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HIGHLY ELEVATED LEVELS OF PROSTAGLANDIN D SYNTHASE IN THE SERUM OF PATIENTS WITH RENAL FAILURE

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ABSTRACT

Objectives. To investigate whether prostaglandin D (PGD) synthase levels differ in the serum of patients with or without renal dysfunction. PGD synthase or beta-trace protein is a major constituent (approximately 3% of total protein) of human cerebrospinal fluid (CSF). We previously reported that PGD synthase levels in serum are approximately 40- to 60-fold lower than those in CSF.

Methods. We measured the PGD synthase concentration in various sera with a highly sensitive and specific immunofluorometric assay along with the serum creatinine level. Analysis for PGD synthase and creatinine was performed in 30 sera from non-renal failure subjects, in 7 sera from patients treated with continuous ambulatory peritoneal dialysis, and in 34 sera that were before and after hemodialysis samples from 17 patients with renal failure.

Results. Elevated creatinine concentration was observed in patients with renal insufficiency, as expected (Mann-Whitney $P < 0.0001$; chi-square $P < 0.0001$). We found that serum PGD synthase concentration from patients with renal failure is significantly elevated compared with the serum PGD synthase concentration from non-renal failure subjects (Mann-Whitney $P < 0.0001$; chi-square $P < 0.0001$). Approximately a 35-fold increase of serum PGD synthase is observed for patients with renal failure compared with non-renal failure subjects. Serum PGD synthase concentration is not affected by hemodialysis in acute renal failure patients (Mann-Whitney $P = 0.918$), unlike serum creatinine levels, which were decreased significantly after hemodialysis (Mann-Whitney $P = 0.0001$).

Conclusions. We conclude that renal impairment is highly associated with elevated serum PGD synthase levels. Measurement of PGD synthase in serum is a new biochemical marker of renal insufficiency.

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Prostaglandin D (PGD) synthase, originally identified and termed as beta-trace protein (beta-trace) by Clausen, is a major constituent of the human cerebrospinal fluid (CSF), representing approximately 3% of total CSF protein.[1] Recently, there is renewed interest for the origin and function of beta-trace protein since several investigators described its structural identity with human PGD synthase.[2–4] PGD synthase is an enzyme that catalyzes the conversion of PGH_2 to PGD_2 in the brain, in the presence of various sulfhydryl compounds.[5] Experimental evidence associates PGD_2 with sleep induction.[6] In addition, PGD synthase belongs to the lipocalin superfamily and shares characteristics similar to those of other lipocalins.[7] Lipocalins are secretory proteins thought to transport small hydrophobic ligands. Postnatally, the cellular location of PGD synthase changes, and the enzyme is thought to play important roles in both maturation and maintenance of the central nervous system (CNS).[8]

We previously purified PGD synthase to homogeneity from human amniotic fluid.[9] Studies conducted with a specific and sensitive immunoflurometric assay for PGD synthase[10] did not associate CSF or serum PGD synthase concentration with specific neurologic disorders.[11] Our results indicate that CSF and amniotic fluid PGD synthase concentration is approximately 40- to 60-fold and

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TABLE I. *Distributions of serum PGD synthase, serum creatinine, age, and gender from non-renal failure and renal failure subjects*

	No. of Samples	Range	Mean (SD)	25th Percentile	Median	75th Percentile
Serum PGD synthase (μg/L)						
Non-renal failure subjects	30	102–426	239 (94)	164	226	324
Prehemodialysis patients	17	2,948–12,878	8,503 (2,711)	7,069	9,262	10,403
Posthemodialysis patients	17	3,189–14,925	8,592 (2,906)	7,100	8,797	11,166
CAPD patients	7	4,768–13,387	9,090 (3,360)	4,793	8,934	11,983
Serum creatinine (μmol/L)						
Non-renal failure subjects	30	20–119	77 (26)	60	87	96
Prehemodialysis patients	17	322–1,300	680 (302)	412	499	952
Posthemodialysis patients	17	126–599	288 (135)	169	259	393
CAPD patients	7	481–1,182	793 (238)	590	771	1,002
Age						
Non-renal failure subjects	30*	26–63	44 (9)	36	45	51
Renal failure patients†	24‡	29–56	42 (8)	36	42	50

KEY: PGD = prostaglandin D; SD = standard deviation; CAPD = continuous ambulatory peritoneal dialysis.
* *Gender distribution: 18 men and 12 women.*
† *Includes CAPD patients.*
‡ *Gender distribution: 13 men and 11 women.*

approximately three-fold higher than the serum concentration, respectively.[10,11]

An observation of a patient with renal failure who had serum PGD synthase concentration 5 to 10 times higher than normal controls encouraged us to investigate the distribution of PGD synthase concentration in the serum of patients with or without renal impairment. The objective of this study is to determine whether PGD synthase concentration differs in the serum of patients with or without renal dysfunction.

MATERIAL AND METHODS

Clinical Specimens

Sera obtained from patients with renal failure were collected at the Wellesley Hospital, Toronto, and stored at −20°C until analysis. Seven sera were from patients undergoing continuous ambulatory peritoneal dialysis (CAPD). Thirty-four sera were before and after hemodialysis samples from 17 patients with renal failure. Sera corresponding to 30 hospitalized subjects with normal serum creatinine and no history of renal disease were selected as a control group. The control group was matched for age and gender with the renal failure group.

Methodology

PGD synthase determinations were performed using a highly sensitive and specific immunofluorometric procedure described in detail elsewhere.[10] Briefly, the PGD synthase assay uses a mouse monoclonal anti-PGD synthase capture antibody coated to polystyrene microtiter wells, a biotinylated mouse monoclonal anti-PGD synthase detection antibody, and alkaline phosphatase-labeled streptavidin (SA-ALP). In the assay, 50 μL of diluted serum sample in a 6% bovine serum albumin (BSA) diluent are incubated with the coating antibody in the presence of 50 μL of assay buffer containing the detection antibody. Serum samples from the control group were diluted 100-fold in 6% BSA. Serum samples from the renal failure group were diluted 500-fold in 6% BSA. After a 2-hour incubation followed by washing 6 times, the SA-ALP conjugate is added for 15 minutes, followed by another washing 6 times. The activity of ALP is then measured by adding the substrate 5-fluorosalicylphosphate, incubating for 10 minutes, and then by adding a Tb^{3+} and ethylenediaminetetroaacetic acid (EDTA)-containing developing solution. After 1 minute the fluorescence is measured in the time-resolved fluorometric mode. This assay has a detection limit of 0.2 μg/L of PGD synthase. Serum creatinine was quantitatively determined for all samples with the COBAS INTEGRA anlyzer (Roche Diagnostics, Basel, Switzerland) based on the Jaffé reaction. All assays were run in duplicate.

RESULTS

Determination for PGD synthase and creatinine was performed in 30 sera from non-renal failure subjects, in 7 sera from patients with CAPD, and in 34 sera from before and after hemodialysis samples corresponding to 17 patients with renal failure. The distribution of PGD synthase and creatinine concentration in all sera tested from non-renal and renal failure subjects, along with their age and gender, are shown in Table I. PGD synthase was detectable in all sera analyzed. Similar data, as shown in Table I, were obtained when the samples were diluted anywhere from 10- to 100-fold (controls) or from 200- to 2000-fold (renal failure).

Increased serum creatinine concentration is indicative of renal impairment.[12] Comparison of creatinine concentration of the prehemodialysis sera from the 17 patients with renal failure and of the sera from 7 patients with CAPD with the 30 sera from the non-renal failure subjects indicated the expected significant difference (Mann-Whitney $P < 0.0001$, chi-square $P < 0.0001$). Elevated creatinine concentration was observed in patients with renal insufficiency (Tables II, III and Fig. 1B). To examine whether the creatinine concentration

TABLE II. *Comparison of PGD synthase and creatinine concentration in serum between non-renal failure and renal failure patients

Patient Group	No.	Median PGD Synthase (μg/L)	Median Creatinine (μmol/L)	*P* Value†
Non-renal failure	30	226	87	
Renal failure	24	9098	716	<0.0001

Key: Abbreviations as in Table I.
** Renal failure patients include CAPD patients.*
† Mann-Whitney P value calculated at the 95% confidence level.

changes after hemodialysis, we compared the creatinine concentration of the before and after hemodialysis sera from the 17 patients with renal failure. Our results indicate a significant difference of the creatinine concentration between the before and after hemodialyzed sera from the 17 patients with renal failure (Mann-Whitney $P = 0.0001$). Serum creatinine concentration decreased significantly after hemodialysis of the 17 patients with renal failure (Table IV, Fig. 1B), as expected.

To determine whether the serum PGD synthase concentration of the non-renal failure subjects is different from the serum PGD synthase concentration of the patients with renal failure, we used both the Mann-Whitney and chi-square tests (Tables II and III). Serum PGD synthase concentrations from patients with renal failure are significantly elevated compared with the serum PGD synthase concentrations from non-renal failure subjects (Mann-Whitney $P < 0.0001$, chi-square $P < 0.0001$). Approximately a 30-fold increase of serum PGD synthase is observed for patients with renal failure compared to non-renal failure subjects. Elevated levels of PGD synthase were observed for both patients with renal failure (before and after hemodialysis sera) and patients with CAPD compared with the PGD synthase levels from the non-renal failure subjects (Fig. 1A). To study whether the PGD synthase concentration changes after hemodialysis, we compared the PGD synthase concentration of the before and after hemodialyzed sera from the 17 patients with renal failure (Table IV). Our results show that there is no difference of the PGD synthase concentration between the before and after hemodialysis sera from the 17 patients with renal failure (Mann-Whitney $P = 0.92$) (Fig. 1A).

To investigate the presence of an association between serum creatinine and serum PGD synthase, we have correlated the serum concentration of creatinine and PGD synthase of the 30 non-renal failure subjects and of the sera from the 24 patients with renal failure. Regression analysis indicated a weak positive linear association between serum creatinine and serum PGD synthase concentration from the non-renal failure group (n = 30, Pearson correlation coefficient $r = 0.37$, $P = 0.046$). There was no statistical linear association among the renal failure group (n = 24, Pearson corralation coefficient $r = 0.20$, $P = 0.35$). Regression analysis results after separating this group into CAPD and renal failure groups indicated a statistical linear but weak association between the creatinine concentration and the PGD synthase concentration of the sera from the CAPD patients (n = 7, Pearson correlation coefficient $r = 0.75$, $P = 0.051$). It appears that the PGD synthase concentration in the serum of the non-renal failure subjects and patients with CAPD increases with increasing serum creatinine concentration.

COMMENT

There is a renewed interest for the study of PGD synthase's site of expression and biologic function, because its potential capability to act as an enzyme and as a lipophilic transporter is unique and well accepted.[13] Quantification of the PGD synthase distribution in various human fluids indicated that PGD synthase concentration is highest in CSF followed by the levels in seminal plasma, amniotic fluid, and urine.[10] Attempts to elucidate the site of expression and secretion of PGD synthase in the CNS show that the epithelial cells of the choroid plexus are the major site of PGD synthase production, although cultured astrocytes and leptomeningeal cells also have the potential to synthesize it.[14,15] Because of its highly specific expression pattern at blood-tissue barriers during embryogenesis, it was recently suggested that PGD synthase could have a role in the maturation and/or maintenance of the blood-cerebrospinal, blood-retina, blood-aqueous humor, and blood-testis barriers.[16,17]

Determination of PGD synthase in the CSF and serum of patients with various neurologic disorders revealed no concrete associations.[11] Our results in this study demonstrate that PGD synthase concentration is significantly elevated in the serum of patients with renal impairment. Patients with renal impairment had approximately a 35-fold increase in serum PGD synthase levels compared with control subjects (Fig. 1A). This increase is not altered after hemodialysis, unlike the creatinine levels, which decrease significantly (Fig. 1B). The difference in clearance between creatinine and PGD synthase by hemodialysis is likely due to their differences in size. Creatinine can easily diffuse through the pores of the hemodialysis membrane, but PGD synthase, like many other macromolecules of similar size, cannot.

TABLE III. *Association of PGD synthase and creatinine concentration in serum between non-renal failure and renal failure patients* *

	Serum PGD Synthase (μg/L)‡				Serum Creatinine (μmol/L)§			
Patient Group†	0–206	207–359	360–8,812	8,813–13,387	0–81	82–100	101–692	693–1,300
Non-renal failure (n = 30)	14	13	3	0	13	14	3	0
Renal failure (n = 24)	0	0	11	13	0	0	11	13

Key: Abbreviations as in Table I.
* *Chi-square = 44.45; degrees of freedom = 3; P < 0.0001.*
† *The renal failure patient group includes CAPD patients.*
‡ *The four categories are the quartiles of the serum concentration of PGD synthase for all cases.*
§ *The four categories are the quartiles of the serum concentration of creatinine for all cases.*

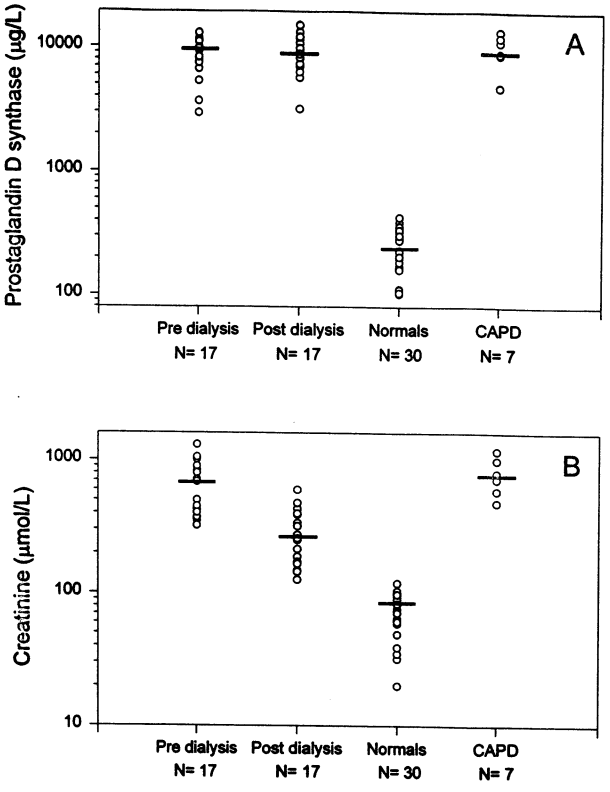

FIGURE 1. *Distribution of PGD synthase (A) and creatinine (B) concentration in serum from patients with renal failure before and after hemodialysis, from non-renal failure subjects, and from CAPD patients. Concentration values are plotted on a logarithmic scale. The median for each dot plot is represented by a horizontal bar.*

Our correlation studies have indicated a weak positive correlation between serum creatinine and serum PGD synthase concentration in the group of control subjects with normal renal function. However, no such correlation was seen when all renal patients, including those with CAPD treatment, were examined. When the patients with renal failure were seperated into groups receiving hemodialysis or CAPD, a correlation was noted between serum creatinine and serum PGD synthase in the latter group only. Although the patient numbers are small and the data need confirmation, we speculate that there is no correlation between creatinine and PGD synthase in the hemodialysis group because creatinine is removed efficiently during hemodialysis, whereas PGD synthase is not (Table IV, Fig. 1). In CAPD, removal of large molecules is more efficient, as is the case with beta-2-microglobulin.[18] Alternatively, patients with CAPD may have some residual renal function.

Olsson and colleagues[19] pioneered the studies of beta-trace or PGD synthase metabolism in human serum. Their studies conducted with ^{125}I-labeled beta-trace protein indicated that it has a short turnover time of approximately 1.2 hours, and approximately 240 mg is metabolized per 24 hours. Almost all of the protein is excreted in urine. In this study we report dramatic elevations of PGD synthase in the serum of individuals with renal dysfunction. Previously, another study suggested that PGD synthase concentration is elevated in the serum of patients with renal dysfunction, but the report was based on only 6 patients, and the technique used, immunoaffinity chromatography, is not suitable for routine use.[20] In our study we used a method that can be easily adapted to routine laboratories and to automated immunoassay analyzers. Moreover, we report that this molecule is not efficiently cleared by either hemodialysis or CAPD.

Our findings can be explained by proposing a model that includes PGD synthase production in the CNS and its accumulation in the CSF at relatively high levels (approximately 7,000 to 27,000 μg/L).[10] From the CSF, PGD synthase diffuses into the serum (levels approximately 100 to 400 μg/L)[10] and then is cleared by the kidneys into the urine (levels approximately 600 to 1600 μg/L).[10] In renal failure, PGD synthase clearance is reduced, and the protein accumulates in serum at levels approaching those of CSF in patients who are either on hemodialysis or CAPD. Alternatively, PGD synthase may be produced by the diseased kidney, but there is no literature supporting this speculation.

Like many other medium and large size molecules, it appears that PGD synthase (molecular

TABLE IV. ***Comparisons of PGD synthase and creatinine concentration between before and after hemodialysis sera from the patients with acute renal failure***

Sample Type	No.	Median PGD Synthase (μg/L)	*P* Value*	Median Creatinine (μmol/L)	*P* Value*
Prehemodialysis	17	9262		686	
Posthemodialysis	17	8797	0.918	259	0.0001

KEY: Abbreviations as in Table I.
** Mann-Whitney P value calculated at the 95% confidence level.*

mass approximately 25 kDa) accumulates in serum of patients with renal failure at much higher levels than in normals. For one of these molecules, beta-2-microglobulin, it is certain that its accumulation and inefficient clearance during hemodialysis or CAPD causes severe chronic complications like amyloidosis.[21–23] The possible chronic complications of PGD synthase accumulation are worth examining because of the knowledge that this molecule is a lipophilic transporter,[24] and its site of production is mainly the CNS. Although we do not have any data, we would expect that the interference of diffusion of PGD synthase from CNS to serum may induce accumulation and possibly deposition of PGD synthase in CNS with possible adverse effects.

In summary, we report dramatic elevation of PGD synthase concentration in serum of patients with renal failure. It will be worth examining if the levels of PGD synthase in serum correlate with the degree of renal sufficiency and if this marker is more sensitive than creatinine in assessing early or minimal renal impairment or renal rejection after transplantation. Given that this molecule is not cleared by hemodialysis, it would be important to examine possible chronic complications related to PGD synthase accumulation in serum or CNS. Furthermore, because our patient population is relatively small, we recommend larger studies to further confirm these potentially important data.

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