B. Agroyannis, MD; A. Paraskevopoulos, MD; C. Fourtounas, MD; H. Tzanatos, MD; A. Tsomi, MD; D. Vlahakos, MD; A. Dalamangas, MD

Effect of L-Carnitine on the Calcium Content of Erythrocytes of Uremic Patients on Hemodialysis

The purpose of this study was to determine the calcium content of erythrocytes in uremic patients under regular hemodialysis (HD) and to evaluate the effect of L-carnitine (L-C) on this cellular ion. In 36 hemodialyzed uremic patients—18 under treatment with L-C and 18 without L-C—serum and erythrocytic calcium were determined pre- and post-HD. Erythrocytic calcium was also determined in 21 normal subjects (controls). The mean value (±SD) of erythrocytic calcium in patients under L-C was 2.00 ± 0.18 μg/ml pre-HD vs. 1.96 ± 0.17 μg/ml post-HD. The mean value without L-C was 2.21 ± 0.18 μg/ml pre-HD vs. 2.20 ± 0.25 μg/ml post-HD. The control group had a mean value of 0.60 ± 0.13 μg/ml. These values show that uremic patients in both groups, pre- and post-HD, have significantly higher erythrocytic calcium levels in comparison to the controls ($p<0.0001$). However, the comparison of values between the two groups of HD patients shows that erythrocytic calcium in patients treated with L-C was significantly lower pre- and post-HD than in patients without L-C ($p<0.01$). The mean serum calcium level in patients treated with L-C was 10.60 ± 0.60 mg/dl pre-HD vs. 12.10 ± 0.89 mg/dl post-HD. The mean value without L-C was 10.57 ± 0.61 mg/dl pre-HD vs. 12.18 ± 0.73 mg/dl post-HD. These values were not significantly different between the two groups. In conclusion, uremic patients present with high erythrocytic calcium pre- and post-HD. The administration of L-C in these patients may maintain erythrocytic calcium at lower levels.

Among the factors contributing to anemia in uremic patients are a deficiency in erythropoietin and a shortened life span of erythrocytes. Among the factors that contribute to the shortened survival are increased osmotic fragility, decreased deformability, and abnormal erythrocytes.

Calcium is an important ion in the normal functioning and survival of animal cells. Calcium accumulation in erythrocytes has been associated with a wide range of toxic factors. A rise in the intracellular calcium concentration of erythrocytes may cause decreased deformability and abnormal erythrocytes (echinocytic transformation).[1-3]

It has been suggested that L-carnitine (L-C) stabilizes erythrocyte membranes, protects their deformability, and improves the anemia in uremic patients.[4,5]

The purpose of the present study was to determine the erythrocytic calcium levels in hemodialyzed uremic patients under treatment with L-C and without L-C.

PATIENTS AND METHODS

This study involved 36 hemodialyzed uremic patients, 18 of whom were treated with L-C and 18 of whom were not.

Patients under L-C had a mean age of 65 yr (range, 35–81 yr), a mean hematocrit (Hct) of 34.5%, and a mean duration on HD of 62 mo (range, 18–116 mo). L-C—used for cramps and muscle weakness—was administered post-HD (1 g IV).

The authors are with the Department of Nephrology, Aretaieion University Hospital, Athens, Greece.

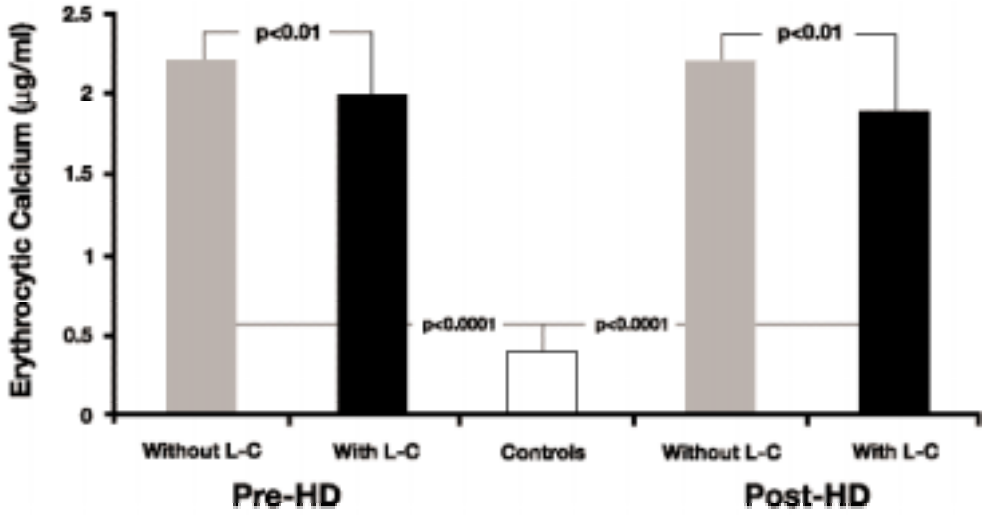

Figure 1. Erythrocytic calcium levels pre-HD and post-HD in uremic patients treated with L-carnitine (L-C) and without L-C, and in controls. As shown, uremic patients either with or without L-C have significantly higher erythrocytic calcium levels than do the controls ($p<0.0001$). Uremic patients treated with L-C, however, have significantly lower erythrocytic calcium levels pre- and post-HD than do patients not receiving L-C ($p<0.01$).

Patients without L-C had a mean age of 59 yr (range, 32–78 yr), a mean Hct of 33.5%, and a mean duration on HD of 50 mo (range, 14–118 mo).

The HD procedure consisted of bicarbonate dialysate, a blood flow rate of about 300 ml/min, a transmembrane pressure of 110–170 mm Hg, and a weight loss of 1.8–3.8 kg.

Erythrocytic and serum calcium levels were determined from blood samples drawn from the arterial bloodline pre- and post-HD. Erythrocytic calcium was also determined in 21 normal subjects (controls). For the determination of erythrocytic calcium, the heparinized blood samples were centrifuged for 10 min at 2,500 G and plasma was removed. The cells were washed 3 times with normal saline, and hemolysis of the erythrocytes was obtained by the addition of distilled water.

Erythrocytic calcium was determined by atomic absorption spectrophotometry. By this method, the mean value for erythrocytic calcium in normal subjects is 0.634 µg/ml.[6] In our controls it was 0.60 ± 0.13 µg/ml.

The results are presented as the mean ±SD. An unpaired *t*-test was used for comparison between the values.

RESULTS

According to our findings, uremic patients under hemodialysis have high erythrocytic calcium levels both pre- and post-HD. However, the administration of L-carnitine may maintain erythrocytic calcium at lower levels, as our results show.

According to our findings, uremic patients under hemodialysis have high erythrocytic calcium levels both pre- and post-HD. However, the administration of L-carnitine may maintain erythrocytic calcium at lower levels, as our results show.

In patients treated with L-C, erythrocytic calcium was 2.00 ± 0.18 µg/ml pre-HD vs. 1.96 ± 0.17 µg/ml post-HD. In patients without L-C, it was 2.21 ± 0.18 µg/ml pre-HD vs. 2.20 ± 0.25 µg/ml post-HD. In the controls, the erythrocytic calcium was 0.60 ± 0.13 µg/ml.

Figure 1 shows that uremic patients pre- and post-HD, with and without L-C, have significantly higher erythrocytic calcium levels than do the controls ($p<0.0001$). However, patients treated with L-C have significantly lower erythrocytic calcium levels pre- and post-HD than do patients not receiving L-C ($p<0.01$).

Figure 2 shows that the serum calcium levels in patients treated with L-C and in patients without L-C are not significantly different pre- or post-HD between the two groups of patients; as expected, serum calcium increases post-HD in both groups.

DISCUSSION

Calcium is thought to be important for the normal functioning and survival of animal cells. It is recognized as an important ion for modulating many critical cellular functions such as

membrane plasticity, cation exchange, and even cell death. The calcium content of human erythrocytes is strictly controlled by a membrane that normally is not readily permeable to calcium, and by an effective Ca-Mg ATPase that accounts for calcium efflux.

It has been suggested that the high erythrocytic calcium levels in dialysis patients may be due to increased membrane permeability and to decreased Ca-ATPase within the cell.[1-3] In this study, it was found that uremic patients undergoing regular HD present high erythrocytic calcium levels both pre- and post-HD. Our results are in agreement with those of Gafter et al. who used the same atomic absorption spectrophotometric method and found that total erythrocytic calcium levels in uremic individuals were about 4-fold higher than in normal controls. Similarly, Corry et al., using a fluorometric method, found that the cytosolic erythrocytic calcium levels were 2-fold higher in uremic patients than in normal controls.[7,8]

In uremic patients, the increase of membrane permeability may be due to the changes in the erythrocytes' membrane lipid composition, to the alterations in cellular deformability, to the shearing stress brought on by the extracorporeal circuit, and to complement activation. It has been proposed that under conditions of limited complement activation, an increase of cell membrane permeability is caused and the calcium is freely diffusable through the fixed complement pore. A rise in intracellular calcium activates K^+, Cl^-, and water loss (Gardos effect), thereby preventing colloidosmotic swelling and lysis of the cells.

L-carnitine is suspected to play an important role in maintaining erythrocyte membrane function. It protects membrane lipids from oxidative stress and increases membrane stability, mainly when erythrocytes are subjected to high shearing stress.

Despite the correction of Ca-ATPase activity during HD, which accounts for calcium efflux, the intracellular calcium remains high post-HD. The suggestion is that many factors—such as complement activation, shearing stress, and peroxidative damage of the membranes' lipid—may increase the erythrocytes' membrane permeability during HD and favor calcium influx.[7-12]

The importance of our findings is that uremic patients treated with L-C have lower erythrocytic calcium levels than do patients without L-C treatment. A low cell calcium level is crucial for the preservation of normal shape, membrane deformability, and survival of the erythrocytes.

L-carnitine is suspected to play an important role in maintaining erythrocyte membrane function. It protects membrane lipids from oxidative stress and increases membrane stability, mainly when erythrocytes are subjected to high shearing stress.

Impaired mechanical stability of erythrocytes improves with the use of L-C. Deformability remains unaffected and the activity of the Na^+–K^+ pump increases. Erythrocytes from patients with low free-carnitine may have a tendency for accelerated osmotic fragility. According to our results, the erythrocytes in uremic patients treated by L-C may be protected from the toxicity of high intracellular calcium. This is probably one of the mechanisms by which carnitine may reduce the erythropoietin requirements in a subgroup of hemodialysis patients.

It has been suggested that free-carnitine, as well as the ratios of free-carnitine to total-carnitine and free-carnitine to acelcarnitine, are decreased in dialysis patients. The administration of L-C in these patients may preserve the functional properties of the erythrocytic membrane,

continued on page 127

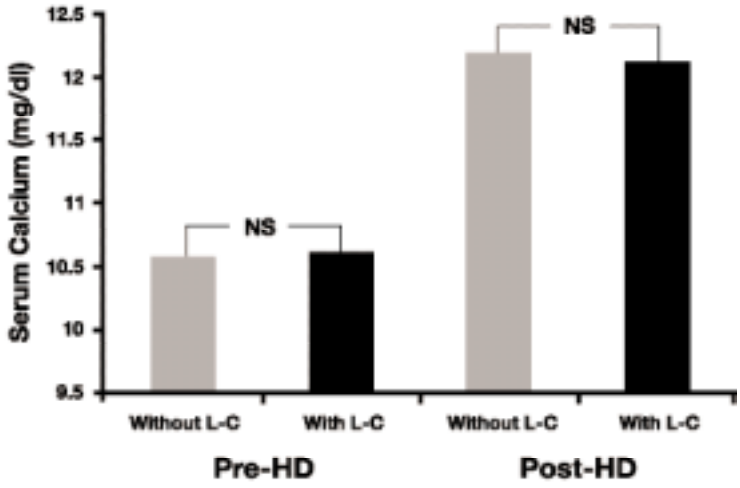

Figure 2. Serum calcium levels pre-HD and post-HD in uremic patients treated with L-carnitine (L-C) and without L-C. As shown, the levels are not significantly different pre- or post-HD between the two groups of patients.

continued from page 109

such as permeability, and protect the erythrocyte from toxic increases of intracellular calcium.[4,5,13] In conclusion, it is suspected from our results that L-carnitine plays an important role in maintaining the erythrocytes' membrane function, and may contribute to the longer life span of the erythrocytes.

References

1. Udden MM, O'Reazed A, Kegel H, McIntize LV, Lynch EC. Decreased deformability of erythrocytes and increased intracellular calcium in patients with chronic renal failure. *Clin Hemorheol* 1984; 4:473-481.
2. Morgan BP, Luzio IP, Cambell AK. Intracellular Ca^{++} and cell injury: A paradoxical role of Ca^{++} in complement membrane attack. *Cell Calcium* 1986; 7:399-411.
3. Agroyannis B, Fourtounas C, Kopelias I, Paraskevopoulos A, Bougatsos G, Katsoudas S, Tzanatos H, Tsomi A, Voudiclari S. Changes in erythrocytes Ca^{++}, K^{+}, Mg^{++} content in uremic patients on CAPD [Abstract]. *Perit Dial Int* 2000; 20(Suppl 1):56.
4. Arduini A, Rossi M, Mancineili G, Beigiglio M, Scurti R, Radatti GL, Shohet SB. Effect of L-carnitine and acetyl-L-carnitine on the human erythrocyte membrane stability and deformability. *Life Sci* 1990; 47:2395-2400.
5. Matsumura M, Hatakeyama S, Koni J, Mabuchi I, Muramoto H. Correlation between serum carnitine levels and erythrocytes osmotic fragility in hemodialysis patients. *Nephron* 1996; 72:574-578.
6. Harrison DG, Long C. The calcium content of human erythrocytes. *J Physiol* 1968; 199:367-381.
7. Gafter U, Malachi T, Barak H, Djaldeth M, Levi J. Red blood cell calcium homeostasis in patients with end-stage renal disease. *J Lab Clin Med* 1989; 114:222-231.
8. Corry DB, Ellis CC, Tuck ML. Increased inward passive permeability in vitro to sodium in uraemic erythrocytes. *Clin Sci* 1996; 90:3-8.
9. Hashimoto H, Mio T, Sumino K. Lipid abnormalities of erythrocytes membranes in hemodialysis patients with chronic renal failure. *Clin Chim Acta* 1996; 2S2:137-145
10. Larsen FL, Katz S, Roufogalis BD, Brooks DT. Physiological shear stresses enhance the Ca^{2+} permeability of human erythrocytes. *Nature* 1981; 294:667-668.
11. Halperin JA, Brugara C, Nicholson-Weller A. Ca^{2+} activated K^{+} efflux limits complement-mediated lysis of human erythrocytes. *J Clin Invest* 1989; 83: 1466-1471.
12. Luciak M, Tiznadel K. Free oxygen species metabolism during hemodialysis with different membranes. *Nephrol Dial Transplant* 1991; 6: 66-70.
13. Bommer J. Saving erythropoietin by administering L-carnitine? *Nephrol Dial Transplant* 1999; 14:2819-2821. D&T