Erythrocyte Survival in Chronic Renal Failure

Role of Secondary Hyperparathyroidism

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Abstract

The human erythrocyte (RBC) is a target organ for parathyroid hormone (PTH) and the hormone increases RBC osmotic fragility and induces their hemolysis. The present study was undertaken to examine whether elevated blood levels of PTH affect RBC survival, and therefore whether PTH, being an extracorpuscular factor, is responsible for the shortened RBC survival in chronic renal failure. ^{51}Cr-labeled RBC survival was elevated in six normal dogs, in six animals with chronic renal failure and secondary hyperparathyroidism (NPX), and in six thyroparathyroidectomized dogs (NPX-TPTX) with comparable degree and duration of chronic renal failure. In the normal dogs, ^{51}Cr-labeled RBC survival ranged between 22 and 35 (25.6±1.9) d. In the NPX dogs, ^{51}Cr-labeled RBC survival was shortened and the values ranged between 16 and 20 (18.4±0.6) d, a value significantly ($P < 0.01$) lower than normal dogs. In NPX-TPTX dogs, ^{51}Cr-labeled RBC survival ranged between 20 and 33 (25.2±1.8) d, a value not different from that in normal dogs but significantly higher ($P < 0.01$) than that in NPX animals.

Our data demonstrate that excess blood levels of PTH and not other consequences of the uremic state are responsible for the shortened RBC survival in chronic renal failure.

Introduction

Survival of erythrocytes (RBC)[1] is shortened in patients with advanced renal failure (1–7). This derangement has been attributed to an extracorpuscular factor in that RBC from uremic patients have normal survival when infused into a normal person whereas RBC from normal subjects display shortened survival when given to a uremic patient (8, 9). The nature of this factor is not known. Furthermore, most studies to date have shown that hemodialysis (1, 3) or chronic ambulatory peritoneal dialysis (7) does not improve RBC survival, suggesting that the factor responsible for this abnormality is not dialyzable.

We have previously demonstrated that the RBC is a target organ for parathyroid hormone (PTH) and the latter increases osmotic fragility of RBC and induces hemolysis (10). Patients with advanced renal failure have secondary hyperparathyroidism and elevated blood levels of PTH (11–13). Thus, it is theoretically possible that the in vitro effect of PTH on osmotic fragility of RBC has clinical relevance in that the excess blood levels of PTH in the uremic patients are partially or totally responsible for the shortened survival of RBC.

Saltissi and Carter (14) recently reported a significant inverse relationship ($r = -0.67$, $P < 0.01$) between RBC survival, as measured by ^{51}Cr, and the blood levels of PTH in 27 hemodialysis patients. They suggested that the secondary hyperparathyroidism of renal failure plays a major role in the genesis of the reduced RBC survival in these patients. However, these observations do not provide definite proof for a role of PTH in this RBC abnormality. Indeed, in advanced uremia negative correlations between RBC survival and blood urea, creatinine and phosphate (2, 6) have been reported but none of these compounds has been proven to be the culprit for the reduced RBC survival. It is possible that another, as yet unidentified, factor(s) responsible for the shortened RBC survival, accumulates in the blood of uremic patients as PTH does, and that the elevated blood levels of PTH represent an index for the levels of this other compound(s).

In order to definitely incriminate PTH in the genesis of the shortened survival in uremia, one must document that RBC survival is normal in a uremic state without excess PTH. The present study was undertaken to examine this question.

Methods

Female mongrel dogs weighing 18–25 kg (21±0.5 [SE]) were studied. All dogs were subjected to episiotomy to permit easy access to the bladder for the measurement of creatinine clearance in the awake state. The animals received the same diet providing 78 g of protein, 60 g of fat, 5 g of calcium, and 3 g of phosphorus per day (Kal-Kan, Kal-Kan Food Company, Inc., Vernon, CA). Base-line studies included the measurements of the plasma concentrations of sodium, potassium, bicarbonate, calcium, magnesium, phosphorus, and serum PTH, and free thyroxin index, and the determination of endogenous creatinine clearance. After these studies, all dogs underwent left subtotal renal infarction by ligation of five of the six branches of the left renal artery, and in half of the dogs, thyroparathyroidectomy was also performed at the same time. The success of the procedure was ascertained by a fall in the plasma concentration of calcium of at least 2 mg/dl. The diet of the thyroparathyroidectomized dogs was supplemented with 1–5 g of calcium carbonate/day to maintain normocalcemia; these dogs also received standardized thyroid extract in the form of thyroid tablets (60 mg) (Armour thyroid tablets), three times per week. Thus, this protocol provided two groups of dogs with chronic renal failure (CRF): one with intact parathyroid glands (NPX) and the

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1. *Abbreviations used in this paper:* CRF, chronic renal failure; NPX, hyperparathyroidism; NPX-TPTX, thyroparathyroidectomy; PTH, parathyroid hormone; RBC, erythrocyte(s).

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Table I. Creatinine Clearance, Serum Electrolytes, and PTH in Dogs with Intact Parathyroid Glands (NPX) and Thyroparathyroidectomized Animals (NPX-TPX) before and after Various Duration of CRF

	Duration of CRF	Creatinine clearance		Sodium		Potassium		HCO_3	
		B	A	B	A	B	A	B	A
	wk	*ml/min*	*ml/min*	*meq/liter*	*meq/liter*	*meq/liter*	*meq/liter*	*meq/liter*	*meq/liter*
NPX	80	58	11	147	149	4.2	4.7	22	21
NPX-TPTX	80	65	10	149	150	4.4	4.7	24	22
NPX	70	65	8	150	147	3.5	4.1	19	18
NPX-TPTX	70	60	9	150	148	4.2	4.0	23	22
NPX	70	60	30	151	149	4.3	4.1	20	19
NPX-TPTX	70	64	27	150	148	4.9	4.5	21	20
NPX	52	62	10	148	146	3.9	4.2	22	20
NPX-TPTX	50	60	12	146	147	3.9	4.0	20	19
NPX	29	55	12	150	148	4.5	4.8	19	18
NPX-TPTX	32	51	13	147	148	4.3	4.5	21	20
NPX	19	52	13	145	144	4.1	4.3	23	21
NPX-TPTX	18	50	11	152	151	4.1	4.4	22	20
NPX (n = 6)	53.3±10.1	59.0±1.9	14.0±3.3	148.5±1.0	148.3±2.4	4.1±0.1	4.4±0.1	21.0±0.7	20±0.6
NPX-TPTX (n = 6)	53.3±10.0	58.0±2.4	14.0±2.7	147.3±1.3	148.7±0.6	4.3±0.1	4.4±0.1	22.0±0.7	21±0.5
Normal (n = 6)		58.0±1.5		153.0±1.1		4.3±0.08		23.0±0.6	

There was no significant difference in these parameters in all three groups except for serum PTH levels which were significantly higher in NPX group than normal dogs and were undetectable in NPX-TPTX group. Abbreviations: B, before chronic renal failure; A, after chronic renal failure; UD, undetermined.

other without parathyroid glands (NPX-TPTX). All animals were followed carefully thereafter for several months during which measurements of plasma concentrations of electrolytes and PTH were determined several times and endogenous creatinine clearance was measured every month. We did not encounter difficulty in maintaining the NPX and NPX-TPTX dogs and the mortality was ~15%.

The survival of RBC was studied in six normal dogs, six NPX dogs and six NPX-TPTX animals utilizing the ^{51}Cr technique (15). Whole blood in the amount of 9.5 ml was added to 2 ml of acid citrate dextrose. Approximately 50 μCi of ^{51}Cr as $Na^{51}CrO_4$ was added to the blood mixture, which was then incubated at room temperature for 30 min with occasional swirling. An amount of 0.5 ml of 50 mg of ascorbic acid/10 ml was then added to the blood mixture, which was swirled for 5 min and then centrifuged at 700 *g* at 4°C for 10 min. The labeled RBC were washed with 4–5 ml N saline and centrifuged twice. The final pellet of labeled RBC was reconstituted to a total volume of 10 ml with normal saline which was then injected into the dog from which the blood was obtained. Thereafter, blood samples were obtained at 1, 3, 5, 7, 9, 10, 12, 14, 17, and 21 d. The blood samples were analyzed for radioactivity with a scintillation counter (Biomedical Equipment). Exactly 2.5 ml of each sample was counted and the radioactivity of all samples was measured on the same day, thus enabling correction for radioactive decay using the data for the measurement of the day 1 sample as 100%. The $t_{½}$ ^{51}Cr was calculated by linear regression analysis of the data from each dog using logarithms of the counts per minute. The correlation coefficient was 0.95 or better (0.98±0.003).

The concentrations of phosphorus, creatinine, and bicarbonate were determined by Technicon Autoanalyzer (Technicon Instrument Corp., Tarrytown, NY), those of sodium and potassium with Instrumentation Laboratory flame photometer (model 343, Instrumentation Laboratories, Inc., Lexington, MA) and of calcium and magnesium with Perkin-Elmer atomic absorption spectrophotometer model 503 (Perkin-Elmer Co., Norwalk, CT), and ionized calcium was measured with Orion Electrode model SS-20 (Orion Biomedical, Cambridge, MA). PTH was determined with radioimmunoassay using sheep antiserum 478 (kindly supported by Dr. Claude Arnaud), ^{125}I-labeled bovine PTH, and pooled sera from dogs with CRF as a standard. This antibody reacts predominantly with an immunological determinant in the carboxyl region of PTH, and it will detect both intact hormone and its carboxy-terminal fragment. With this assay, PTH was detected in 15 of 19 normal dogs; the limit of detectability was 12 μleq/ml and the values ranged from 12 to 24 (17.8±1.2) μleq/ml. Elevated PTH levels were found in all 10 animals with CRF studied with the range being 64–350 μeq/ml. The free tyroxin index was calculated as the product of T_3 uptake ratio and T_4. All data are presented as the mean±SE. Statistical comparisons utilized Student's t test.

Results

The biochemical data before and after CRF in NPX and NPX-TPTX dogs are given in Table I. The 5/6 nephrectomy resulted in a significant decrease ($P < 0.01$) in creatinine clearance of about 76±2.4% in NPX and 77±4.1% in NPX-TPTX of baseline values, and there were no significant differences between the creatinine clearance values of NPX and NPX-TPTX dogs. The serum levels of PTH were markedly elevated to values ranging from 82 to 280 μleq/ml in NPX animals and were undetectable in NPX-TPTX animals. There were no significant differences in the other parameters between the values observed before and after the induction of CRF. Similarly, there were no significant differences between these parameters after the induction of CRF in NPX and NPX-PTX except for the high serum levels of PTH in NPX dogs.

Fig. 1 depicts the RBC survival curves for normal NPX and NPX-TPTX dogs. The curves for the normal and NPX-TPTX dogs were almost identical and their slopes were significantly different ($P < 0.05$) from that of the NPX dogs.

The values for the RBC survival in the normal dogs, NPX animals, and NPX-TPTX dogs are shown in Fig. 2. In the normal dogs, the values for ^{51}Cr-labeled RBC survival ranged between

Total calcium		Ionized calcium		Phosphorus		Magnesium		PTH after CRF	Free T_4 index	
B	A	B	A	B	A	B	A		B	A
mg/dl	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*μleq/ml*		
9.7	10.1	4.6	4.4	4.0	4.9	1.8	1.7	82	2.2	2.4
10.4	10.2	4.2	4.0	4.0	4.0	2.1	1.8	UD	2.0	2.2
10.1	9.9	4.8	4.6	4.6	4.0	2.0	1.8	90	2.3	2.2
10.3	10.0	5.0	4.2	4.4	4.6	1.9	1.8	UD	2.1	2.0
10.9	9.8	5.0	4.6	4.0	3.8	2.1	1.9	190	2.1	2.1
10.8	9.2	5.0	4.4	5.0	4.7	1.7	1.8	UD	2.0	1.9
9.8	10.2	4.4	4.8	3.8	3.7	1.8	1.7	280	1.9	1.8
10.0	10.4	4.2	4.4	4.2	4.3	2.0	1.9	UD	2.2	2.3
9.5	10.6	4.6	4.8	5.1	4.8	1.9	1.8	270	1.8	1.8
9.7	10.0	4.6	4.8	3.6	3.8	2.2	2.1	UD	1.8	1.9
9.5	10.4	4.6	4.4	4.2	4.0	1.9	2.1	280	2.0	2.1
10.0	10.2	3.8	3.9	5.0	4.6	1.9	1.8	UD	2.0	1.9
9.9±0.2	10.2±0.1	4.6±0.1	4.6±0.1	4.3±0.1	4.2±0.2	1.9±0.04	1.8±0.06	199±38	2.0±0.08	2.1±0.07
10.2±0.2	10.0±0.2	4.5±0.2	4.3±0.1	4.4±0.2	4.3±0.1	2.0±0.07	1.9±0.05	UD	2.0±0.05	2.0±0.07
9.9±0.04		4.6±0.2		4.2±0.2		1.9±0.06		17.8±1.2	2.1±0.09	

22 and 35 d with a mean value of 25.6±1.9 d. In the NPX dogs ^{51}Cr-labeled RBC survival was shortened and the values ranged between 16 and 20 d, with a mean value of 18.4±0.6 d which is significantly ($P < 0.01$) lower than in normal dogs. In the NPX-TPTX animals, the ^{51}Cr-labeled RBC survival ranged between 20 and 33 d with a mean value of 25.2±1.8 d; this value was not different from that in normal dogs and significantly ($P < 0.01$) higher than that in NPX animals.

Discussion

The results of the present study demonstrate that $t_{½}$ ^{51}Cr in the normal dogs is comparable to that observed by several investigators in normal subjects (1, 2, 5, 6). The data further show that dogs with CRF and secondary hyperparathyroidism have a significantly reduced $t_{½}$ ^{51}Cr, and the values of the latter are comparable to those reported in patients with chronic renal failure (1, 2, 5, 6). Thus, the dog provides a good experimental animal for the study of RBC survival in CRF, and data obtained in dogs are applicable to humans in this regard.

Our data clearly show that $t_{½}$ ^{51}Cr is normal in dogs with CRF but without secondary hyperparathyroidism. Thus, it appears that the shortened RBC survival in NPX dogs is related to the excess blood levels of PTH and not to other consequences of chronic renal failure. Our observations and those of Saltissi

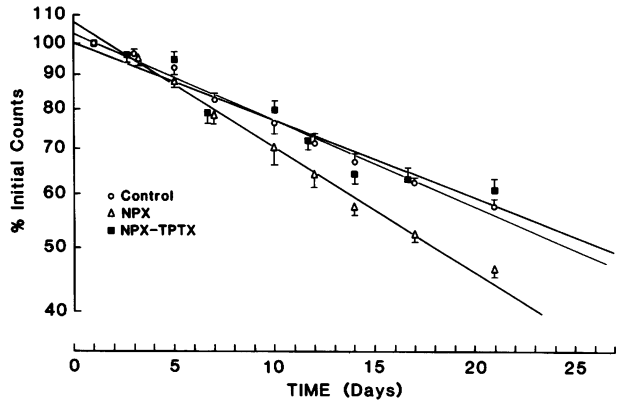

Figure 1. The RBC survival curves in normal, NPX, and NPX-TPTX dogs. Each data point represents the mean of six studies and the brackets denote 1 SE. The slope of the curve for NPX is significantly different (<0.05) from those of normal and NPX-TPTX.

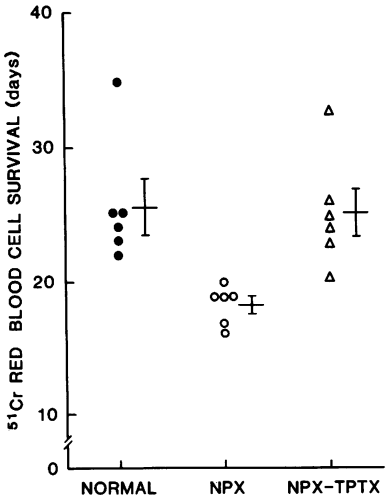

Figure 2. ^{51}Cr-labeled RBC survival in normal dogs, animals with CRF and excess blood levels of PTH (NPX) and in thyroparathyroidectomized dogs with CRF (NPX-TPTX).

and Carter (14) provide strong evidence that the extracorpuscular factor responsible for the shortened RBC survival in uremia is PTH. Such a notion would explain the finding that dialysis therapy does not improve RBC survival (1, 3, 7) inasmuch as PTH is not a dialyzable compound.

It is theoretically possible that some other, as yet unidentified, compound could also be present in the blood of the uremic animals or humans and it could contribute to the shortened RBC survival. However, this possibility seems remote unless one postulates that the production and/or accumulation of such a compound depends on the state of secondary hyperparathyroidism. Even if such a possibility does exist, one must still conclude that the excess blood levels of PTH are of paramount importance in the genesis of the reduced RBC survival in uremia. Our previous studies (10) demonstrating that both the intact PTH (1-84 PTH) and its amino-terminal fragment (1-34 PTH) increased osmotic fragility of human RBC and caused their hemolysis are consistent with the notion that excess blood levels of PTH in chronic renal failure have a direct effect on RBC survival.

The mechanisms of action of PTH on human RBC have been previously investigated in our laboratory (10, 16). These studies showed that the effect of PTH on osmotic fragility is due to alteration in the shape of the RBC; this effect is dependent on enhanced calcium entry in RBC and on stimulation of Ca-activated ATPase of RBC membrane (10). PTH also affects phospholipid turnover of human RBC (16). These events may affect the spectrin-actin of the cystoskeletal network of RBC and may alter the stability and integrity of the cell membrane.

The data of the present study and other results from our laboratory (10) as well as those of others (14) demonstrate that PTH satisfies most of the criteria for a uremic toxin (17) in regard to its action on the RBC in chronic renal failure. Its blood levels are elevated; it affects RBC in vitro by increasing their osmotic fragility; there is a direct relation between blood levels of PTH and RBC survival in uremic patients; and RBC survival is normal in states of CRF without secondary hyperparathyroidism.

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