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Possible Diagnostic Value of Plasma Prostaglandin Levels in Forensic Autopsy Cases : The Significantly Low Ratio of 6-keto-Prostaglandin F_1 Alpha to Thromboxane B_2 in Person Dying of Cardiovascular Disease

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ABSTRACT

In the present study, the concentrations of eight kinds of prostagladin (PG) in plasma samples obtained at forensic autopsy of 73 persons aged between 0 to 84 years were analyzed with gas chromatography/mass spectrometry. Possible differences in PG behavior in relation to the cause of death and the postmortem interval was clarified by studying concentrations of 6-keto-prostaglandin F_1 alpha (6-keto-$PGF_1\alpha$), the stable metabolite of prostacyclin (PGI_2), and of thromboxane (TX) B_2, the stable metabolite of TXA_2. The measurements of PGs with gas chromatography/mass spectrometry were not affected by the postmortem interval or by age, and the ratios of 6-keto-$PGF_1\alpha$ to TXB_2 in persons dying of cardiovascular disease were significantly lower than in other persons. (Jikeikai Med J 2006 ; 53 : 131-9)

Key words : prostaglandin, 6-keto-prostaglandin F_1 alpha, thromboxane B_2, cardiovascular disease, forensic autopsy

INTRODUCTION

Prostaglandins (PGs) and thromboxanes (TXs) play important roles, particularly in cardiovascular diseases. The balance of TXA_2 and prostacyclin (PGI_2) regulates interactions between platelets and the vessel wall[1].

Many clinical studies have examined the pathophysiological behavior of PGs. However, quantitative analysis of autopsy samples in forensic medicine is considered difficult because the postmortem interval (PMI) and inevitable degenerative changes in PGs were believed to affect the accuracy of measurements. Changes over time in the concentrations of PGE_1 and $PGF_2\alpha$ in postmortem animal tissues have been observed[2] ; however, PGs in samples obtained at forensic autopsy have not been reported.

In the present study, plasma concentrations of PGs in cadaveric blood obtained at forensic autopsy were measured with gas chromatography (GC)/mass spectrometry (MS) and analyzed. The findings suggest concentrations of 8 kinds of PGs in plasma samples obtained at forensic autopsy of 73 persons and an interesting relation between the ratio of the concentrations of TXB_2 and 6-keto-$PGF_1\alpha$ and the cause of death.

MATERIALS AND METHODS

The subjects of this study were 73 forensic autopsy cases examined at the Department of Forensic Medicine, The Jikei University School of Medicine. Age at death ranged from 0 to 84 years. The causes of deaths were determined after complete

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forensic autopsies, including toxicological and histopathological examinations of tissues and fluids.

Blood samples were obtained at autopsy from the right atrium using a sterilized injection syringe. Each blood sample was centrifuged for 15 minutes at 3,000 rpm to separate the plasma. Immediately there after, 0.3 mM of indomethacin was added to each plasma sample, which was thoroughly mixed, and then stored at −80°C until assay.

The extraction and derivatization of PGs were performed according to the procedure of Obata et al[3]. Eight kinds of PGs in plasma were assayed with a GC/MS/selected ion monitoring (SIM) system : TXB_2, 6-keto-$PGF_1\alpha$, $PGF_2\alpha$, $9\alpha,11\beta$-PGF_2, 8-epi-$PGF_2\alpha$, PGD_2, PGE_1, PGE_2.

A GC/MS apparatus (JMS-DX 303, JEOL, Tokyo Japan) equipped with a data-processing system (JMA-DA 5000, JEOL) was used. The column was a 30 m × 0.32 mm internal diameter fused silica capillary (film thickness, 0.1 μm ; DB-1, J&W Scientific, Folsom, CA, USA). The temperature of the column oven was programmed in two-step gradient conditions. The first step was an increase from 100°C to 220°C at 32°C/minute, and the second step was an increase from 220°C to 300°C at 4°C/minute. A solventless injector was set on the injection block of the GC apparatus. The temperature of the injection port and the separator block was 300°C, and the ionization source was maintained at 200°C. Helium was used as a carrier gas. The ionization energy was 70 eV at a mass spectral resolution of 3000. The selected ions monitored were TXB_2 and 6-keto-$PGF_1\alpha$: 670.44 ; $PGF_2\alpha$, $9\alpha,11\beta$-PGF_2, and 8-epi-$PGF_2\alpha$: 625.41 ; PGE_1 : 554.37 ; PGD_2 and PGE_2 : 552.35 ; tetradeuterated TXB_2, tetradeuterated 6-keto-$PGF_1\alpha$: 674.46 ; pentadeuterated $PGF_2\alpha$: 630.45 ; and tetradeuterated PGE_2 : 556.38.

This study was approved by the Ethics Committee of The Jikei University School of Medicine, and samples were anonymized in an unlinkable fashion.

RESULTS

1. *Analytical value of forensic autopsy specimen*

Simultaneous quantitative analysis of 8 kinds of PGs was performed in plasma specimens obtained at autopsy in 73 cases. Death was due to internal causes in 45 cases (61.6%) and to external causes in 28 cases (38.4%, Table 1). Table 2 shows PG concentrations in plasma specimens obtained at forensic autopsy in the 73 cases. Table 3 shows the mean concentrations and median values of the PGs in the 73 plasma specimens.

2. *Reference of each PGs concentration in plasma preparations and PMI*

No correlations were found between the plasma concentrations of each PG and the PMI (5 to 72 hours, Fig. 1).

3. *Reference of each PGs concentration in plasma preparations and age*

No correlations were found between the plasma concentrations of each PG and subject age (0 to 84 years ; Fig. 2).

4. *6-keto-$PGF_1\alpha$ to TXB_2 ratio in a group of cardiovascular disease*

Of 45 deaths due to internal causes, 21 were attributable to cardiovascular disease and 24 were attributable to other diseases. The concentration of TXB_2 was higher than that of 6-keto-$PGF_1\alpha$ in 14 (66.7%) of 21 cases of cardiovascular disease and in 7 (29.2%) of 24 cases of noncardiovascular disease. The mean 6-keto-$PGF_1\alpha$ to TXB_2 ratio was significantly lower in cases of cardiovascular disease (2.40) than in cases of noncardiovascular disease (10.05 ; t-test, $p < 0.05$; Fig. 3).

DISCUSSION

Simultaneous assays of PGs could be performed with GC/MS/SIM in specimens of cadaveric human plasma obtained at autopsy. Although various values of PG in healthy adults have been reported, most values obtained in the present study were higher than those obtained in previous studies[4].

Tissue and blood samples are often collected at forensic autopsy after a long PMI, which has been thought to affect plasma concentrations of PGs

Table 1. Distribution according to the cause of death of 73 forensic autopsies

Cause of death	Number
Intrinsic death	45
Cardiovascular disease	21
Ischemic heart disease	17
Interstitial myocarditis	2
Hypertrophic idiopathic cardiomyopathy	1
Other	1
Central nervous system disease	4
Cerebrovascular disorder	2
Purulent meningitis	1
Other	1
Respiratory system disorder	14
Pneumonia	6
Bronchial asthma	3
Pulmonary thromboembolism	2
Other	3
Digestive disease	4
Alcoholic liver disease	2
Intestinal atresia	2
Other intrinsic death	2
Exsiccation	1
Sudden death	1
Exogenous death	28
Suffocation	6
Poisoning	5
Hemorrhagic shock	3
Intracranial injury	3
Fire	2
Exsanguination	2
Subdural hematoma	2
Drowning	1
Brainstem injury	1
Brain contusion	1
A cervical cord injury	1
Flail chest	1

Table 3. Mean and median value of prostaglandin concentration out of plasma in 73 forensic autopsies

	TXB_2	6-keto-$PGF_1\alpha$	$PGF_2\alpha$	9α,11β-PGF_2	8-epi-$PGF_2\alpha$	PGD_2	PGE_1	PGE_2	(ng/ml)
Mean	4.5881	8.6620	1.0457	0.2789	0.7004	1.2938	0.9072	0.8616	
Standard deviation	8.0306	17.3182	1.7639	0.9794	3.6344	4.0509	4.2169	1.8406	
Median value	0.8562	1.3438	0.4592	0.0533	0.1855	0.0938	0.0596	0.2014	

through metabolism and degeneration and thereby complicate the practical application of their analysis. However, the concentrations of 8 kinds of PGs in cadeveric plasma obtained at 73 forensic autopsies and analyzed simultaneously with GC/MS/SIM showed no correlation with PMI (5 to 72 hours; Fig. 1).

In forensic medicine, concentrations of PGE_1 and

Table 2. PG concentrations in plasma from 73 forensic autopsies

Case No.	Age (years)	Sex	Cause of death	PMI (hours)	Concentration of PG (ng/ml) (ND ; not detected)							
					TXB_2	6-keto-$PGF_1\alpha$	$PGF_2\alpha$	9α,11β-PGF_2	8-epi-$PGF_2\alpha$	PGD_2	PGE_1	PGE_2
1	28	M	Ischemic heart disease	14	0.3990	1.6950	0.0788	N.D.	0.1408	0.5562	N.D.	0.4492
2	38	M	Ischemic heart disease	19	15.6379	0.1741	1.6497	0.0175	0.3301	2.7575	0.1938	1.2890
3	38	M	Ischemic heart disease	13	29.9153	2.2051	3.3663	0.0038	0.5715	0.0525	0.1060	6.7420
4	39	M	Ischemic heart disease	14	5.8482	0.6222	0.6356	0.0653	0.0356	2.0721	0.2051	0.0843
5	39	M	Ischemic heart disease	12	0.5208	0.2618	0.0490	0.0022	0.1016	N.D.	0.0157	0.1844
6	40	M	Ischemic heart disease	8	7.9191	2.3215	0.3699	0.0576	0.0743	0.1224	0.0108	0.3912
7	56	M	Ischemic heart disease	13	20.2412	8.5413	1.6097	N.D.	0.3592	0.0008	0.1400	2.2249
8	57	M	Ischemic heart disease	72	0.5896	0.7416	0.0526	0.0526	0.0364	1.3133	0.0100	0.1070
9	58	M	Ischemic heart disease	16	0.6322	0.2866	1.1294	0.0023	0.1708	0.7932	0.2601	0.1545
10	59	M	Ischemic heart disease	48	0.2349	4.8515	0.6852	0.0203	0.2350	0.2846	0.5764	0.0377
11	60	M	Ischemic heart disease	21	0.2647	0.2001	3.3426	0.1298	0.5462	0.5466	0.0433	0.1199
12	61	M	Ischemic heart disease	21	4.5016	4.1412	1.0725	N.D.	0.6378	1.0436	0.4544	0.2014
13	64	F	Ischemic heart disease	19	3.1355	0.5185	1.4472	0.0102	0.2943	0.0006	0.0391	0.2026
14	64	M	Ischemic heart disease	21	1.4117	4.1206	2.1233	0.2305	0.4014	2.7114	0.5819	0.5228
15	70	F	Ischemic heart disease	22	0.0730	0.0469	0.2454	0.2785	0.5030	0.4644	0.3370	0.1160
16	70	M	Ischemic heart disease	16	0.0661	0.3154	0.0613	0.0068	0.0469	0.0052	0.0596	0.0129
17	79	M	Ischemic heart disease	23	0.4841	0.2071	0.4097	0.0220	0.0331	0.0459	0.1161	0.0170
18	23	M	Interstitial myocarditis	24	2.4539	0.1660	8.6940	8.1214	0.0204	2.4273	0.3903	0.4170
19	41	F	Interstitial myocarditis	11	0.5120	0.3799	0.0598	0.0022	0.1535	0.4747	0.0348	0.2432
20	31	M	Hypertrophic idiopathic cardiomyopathy	22	21.3694	35.5511	1.0881	0.1563	0.3654	0.3123	0.6712	0.8662
21	44	M	Cardiovascular disease/other	8	0.2340	2.1595	0.1607	0.0007	0.1075	0.0491	0.0020	0.0962
22	28	F	Cerebrovascular disorder	8	2.3372	0.3310	0.8558	0.3136	N.D.	N.D.	N.D.	1.8472
23	55	F	Cerebral hemorrhage	16	5.3755	3.2486	0.6704	0.0152	0.1257	0.0053	0.0661	0.6238
24	58	M	Purulent meningitis	10	1.4389	14.1594	1.2370	0.0533	0.2879	0.5479	0.9642	0.3832
25	46	F	Central disorder /other	23	0.1305	9.1648	0.4381	0.1362	0.2129	3.2370	0.0891	0.2097
26	12	M	Pneumonia	8	0.1418	1.9342	2.3586	0.1603	0.3154	0.0234	1.2832	2.1833
27	26	M	Pneumonia	48	0.1136	0.1686	0.2450	N.D.	0.4690	N.D.	N.D.	0.1934
28	44	F	Pneumonia	8	0.0572	4.0707	0.3513	0.0104	0.1263	1.1036	0.1227	0.0862
29	10M	F	Pneumonia	7	0.7016	0.6848	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
30	3M	M	Pneumonia	16	3.2466	0.2176	0.0248	0.1990	N.D.	N.D.	N.D.	N.D.
31	4M	M	Pneumonia	22	1.3942	10.7272	0.7536	1.3344	0.3770	N.D.	N.D.	N.D.
32	13	F	Bronchial asthma	22	0.7996	5.9214	0.1256	N.D.	0.1306	N.D.	N.D.	0.2356
33	38	F	Bronchial asthma	20	10.8820	3.0980	1.1944	0.5028	0.6226	15.8548	N.D.	3.4226
34	68	F	Bronchial asthma	10	0.1853	0.2880	0.0015	0.0024	0.4350	0.0006	0.0532	1.6376
35	23	M	Pulmonary thromboembolism	14	1.0615	1.3278	0.0676	0.1385	0.1249	0.0258	0.0047	0.2458
36	42	F	Pulmonary thromboembolism	10	0.1499	0.3168	0.0049	N.D.	0.2732	0.0021	0.0346	0.0581
37	3	M	Respiratory system disorder/other	38	0.7727	2.5291	0.3252	0.3669	0.0942	0.0108	0.0199	0.1103

Table 2 (Continued)

Case No.	Age (years)	Sex	Cause of death	PMI (hours)	Concentration of PG (ng/ml) (ND ; not detected)							
					TXB_2	6-keto-$PGF_1\alpha$	$PGF_2\alpha$	9α,11β-PGF_2	8-epi-$PGF_2\alpha$	PGD_2	PGE_1	PGE_2
38	25	M	Respiratory system disorder/other	8	9.8986	38.8820	1.3141	1.3967	0.4723	4.6733	1.4150	12.7826
39	38	F	Respiratory system disorder/other	14	0.0202	0.1006	N.D.	N.D.	N.D.	0.0202	N.D.	0.0560
40	31	M	Alcoholic liver disease	11	4.8717	2.2153	0.3983	0.2156	0.1448	0.0830	0.0883	0.0304
41	52	M	Alcoholic liver disease	8	0.0290	0.5232	0.1011	0.0031	0.1277	0.0639	0.0052	0.0042
42	29	M	Intestinal atresia	15	0.3129	0.1592	0.2943	0.0057	0.1156	0.0332	0.0159	0.0065
43	74	F	Intestinal atresia	23	11.1924	20.4755	0.5004	0.0372	0.2040	0.0747	0.0961	1.6488
44	84	M	Exsiccation	21	1.6163	7.1357	5.9000	0.2168	1.4282	0.8465	0.7829	0.2787
45	42	M	Sudden death	24	0.5093	7.8075	0.3073	0.0028	0.2150	0.1593	0.0521	0.4256
46	2	F	Suffocation	41	23.4550	53.2514	1.3883	0.1241	0.3204	0.2643	0.5058	1.2873
47	4	M	Suffocation	36	6.3703	20.8165	10.2757	0.9028	2.2503	0.4192	0.7964	5.0704
48	47	F	Suffocation	18	38.0740	1.2938	0.9950	0.3750	0.5008	10.6236	N.D.	1.8708
49	49	M	Suffocation	21	3.4925	1.5959	0.5772	0.0023	0.0560	0.0666	0.0585	0.0633
50	61	M	Suffocation	17	1.7665	1.1097	0.4242	0.0036	0.1677	0.0075	0.0701	0.3280
51	2M	M	Suffocation	21	2.4594	50.8520	0.3960	N.D.	0.1926	N.D.	27.6106	N.D.
52	16	M	Poisoning	18	0.2158	1.8382	N.D.	0.0560	0.2774	0.1244	N.D.	0.5210
53	22	M	Poisoning	26	0.5949	0.1743	1.4655	0.0547	0.3323	0.3446	0.0862	0.1976
54	54	M	Poisoning	7	0.0584	0.0618	0.0023	0.1117	0.1204	0.0907	0.0216	0.0051
55	57	M	Poisoning	8	0.0368	1.1238	0.0640	N.D.	N.D.	N.D.	N.D.	0.4690
56	84	F	Poisoning	20	6.3896	5.7213	0.1760	0.2027	0.1109	0.0481	0.0037	0.5694
57	44	M	Hemorrhagic shock	39	0.1274	0.0924	0.0254	N.D.	N.D.	N.D.	N.D.	N.D.
58	78	M	Hemorrhagic shock	9	1.0856	0.3806	0.2327	0.1732	0.0594	0.0520	0.0946	0.0205
59	78	M	Hemorrhagic shock	24	0.0366	0.0305	1.3247	0.1179	0.7114	1.1643	0.1428	0.0507
60	21	F	Intracranial injury	20	5.7816	55.1686	N.D.	N.D.	0.0626	N.D.	N.D.	N.D.
61	57	M	Intracranial injury	39	14.0714	3.3945	1.2276	1.1608	0.5377	0.1149	0.1781	2.5556
62	69	M	Intracranial injury	5	0.0104	1.3438	0.2060	0.0023	0.1469	0.0224	0.0110	0.1925
63	37	F	Fire	7	1.3286	0.8451	0.9221	0.2542	0.1150	4.8543	0.0335	0.0509
64	42	M	Fire	10	32.3362	24.1030	0.1766	0.2096	0.3028	0.0938	23.8022	0.1612
65	48	M	Exsanguination	24	0.0350	0.0400	0.9390	0.2067	0.2938	0.8090	0.1411	0.0176
66	60	M	Exsanguination	7	0.2403	26.3922	0.4645	0.3858	0.1298	0.6281	1.1788	2.0378
67	6	F	Subdural hematoma	12	0.2218	0.5029	0.4592	0.0229	0.0397	0.0150	0.0660	0.0338
68	62	M	Subdural hematoma	19	3.7646	22.8147	3.2683	0.3392	0.7299	0.8630	0.0504	1.9721
69	33	M	Drowning	6	0.0617	0.1279	0.6288	0.0432	0.1855	29.0932	0.4406	0.0327
70	46	M	Brainstem injury	29	4.1096	92.8884	3.4964	1.1551	0.5268	0.9898	1.3738	1.9577
71	33	M	Brain contusion	6	14.2178	61.1807	0.4755	0.1139	0.0969	0.3375	0.0242	2.0210
72	60	M	Cervical cord injury	12	0.0483	0.1463	0.7543	0.0538	0.1979	0.6188	0.1954	0.1222
73	50	M	Flail chest	12	0.8562	0.0402	0.1682	N.D.	31.2000	N.D.	N.D.	0.3362

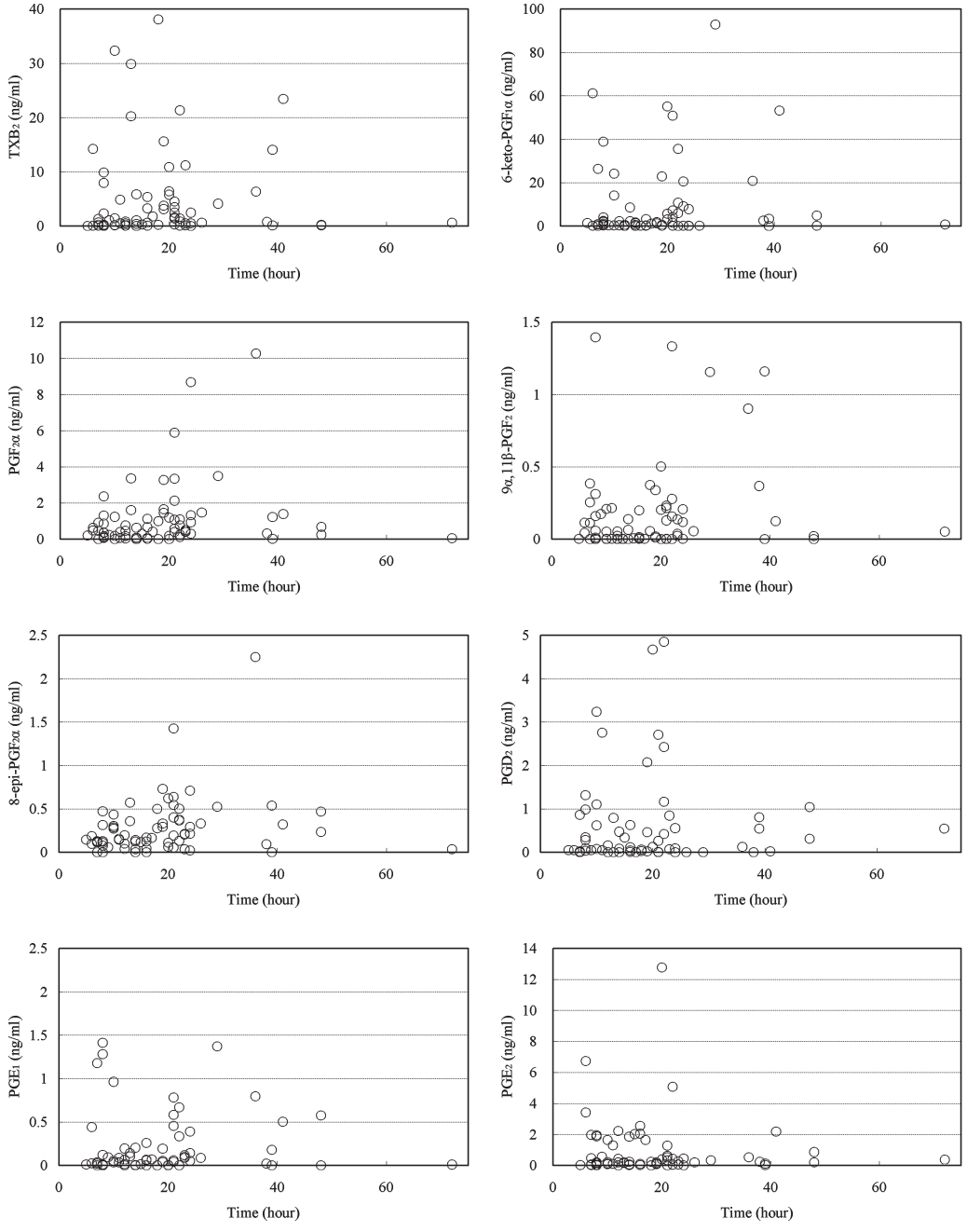

Fig. 1. The relation between PG concentrations of plasma and the PMI (5 to 72 hours) for 73 cases in forensic autopsy. PG levels were not correlated with the PMI.

$PGF_{2}\alpha$ in rat organs have been measured with radioimmunoassy by Mitsuhashi[2]. Mitsuhashi found a pattern of the time courses of changes in concentration of PGE_1 and $PGF_{2}\alpha$ according to the organ (brain, heart and kidney) and the cause of death (potassium chloride intravenous infusion, suxamethonium chloride intravenous infusion, and acute carbon monoxide poisoning). Although the present study differed from that of Mitsuhashi, both studies have found that the PMI does not affect PG values.

In addition, production of PGI_2 has been reported to decrease with aging[5], but no such decrease was found in the present study (Fig. 2). It was thought that disease state affected PG value than age. There-

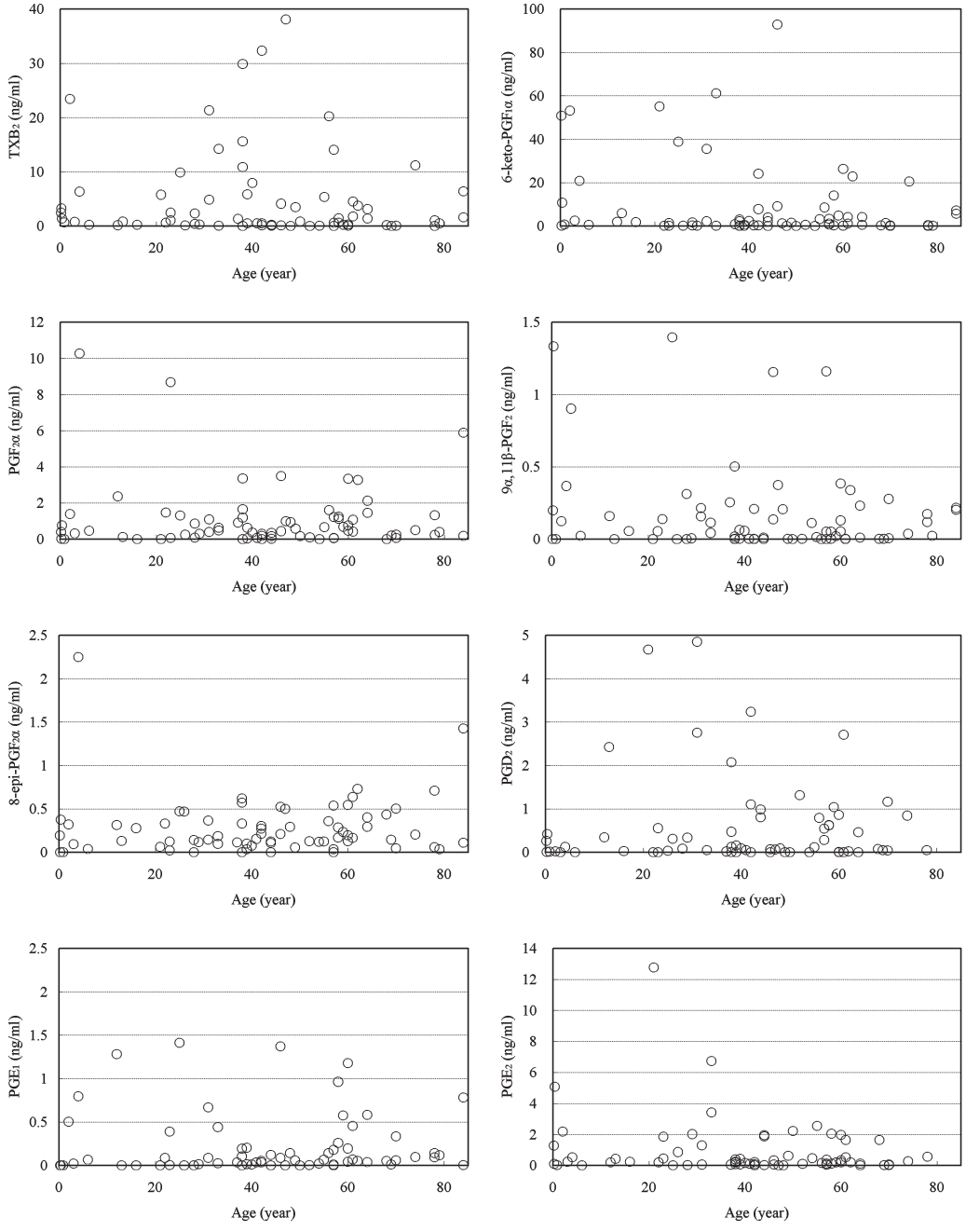

Fig. 2. The relation between PG concentrations of plasma and subject age (0 to 84 years) for 73 cases in forensic autopsy. PG levels were not correlated with subject age.

fore, the present study suggests that plasma concentrations of PGs can be applied to pathophysiological analysis.

PGs play important roles, particularly in cardiovascular diseases. The balance of TXA_2 and PGI_2 regulates interactions between platelets and the vessel wall[1]. Comparisons of the ratio, rather than of absolute values, of TXB_2 and 6-keto-$PGF_1\alpha$, metabolites of TXA_2 and PGI_2, have been reported, with TXB_2 concentrations, ranging from 10 pg/ml to 10 ng/ml (4, 6–9). The yield of TXA_2 is enhanced and that of PGI_2 decreases in cases of acute coronary disease, particularly myocardial infarction[10,11]. In addition, a change in the balance 6-keto-$PGF_1\alpha$ and TXB_2 is

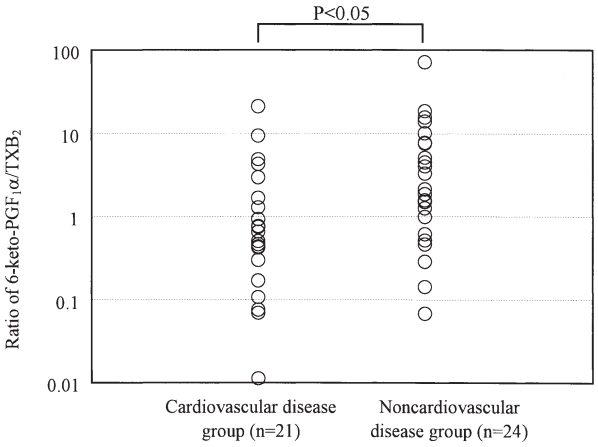

Fig. 3. The relation between ratios of 6-keto-$PGF_1\alpha$ to TXB_2 in the cardiovascular disease group (21 cases) and the noncardiovascular disease group (24 cases). In the cardiovascular disease group, the mean 6-keto-$PGF_1\alpha$ to TXB_2 ratio was significantly lower (2.40), than in the noncardiovascular disease group (10.05 ; t-test, $p<0.05$).

reported about arteriosclerosis, myocardial infarction[12], and vascular diseases[13]. Murota reported that arterial sclerosis is inhibited if PGI_2 levels are high[5].

As shown in Figure 3, the mean 6-keto-$PGF_1\alpha$ to TXB_2 ratio in persons dying of cardiovascular disease (2.40) was significantly lower than that in persons dying of noncardiovascular diseases (10.05 ; t-test, $p<0.05$). Although levels of 6-keto-$PGF_1\alpha$ value are generally higher than those of TXB_2 in healthy adults, levels of TXB_2 were higher than those of 6-keto-$PGF_1\alpha$ value in two thirds of persons dying of cardiovascular disease group in the present study. This finding indicates that the amount of TXB_2 was less than that of 6-keto-$PGF_1\alpha$ in persons dying of noncardiovascular diseases but was greater than that of 6-keto-$PGF_1\alpha$ in persons dying of cardiovascular diseases. From TXB_2 and 6-keto-$PGF_1\alpha$ being metabolite of TXA_2 and PGI_2, this finding is consistent with findings of previous reports.

These results suggest that the determination of PG levels can be applied to forensic medicine.

CONCLUSION

Quantitative PG analysis in forensic samples is rarely done, because PG concentrations are believed to be modified by the metabolism of PGs and physical changes due to the PMI. In this study, 8 kinds of PGs (TXB_2, 6-keto-$PGF_1\alpha$, $PGF_2\alpha$, 9α,11β-PGF_2, 8-epi-$PGF_2\alpha$, PGD_2, PGE_1, and PGE_2) were assayed with GC/MS in plasma samples obtained at autopsy. The results show that the concentrations of these PGs are not affected by subject age or PMI.

The present study has also found that the ratio of 6-keto-$PGF_1\alpha$ to TXB_2 in plasma is significantly lower in persons who have died of cardiovascular disease. This finding suggests that measurements of 6-keto-$PGF_1\alpha$ and TXB_2 might be used to diagnose suspected cardiovascular disease at autopsy.

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REFERENCES

1. Muller B. Pharmacology of thromboxane A_2, prostacyclin and other eicosanoids in the cardiovascular system. Therapie 1991 ; 46 : 217-21.
2. Mitsuhashi H. Time course of changes in concentration of prostaglandin E_1 and $F_2\alpha$ in postmortem tissues in deaths from various causes (in Japanese). Jpn J Legal Med 1979 ; 33 : 252-8.
3. Obata T, Nagakura T, Kammuri M, Masaki T, Maekawa K, Yamashita K. Determination of 9α, 11β-prostaglandin F_2 in human urine and plasma by gas chromatography-mass spectrometry. J Chromatogr 1994 ; B 655 : 173-8.
4. Nagakura T, Obata T. Positioning in disease state of fat mediator : prostaglandin (in Japanese). Gendaiiryou 1997 ; 29 Suppl IV : 2857-63.
5. Murota. S. Prostaglandin (in Japanese). Gendaikagaku 1980 ; 114 : 12-22.
6. Hornych A, Krief C, Bariety J. Radioimmunoassay

measurement of thromboxane B_2 in human plasma and urine. Prostaglandins Leukot Med 1982 ; 8 : 467–80.
7. Rubin PD, Murthy VS, Hansen RG, Pietrowiak C. Dissociation and diurnal variation of prostaglandin E_2 6-keto-prostaglandin $F_1\alpha$, and thromboxane B_2 excretion in healthy females. Prostaglandins Leukot Med 1987 ; 27 : 105–17.
8. Westlund P, Granstrom E, Kumlin M, Nordenstrom A. Identification of 11-dehydro-TXB_2 as a suitable parameter for monitoring thromboxane production in the human. Prostaglandins 1986 ; 31 : 929–60.
9. Kurimoto F. Thromboxane B_2 assay and the clinical significance. Jpn J Clin Med 1990 ; 48 (Suppl) : 167–70.
10. Kawai C, Fujiwara H. Pathogenesis of acute myocardial infarction and new regulatory mechanism of vasoactive substances. Ther Res 1991 ; 12 : 3789–812.
11. Tada M, Kazuya T, Inoue M, Kodama K, Mishima M, Yamada M, et al. Elevation of thromboxane B_2 levels in patients with classic and variant angina pectoris. Circulation 1981 ; 64 : 1107–15.
12. Samuelsson B, Goldyne M, Granstrom E, Hamberg M, Hammarstrom S, Malmsten C. Prostaglandins and thromboxanes. Annu Rev Biochem 1978 ; 47 : 997–1029.
13. Mizugaki M. Establishment of microanalysis of prostaglandin metabolites by GC/MS and its clinical application (in Japanese). Yakugaku Zasshi 1999 ; 119 : 61–80.