach, thymus, thyroid, trachea, vessels. Mineralization almost always more frequent in males, and sometimes accompanied by blood cell (mononuclear) infiltrate.
  Kidney:
  Mineralization and tubular dilatation and regeneration in some/all animals of iv- and sc-treated 19-nor mid/high dose and calcitriol groups. Kidney degeneration, fibrosis, inflammation in iv calcitriol group (mostly males). Kidney atrophy, fibrosis, necrosis, mineral cast in sc-treated 19-nor high dose males.
  Femur hyperostosis in iv calcitriol and sc 19-nor high dose groups, mostly in males.
  Lung granulomas in sc 19-nor mid and high dose groups
  Prostate inflammation in a few iv and sc 19-nor high dose animals, mineralization in iv calcitriol-treated and concretion in iv 19-nor high dose and calcitriol-treated.
  Findings in iv study only: *Liver* cell changes, *trachea/lung* osseous metaplasia, *uterus* dilatation in a few mid/high dose 19-nor and calcitriol-treated animals. *Spleen* extramedullary hematopoiesis, *stomach* degeneration and gland dilatation, and *bladder* microlith in a few calcitriol-treated.

C. Mouse (3-month s.c. study: 0.1, 0.5, 3, 10 μkd)

- Hematology: WBC count ↓ in mid-high and high dose males, and in all treated females.
- Clinical chemistry: Serum Ca and P ↑ in all treated. ALP ↑ in mid-high and high dose females.
- Pathology: Skin discoloration in all treated. Kidney inflammation and mineralization in all dose-treated, with higher incidence in m.

Comments

- The pharmacological effect of 19-nor vitD2 analog is reflected by hypercalcemia, hypercalciuria and resulting decrease in circulating PTH levels, in both dog, rat and

mouse. In the rodents, hyperphosphatemia is also seen.

- Hypercalcemia and high Ca flux causes ectopic mineralization in many organs, particularly in kidney. Other pathologic changes in kidney include degeneration, inflammation, and tubular dilatation and regeneration.
- At high doses, the hypercalcemia and kidney pathology causes morbidity and mortality.

- Hematologic changes include an increase in WBC in the female rat, and a decrease in WBC in the mouse. The neutropenia seen in the dog does not occur in the rodent.
- The cause of the serum ALP and ALT changes in dog and mouse, and the serum bilirubin increase in the dog is unclear.
- Aciduria and proteinuria are observed in the rat, not in the dog.

- The prostate is affected by atrophy in the dog, and inflammation or mineralization in the rat.
- The testicular degeneration and sperm cell abnormalities in the dog are not seen in the rodent.
- The thymus atrophy in the dog is not seen in the rodent.

GENETIC TOXICOLOGY

- 19-nor-1α,25-dihydroxyvitamin D_2 was non-mutagenic in the human peripheral blood lymphocyte chromosome aberration assay.

G. RECOMMENDATIONS TO SPONSOR

No action indicated.

/S/

Gemma A. Kuijpers, Ph.D.

Kuijpers

MAR 20 1997

March 14, 1997

Sponsor: Abbott Laboratories, Abbott Park, IL 60064-3537
Date submitted: December 1, 1995
Serial Nr: 010

PHARMACOLOGY/TOXICOLOGY
RAT AND MOUSE CARCINOGENICITY STUDY DOSE SELECTION :
DOCUMENT FOR EXECUTIVE CAC COMMITTEE AND MEETING REPORT

Drug: 19-nor-1,25-dihydroxyvitamin D_2 Analog Injection
Category: Hormone
Indication: Hypocalcemia and elevated PTH levels in end-stage renal disease

Contents

/S/

Gemma A. Kuijpers, Ph.D.

/S/ 3/20/97

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The following document was sent to the Executive CAC (S. Olmstead) on 3/11/96:

A. DOCUMENT FOR EXECUTIVE CAC COMMITTEE

This _____ for the parenteral use of **19-nor-1,25-dihydroxy-vitamin D2** for the treatment of hypocalcemia and elevated PTH levels in end-stage renal disease. These patients suffer from stage II - stage IV renal insufficiency, are hypocalcemic and hyperphosphatemic, have compensatory, largely increased plasma parathyroid hormone levels, and are usually on renal dialysis.

A dose-selection for 2-year subcutaneous carcinogenicity studies of the test compound in rats and mice was submitted to the FDA _____
The subcutaneous administration route was chosen for reasons of feasibility. Animals will be dosed three times per week, to mimic the intended clinical dosing regimen. The maximum intended human dose is 0.3 μg/kg. So far, the highest human dose used has been 0.16 μg/kg.

The doses proposed are:

1) for the rat:	0	0.15	0.5	1.5 μg/kg/dose (s.c., 3x per week)
2) for the mouse:	0	1.0	3.0	10 μg/kg/dose (s.c., 3x per week)

1. RAT STUDY

Doses selected were based on the results of a 3-month s.c. maximum-tolerated dosage study in Sprague Dawley rats. Dosages were **0, 0.1, 0.5, 3 μg/kg/dose** (control, LD, MD, HD). Results of a 3-month i.v. rat study were compared with the 3-month s.c. rat study, carried out with the same doses in μg/kg, with regard to changes in serum PTH, calcium and phosphorus levels. It was concluded by the Sponsor that s.c. administration has similar effects as i.v. administration (see discussion of PK data below).

The most prominent and statistically significant effects observed in the s.c. study were a decrease in body weight of up to 10% in HD males, and a marked dose-dependent decrease in serum PTH levels in males and females down to less than 6% of the control value. Also, there was a significant dose-dependent increase in serum calcium from ca. 9.5 mg/dl in controls up to 12.5 mg/dl in HD males, and a slightly dose-dependent increase in serum phosporus from ca. 7 mg/dl in control males and 5.5 mg/dl in control females, to 8.5 mg/dl in HD males and 7.5 mg/dl in HD females. Urinary calcium and fractional calcium excretion were greatly increased in both males and females. Aciduria developed in both sexes. Adrenal weights were increased in males. Histopathologically, the main effect was soft tissue mineralization, mineralization-related nephropathology, and hyperostosis of the bone. The latter effects were larger in males:

Hyperostosis of the femur was seen in a majority of the HD males, but only in 1/10 HD females. Mineralization was mostly observed in the kidney, and in the MD dose groups, 3/10 males and 9/10 females were affected. In HD groups, 10/10 males and 9/10 HD females were affected. The kidney mineralization in the HD groups was minimal (5/10), mild (4/10), or moderate (1/10) in HD males, and minimal (8/10) or mild (1/10) in HD females. In 1/10 to 6/10 HD males, minimal to moderate, and in 1/10 HD male severe mineralization was seen in multiple tissues, while in 1/10 HD females minimal mineralization was evident in mesentery, pancreas, stomach and/or salivary gland. Kidney tubule dilatation was seen in 2/10 MD males and 2/10 MD females, and in 6/10 HD males and 1/10 HD females. In the HD groups, dilatation was minimal (4/10), mild (1/10) or moderate (1/10) in males, and minimal (1/10) in females.

On the basis of the histopathology results, in particular the soft tissue mineralization, the Sponsor believes that long-term treatment with the high dose (3 μg/kg) has "a significant possibility of decreasing the animal's lifespan". The medium dose (0.5 μg/kg) is considered the dose that is "tolerated without apparent significant physiological dysfunction". The highest dose for the carcinogenicity studies, for both males and females, was set at 1.5 μg/kg, the middle dose at 0.5 μg/kg, and the low dose at 0.1 μg/kg.

Preliminary assessment of plasma levels of the 19-nor vitamin D2 analog in rats and humans was done and the results are shown in Table 1 (Results submitted as fax to reviewer on February 28, 1996). Extrapolation of these results suggests that in the rats receiving the highest dose selected for the carcinogenicity studies (1.5 μg/kg), the plasma AUC is approximately 2x the AUC in humans receiving the maximum intended dose of 0.3 μg/kg. From the PK data we can also conclude that s.c. dosing results in a relatively higher exposure than i.v. dosing. The latter is in accordance with the increased sensitivity of the rats to s.c. dosing with regard to changes in serum PTH, Ca and P, and tissue mineralization.

Preliminary PK data (Submissions October 31, 1995, and January 19, 1996) indicate that in the rat, the 19-nor-vitaminD2 analog in eliminated mainly in feces, through biliary excretion (fecal excretion >90% at 72h after a single dose of 1.7 ug/kg i.v.). The $T_{½}$ is 7.5h, the $AUC_{(0\text{-}12h)}$ 25 ng.h/ml. The compound 19-nor-vitD2 is extensively bound to proteins in both mouse, rat, dog, monkey and human plasma.

2. MOUSE STUDY

Doses selected were based on the results of a 3-month s.c. maximum-tolerated dosage study in ICR mice (Submission January 19, 1996, Vol 5.2, Serial Nr. 012). Dosages were 0, 0.1, 0.5, 3, 10 μg/kg/dose. In mice, there were no effects on body weight, but there was an unexplained leukopenia seen at mid and high dosages in both sexes. Serum PTH levels were again sharply and dose-dependently decreased, although somewhat less than in the rat. Calcium was increased dose-dependently, significantly so in MHD and HD males and in HD females, and phosphorus was increased rather non-dose-dependently, in all male and female dose groups.

Histopathologic changes in mice consisted of kidney mineralization and ensuing inflammation, and were much more pronounced in males than in females; in MHD groups, nephrocalcinosis was seen that was minimal in 5/10 males, and minimal in 1/10 females. In HD groups, it was minimal (7/10) or mild (1/10) in males, and minimal (1/10) in females. The sponsor concludes that the high dose (10 μg/kg/dose) is "tolerated by the mice without causing changes that would compromise long-term survival." Therefore, the high dose selected for the carcinogenicity study in mice is **10 μg/kg**, the middle dose **3 μg/kg**, and the low dose **1 μg/kg**.

3. COMMENTS

A. The changes in PTH, Ca and P are expected changes due to the stimulation of intestinal Ca (and P) absorption by the vitamin D2 analog. This is the anticipated and desired pharmacologic effect of the test compound. The main toxicity that is revealed in the rat and mouse toxicity studies, at the doses used, is vitamin D toxicity (hypercalcemia, hypoparathyroidism), and the MTD is based on this toxicity. To be able to test higher drug doses, the Sponsor already decided to give the animals in these studies a diet with lower than standard Ca and P contents (0.5% Ca, 0.4% P, instead of the usual 0.9% Ca, 0.7% P) - that still meets the minimal Ca and P requirements of the laboratory animals - thus attenuating the vitamin D toxicity of the test compound. The dosing still appears to be limited by this pharmacological/toxic effect of the vitamin D2 analog.

B. Data
In the 3-month s.c rat study, there is a large variability in serum K, BUN, ALP and AST values between animals and between values measured at 55 days and 92 days. For example, some animals in all dose groups have serum K values of 8 mmol/L or higher, are thus severely hyperkalemic and would not be able to live. This diminishes the reliability of the data.

C. Dose selection/Pathology
Dose selection for carcinogenicity studies was done on the basis of MTD, and the MTD was determined on the basis of the histopathology results in male rats and mice. Upon review of these data, this reviewer feels that the highest dose selected can be increased, at least in female rats, and in male and female mice. Although the 3-month s.c. mouse study was not submitted until January 19, 1996, the Sponsor already started the carcinogenicity studies in January 1996. Thus, it seems recommendable that another dose group for both males and females, of rats and mice, should be added.

March 11, 1996
Gemma A. Kuijpers, Ph.D.
HFD-510

Table 1. Pharmacokinetic parameters for 19-nor-vitaminD2 in rats and humans.

	Dose (µg/kg)	C_{max} (ng/mL)*	$AUC_{(x-y)}$*	x-y (h)	note
Rat (N=8)	3.0, i.v.	11.7	43	0-24	values: females<males
Rat (N=8)	3.0, s.c.	6.5	71	0-24	females<males
Rat (extrapolated)	1.5, s.c. (HD carcino study)	3.3	21.5	0-24	
Human (N=4, males)	0.16, i.v.	1.15	5.4	0-∞	
Human (extrapolated)	0.3, i.v.	2.15	10	0-∞	

* Standard deviations of C_{max} and AUC values were between 50 and 80% in rats (N=4/sex), and ca. 15% in humans (N=4 males).

B. REPORT OF MEETING WITH EXEC CAC

Meeting minutes drafted by the Executive CAC, and signed by Dr. R.Osterberg were sent to me (G. Kuijpers) in the beginning of August, 1996 (Attachment II). Since there was an error in these minutes I sent a correction to S. Olmstead by e-mail, who replied she would resolve the discrepancy (Attachment II).

The following are the reviewers' meeting minutes, that were not circulated formally to the Exec CAC:

Executive CAC Committee
March 19, 1996

19-nor-1,25-dihydroxyvitamin D_2
Abbott

Committee members: Robert Osterberg, Ph.D., HFD-520, Joseph Contrera, Ph.D., HFD-900, Abby Jacobs, Ph.D., HFD-540, Alex Jordan, Ph.D., HFD-510, Sharon Olmstead, HFD-006.

Committee Recommendations:

- The high doses chosen by the Sponsor for both the rat and mouse carcinogenicity study (rat 1.5 μkd, mouse 10 μkd) are likely to be doses that lead to problems over the long term because of findings of hypercalcemia, kidney mineralization, aciduria (rat) and leukopenia (mouse).
- The Committee does not clearly agree with the reviewer that higher doses can be chosen for the female rats or for male and female mice.
- The Committee concurs with the dose selection and the dosing regimen proposed by the Sponsor.

Conclusion:

- Reviewer followed Exec CAC's advice and study is proceeding as described in the Sponsor's protocol (Submission December 1, 1995)

/S/

Gemma Kuijpers, Ph.D.
Pharmacology Reviewer
HFD-510

C. ATTACHMENT I

THREE-MONTH S.C. MAXIMUM-TOLERATED DOSAGE STUDY IN RATS (Vol. 3.2)
Abbott Study Nr. TA94-370 (Abbott Laboratories, IL). Study period March - June 1995.
Lot Nr. 95-0135.

PURPOSE - Determine a MTD for an anticipated _____.

PROCEDURES - Crl:CDR(SD)BR rats (10/sex/dose group), age_____, weight_____ were dosed subcutaneously, three times a week, 2 or 3 days apart, with **0, 0.1, 0.5, 3.0 µg/kg/day** (control, LD, MD, HD) of the 19-nor-vitD2 analog in 30% (v/v) propylene glycol and 20% (v/v) ethanol in water (1ml/kg) for 13 weeks. From 2 weeks before dosing, rats were fed a diet containing ca. 0.5% Ca, 0.4% P, and 1.0 IU/g vitamin D. In addition, five satellite rats/sex/dose group of 19-nor-vitD2 were used for measurement of plasma drug concentrations on Day 0 and Day 77.

RESULTS -

Mortality - Of the satellite rats, 6 died during blood collection, 1 HD died on Day 72 with cause unknown.

Clinical signs - No treatment-related effects

Body Weight - From Day 40, decrease in BW in HD males (m) as compared to controls, up to 10% at end of study. Decrease in BW gain in HD m ca. 17%. Also, trend to small decrease of BW in LD and MD m.

Food Consumption - No treatment-related effects.

Vital Signs - Not examined

Ophthalmoscopy - Ocular opacity in 3 HD m at time of necropsy.

Hematology - No treatment-related effects.

Coagulation - Slight, dose-dependent (dd) reduction of APTT in male dose groups, significant in MD and HD m.

Clinical Chemistry - Creatinine minimally decreased in LD, MD, HD m and f. Ca and P increased dd in all m and f dose groups.

Serum PTH - PTH levels decreased significantly in all treated, down from _____ _____ in HD m, and from _____ in HD f. In LD and MD, values after 3 mo were lower than after 1.5 mo.

Urinalysis - Urinary Ca values dd and sharply increased in all treated, from _____ in HD m, and from _____ in HD f. Urinary pH decreased dd from _____ in controls

to 5 in HD m and f. In HD m, there was an occurrence of single animals with multiple Ca or P crystals in urine.

Organ Weights -
Heart, Liver, Kidney, Spleen - Slight dd increase in relative weight of all organs in males, and slight increases of absolute and relative kidney and spleen weight in females. Changes were significant only in HD groups.
Adrenal - Slight to moderate dd increases of absolute and relative weight in males, significant in MD, HD.
Prostate - Slight decrease of absolute weight in HD.

Gross Pathology - *Numbers in parentheses indicate incidence ($N_{examined}$ = 20-20-20-20)*
Stomach - discoloration (0-0-0-1)
Eye - opacity (0-0-0-3), small (0-0-1-1)
Kidney - abnormal surface (0-0-0-1)
Uterus - fluid-filled (1-2-0-1)

Histopathology - *Numbers in parentheses indicate incidence [$N_{examined}$= (m 10 - either 0 or 10 - either 0 or 10 - 10) (f 10 - either 0 or 10 - either 0 or 10 - 10)]*
Adrenal - extramedullary hematopoiesis (m 0-?-?-1) (? = 0 animals examined)
Aorta - mineralization (m 0-?-0-6)
Femur - hyperostosis (m 0-?-0-6/9) (f 0-?-0-1)
Bronchus - mineralization (m 0-?-?-1/1)
Eye - mineralization (m 0-?-?-1)
Heart - mineralization (m 0-?-0-3)
Kidney - atrophy (m 0-?-0-3), fibrosis (m 0-?-0-1), mineralization (m 0-1-3-10) (f 2-2-9-9), necrosis (0-0-0-1), cast (m 0-1-1-2), tubular dilatation (m 0-0-2-7) (f 0-0-2-1)
Lung - osseous metaplasia (m 2-?-0-0) (f 0-?-0-1), mineralization (m 1-?-1-1) (f 1-?-4-1)
Mesentery - mineralization (m 0-?-0-2) (f 0-?-1-0)
Pancreas - mineralization (m 0-?-?-2)
Prostate - inflammation (1-?-?-3)
Salivary gland - mineralization (m 0-?-0-1)
Skeletal muscle - mineralization (m 0-?-0-1)
Stomach - mineralization (m 0-?-0-3) (f 0-?-0-1)
Trachea - mineralization (m 0-?-0-4)
Vessels - mineralization (m 1-0-2-6) (f 1-0-0-4)

Note: Drug-related changes were mostly seen in males. A few animals of the HD m group were found with changes in many organs, while in most HD m and f only a few organs were affected.

THREE-MONTH S.C. MAXIMUM-TOLERATED DOSAGE STUDY IN MICE (Vol. 5.2)
Abbott Study Nr. TD94-381 (Abbott Laboratories, IL). Study period March - June 1995. Lot Nr. 95-0135.

PURPOSE - Determine a MTD for an anticipated _____

PROCEDURES - Crl:CD^{R}-1(ICR)BR mice (10/sex/dose group), age_____, weight_____ were dosed subcutaneously, three times a week, 2 or 3 days apart, with **0, 0.1, 0.5, 3.0, 10.0 µg/kg/day** (control, LD, MLD, MHD, HD) of the 19-nor-vitD2 analog in 30% (v/v) propylene glycol and 20% (v/v) ethanol in water (2ml/kg) for 13 weeks. From 2 weeks before dosing, rats were fed a diet containing ca. 0.5% Ca, 0.4% P, and 1.0 IU/g vitamin D. In addition, 5 (control) or 25 (drug dose groups) satellite mice/sex/dose group of 19-nor-vitD2 were used for measurement of plasma drug levels at the end of the treatment period.

RESULTS -

Mortality - 1 MLD f died, probably related to ovarian teratoma. Scabs on skin seen in all groups including control, probably due to repeated s.c. dosing.

Clinical signs - No effects.

Body Weight - No effects.

Food Consumption - No effects.

Ophthalmoscopy - No abnormalities.

Hematology - Moderate, dose-dependent (dd) decrease of WBC count in MHD and HD m, and in all f dose groups.

Coagulation - No changes.

Clinical Chemistry - Serum Ca increased dd in all m and f dose groups. Increase was somewhat more pronounced in m than in f. Serum P increased non-dd in all m and f dose groups. Serum ALP mildly increased in MHD and HD f.

Serum PTH - After 92 days, PTH levels decreased significantly in all treated, from_____ _____in HD m, and from_____ _____in HD f.

Urinalysis - No data.

Organ Weights - No changes.

Gross Pathology - *Numbers in parentheses indicate incidence ($N_{examined}$ = 20-20-20-20-20)*
Skin - discoloration (2-6-6-6-5), scab (4-3-6-4-2)

Histopathology - *Numbers in parentheses indicate incidence ($N_{examined}$ = 10-10-10-10-10)*
Kidney - Inflammation (m 0-1-1-5-5) (f 0-1-1-0-1), mineralization (m 0-1-1-5-8) (f 0-2-0-1-1). *Note*: Kidney changes localized in renal medulla, especially outer medullary stripe (thin loops of Henle).