Author's response to reviews

Title: Acetate free citrate-containing dialysate increase intact-PTH and BAP levels in the patients with low intact-PTH.

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Version: 3 Date: 22 December 2012

Author's response to reviews: see over
Dear Dr. Hayley Henderson

Thank you very much for reviewing our manuscript and providing us with valuable comments for its improvement. The comments from the reviewer have been helpful in allowing us to revise our manuscript. We appreciate the time you have taken to suggest improvements for the manuscript.

Response to Dr. Piergiorgio G Bolasco.

Thank you for your constructive comments. We have revised the manuscript in accordance with your suggestions.

(Major compulsory revision)

Single-session study:

1) As Dr. Piergiorgio G Bolasco suggested, the change in pH following the hemodialysis session may alter the ionised calcium bioavailability. Thus, we have added the following sentence to the Discussion section: ‘The primary limitations of this study are the small patient population, the lack of values for pharmacological parameters such as the blood levels for citrate and acetate, and our inability to specify a single type of dialysis membrane, the glucose concentration or the pH in the dialysate’.

2) As Dr. Piergiorgio G Bolasco suggested, the difference in dialysis membrane may influence the ionised calcium mass transfer. However, we usually select the dialysis membrane according to the clinical characteristics of the patient, making the selection of a single dialysis membrane for all patients ethically difficult during the study period. Thus, we have added a sentence to the Discussion section, as mentioned in the response to item 1.

3) As Dr. Piergiorgio G Bolasco suggested, the glucose concentration in the dialysate may affect the ionised calcium. Thus, we have added a sentence in the Discussion section, as mentioned in the response to item 1.

4) Ionised calcium does not follow consensus in that the total calcium is not clear. We mentioned the following sentence in the Discussion section: ‘There were no significant differences in the pre-dialysis t-calcium and i-calcium levels during the cross-over study. In contrast, in the single-session study, we found significant increases in the i-calcium and t-calcium and decreases in the int-PTH levels after HD with A(+)D, while no significant differences were noted in the i-calcium or int-PTH between the pre- and
post-HD levels with A(−)D. Based on these results, we presumed that the citrate in the dialysate could chelate the i-calcium during the HD sessions with A(−)D’.

Furthermore, we removed the following phrase from the Discussion section as suggested by Dr. Piergiorgio G Bolasco.

‘… and affect the PTH levels in the MHD patients, which might cause an increase in BAP’.

5) As Dr. Piergiorgio G Bolasco suggested, treatment time is important for the evaluation of the total or ionised calcium balance. We mentioned the following sentence in the Results section: ‘All patients were treated with HD three times weekly for 3–5 hrs/session for an average of 3.6 ± 0.3 hours’. Furthermore, we have added the following comment in the Results section: ‘The treatment time was unchanged for each patient during the study’.

6) Saline infusion: As Dr. Piergiorgio G Bolasco suggested, saline infusion during a hemodialysis session may affect a patient’s pH level. However, in the single-session study, no patients received a saline infusion.

Sampling time: As Reviewer 2 suggested, we have added the following sentence regarding the measurement of time to the Methods section: ‘The blood levels of pH, HCO3−, t-calcium, and i-calcium were measured immediately after sampling from patients. The remaining blood samples were stored at −30°C until the BAP and int-PTH were measured’.

Cross-over study:

7) As mentioned in the response to item 2, we have added a sentence to the Discussion section, as mentioned in the response to item 1.

8) Treatment time: We provided the following sentence in the Results section: ‘All patients were treated with HD three times weekly for 3–5 hrs/session for an average of 3.8 ± 0.5 hours’. Furthermore, we have added the following comment to the Results section: ‘The treatment time for each patient was unchanged during the study’.

Saline infusion: Unfortunately, we did not confirm the saline dose in the cross-over study.

Ultrafiltration rates: As Dr. Piergiorgio G Bolasco suggested, we have added the following comment to the Results section: ‘Ultrafiltration was subsequently adjusted according to each patient’s condition. The mean ultrafiltration per session was 1794 ±
560 mL in the first A(+)D period, 1944 ± 560 mL in the A(−)D period, and 1878 ± 565 mL in the second A(−)D period. However, no significant differences were noted in the total ultrafiltration in the three periods.

9) As mentioned in the Discussion section, a target int-PTH level of 60-180 pg/mL is recommended by the 2006 JSDT guidelines for the management of secondary hyperparathyroidism in chronic dialysis patients because low (<60 pg/mL) or high (>180 pg/mL) PTH levels result in poor prognosis for the patient. Thus, we conducted a stratified analysis, according to int-PTH levels.

10) As Dr. Piergiorgio G Bolasco suggested, we have revised the Discussion section.

11) As Dr. Piergiorgio G Bolasco suggested, we have added the following text to the Discussion section: ‘Inflammatory condition and bone metabolism: Acetate induces the expression of pro-inflammatory cytokines in monocytes and polymorphonuclear neutrophil leukocytes, which may be responsible for chronic inflammation in HD patients (20). The removal of acetate from the dialysates and the purification of the dialysates would lead to a possible attenuation of chronic inflammatory conditions in MHD patients. Recently, Eleftheriadis T et al. demonstrated the negative correlation between serum IL-6 levels and bone turnover osteocalcin or the beta-isomerised C-terminal cross-linked peptide of collagen type I, which suggests a relationship between bone turnover and chronic inflammation in MHD patients (21). These reports indicate that the attenuation of a chronic inflammatory condition may improve low bone turnover in MHD patients. Although, this study did not compare the inflammatory conditions of A(−)D and A(+)D, the improvement in bone turnover found in this study could be caused by the attenuation of the chronic inflammatory condition by A(−)D.’

12) As Dr. Piergiorgio G Bolasco suggested, the calcium-based phosphor-chelates may affect the ionised calcium or int-PTH levels. However, if we exclude the patients taking calcium-based phosphor-chelates from this study, the study population becomes small. Furthermore, the phosphate binder dose (including calcium acetate or sevelamer) was unchanged during the period. Therefore, we included these patients in the study.

13) Dr. Piergiorgio G Bolasco suggested, we removed the following comment from the Discussion section: ‘It has been reported that metabolic acidosis has detrimental effects on protein and amino acid metabolism and causes malnutrition in HMD patients (20) (21). Low levels of serum albumin and urea nitrogen increased the odds ratio for developing hypoparathyroidism (22). Thus, there is a possibility that malnutritional
conditions should be considered among the causes of decreased serum PTH levels and the aggravation of low bone turnover disease. In our previous study (1), after 4 months of HD with A(-)D, in the patients with low serum albumin levels (< 3.8 g/dL), we found a significant increase in serum albumin levels. From these reports and our results that showed a significant increase in serum albumin in the patients with malnutrition by using A(-)D, we concluded that the attenuation of malnutrition with A(-)D might affect serum int-PTH and BAP levels.

14) As Dr. Piergiorgio G Bolasco suggested, we have added the following comment to the Introduction section, regarding the purpose of this study: ‘The purpose of this study is to determine the effect of varying the dialysate composition on the calcium and int-PTH metabolism in a single HD session and as part of an earlier cross-over study’.

(Minor essential revision)

15) The first A(+) period and the second A(+) period are set as observational periods for comparison with the A(−) period. We believe that the changes in various parameters during each period are important to this study. Therefore, we believe that four columns are required in figures 3-5.

16) Dr. Piergiorgio G Bolasco suggested, we have added figure numbers to figures 1, 2, and 3.

17) As Dr. Piergiorgio G Bolasco suggested, we have changed the subtitle in Figure 3 from ‘Patients with HCO3− < 20 mEq/L’ to ‘Patients with HCO3− ≥ 20 mEq/L’.