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Removal of Parathyroid Hormones and β_2-microglobulin Using PMMA Membranes

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Introduction

By performing hemofiltration and hemodialysis using polysulfone membranes, Wizemann, et al., showed that C-PTH can be filtered. [1] As to PTH removability by adsorptive filters, there have been *in vitro* reports by Nakamura, et al., and *in vivo* reports by us.[3] We reported that, with PMMA (polymethylmethacrylate) membranes (Toray B1 series dialyzers), the reduction rate of C-PTH was great. Changes in M-PTH (mid-region PTH) and intact-PTH were not studied enough in our earlier work. We will discuss them in this report.

Subjects and Methods

The subjects were randomly selected from approximately 200 hemodialysis patients with C-PTH levels of 4 ng/ml or more.

1) Two hemodialysis treatments were performed on two individuals, using PMMA (BK-1.6U) and cuprammonium rayon (AM neo 2000L, hereafter Cu). Pre- and post-dialysis levels of serum calcium (S-Ca), β_2 -microglobulin (β_2-MG) and various PTH's were measured. Pre C-PTH levels for each patient were 9.15 and 9.4ng/ml, respectively. Five hours hemodialysis was done at Q_B200ml/min, Q_D500ml/min, with total fluid removal of 3.4—3.8L.
2) Intra-and inter-dialytic changes in serum PTH concentrations were studied for 5 patients. The BK-1.6U dialyzers were used for 4 patients and BK-2.1U for one patient. Each patient was on hemodialysis for 5 hours at Q_B200ml/min, Q_D500ml/min. The total fluid removal was 2.4—3.65L. C-PTH levels for these 5 patients were 6.0—25.4 ng/ml.
3) Three patients were switched to BK-1.6U dialyzers (1.6m^2 surface area and high β_2-MG removal) from dialyzers with little or no β_2-MG removal (B2-1.0H, RENAK-A 10H, and KF201-15C). Three additional patients who had been treated with B1-2.0 dialyzers (2.0m^2, relatively high β_2-MG removal) were switched to BK-2.1U dialyzers (2.1m^2, higher β_2-MG removal). PTH and β_2-MG were regularly measured commencing one month prior to the switch in dialyzer type until 4 months following the switch. The 6 patients included 5 males and 1 female with ages ranging from 25 to 59 years old, and duration on hemodialysis from 2.7 to 12.8 years. C-PTH concentrations ranged from 4.5 to 36.1 ng/ml.

C-PTH$_{65\text{-}84}$ and intact-PTH$_{1\text{-}84}$ were measured with the RIA double antibody method (INC kit) by Bristol-Myers Laboratory. M-PTH$_{44\text{-}68}$ was measured by the laboratory of Yamasa Soy Sauce Co., Ltd., using the Yamasa kit.

β_2-MG was measured by RIA double antibody method (Pharmacia). The data are shown as mean $\pm$standard deviation, and the significant difference was judged with Student's t-test.

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Results

1) As shown in **Figure 1,** removal rates using PMMA membranes (BK-1.6U) were as follows:

β_2-MG	55.0±12.7%
S-Ca	−22.5±3.5%
C-PTH	74.7±3.6%
M-PTH	60.8±5.1%
intact-PTH	74.8±8.6%

Removal rates using Cu membranes (AM neo 2000L) were as follows:

β_2-MG	−29.5±11.6%
S-Ca	−19.3±4.6%
C-PTH	5.0±5.8%
M-PTH	6.7±5.5%
intact-PTH	57.6±19.5%

Although there was no difference in the removal rates of S-Ca and intact-PTH between the two dialyzers, significant differences were observed in the removal rates of C-PTH and M-PTH.

2) As shown in **Figure 2** using the PMMA dialyzer, M-PTH levels were significantly lowered by hemodialysis. The predialysis level of 56.3±23.4ng/ml was lowered to 38.2±13.5ng/ml after 1 hour of treatment and to 23.6±7.1ng/ml 5 hours after the start of hemodialysis. However, 3 hours after the conclusion of treatment the level rapidly rose to 37.9±15.0ng/ml, and at 48 hours to 52.9±21.1ng/ml. C-PTH exhibited a similar change. On the other hand, intact-PTH exhibited a pattern of change that was different from those of C-PTH and M-PTH.

The level of intact-PTH (343±159pg/ml pre-dialysis) decreased to 227±124pg/ml at 1 hour and continued to significantly decrease during the initial 3 hours of treatment. However, the intact-PTH level scarcely decreased thereafter, reaching 254±182pg/ml at the conclusion of treatment (5 hours). Moreover, 3 hours

Fig.1 Comparison in various removal (reduction) rates between dialyzers

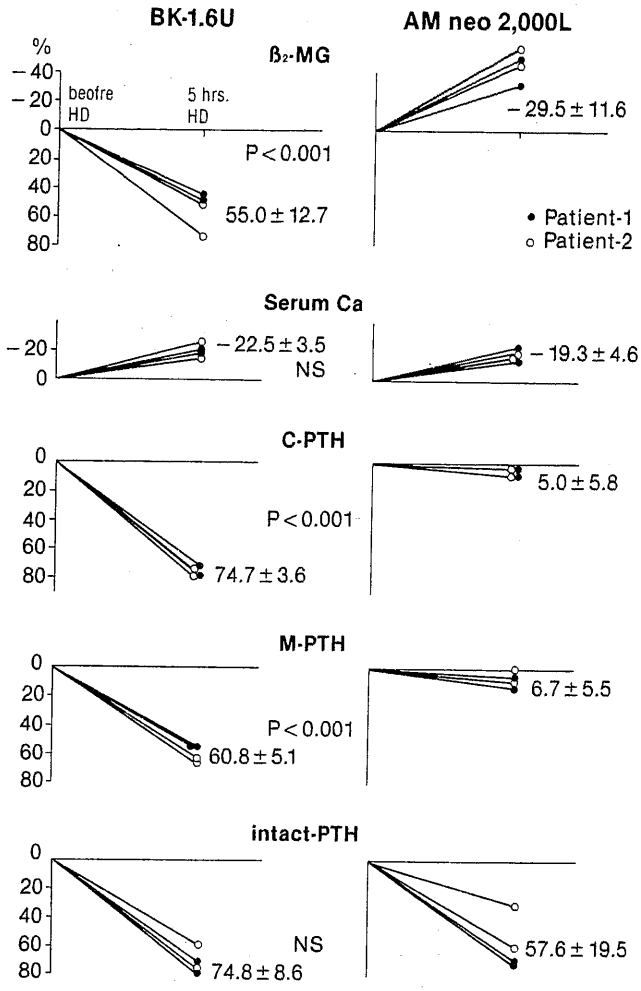
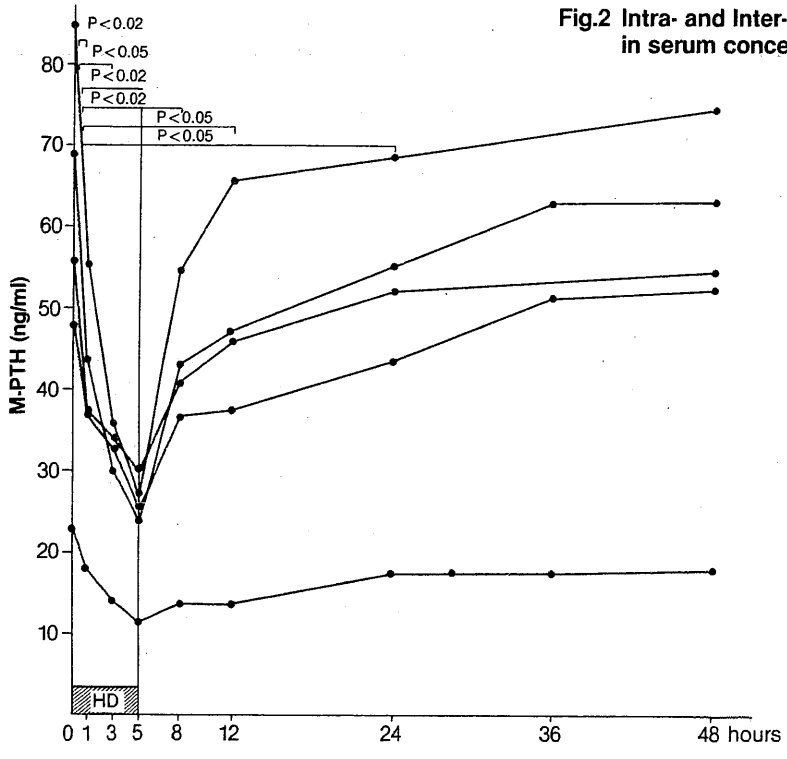


Fig.2 Intra- and Inter-dialytic changes in serum concentration of M-PTH



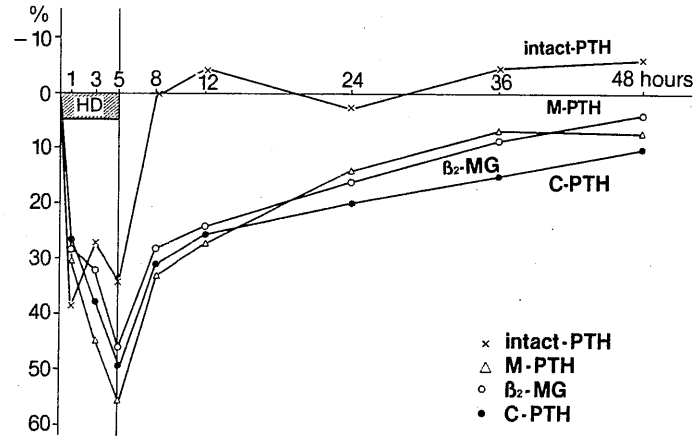


Fig.3 Intra- and Inter-dialytic changes in removal (reduction) rates for various types of PTH and ß2-MG (BK-1.6U and BK-2.1U dialyzers were used, mean level of 5 patients)

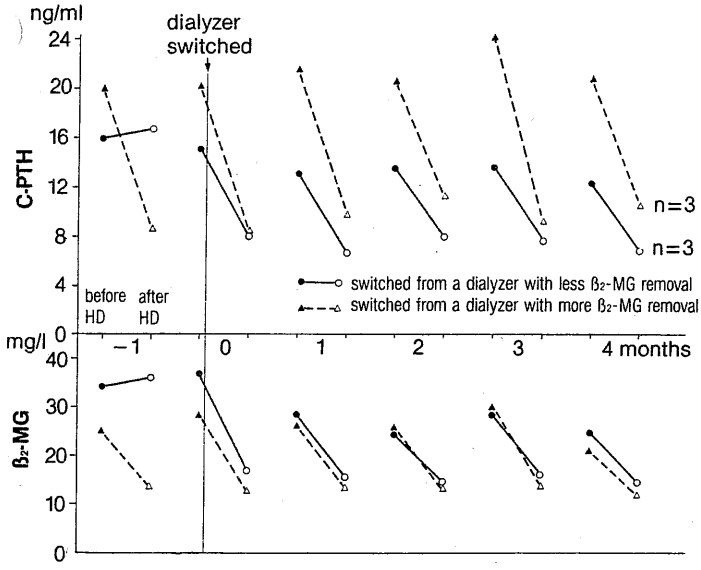


Fig.4 Changes of C-PTH and ß2-MG caused by the switch of dialyzers

following the end of treatment there was a rebound to a level of 361 ± 197pg/ml (higher than pre-dialysis) and to 373 ± 182pg/ml at 48 hours after the initiation of treatment. The level of ß2-MG, which was 30.7 ± 10.7 mg/L at pre-dialysis, decreased to 16.0 ± 6.2mg/L in 5 hours, but rapidly rose to 21.5 ± 6.7mg/L in 8 hours, continuing to rise, gradually reaching 28.3 ± 5.7mg/L at 48 hours after the start of hemodialysis. **Figure 3** illustrates described results as the removal (reduction) rates. C-PTH, M-PTH and ß2-MG decreased at 5 hours after the start of hemodialysis to 49.5 ± 14.9%, 55.5 ± 10.4% and 47.0 ± 14.8%, but at 8 hours rose to 31.2 ± 13.7%, 32.8 ± 7.8% and 28.3 ± 13.2%, respectively, and thereafter slowly returned to their original levels, demonstrating the two-phase change. On the other hand, intact-PTH decreased to 34.0 ± 26.8% in 5 hours after the start of hemodialysis, but rose back to 0.6 ± 30.6%, almost the same as the pre-dialysis level, in 8 hours, scarcely changing thereafter. This pattern was entirely different from those of the other substances we have discussed.

3) **Figure 4** illustrates the changes in pre- and post ß2-MG and C-PTH values during the study period. In the group where a dialyzer with little or no ß2-MG removal was changed to the BK-1.6U dialyzer, the predialysis level of C-PTH was lowered from 16.1 ± 8.7ng/ml before the switch in dialyzers to 12.5 ± 7.3ng/ml 4 months after switching. At the same time the predialysis ß2-MG level also showed a downward trend from 34.3 ± 10.1ng/ml to 25.3 ± 1.5ng/ml. As for intact-PTH, no change was observed.
   On the other hand, in the group where the B1-2.0 was switched to BK-2.1U, neither C-PTH, ß2-MG nor intact-PTH changed.
4) No significant changes in S-Ca or P were observed during the study period for either group.

Discussion

PTHs in blood do not exist freely as a single molecule but are heterogeneous.[4] Namely, C terminal PTH and M-PTH are measured in such a way where various fragments having C terminal structure or mid-region of PTH are included, respectively. In renal failure, mainly fragments with molecular weights of 5,500-7,500 are measured. However, these fragments do not possess biological activity. On the other hand, N terminal PTH with a molecular weight of 4,100 and intact-PTH with a molecular weight of 9,500, both possess biological activity. In addition, the half-life of C terminal fragments in blood is 1 hour or more, while that of intact-PTH is as short as 10 minutes or less.[4] Therefore, the measurement of C-PTH and M-PTH is an inappropriate method for observing the rapid change in secretion, while measurement of intact-PTH and N-PTH is useful[5].

First we observed the change, before and after hemodialysis, of various PTHs with PMMA and Cu dialyzers. While C-PTH and M-PTH were significantly reduced with a PMMA membrane, the reduction rate with a Cu membrane was extremely small. However, the increase rate of S-Ca was the same for both types of membranes. For intact-PTH, both dialyzers showed similar reduction rates. The above observations show that obvious differences exist between PMMA membranes and Cu membranes in their ability to remove various PTHs. They also show that C-PTH and M-PTH are not directly related to the change in S-Ca during hemodialysis. The fact that intact PTH, with a high molecular weight, was also reduced by dialysis with Cu, suggests that the rapid inhibition of intact-PTH secretion is caused by the rise in S-Ca level during hemodialysis. However, besides inhibition of PTH we were not able to clarify to what extent membrane adsorption may have also contributed to the intact-PTH reduction observed by using PMMA membranes.

As previously reported for ß2-MG[6], C-PTH and M-PTH also show a two phase change in serum concentration—rapid increase just after hemodialysis followed by a continuous gradual increase until the next hemodialysis treatment. In order to explain these phenomena for C-PTH and M-PTH, the existence of an extracellular pool may be inferred, similar to ß2-MG. On the other hand, intact-PTH which is metabolized more quickly returned to the pre-dialysis level just 3 hours after the end of the

hemodialysis session, maintaining an almost constant serum level until the next treatment. This suggests that hemodialysis was effective in maintaining a low concentration of PTH with biological activity during hemodialysis and for a short period of time afterwards.

Patients using a membrane with a low β_2-MG removal rate were switched to a PMMA membrane. They were observed for 4 months, during which the level of C-PTH was lowered. However, serum concentrations of intact-PTH did not show significant change. Therefore, it is assumed that dialyzers with higher β_2-MG removability are more effective in removing C-PTH and M-PTH which have an accumulative tendency and long half-lives. Nevertheless, it is not clear whether the use of those dialyzers may lead to the prevention and treatment of secondary hyperparathyroidism. On the other hand the control of serum P and S-Ca levels are considered to be far more important factors.

Conclusion

Our study on the removal of various PTHs by hemodialysis showed the following:

1) C-PTH and M-PTH exhibited changes in serum concentration in a pattern remarkably similar to that of β_2-MG. Using a high-flux PMMA membrane they showed a two-phase rebound pattern after hemodialysis.
2) Intact-PTH returned to the pre-dialysis level as early as 3 hours following the end of treatment. In dialysis with a Cu membrane, the concentration of intact-PTH was also lowered at the end of treatment, suggesting that the secretion of intact-PTH was inhibited in accordance with the rise of S-Ca.
3) It is possible to lower C-PTH level by switching from a dialyzer with low β_2-MG removal to a PMMA B1 or a BK dialyzer and also maintaining serum P level at a lower level.

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