Annals of Nuclear Medicine Vol. 8, No. 4, 231–237, 1994

Circulating forms of immunoreactive parathyroid hormone-related protein for identifying patients with humoral hypercalcemia of malignancy: A comparative study with C-terminal(109-141)- and N-terminal(1-86)-region-specific PTHrP radioassay

Mitsuko SUEHIRO, Minoru MURAKAMI and Minoru FUKUCHI

Department of Nuclear Medicine, Hyogo College of Medicine

We evaluated the circulating forms of immunoreactive PTHrP in 115 healthy subjects and 122 patients with malignant diseases by using radioassay systems (RAS) specific for the C-terminal (109-141) fragment of PTHrP (C-RAS) and for the N-terminal(1-86) (N-RAS). PTHrP levels in healthy controls ranged from 1.5 to 38.2 (mean: 24.5) pmol/L with the C-RAS and from 0.9 to 2.5 (mean: 1.7) pmol/L with the N-RAS. The ratio of circulating N-terminal fragment (N) to C-terminal fragment (C) of PTHrP was calculated to be about 1: 14.4 in the healthy subjects. Of the 122 patients with malignant diseases, 40 (32.8%) had circulating PTHrP levels undetectable with the N-RAS, but only 11 (9.0%) patients had levels undetectable with the C-RAS. Of the former 122 patients, 41 (33.6%) had high PTHrP as determined with the C-RAS, and 10 (8.2%) had high PTHrP as determined with the N-RAS. The former of these included only 8 (19.5%) HHM patients, while the latter included 8 (80.0%) HHM patients. The circulating N to C ratio was about 1: 70.7 in the HHM patients. The N and C obtained with the different RASs showed a close correlation ($r = 0.86$). The values also showed a close correlation with serum Ca; $r = 0.75$ for C-RAS and $r = 0.81$ for N-RAS. In addition, the correlations between the PTHrP reading obtained with the different RASs and serum Cr were: $r = 0.42$ with C-RAS and $r = 0.26$ with N-RAS. The circulating form of immunoreactive PTHrP fragments is therefore comprised mainly of PTHrP(109-141). In contrast, circulating concentrations of the PTHrP(1-86) fragment are very low, but detection of the PTHrP(1-86) fragment with the N-RAS is a more useful indicator of HHM with fewer false positive results and is less likely to be influenced by renal function than the detection of the PTHrP(109-141) fragment with C-RAS.

Key words: parathyroid hormone-related protein, humoral hypercalcemia of malignancy, radioassay, serum calceum

INTRODUCTION

HUMORAL HYPERCALCEMIA OF MALIGNANCY (HHM) is the most common paraneoplastic syndrome because the tumor produces a humoral factor that interacts with parathyroid hormone receptors.[1-4] Parathyroid hormone-related protein (PTHrP) is now known to play a major role in the pathogenesis of HHM.[5,6] Measurement of circulating PTHrP concentrations is therefore clinically very important for identifying patients with HHM, but the existence of a variety of circulating forms of PTHrP has been suggested by sequence analysis.[7,8] Very little is known about the circulating forms of PTHrP, especially their metabolic status in blood after secretion from the tumor. Recently there have been reports of several radioassay systems (RASs) for circulating immunoreactive PTHrP fragments using antibodies to synthetic fragments of human PTHrP, including human PTHrP(1-34),[9-11] PTHrP(37-67),[10] PTHrP(1-74),[13,14] PTHrP(1-86),[11,12] PTHrP(109-138),[14] PTHrP(127-141),[15] and PTHrP(109-141).[16] Due to the lack of information concerning the circulating molecular forms of PTHrP in HHM patients, however, the clinical usefulness for laboratory tests of

Received January 14, 1994, revision accepted May 14, 1994.

For reprint contact: Mitsuko Suehiro, M.D., Department of Nuclear Medicine, Hyogo College of Medicine, 1-1, Mukogawa-cho, Nishinomiya, Hyogo 663, JAPAN.

Table 1 Diagnosis of the 122 patients with malignant diseases

Diagnosis	No. of patients	Diagnosis	No. of patients
Lung cancer	15	Mesothelioma	3
Esophageal cancer	15	Gastric cancer	2
Breast cancer	13	Carcinoma of the tongue	2
Bladder cancer	13	Uterine cancer	1
Hepatocellular carcinoma	11	Acute lymphocytic leukemia	1
Osteocarcinoma	9	Enbryonal carcinoma	1
Colon cancer	7	Ovarian cancer	1
Metastatic cancer*	7	Cholangiocarcinoma	1
Non Hodgkin lymphoma	5	Primary macroglobulinemia	1
Pharyngeal cancer	5	Renal carcinoma	1
Prostatic cancer	4	Thyroid cancer	1
Melanoma	3	Total	122

*= unknown origin

such region-specific RASs is unclear. We therefore evaluated the circulating forms of immunoreactive PTHrP in patients with malignant diseases by two different region-specific radioimmunoassays, one for the C-terminal region of PTHrP from amino acid 109 through amino acid 141: PTHrP(109-141) (C-RAS); and a two-site immunoradiometric assay for the N-terminal fragment from amino acid 1 through amino acid 86: PTHrP(1-86) (N-RAS), to study their clinical usefulness in laboratory tests for identifying patients with HHM.

MATERIALS AND METHODS

Patients

Plasma was obtained from 122 patients with malignant diseases with or without HHM (54 females, 68 males; mean age 61.2 years, range 6–87). Final diagnoses of the patients are listed in Table 1. Circulating PTHrP levels were measured simultaneously by the two RASs. Serum calcium (Ca) and serum creatinine (Cr) concentrations were also measured by routine methods in our hospital. Serum Cr concentrations were adjusted with their serum albumin values. One hundred and fifteen normal healthy volunteers (our laboratory staff and medical students: 77 female, 38 male; mean age 30.1 years, range 21–58; all normocalcemic and taking no medication known to affect Ca metabolism) also served as controls. The results obtained from the two RASs in the 122 patients were compared. In addition, the PTHrP levels determined with the two RASs, serum Ca and serum Cr concentrations were also analyzed comparatively.

Radioassays

(A) Radioassay for PTHrP(109-141)

C-RAS, which was developed by Daiichi Radioisotopes Laboratory Ltd., Chiba, Japan, utilizes sheep antiserum to synthetic human PTHrP(109-141) and ^{125}I-labeled PTHrP synthetic human PTHrP(109-141). The following fundamental data for this C-RAS were obtained by the usual assay procedure in our laboratory. The minimal detectable level of circulating PTHrP(109-141) was 8.0 pmol/L. No significant effect was observed when human synthetic PTHrP(1-86), PTH(1-84), and PTH(44-68) fragments were added to the assay system. Multiple dilutions of plasma from the HHM patients resulted in curves parallel to those obtained with standards for PTHrP(109-141). The recovery of PTHrP(109-141) added to test plasma was 98.1 ± 3.8% (mean and SD). The interassay and intraassay coefficients of variation were 5.7% and 3.9%, respectively.

(B) Radioassay for PTHrP(1-86)

N-RAS, which was developed by Nichols Institute Diagnostics, San Juan Capistrano, CA, U.S.A., utilizes a ^{125}I-labeled anti-PTHrP(1-40) polyclonal antibody (PoAb) and a biotinylated anti-PTHrP(60-72) PoAb in addition to synthetic PTHrP(1-86) as a standard. These N-RAS kits were kind gifts from Nihon Medi-physics Co., Ltd., Hyogo, Japan. The following fundamental data for this N-RAS were obtained by the usual assay procedure in our laboratory. The minimal detectable level of circulating PTHrP(1-86) was 0.3 pmol/L. No significant effects were observed when human synthetic PTHrP(109-141), PTH(1-84), and PTH(44-68) fragments were added to the assay system. Multiple dilutions of plasma from the HHM patients resulted in curves parallel to those obtained with standard synthetic PTHrP(1-86). The recovery of PTHrP(1-86) added to test plasma was 105.3 ± 11.4% (mean and SD). The interassay and intraassay coefficients of variation were 10.3% and 6.2%, respectively.

(C) Sample collection and handling

Blood was collected from healthy subjects and from patients with malignant diseases with or without HHM into tubes containing trasylol-EDTA. The blood was mixed well after collection and then kept at 4°C. The plasma was separated within a maximum of 60 minutes after collection, then stored at −20°C until assay.

Table 2 Assay results of circulating PTHrP fragments and serum Ca levels in the 115 normal subjects, 122 patients with malignant diseases and 8 patients with HHM

Subjects	Number of subjects	Circulating PTHrP		Serum Ca levels (mg/dl)
		C-terminal (pmol/L)	N-terminal (pmol/L)	
Healthy controls	115	15.1 – 38.2 (24.5 ± 6.9)	0.9 – 2.5 (1.7 ± 0.4)	—
Malignant diseases	122#	< 8.0 – 951.5 (62.7 ± 128.0)*	< 0.3 – 9.1 (1.6 ± 1.6)*	7.7 – 16.1 (9.5 ± 1.2)
Patients with HHM	8	80.5 – 951.5 (410.3 ± 302.8)	3.0 – 9.1 (5.8 ± 2.2)	11.4 – 16.1 (13.0 ± 1.5)

#: including the 8 HHM patients; ( ): Mean and SD; ( )*: Mean and SD excluding patients with undetectable levels of PTHrP; PTHrP: Parathyroid hormone-related protein; HHM: Humoral hypercalcemia of malignancy.

RESULTS

Circulating PTHrP fragment levels in the healthy controls ranged from 15.1 to 38.2 (mean: 24.5) pmol/L with the C-RAS and from 0.9 to 2.5 (mean: 1.7) pmol/L with the N-RAS (Table 2). In contrast, those in the 122 malignant disease patients (including the 8 HHM patients) ranged from < 8.0 to 951.5 pmol/L with the C-RAS and from < 0.3 to 9.1 pmol/L with the N-RAS (Table 2). From the two RASs, the ratio of circulating immunoreactive PTHrP(1-86) fragment (N) to PTHrP(109-141) fragment (C) was calculated to be about 1 : 14.4 in the healthy controls. The PTHrP levels obtained with the two RASs (n = 73, excluding patients with levels undetectable with either C-RAS or N-RAS) correlated well (Fig. 1). Of the 122 patients, 63 (51.6%) had normal PTHrP (26.4 ± 5.6 pmol/L, mean and SD; CN group), 18 (14.8%) had lower than normal levels, including 11 (9.0%) patients with undetectable levels (CL group), and 41 (33.6%) had high PTHrP (126.9 ± 194.2 pmol/L; CH group) with the C-RAS. The latter 41 patients included 8 (19.5%) HHM patients, whose PTHrP ranged from 80.5 to 951.5 pmol/L (mean: 410.3) with the C-RAS (Table 2). In contrast, with the N-RAS, 44 (36.1%) of these 122 patients had normal PTHrP (1.4 ± 0.4 pmol/L; NN group), 68 (55.7%) had lower than normal levels, including 40 (32.8%) patients with undetectable levels (NL group), and 10 (8.2%) had high PTHrP (5.3 ± 4.1 pmol/L; NH group). The latter 10 patients included 8 (80.0%) HHM patients, whose PTHrP ranged from 3.0 to 9.1 pmol/L (mean: 5.8) with the N-RAS (Table 2). From the two RASs, the ratio of N and C was calculated to be about 1: 70.7 in the HHM patients. As shown in Table 2, the serum Ca in the 122 patients with malignant diseases ranged from 7.7 to 16.1 mg/dl (9.5 ± 1.2, mean and SD) and they were distinguishable as follows: 9.3 ± 0.6 mg/dl in the CN group, 9.4 ± 0.5 mg/dl in the NN group, 9.3 ± 0.5 mg/dl in the CL group, and 9.1 ± 0.6 mg/dl in the NL group. In contrast, the serum Ca concentrations in the CH group and the NH group were

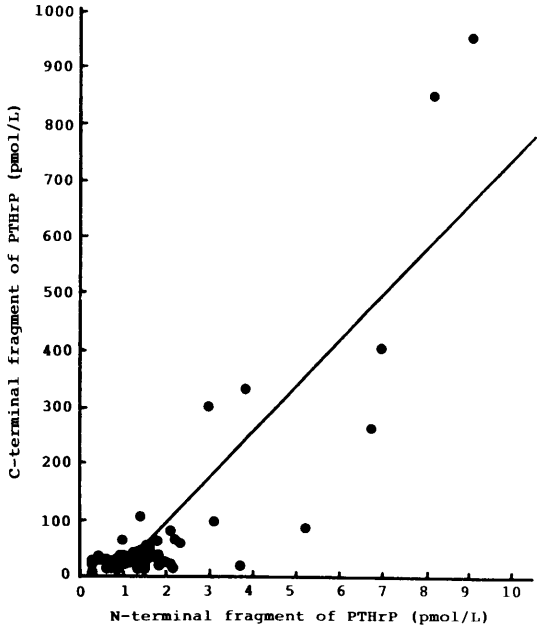

Fig. 1 Correlation between levels of C-terminal and N-terminal fragments of PTHrP in patients with malignant diseases (n = 73, r = 0.86, p < 0.001, $y = 78.4x - 56.9$).

9.9 ± 1.8 mg/dl and 12.3 ± 1.9 mg/dl, respectively. In addition, the serum Ca concentrations in 8 of the HHM patients ranged from 11.4 to 16.1 mg/dl (mean: 13.0) as shown in Table 2. The coefficient of correlation (CC) between the PTHrP reading obtained with the C-RAS and serum Ca were follows: r = 0.75 (p < 0.001), $y = 79.4x - 693.9$ (Fig. 2). However, CC between the PTHrP and serum Cr concentrations in 111 patients with malignant diseases including or excluding the 8 HHM patients were as follows: r = 0.02 (not significant) including the 8 HHM patients and r = 0.42 (p < 0.01), $y = 18.6x + 14.3$ excluding the 8 HHM patients. In contrast, the CC between the PTHrP readings obtained with the N-RAS and serum Ca were as follows: r = 0.81 (p < 0.001), $y = 1.1x - 8.8$ (Fig. 3), but CC between the PTHrP and serum Cr concentrations in 82 patients with malignant diseases (excluding

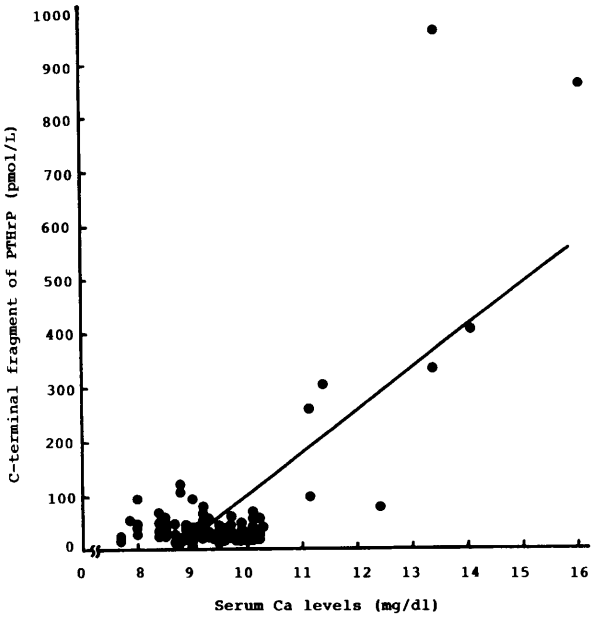

Fig. 2 Correlation between PTHrP C-terminal fragment levels and serum Ca levels in patients with malignant diseases (n = 111, r = 0.75, p < 0.001, $y = 79.4\,x - 693.9$).

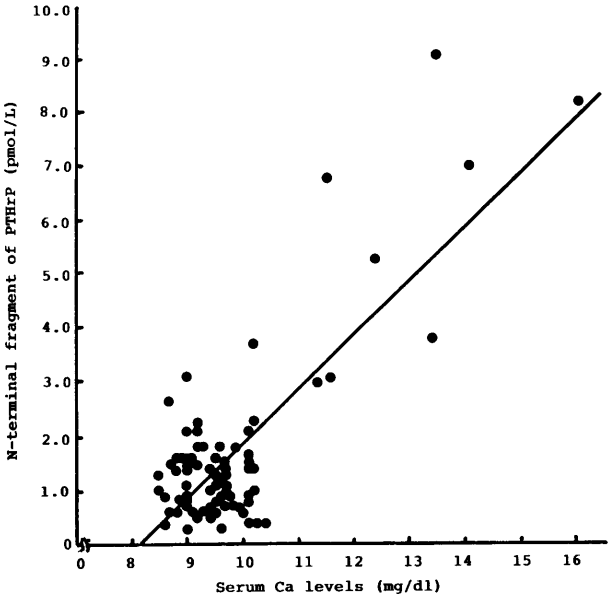

Fig. 3 Correlation between PTHrP N-terminal fragment levels and serum Ca levels in patients with malignant diseases (n = 82, r = 0.81, p < 0.001, $y = 1.1\,x - 8.8$).

Table 3 Clinical profiles of the 8 patients with HHM

Patients Age/Sex	Circulating PTHrP		Serum Ca levels (mg/dl)	Serum Cr levels (mg/dl)	Diagnosis
	C-terminal	N-terminal			
1 60/M	95.5	3.1	11.6	0.9	Esophageal cancer
2 54/M	951.5	9.1	13.5	1.1	Esophageal cancer
3 59/M	851.1	8.2	16.1	1.2	Esophageal cancer
4 60/M	80.5	5.3	12.4	0.9	Pharyngeal cancer
5 60/M	263.7	6.8	11.6	0.9	Pharyngeal cancer
6 79/M	405.9	7.0	14.1	1.4	Lung cancer
7 70/F	330.7	3.8	13.4	0.8	PMG*
8 6/F	301.7	3.0	11.4	0.3	Non-Hodgkin lymphoma

*= primary macroglobulinemia

40 malignant disease patients with undetectable PTHrP) including or excluding the 8 HHM patients were as follows: r = 0.002 (not significant) including the 8 HHM patients and r = 0.26 (p < 0.05), $y = 0.4\,x + 0.7$ excluding the 8 HHM patients. In the present study, as shown in Table 3, eight HHM patients were identified among the 122 patients with malignant diseases.

DISCUSSION

In the present study, we used C-terminal(109-141) region and N-terminal(1-86) region-specific RASs to evaluate the circulating forms of PTHrP in healthy subjects and in patients with a variety of malignant diseases with or without HHM, because we supposed that both the C-terminal and N-terminal fragments of PTHrP, but probably not the intact peptide (PTHrP1-141), are present in the circulation in such patients. In the healthy subjects (mean age 30.1 years; 77 females and 38 males), circulating PTHrP was detected in all samples, not only with the C-RAS but also with the N-RAS. In contrast, of the patients with malignant diseases with or without HHM (mean age 61.2 years; 54 females and 68 males), 9.0% had PTHrP undetectable with the C-RAS, and 32.8% had PTHrP undetectable with the N-RAS. This latter 32.8% of the patients (40 cases; mean age 57.7 years; 21 females and 19 males) included 2 cases who had PTHrP also undetectable with the C-RAS, while the remaining 38 patients showed concentrations of 41.3 ± 23.3 pmol/L with the C-RAS. This level is not significantly different from that (31.8 ± 17.2 pmol/L) of the patients without HHM detectable with the N-RAS. The reasons for this correlation between the level of detection with the N-RAS in healthy subjects and patients with malignant diseases was not clear. In our basic studies with the N-RAS, the assay procedure was simple and there were no problems

in reproducibility, dilution test or recovery test, as mentioned in the present report. On the other hand, it is well known that PTHrP(1-72) and PTHrP(1-86) are rapidly degraded in collected samples and must, therefore, be collected in tubes containing proteinase inhibitors.[12,17] Recently, Nichols Institute Diagnostics also proposed the use of tubes containing proteinase inhibitors for collecting samples for use in the N-RAS. However, in the present study, all assay samples were collected in tubes containing trasylol-EDTA, and stored at −20°C until assay with either the N-RAS or C-RAS. Our sample collection and handling procedures may therefore not have been ideal. But, in our preliminary studies on the stability of assay samples, i.e. PTHrP(1-86)-supplemented plasma, we confirmed uniform stability when samples were stored at −20° for 6 months, not only utilizing tubes containing proteinase inhibitors (105.7 ± 10.3% of the initial value), but also using tubes containing trasylol-EDTA (106.4 ± 3.7% of the initial value). Patients' blood was also stable at 4°C for 60 minutes after collection, not only utilizing tubes containing proteinase inhibitors (92.0 ± 5.3% of the initial value), but also using tubes containing trasylol-EDTA (93.6 ± 9.2% of the initial value). In addition, as many as three cycles of freeze and thawing had no significant effect on the assay results for patients' plasma with the N-RAS (97.4 ± 3.3% of the initial value). The sample collection and handling procedures used in the present study therefore probably did not affect the results of the assay. Fraser et al.[18] suggested the existence of PTHrP fragments that are not detectable by the N-RAS or that another as yet uncharacterized molecule is being produced in HHM patients which has a hypercalcemic effect and can stimulate NcAMP production. In addition, several authors have previously reported the detection of different fragments of the N-terminal region of PTHrP such as PTHrP(56-86) and PTHrP(52-61) circulating in the blood,[10,19] but serum Ca concentrations of the patients in whom PTHrP was undetectable with the N-RAS were 8.9 ± 0.6 mg/d*l*, significantly lower than the values in the malignant disease patients with N-RAS-detectable PTHrP (9.5 ± 0.5 mg/d*l*) and in patients with HHM (12.3 ± 1.9 mg/d*l*). Thus, as serum Ca decreased in parallel with PTHrP(1-86), it seems likely that our patients who had concentrations of PTHrP undetectable with the N-RAS probably did not have concentrations of immunologically undetectable but biologically active PTHrP fragments. Furthermore, all our patients who had undetectable levels of PTHrP with the N-RAS were receiving chemotherapy for their illness at the time of sample collection. Blind et al.[11] reported that PTHrP(53-84) and PTHrP(1-86) fragments decreased significantly during chemotherapy in parallel with the normalization of serum Ca in a patient with HHM. It is therefore possible that in our patients PTHrP concentrations detectable with the N-RAS were affected by chemotherapy. For reference, serum Cr concentrations of these were found to be 1.1 ± 0.5 mg/d*l*, not significantly different when compared to those (1.1 ± 0.2 mg/d*l*) of patients in whom PTHrP was detectable by the N-RAS. In the present study, we detected high PTHrP(1-86) in 10 patients with the N-RAS. Eight of these 10 patients had HHM. The remaining 2 patients' clinical diagnoses were adenocarcinoma of the lung (PTHrP not detectable with the C-RAS, 3.1 pmol/L with the N-RAS, serum Ca: 9.0 mg/d*l*, and serum Cr: 1.1 mg/d*l*) and osteocarcinoma (17.8 pmol/L with the C-RAS, 3.7 pmol/L with the N-RAS, serum Ca: 10.2 mg/d*l*, and serum Cr: 1.2 mg/d*l*). The latter patient showed had a high serum Ca concentrations (12.1 mg/d*l*) about one month after the study and was clinically diagnosed as having HHM, but the former patient died due to severity of the illness a short time later. These results suggest that most patients who had high PTHrP with the N-RAS finally progress to HHM. In contrast, there were 41 patients who showed high PTHrP with the C-RAS in the present study, and 8 of these 41 patients were identified as having HHM. The remaining 33 patients had 1.2 ± 0.6 mg/d*l* serum Cr, significantly higher than (0.9 ± 0.3 mg/d*l*) in the HHM patients. In addition, these 33 patients had 9.0 ± 0.7 mg/d*l* serum Ca, significantly lower than the 9.3 ± 0.6 mg/d*l* of the patients in the CN group, and the 9.3 ± 0.5 mg/d*l* of the patients who had undetectable PTHrP with the C-RAS. Furthermore, of these 33 patients, 18 (54.5%) had undetectable PTHrP with the N-RAS. These findings suggest that circulating PTHrP(1-86) in these patients may be degraded or metabolized, thus reducing the amount of PTHrP(1-86) fragment detectable with the N-RAS in parallel with a decrease in serum Ca but that the PTHrP(109-141) fragment remained in the blood due to renal dysfunction, as it is has been shown that urinary PTHrP(109-141) can be detected with the C-RAS without degradation at concentrations significantly higher (about 10.3-fold) than in the blood.[16] But it is not yet clear if this is so, although the matter is obviously important and requires further research and validation with sensitive and specific RASs for various PTHrP fragments circulating in the blood. Very little is known about the molar ratio of circulating PTHrP(1-86) to PTHrP(109-141). In the present study, the ratio of circulating PTHrP(1-86) fragment to PTHrP(109-141) fragment was calculated to be about 1 : 14.4 in healthy subjects and 1 : 70.7 in HHM patients, but we could not calculate the molar ratio in the malignant disease patients without HHM, because there were many cases who had concentrations of PTHrP which were undetectable with the RASs used, especially with the N-RAS. Kasahara et al.[16] reported previously that the circulating concentrations of PTHrP(109-141) in healthy subjects are 24.6 ± 13.8 pM or 30.7 ± 12.0 pM, and that these are high in patients with HHM when compared to healthy subjects (about 12.7-fold or 29.9-fold; 305.2 ± 158.7 pM or 642.0 ± 195.6 pM). These results indicate that the circulating concentrations of the PTHrP(109-141) fragment are significantly

higher than those of the PTHrP(1-86) fragment, both as previously reported elsewhere,[11,12] and in the present study, not only in HHM patients but also in healthy subjects. In addition, the circulating PTHrP concentrations were calculated to be about 3.4-fold higher for the N-terminal(1-86) fragment and about 16.7-fold higher for the C-terminal(109-141) fragment in the HHM patients when compared to the healthy subjects. It is therefore likely that our results concerning the molar ratio of the PTHrP(1-86) and PTHrP(109-141) fragments are correct. Of the 122 patients with malignant diseases, 41 (33.6%) had high PTHrP with the C-RAS, whereas only 10 (8.2%) had high concentrations with the N-RAS. However, the former 41 patients included only 8 (19.5%) HHM patients, while the latter 10 patients also included 8 (80.0%) HHM patients. This indicates that the two RASs are clinically useful as a screening test for HHM patient, because there was a close correlation between the assay results and serum Ca concentrations, as shown in Figures 2 and 3. However, the N-RAS was more useful as it gave fewer false positive result than the C-RAS for identifying patients with HHM. In addition, CC between assay results and serum Cr concentrations in malignant disease patients without HHM were $r = 0.26$ ($p < 0.05$) with the N-RAS, but $r = 0.42$ ($p < 0.01$) with the C-RAS. Therefore, it seems that the results of the N-RAS are less likely to be influenced by renal function.

In conclusion, the concentrations of both circulating PTHrP fragments, PTHrP(1-86) and PTHrP(109-141), showed a close correlation with serum Ca concentrations. The PTHrP in the circulation is mainly comprised of PTHrP(109-141) fragments when concentrations are compared with those of the PTHrP(1-86) fragment. The PTHrP(109-141) fragment is very stable in the blood, and its detection is very easy in clinical laboratory tests with the C-RAS. In contrast, circulating PTHrP(1-86) fragments are present at very low levels when compared to the concentration of circulating PTHrP(109-141) fragments in the blood, not only in HHM patients but also in healthy subjects. However, detection of the PTHrP(1-86) fragments was a very useful clinical indicator of HHM with fewer false positive results and less likelihood of being influenced by renal function than detection of PTHrP(109-141) fragments.

ACKNOWLEDGMENTS

We wish to thank Daiichi Radioisotopes Laboratory, Ltd. Chiba, Japan for kindly providing PTHrP(109-141) RIA. We also thank Nihon Medi-Physics Co., Ltd., Hyogo, Japan for kindly supplying PTHrP(1-86) IRMA.

This paper was presented in part at the 40th Annual Meeting of the Society of Nuclear Medicine, held in Toronto, Ontario, Canada, June 8–11, 1993.

REFERENCES

1. Bilezikian JP. Parathyroid hormone-related peptide in sickness and in health (editorial). *N Engl J Med* 322: 1151–1153, 1990.
2. Stewart AF, Horst R, Deftos LJ, Cadman EC, Lang R, Broadus AE. Biochemical evaluation of patients with cancer-associated hypercalcemia: evidence for humoral and nonhumoral groups. *N Engl J Med* 303: 1377–1383, 1980.
3. Goltzman D, Stewart AF, Broadus AE. Malignancy-associated hypercalcemia: evaluation with a cytochemical bioassay for parathyroid hormone. *J Clin Endocrinol Metab* 53: 899–904, 1981.
4. Stewart AF, Insogna KL, Goltzman D, Broadus AE. Identification of adenylate cyclase-stimulating activity and cytochemical glucose-6-phosphate dehydrogenase-stimulating activity in extracts of tumors from patients with humoral hypercalcemia of malignancy. *Proc Natl Acad Sci USA* 80: 1454–1458, 1983.
5. Broadus AE, Mangin M, Ikeda K, Insogna KL, Weir EC, Burtis WJ, Stewart AF. Humoral hypercalcemia of cancer. Identification of a novel parathyroid-like peptide. *N Engl J Med* 319: 556–563, 1988.
6. Budayr AA, Nissenson RA, Klein RF, Pun KK, Clark OH, Diep D, et al. Increased serum levels of a parathyroid hormone-like protein in malignancy-associated hypercalcemia. *Ann Int Med* 111: 807–812, 1989.
7. Suva LJ, Winslow GA, Wettenhall REH, Hammonds RG, Mosely JM, Diefenbach-Jagger H, et al. A parathyroid hormone-related protein implicated in malignant hypercalcemia: Cloning and expression. *Science* 237: 893–896, 1987.
8. Burtis WJ, Brady TG, Orloff JJ, Ersbak JB, Warrell RJ, Olson BR, Wu TL, Mitnick ME, Broadus AE, Stewart AF: Immunochemical characterization of circulating parathyroid hormone-related protein in patients with humoral hypercalcemia of cancer. *N Engl J Med* 322: 1106–1112, 1990.
9. Ratcliffe WA, Hughes S, Gilian MG, Heath DA, Ratcliffe JG. Production and characterization of monoclonal antibodies to parathyroid hormone-related protein. *J Immunol Methods* 127: 109–116, 1990.
10. Ratcliffe WA, Norbury S, Stott RA, Heath DA, Ratcliffe JG. Immunoreactivity of plasma parathyrin-related peptide: three region-specific radioassays and a two-site immunoradiometric assay compared. *Clin Chem* 37: 1781–1787, 1991.
11. Blind E, Raue F, Meinel T, Bucher M, Manegold C, Ebert W, et al. Levels of parathyroid hormone-related protein in hypercalcemia of malignancy: comparison of midregional radioimmunoassay and two-site immunoradiometric assay. *Clin Investig* 71: 31–36, 1993.
12. Ratcliffe WA, Norbury S, Heath DA, Ratcliffe JG. Development and validation of an immunoradiometric assay of parathyrin-related protein in unextracted plasma. *Clin Chem* 37: 678–685, 1991.
13. Gaich G, Burtis WJ. Measurement of circulating parathyroid hormone-related protein in rats with humoral hypercalcemia of malignancy using a two-site immunoradiometric assay. *Endocrinology* 127: 1444–1449, 1990.
14. Burtis WJ, Brady TG, Orloff JJ, Ersback JB, Warrell RP,

Olson BR, et al. Immunochemical characterization of circulating parathyroid hormone-related protein in patients with humoral hypercalcemia of cancer. *N Engl J Med* 322: 1106–1112, 1990.
15. Imamura H, Sato K, Shizume K, Satoh T, Kasono K, Ozawa M, et al. Urinary excretion of parathyroid hormone-related protein fragments in patients with humoral hypercalcemia of malignancy and hypercalcemic tumor-bearing nude mice. *J Bone mineral Res* 6: 77–84, 1991.
16. Kasahara H, Tsuchiya M, Adachi R, Horikawa S, Tanaka S, Tachibana S. Development of a C-terminal-region-specific radioimmunoassay of parathyroid hormone-related protein. *Biomed Res* 13: 155–161, 1992.
17. Pandian MR, Morgan CH, Carton E, Segre GV. Modified immunoradiometric assay of parathyroid hormone-related protein: clinical application in the differential diagnosis of hypercalcemia. *Clin Chem* 38: 282–288, 1992.
18. Fraser WD, Robinson J, Lawton R, Durham B, Gallacher ST, Boyle IT, et al. Clinical and laboratory studies of a new immunoradiometric assay of parathyroid hormone-related protein. *Clin Chem* 39: 414–419, 1993.
19. Mitlak BH, Hutchinson JS, Kaufman SD, Nussbaum SR. Parathyroid hormone-related peptide mediates hypercalcemia in an islet tumor of the pancreas. *Horm Metab Res* 23: 344–346, 1991.