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Verapamil protects against progression of experimental chronic renal failure

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Verapamil protects against progression of experimental chronic renal failure. Chronic administration of verapamil (Ver) decreases nephrocalcinosis and tubular ultrastructural abnormalities in the remnant model of chronic renal disease. In the present study, the effect of chronic Ver administration on renal function, renal histology and mortality after subtotal nephrectomy was examined. Fourteen days after staged subtotal nephrectomy rats were paired according to renal functional impairment, mean arterial pressure (MAP), and body weight. Rats were pair fed and received either Ver (0.1 μg/g sc bid, $N = 10$) or saline (0.1 ml sc bid, $N = 10$) for up to 23 weeks. Both members of each pair were sacrificed shortly before the uremic death of controls. At sacrifice, rats treated with Ver had a lower serum creatinine (2.29 vs. 2.99 mg/dl, $P < 0.05$) and a higher creatinine clearance (318 vs. 164 μl/min, $P < 0.05$) than controls. In a second experiment, survival was superior in rats treated with Ver than in controls from week seven ($P < 0.0025$ by week 14). Serum creatinine was higher at week 10 in control rats (1.68 vs. 1.10 mg/dl, $P < 0.05$). MAP was no different between the two groups, irrespective of the time between Ver administration and the measurement of MAP. Histological damage and nephrocalcinosis were worse, and renal and myocardial calcium content was higher in controls. In conclusion, independent of any effect on systematic MAP, chronic administration of Ver protects against renal dysfunction, histological damage, nephrocalcinosis and myocardial calcification, and improves survival in the remnant model of chronic renal disease.

Verapamil (Ver) is effective as a protective agent in various models of ischemic acute renal failure, including the norepinephrine model of ischemia in the dog [1] and the isolated perfused rat kidney [2], and this calcium entry blocker may be beneficial in toxic ARF [3, 4]. The protective effect in ischemic ARF has been associated with concomitant protection against mitochondrial Ca^{2+} accumulation and respiratory dysfunction [1].

Goligorsky and colleagues [5] have shown that chronic Ver administration is protective against nephrocalcinosis, abnormal tubular cell Ca^{2+} kinetics and mitochondrial and tubular basement membrane morphological changes, which were found three weeks after partial nephrectomy in the rat. At this early stage, however, no changes in renal function had occurred; there were no differences in serum creatinine in the Ver-treated and -untreated animals.

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Nephrocalcinosis may well be important in the progression of chronic renal failure. Phosphate restriction is effective in slowing the progression of various models of chronic renal disease [6, 7] and this protection is accompanied, and possibly mediated in part, by a reduction in nephrocalcinosis.

In view of the three week observations on the beneficial effect of chronic Ver administration on nephrocalcinosis, we have examined for 15 weeks the effect of Ver on functional deterioration, histological damage, nephrocalcinosis, and survival in the remnant model of chronic renal failure.

Methods

Two groups of experiments were performed: in the first (Group I) renal function was studied, and in the second (Group II) survival and blood pressure were examined. Renal histology and calcium contents were studied in both groups of experiments.

Renal function (Group I)

Male Sprague–Dawley rats weighing 230 to 340 g were used in all experiments. One week after removal of 2/3 of the left kidney and electrocautery of the remaining 1/3, the right kidney was removed.

For the initial 14 days, post-nephrectomy rats were allowed to recover on normal rat chow (Wayne Lab-blox) and water ad libitum. At 14 days they were paired into two groups according to serum creatinine, creatinine clearance, body weight, and blood pressure. From day 14 the experimental group ($N = 10$) received Ver (0.1 μg/g body wt) twice daily subcutaneously (Calcan®, 2.5 mg/ml), while the control group ($N = 10$) received normal saline in the same dose.

Rats were housed in individual metabolic cages and were pair fed on normal rat chow. Urine output was measured daily and the rats were weighed weekly. Every one to two weeks the rats were lightly anesthetized with ether and mean arterial pressure was measured in the tail artery using a 25-gauge needle connected to a Statham transducer (Statham Instruments) and recorded on a Gilson polygraph. Through the same needle blood was obtained for the measurement of hematocrit and plasma creatinine, urea nitrogen, calcium, phosphorus, albumin, cholesterol, and triglycerides. Prior to each of these days, two consecutive 24-hour urine collections were made and the urine concentrations of protein, calcium, phosphorus, creatinine, urea nitrogen, sodium, potassium, and osmolality were obtained.

Each rat was sacrificed with its partner at the time of pre-terminal azotemia of either rat; this was determined by cessation of eating and confirmed by measurement of plasma creatinine. Kidney slices and the thoracic aorta were immediately placed in 10% neutral buffered formalin for light microscopy and renal cortical squares in 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, for electron microscopy. In addition, pieces of renal cortex (with careful exclusion of tissue scarred by electrocautery), abdominal aorta, myocardium, lung, liver, spleen, and dorsal abdominal skeletal muscle were removed for measurement of Ca^{2+} content. Inulin clearance was measured by conventional methods in four rats to compare inulin and creatinine clearance.

Survival and blood pressure (Group II)

In a second group of experiments (Group II), survival was studied. Rats (N = 22) were prepared, paired and treated in a method identical to that of the first group. In addition, mean arterial pressure was measured at varying times after the dose of Ver or saline to determine if and for how long treatment with Ver altered mean arterial pressure. The choice of time interval between Ver administration and mean arterial pressure recording at each two week period was random and varied between 0 and 5-1/2 hours.

Histology

At sacrifice, the aorta and portions of kidney were fixed in neutral buffered formalin. Histologic sections were stained with hematoxylin (H) and eosin (E), and by Von Kossa stain for calcium. Kidney sections were also examined following preparation with PAS and trichrome stains. Histologic sections were coded so that the observer did not know which tissues were from control rats and which were from Ver-treated rats. Aortic and renal calcification was graded semiquantitatively from 0 to 3+. The percentage of glomeruli with global or segmental sclerosis was determined; <40% abnormal glomeruli was designated 1+; 40 to 60% was 2+; and >60% was 3+. The severity of tubulointerstitial disease was graded semiquantitatively from 1+ (mild) to 3+ (severe). A renal "severity score" was determined for each rat by adding the scores for tissue calcification, glomerular involvement, and tubulointerstitial disease.

For comparison of histology (and calcium content) the rats from both groups were used. In Group II, all of the rats were examined at week 15.

Chemical analysis

Creatinine and urea nitrogen were measured by automated techniques using a Beckman-2 creatinine and Beckman-2 urea analyzer (Beckman Instruments Inc., Fullerton, California, USA). Plasma cholesterol and triglycerides were measured by enzymatic methods [8, 9], albumin by a colorimetric method [10], sodium and potassium by flame photometry (Instrumentation Labs., Inc., Cidra, Puerto Rico), osmolality by an Advanced osmometer (model 3R, Advanced Instruments, Inc., Needham Heights, Massachusetts, USA), calcium by colorimetric orthocresophthalein complexone [11], phosphorus by colorimetric phosphomolybdate [12], urinary protein by trichloroacetic acid precipitation [13], and inulin by an autoanalyzer method. Tissue calcium content was determined by atomic absorption spectrophotometric methodology (model 290B, Perkin–Elmer, Pasadena, California, USA).

Table 1. Blood pressure, weight and biochemical parameters at time of pairing (day 14) in Group I experiments

Treatment	Verapamil (N = 10)	Control (N = 10)	P value
Plasma creatinine *mg/dl*	1.89 ± 0.22	1.73 ± 0.16	NS
Plasma urea nitrogen *mg/dl*	78.1 ± 9.9	74.9 ± 7.7	NS
Creatinine clearance *μl/min*	91 ± 20	64 ± 22	NS
Proteinuria *mg/day*	73.9 ± 16.4	102.4 ± 31.0	NS
Mean arterial pressure *mm Hg*	93.5 ± 4.7	103.2 ± 10.7	NS
Body weight *g*	279.3 ± 11.9	266.0 ± 12.6	NS

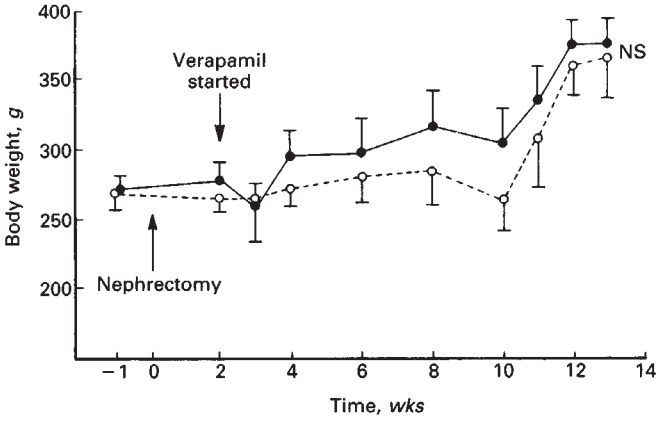

Fig. 1. *Progressive weight gain in rats treated with verapamil (closed circles) or saline (open circles).* Nephrectomy was performed two weeks before treatment was commenced. Mean ± SEM.

Statistical analysis

Comparisons between control and experimental rats were made using the Student's unpaired t-test and chi-squared analysis. Survival was analyzed using the life table method [14]. All results are expressed as the mean ±1 standard error of mean.

Results

Initial renal function and body weight

In both Group I (renal function) and Group II (survival and blood pressure) studies there were no significant differences in plasma creatinine, plasma urea nitrogen, creatinine clearance, mean arterial pressure, proteinuria and body weight between the Ver and saline–treated groups at the time of pairing (day 14). The values for the Group I studies are given in Table 1.

Nutrition

In the Group I studies, there were no significant differences between control and experimental groups in weight gain (Fig. 1) and urinary excretion of water (Fig. 2), sodium, potassium, and total solutes, as expected with pair feeding.

Biochemical parameters

In the Group I studies, treatment with Ver afforded protection against the deterioration in renal function seen in these partially nephrectomized rats. One rat who died early with

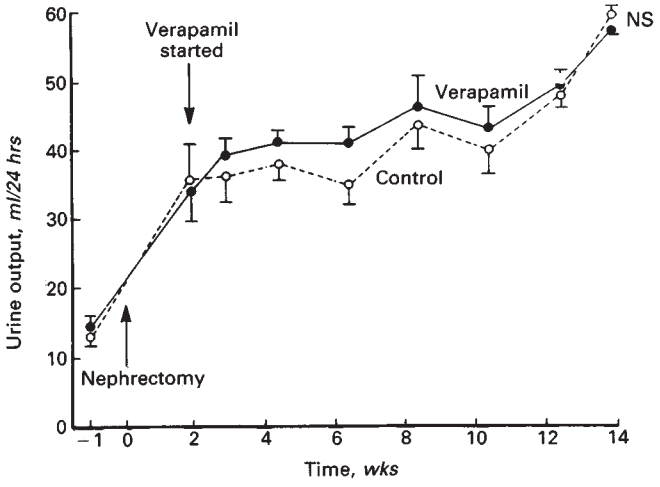

Fig. 2. *Urinary output in rats treated with verapamil (●) or saline (○).* Mean ± SEM.

Table 2. Differences in renal function at sacrifice in Group I experiments

Treatment	Verapamil ($N = 9$)	Control ($N = 9$)	P value
Plasma creatinine *mg/dl*	2.29 ± 0.17	2.99 ± 0.26	<0.05
Plasma urea nitrogen *mg/dl*, $N = 8$	100 ± 14	150 ± 16	<0.05
Creatinine clearance *μl/min*	318 ± 63	164 ± 41	<0.05
Proteinuria *mg/day*	294 ± 67	354 ± 42	NS

Mean ± SEM

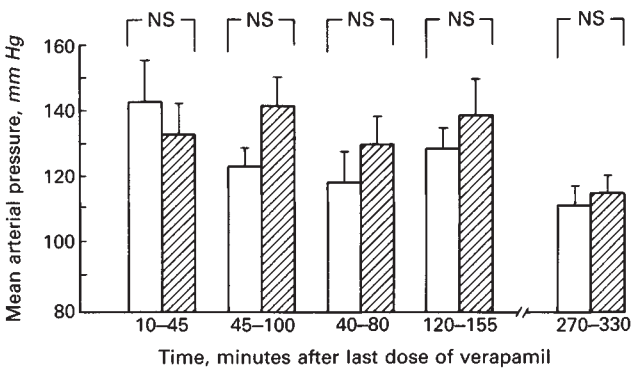

Fig. 3. *Mean arterial blood pressure (MAP) of Group II rats treated with verapamil (▨) or saline (□), taken at varying times after the last dose of verapamil.* There were no significant differences between the MAP of verapamil–treated rats or controls. Mean ± SEM.

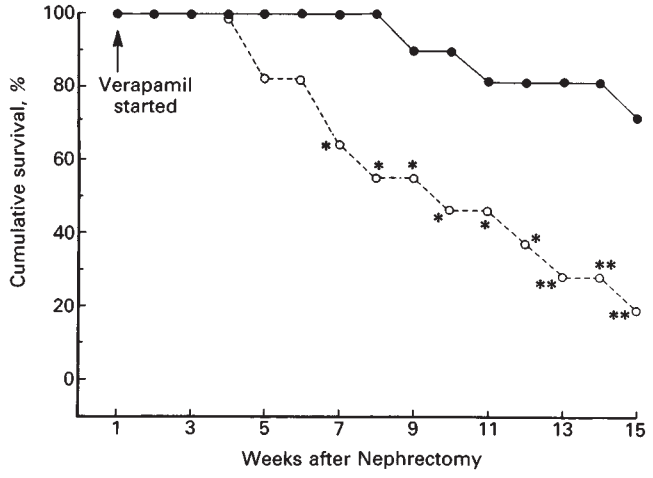

Fig. 4. *Cumulative survival of Group II rats treated with verapamil (closed circles) or saline (open circles).* Symbols are (*) $P < 0.05$; (**) $P < 0.005$.

normal renal function, and his partner, were excluded from analysis. In eight out of the nine remaining pairs of rats, Ver had an obvious protective effect (chi-square analysis, $P < 0.0025$). These significant differences can be seen also when indices of renal function (Table 2) are examined. All tests of renal function were significantly worse in the control rats. In four rats terminal inulin clearance (C_{In}) was compared to simultaneous terminal creatinine clearance (C_{Cr}), and the ratio of C_{In}/C_{Cr} was 0.92 ± 0.20.

Protein excretion increased progressively throughout the study in each rat and no differences in proteinuria could be demonstrated with treatment (mean proteinuria 294 ± 67 vs. 354 ± 42 mg/24 hr, Ver vs. control). Similarly, no effect of Ver on the decrease in hematocrit or increase in triglycerides and cholesterol was seen. Ver-treated animals at week 10 had a numerically, but not significantly lower serum phosphorus (6.76 ± 0.49 vs. 11.63 ± 2.37, NS); no consistent effects on serum calcium, urinary calcium or urinary phosphorus were seen.

Mean arterial pressure

As shown in Figure 3, mean arterial pressure was taken in the Group II experiments at varying times (0 to 5-1/2 hr) after the last dose of Ver or saline; one reading was taken for each rat every two weeks. There was no significant difference between the control and Ver-treated groups. An equal number of rats (6/11) from both groups had readings of >150 mm Hg on one or more occasions. Thus, the protective effect of Ver in the present study appears to be independent of an effect on systemic mean arterial pressure.

Survival

In the Group II experiments, one rat in the Ver group died at week five with a serum creatinine of 1.0 mg/dl; another Ver-treated rat died at week 14 with a large staghorn calculus–causing obstruction. All rats have been included in the survival table (Fig. 4). At week seven, cumulative survival was worse in the control group (64% vs. 100% in Ver-treated rats, $P < .025$). The difference continued until the completion of the study at week 15 (19% vs. 71%, $P < 0.005$).

Histology

Abnormal glomeruli were enlarged and characterized by segmental and global sclerosis, hyalinosis, and adhesions of the tuft to Bowman's capsule. Tubules varied from histologically normal to markedly dilated and tortuous. Some dilated tubules contained casts and had flattened epithelium. There were varying degrees of interstitial fibrosis with tubular atrophy and an interstitial lymphocytic infiltrate. Renal calcification was inter-

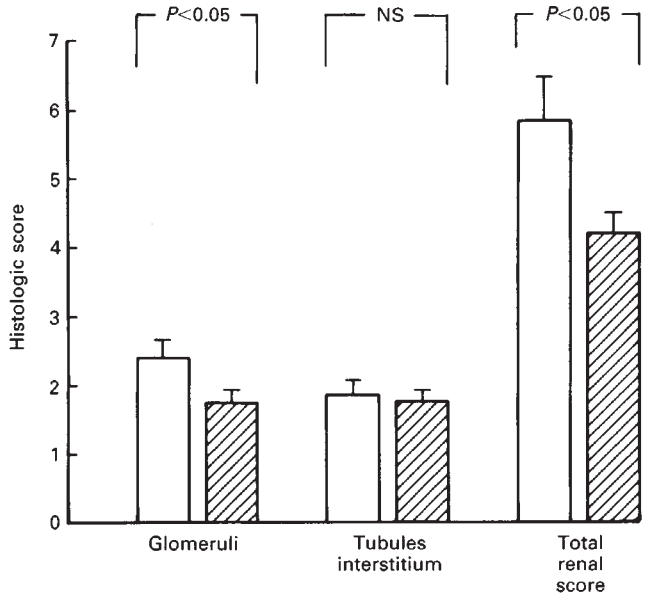

Fig. 5. *Semi-quantitative score for glomerular and tubulo-interstitial histological damage.* Each component is scored on a scale of 0 to 3. Total renal score represents the mean sum of scores for glomerular and tubulo-interstitial damage and nephrocalcinosis. Mean ± SEM. Symbols are described in Figure 3.

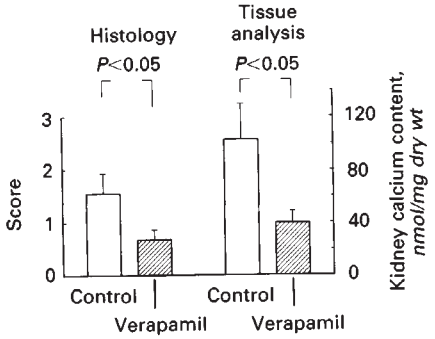

Fig. 6. *Renal calcium accumulation as assessed semi-quantitatively at histology (score) and quantitatively by tissue analysis.* Mean ± SEM.

stitial, either in the form of small nodular masses or linear deposits along tubular basement membranes. Aortic calcification varied from fine, powdery calcific deposits, apparently within smooth muscle cells, to dense calcification of elastic fibers and heavy calcific deposits disrupting and replacing smooth muscle.

When assessed in a blind semi-quantitative manner, glomerular damage, nephrocalcinosis and overall severity of renal damage were significantly worse in control animals (Figs. 5 and 6). Aortic calcification appeared worse in control rats but this did not reach statistical significance.

Calcium content

The mean calcium content was significantly higher in control rats in the kidney and myocardium (Fig. 6, Table 3). Although mean calcium content was higher in the lung and aorta in control rats, this did not reach statistical significance. Calcium content of liver, spleen and muscle was no different between the two groups.

Table 3. Calcium contents in nmol/mg dry weight

	Verapamil (N = 13)	Saline (N = 8)	P value
Kidney	39.4 ± 9.5	102.3 ± 27.7	<0.05
Aorta	23.8 ± 5.3	177.3 ± 131.8	NS
Heart	7.2 ± 0.9	14.0 ± 3.0	<0.05
Lung	17.6 ± 2.7	57.6 ± 28.8	NS

Mean ± SEM values

Discussion

The cost of renal replacement therapy to the community and the patient [15] and the quality of life of patients receiving this therapy [16] argue for prevention rather than treatment of end-stage renal failure. There are several non-specific measures, both dietary and pharmacological, currently available or under investigation that appear to slow the otherwise relentless progression of chronic renal failure. Dietary manipulations include restriction of protein [17], phosphate [6], and perhaps calories [18], acid [19], and lipid [18]. Pharmacological interventions are of less proven benefit and include systemic [20, 21] and glomerular [22, 23] antihypertensive agents, dopamine agonists [24], prostaglandin inhibitors [25], and anticoagulants [26, 27].

Studies by Goligorsky and colleagues [5] have shown that yet another approach may be of value in prevention of progression of chronic renal failure. In their investigations the chronic administration of the calcium entry blocker, Ver, was shown to prevent calcium accumulation in tubular cells and basement membrane, correct abnormal cellular calcium kinetics as well as prevent mitochondrial and tubular basement membrane ultrastructural changes. These observations with Ver were exciting since calcium entry blockers have recently been shown to be protective against acute ischemic [1, 2] and nephrotoxic [3, 4] injury. These findings, therefore, suggested that cellular epithelial and vascular uptake of calcium might be involved in both acute and chronic renal injury. The studies of Goligorsky et al [5], however, involved observations for only three weeks, a time period too short for functional deterioration in this subtotal nephrectomy model of chronic renal failure.

The present studies were therefore undertaken to extend to 15 weeks the observations of Goligorsky et al with the purpose of examining the effect of chronic Ver administration on renal functional deterioration and survival in this model of experimental chronic renal failure. The results demonstrated that chronic Ver significantly attenuated the rate of deterioration of renal function. The protection of renal function occurred despite the absence of any observable effect of verapamil on proteinuria, suggesting that proteinuria and progressive deterioration of renal function are not related directly in this model of chronic renal disease. In addition, the protective effect of chronic Ver treatment was associated with a significant decrease in calcium content of the kidney at the time of sacrifice, an observation supported by the histological study of the kidney. It is of interest that, unlike the studies of Goligorsky et al in which tubular ultrastructural damage was prevented by Ver administration, in the present study histological protection

was limited to glomerular damage and nephrocalcinosis. These findings, therefore, extended the three week observations of Goligorsky et al [5], thus suggesting that prevention of tissue calcium accumulation may be a determinant of the protective effect of Ver in chronic renal failure. Renal tissue calcification is also common in end–stage renal failure in humans [28] and in diseased kidney even before serum creatinine has risen [29], and nephrocalcinosis has been observed in both immunological [7] and non-immunological [6] models of chronic renal failure. In this regard, it is important to emphasize that the protective effect of phosphate restriction has been shown to be associated with diminished renal tissue calcification in both immunological [7] and non-immunological [6] models of chronic renal failure. Taken together, the present and previous results are compatible with increased cellular calcium uptake as a potential factor involved in both acute and chronic renal injury. However, as has been argued previously [18], this does not exclude cellular calcium accumulation secondary to cell death. The enhanced calcium uptake of acute and chronic renal injury may cause cellular damage by several mechanisms including: 1) mitochondrial injury secondary to calcium overload; 2) membrane injury secondary to activation of phospholipases; and 3) enhanced oxygen radical formation. Recent in vivo experiments have confirmed that the calcium entry blockers Ver and nifedipine may afford cellular protection by diminishing cellular calcium uptake. These calcium entry blockers have been shown to protect against anoxic cell injury in cultured proximal and cortical collecting tubules of rabbits [30] and in suspensions of rat proximal tubules [31].

The calcium membrane blockers, Ver and nifedipine, also have been shown to block the systemic pressor response and intrarenal vasoconstriction of angiotensin, norepinephrine and vasopressin [32], to lower systemic blood pressure in normal and hypertensive animals [33, 34], and have been used to treat hypertension in man. In this regard, there can be little doubt that control of hypertension may exert a beneficial effect relative to progression of renal disease in both man [35–37] and experimental animals [20, 21] with chronic renal failure. However, in the present study the beneficial effect of Ver was demonstrated to be independent of differences in blood pressure, suggesting that factors other than systemic hypertension were important in determining the progressive deterioration of renal function. An enhanced solute diuresis has been shown to exert protection in experimental renal failure, and Ver not only increases renal blood flow but also causes a solute diuresis [33, 38]. However, in the present study chronic subcutaneous Ver administration was not associated with differences in solute excretion as compared to the control animals.

The other major pathway whereby Ver may exert its protective effect in this model of chronic renal failure is by altering intrarenal hemodynamics. There is recent evidence that converting enzyme inhibitors may protect against glomerular injury by diminishing the glomerular transcapillary hydraulic–pressure gradient independent of changes in systemic blood pressure [22, 39]. In this regard, Ver has been shown to block both the effect of angiotensin on glomerular microcirculation [40] and mesangial uptake of macromolecules [41].

Thus, in summary, chronic Ver treatment has been shown to retard the progression of chronic renal failure in the partially nephrectomized rat independent of an effect on systemic blood pressure. This protective effect of Ver was shown to be associated with diminished renal tissue calcium as assessed histologically and as measured by atomic absorption spectrophotometric methodology. Not only was there less functional and morphological damage observed in the Ver-treated animals, but a significantly improved survival also occurred. The effect of Ver on tissue calcium uptake, as well as a potential effect on intrarenal hemodynamics, appear most likely to mediate this effect of Ver on chronic renal failure. Thus, calcium entry blockers such as Ver may afford protection not only in acute renal failure [1], but also may attenuate progression of chronic renal disease.

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References

1. Burke TJ, Arnold PE, Gordon JA, Bulger RE, Dobyan DC, Schrier RW: Protective effect of intrarenal calcium membrane blockers before or after renal ischemia. *J. Clin Invest* 74:1830–1841, 1984
2. Shapiro JI, Cheung C, Itabashi A, Schrier RW, Chan L: The effect of verapamil on renal function after warm and cold ischemia in the isolated perfused rat kidney. *Transplantation* 40:596–600, 1985
3. Bakris GL, Burnet JC: A role for calcium in radiocontrast–induced reductions in renal hemodynamics. *Kidney Int* 27:465–468, 1985
4. Eliahou H, Iaina A, Serbon I, Gaveno S, Kapuler S: Verapamil's beneficial effect and cyclic nucleotides in gentamicin–induced acute renal failure (ARF) in rats (abstract). *Proc IX Int Cong Nephrol*, 1984, p. 323A
5. Goligorsky MS, Chaimovitz C, Rapaport J, Goldstein J, Kol R: Calcium metabolism in uremic nephrocalcinosis: Preventive effect of verapamil. *Kidney Int* 27:774–779, 1985
6. Lumlertgul D, Burke TJ, Gillum DM, Alfrey AC, Harris DCH, Hammond WS, Schrier RW: Phosphate depletion arrests progression of chronic renal failure independent of protein intake. *Kidney Int* 29:658–666, 1986
7. Karlinsky ML, Haut L, Buddington B, Schrier NA, Alfrey AC: Preservation of renal function in experimental glomerulonephritis. *Kidney Int* 17:293–302, 1980
8. Fleg, HM: An investigation of the determination of serum cholesterol by an enzymatic method. *Ann Clin Biochem* 10:79–84, 1973
9. Stavropoulous WS, Crouck RD: A new colorimetric procedure for the determination of serum triglycerides. (abstract) *Clin Chem* 20:857, 1974
10. Doumas BT, Watson W, Biggs HG: Albumin standards and measurement of serum albumin with bromocresol green. *Clin Chem Acta* 31:87–96, 1971
11. Connerly HV, Briggs AR: Determination of serum calcium by means of orthocresolphthalein complexone. *Am J Clin Pathol* 45:290–296, 1966
12. Daly JA, Ertinghausen G: Direct method for determining inorganic phosphate in serum with the "Centrifichem." *Clin Chem* 18:263–265, 1972
13. Tinney DJ: A comparison of selected methods for determining urinary protein. *Can J Med Technol* 39:97–112, 1977
14. Cutler SJ, Ederer F: Maximum utilization of the life table method in analyzing survival. *J Chron Dis* 8:699–712, 1958

15. FREEMAN RB: Treatment of chronic renal failure. *N Engl J Med* 312:577–579, 1985
16. EVANS RW, MANNINEN DL, GARRISON LP, HART LG, BLAGG CR, CUTMAN RA, HULL AR, LOWRIE EG: The quality of life of patients with end-stage renal failure. *N Engl J Med* 312:553–559, 1985
17. BRENNER BM, MEYER TW, HOSTETTER TH: Dietary protein intake and the progressive nature of kidney disease: The role of hemodynamically mediated injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation and intrinsic renal disease. *N Engl J Med* 307:652–659, 1982
18. LAOUARI D, KLEINKNECHT C: The role of nutritional factors in the course of experimental renal failure. *Am J Kidney Dis* 3:147–156, 1985
19. NATH KA, HOSTETTER MK, HOSTETTER TH: Pathophysiology of chronic tubulo-interstitial disease in rats: Interactions of dietary acid load, ammonia and complement component C3. *J Clin Invest* 76:667–675, 1985
20. MCQUEEN EC, HODGE JV: Modification of secondary lesions in renal hypertensive rats by control of blood pressure with reserpine. *Q J Med* 30:213–233, 1961
21. NEUGARTEN J, KAMINELSKY B, FEINER H, SCHACHT RG, BALDWIN DS: Nephrotoxic serum nephritis with hypertension: Amelioration by antihypertensive therapy. *Kidney Int* 28:135–139, 1985
22. ANDERSON S, MEYER TW, RENNKE HG, BRENNER BM: Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *J Clin Invest* 76:612–619, 1985
23. ZATZ R, DUNN BR, MEYER TW, ANDERSON S, RENNKE HG, BRENNER BM: Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. *J Clin Invest* 77:1925–1930, 1986
24. STEFONI S, DOCCI D, VANGELISTA A, MOSCONI G, COLI L, PRANDIMI R: Long term treatment of chronic renal insufficiency with ibopamine (SB 7505), a new orally active dopamine-related drug. *Clin Nephrol* 18:168–173, 1982
25. PURKERSON ML, JOIST JH, YATES J, VALDES A, MORRISON A, KLAHR S: Inhibition of thromboxane synthesis ameliorates the progressive kidney disease of rats with subtotal renal ablation. *Proc Natl Acad Sci USA* 82:193–197, 1985
26. PURKERSON ML, JOIST JM, GREENBERG JM, KAY D, HOFFSTEIN PE, KLAHR S: Inhibition by anticoagulant drugs of the progressive hypertension and uremia associated with renal infarction in rats. *Thromb Res* 26:227–240, 1982
27. OLSON JI: Role of heparin as a protective agent following reduction of renal mass. *Kidney Int* 25:376–382, 1984
28. IBELS LS, ALFREY AC, HUFFER WE, CRASWELL, PW, WEIL R: Calcification in end-stage kidneys. *Am J Med* 71:33–37, 1981
29. GIMENEZ L, SOLEZ K, WALKER WG: Renal calcium content, serum phosphorus and serum creatinine in early renal disease (abstract). *Clin Res* 33:484A, 1985
30. SCHWERTSCHLAG U, SCHRIER RW, WILSON P: Beneficial effects of calcium channel blockers and calmodulin binding drugs on in vitro renal cell anoxia. *J Pharm Exp Ther* 238:119–124, 1986
31. BURNIER M, VAN PUTTEN V, WILSON P, BURKE T, SCHRIER R: Beneficial effects of verapamil (V) and nifedipine (N) on Ca influx and cell viability in anoxic renal cortical proximal tubules (CPT). (abstract) *Miner Electrol Metab* 11:390–391, 1985
32. GOLDBERG JP, SCHRIER RW: Effect of calcium membrane blockers on in vivo vasoconstrictor properties of norepinephrine, angiotensin II and vasopressin. *Miner Electrol Metab* 10:178–183, 1984
33. MACLAUGHLIN M, DEMELLO ARIES M, MALNIC G: Verapamil effect on renal function of normotensive and hypertensive rats. *Renal Physiol* 8:112–119, 1985
34. ZIMLICHMAN RR, CHAIMOVITZ C, CHAICHENCO Y, GOLIGORSKY M, RAPAPORT J, KAPLANSKI J: Vascular hypersensitivity to noradrenaline: A possible mechanism of hypertension in rats with chronic uremia. *Clin Sci* 67:161–166, 1984
35. WOODS JW, BLYTHE W, HUFFINES WB: Management of malignant hypertension complicated by renal insufficiency. *N Engl J Med* 192:10–14, 1974
36. POHL JEF, THURSTON H, SWALES JD: Hypertension with renal impairment: Influence of intensive therapy. *Q J Med* 43:569–581, 1974
37. MOGENSEN LE: Antihypertensive treatment inhibiting the progression of diabetic nephropathy, in *Diabetes and Diabetes Treatment III*, Proc 3rd Nordic Symposium Diabetes, edited by DITZEL J. Aakberg, Denmark, Nordisk Insulin Labs, 1979, pp. 103–108
38. MCCROREY HL, BERL T, BURKE TJ, DE TORRENTE A, SCHRIER RW: Effect of calcium transport inhibitors on renal hemodynamics and electrolyte excretion in the dog, in *Hormonal Regulation of Sodium Excretion*, edited by LICHARDUS B, SCHRIER RW, PONCE J. New York, Elsevier/North Holland Biomedical Press, 1980, pp. 133–170
39. ANDERSON S, RENNKE HG, BRENNER BM: Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. *J Clin Invest* 77:1993–2000, 1986
40. ICHIKAWA I, MIELE JF, BRENNER BM: Reversal of renal cortical actions of angiotensin II by verapamil and manganese. *Kidney Int* 16:137–147, 1979
41. RAIJ L, KEANE WF: Glomerular mesangium: Its function and relationship to angiotensin II. *Am J Med* 79 (Suppl 3C):24–30, 1985