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IONIC AND NONIONIC FLUORIDE LEVELS IN BLOOD OF DIALYZED AND UNDIALYZED PATIENTS WITH RENAL FAILURE AND KIDNEY TRANSPLANTED PATIENTS

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SUMMARY: The present study revealed that the total fluoride level in human whole blood is closely related to the renal function. For the undialyzed patients who had not undergone hemodialysis, the total fluoride level in whole blood linearly increased with the increase of creatinine (Cr) value. The increased fluoride was found to be nonionic in the other blood part than serum, while the nonionic fluoride level in serum was almost constant. That is, the hemodialysis treatment finally reduced the nonionic fluoride level in the other blood part than serum. On the other hand, one hemodialysis treatment could excrete ionic fluoride, but not nonionic fluoride. These results suggest that the ionic fluoride is transformed to nonionic fluoride to be accumulated in other blood part than serum and the non-ionic fluoride is transformed to the ionic fluoride to be excreted. Thus the accumulation of nonionic fluoride in other blood part than serum plays a role of the buffer in preventing a too high serum fluoride level.

INTRODUCTION

Since the discovery of nonionic fluoride in plasma by Taves in 1968 (1), many investigations have carried analysis of serum or plasma for nonionic fluoride. In the previous studies, we established an analytical method for determining trace amounts of fluorides present in whole blood by gas chromatography

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(2) and the low temperature oxygen plasma ashing (3). Fluoride analysis of whole blood made by us (3) and Belisle et al. (4) showed that nonionic fluoride is distributed not only in serum or plasma but also in other blood part. Many discussions have been made on ionic fluoride in plasma or serum only, because handling whole blood is tiresome, which makes analysis of whole blood difficult. Our findings indicate that analyses of both serum and the other blood part for ionic and nonionic fluorides are necessary to evaluate fluoride metabolism.

Many reports on in vivo excretion of fluoride tell that most fluoride is rapidly excreted in urine (5). Most of fluoride must be excreted also through the kidney. Therefore, as a part of our studies on the fluoride metabolism in pathological conditions, the amounts of ionic and nonionic fluorides in blood of patients with chronic renal failure were analyzed and compared with those of healthy controls.

MATERIALS AND METHODS

The study was conducted on 40 outpatients (aged 38-84 years; mean = 63 ± 12 years) with chronic renal failure who had not undergone hemodialysis (undialyzed patients), 40 patients (aged 30-78 years; mean = 53 ± 12 years) undergoing hemodialysis (dialyzed patients), and 10 outpatients (aged 16-52 years; mean = 32 ± 13 years) under observation after kidney transplantation (transplanted patients), giving a total of 90 subjects. Standard values of healthy controls (aged 26-32 years; mean = 28 ± 2 years) were measured previously and reported elsewhere (3). From the 40 patients undergoing hemodialysis with prior durations of from 6 to 159 months, blood samples were taken just before performing hemodialysis treatment. Besides, three patients were studied, immediately before and after the first introduction of the hemodialysis treatment. The 10 outpatients with transplanted kidneys were under observation for periods between 5 and 110 months after transplantation.

Ionic fluoride was determined by gas chromatography after silylation with chlorotrimethylsilane. Nonionic fluoride was converted to ionic fluoride by ashing with a low temperature oxygen plasma asher and subjected to gas chromatography. The detailed procedures have been described elsewhere (2,3). The mean recoveries of added sodium fluoride, p-fluorobenzoic acid, perfluorooctanoic acid by the low temperature oxygen plasma ashing were 88 ± 4 , 91 ± 3 , and $80 \pm 3\%$, respectively, in the range of 50 to 150 ng F added to 1 ml of serum. These figures confirmed the reliability of the low temperature oxygen plasma ashing method for the ashing of biological materials as a pretreatment for determination of fluoride in submicrogram to nanogram amounts (3). Four fluoride values, ionic and nonionic fluoride levels in both serum and the other blood part (the packed cell),

were determined. The nonionic fluoride is defined as the difference between fluoride in the ashed sample (total fluoride) and ionic fluoride in the non-ashed sample. The distributions of ionic and nonionic fluorides in the serum and the packed cell parts calculated from the hematocrit value of each patient are expressed as concentrations in 1 ml of whole blood. Serum creatinine (Cr) and blood urea nitrogen (BUN) levels and hematocrit values were measured with a standard blood autoanalyzer.

Numerical values are presented as means $\pm$ SD (n = the number of data points).

RESULTS

The total fluoride levels in whole blood of all patients are plotted against their serum Cr values in Fig. 1. In the undialyzed patients, the total fluoride levels in whole blood linearly increased with the increase of their Cr values. Although the average total fluoride level in whole blood of the transplanted patients [115 ± 42 ng F/ml ($n=10$)] was higher than that of the healthy controls [77 ± 28 ng F/ml ($n=11$)], it was at the bottom of the renal failure groups. The dialyzed patients were in a high Cr region and distinguishable from the other groups of patients.

The average amount of the total fluoride in serum and the packed cell in 1 ml of whole blood for each group are shown in Fig. 2. The undialyzed patients were divided into the following three groups; (i) with Cr values below 3 mg/dl, (ii) with Cr values between 3 and 7 mg/dl, and (iii) with Cr values above 7 mg/dl. Figure 2 shows that in these undialyzed patients the total fluoride levels in serum were almost constant (82, 89, and 87 ng F/ml of whole blood), regardless of their Cr value. The total fluoride level in the serum of dialyzed patients [92 ± 30 ng F/ml of whole blood ($n=40$)] was similar to the average total fluoride level of undialyzed patients [85 ± 17 ng F/ml of whole blood ($n=40$)]. The overall average of the total fluoride level in the serum of healthy controls [46 ± 13 ng F/ml of whole blood ($n=11$)] and the transplanted patients [51 ± 19 ng F/ml of whole blood ($n=10$)] was 48 ± 16 ng F/ml of whole blood ($n=21$), which was much lower than the average values of the groups with renal failure [89 ± 24 ng F/ml of whole blood ($n=80$)]. In contrast, the total fluoride levels in the packed cell increased together with the increase in the Cr value (from 49 to 184 ng F/ml of whole blood). The details of the results revealed that the duration of hemodialysis or the age of patients did not affect the fluoride level.

Fig. 1. The relationship between renal function and total fluoride level in whole blood; ● : undialyzed, ◇ : dialyzed, ○ : kidney transplanted patients (linear regression coefficient for the undialyzed patients was 0.735).

Fig. 2. The comparison of distributions of total fluoride in serum and the packed cell in 1 ml of whole blood of healthy controls and patients with renal failure.

Fig. 3. The comparison of distributions of ionic and nonionic fluorides in 1 ml of whole blood of healthy controls and patients with renal failure.

Fig. 4. The relationship between renal function and serum ionic fluoride level of the undialyzed patients (linear regression coefficient was 0.085).

Table I. Average distribution and types of fluorides in 1 ml whole blood of healthy controls, dialyzed and undialyzed renal failure patients

	Serum / ng F		Packed cell / ng F		Total fluoride
	Ionic F	Nonionic F	Ionic F	Nonionic F	
Healthy controls (n=11)	5±3	40±12	2±4	29±23	77±28
Dialyzed patients (n=40)	36±11	46±13	11±9	31±21	
Undialyzed patients (Cr > 7, n=7)	35±16	55±30	15±7	41±26	144±48
		92±29		52±31	
		52±20	184±78	169±74	271±70
		87±16			

Table II. The changes of fluoride levels in whole blood and the serum Cr and BUN values before and after the first introduction of the hemodialysis treatment

Donors	Dialysis	Cr (mg/dl)	BUN (mg/dl)	Ionic F (ng F/ml)	Nonionic F (ng F/ml)
1	before	5.2	51	33	134
	after	2.0	15	25	132
2	before	8.9	42	23	98
	after	4.1	18	16	96
3	before	6.7	40	30	91
	after	3.2	15	23	91

The distributions of ionic and nonionic fluorides in whole blood are shown in Fig. 3. The average ionic fluoride level in whole blood was 44 ± 16 ng F/ml (n=40) for the undialyzed patients, which was similar to that of dialyzed patients [46 ± 16 ng F/ml (n=40)]. The ionic fluoride level for the transplanted patients (30 ± 10 ng F/ml) was higher than that for the healthy controls (8 ± 4 ng F/ml), but lower than that of the other groups. On the other hand, the nonionic fluoride level of the undialyzed patients increased together with their Cr values (from 95 to 221 ng F/ml). The serum ionic fluoride level of the undialyzed patients was almost constant [45 ± 19 ng F/ml of serum (n=40)] with variation of the Cr values as shown in Fig. 4 ($r=0.085$).

The distributions of ionic and nonionic fluorides in serum and the packed cell in 1 ml of whole blood were calculated by using hematocrit values of each patient and are summarized in Table I. The ionic fluoride levels in both serum and

the packed cell of the dialyzed patients were higher than those of the healthy controls, while their nonionic fluoride levels were not so different each other. On the other hand, the nonionic fluoride level in the packed cell of the undialyzed patients greatly increased from that of the dialyzed patients, while in the others, the ionic fluoride levels in both serum and the packed cell and nonionic fluoride level in serum of the undialyzed patients, were not so different from those of the dialyzed patients.

The changes of the nonionic fluoride level in whole blood immediately before and after the first introduction of the hemodialysis treatment are shown in Table II, along with the changes in the Cr and BUN values. The slight decrease (7-8 ng F/ml) of the total fluoride level for the three patients measured after the first introduction of hemodialysis treatment was mainly due to the decrease in ionic fluoride, while the Cr and BUN values much more significantly decreased after hemodialysis.

DISCUSSION

As shown in Fig. 1, the total fluoride levels in whole blood showed a clear positive linear relation with the Cr values of undialyzed patients ($r=0.735$; $n=40$). On the other hand, the serum ionic fluoride level of the undialyzed patients is almost constant with the variation of the Cr values as shown in Fig. 4, although it is higher than that of the healthy controls, as was previously reported (5). In addition, there was no correlation between the Cr value and the total fluoride level in serum ($r=0.158$) and the ionic fluoride level in whole blood ($r=0.286$). These results indicate that the total fluoride level in whole blood of undialyzed patients is closely related to the renal function, since the Cr value is known as one of the major indexes of the renal function. Thus, the total fluoride level in whole blood may serve as an index of the renal function which is useful for judging the start of hemodialysis and for following up the patients after renal transplantation.

As shown in Fig. 2, the total fluoride level in serum of the undialyzed and dialyzed renal failure groups [89 ± 24 ng F/ml of whole blood ($n=80$)] was almost constant and much higher than that of the healthy controls and the transplanted patients [43 ± 16 ng F/ml of whole blood ($n=21$)]. On the other hand, the total fluoride level in the packed cell increased with the Cr value. This result seems to indicate that the total fluoride level in serum has a maximum value of about 89

ng F/ml of whole blood and excessive ionic and nonionic fluorides are accumulated in the other blood part than serum. Moreover, the accumulated fluoride in blood of undialyzed and dialyzed patients was mainly nonionic fluoride as shown in Fig. 3, while the ionic fluoride level was almost constant at 46 ± 16 ng F/ml of whole blood ($n=80$).

This phenomenon is well understood by comparing the distributions of ionic and nonionic fluorides in serum and the packed cell (Table I). Although all of ionic and nonionic fluoride levels in serum and the packed cell of renal failure patients increased, the nonionic fluoride level in the packed cell of undialyzed patients was markedly high. The other fluorides (ionic and nonionic fluorides in serum, and ionic fluoride in the packed cell) were on nearly the same levels in dialyzed and undialyzed patients. That is, the insufficiency of the renal function caused the increase of the nonionic fluoride level of the other blood part than serum and the hemodialysis treatment finally reduced the nonionic fluoride level in the other blood part than serum. Table II shows, however, that one hemodialysis treatment can excrete ionic fluoride but the nonionic fluoride level was unchanged. These results suggest that the ionic fluoride is transformed to the nonionic fluoride in vivo to be accumulated and nonionic fluoride to ionic fluoride to be excreted. Consequently, repeated hemodialyses gradually reduce nonionic fluoride accumulated in the packed cell.

Why is the fluoride level increased with the decrease in the renal function? What is the nonionic fluoride? If hypouresis decreases the urinary fluoride excretion (5,6), the excretion of chloride should also be decreased in the same manner. Although the increase in the chloride level in serum is sometimes observed (7), the increase by about 2 to 3 times relative to the normal is quite rare. Therefore, fluoride must be retained against chloride in blood by a different mechanism.

We have reported that the nonionic fluoride in blood can be divided into three types, i.e., ionic, ionizable, and covalently bound organic fluorides (3). In this study, nonionic fluoride included ionizable fluoride and covalently bound organic fluoride. There is a possibility that most of nonionic fluoride determined in the present study is actually not covalently bound organic fluoride but is "ionizable" fluoride. That is, the free ionic fluoride above the saturation in serum may have been combined to proteins or other macromolecules. Such fluoride may not have been analyzed as free ionic fluoride. If this is the case, the other blood part than serum and "nonionic" fluoride may play a role of the buffer in preventing a too high serum fluoride level.

The structures of substances classified as nonionic fluoride are still unknown. To explore the transfer and transformation of nonionic fluoride in blood and thus to study fluoride metabolism, finding compounds associated with non-ionic fluoride must be decisive.

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