Monitoring for anti-Xa activity for prophylactic administration of Fondaparinux in patients with artificial joint replacement

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Key words; deep vein thrombosis (DVT), total hip replacement (THR), total knee replacement (TKR), anti-Xa activity, Fondaparinux

Running title; anti-Xa activity in prophylaxis by Fondaparinux

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Abstract

The efficacy of measuring anti-Xa activity was evaluated in major orthopedic surgery patients receiving thrombo-prophylaxis with Fondaparinux. Although 98 orthopedic patients including those receiving total hip replacement (THR) and total knee replacement (TKR), were treated with 1.5 mg of Fondaparinux for prophylaxis of deep vein thrombosis (DVT). Sixteen patients developed DVT, but none were associated with a fatal pulmonary embolism. There was a wide range of anti-Xa activity, but there were no patients with less than 0.15 mg/L or more than 0.90 mg/L. Anti-Xa activity gradually increased from day 1 to day 8 and showed no significant difference between patients with and without DVT. Anti-Xa activity was correlated with weight, height, body mass index, and antithrombin activity. Postoperative plasma levels of D-dimer and soluble (SF) were markedly high, and those were significantly reduced at day 1 and 4 of treatment with Fondaparinux. Plasma levels of SF were significantly reduced at day 8 and 15, but D-dimer was not. These findings suggested that there was continued thrombin generation after the injection of Fondaparinux until day 8 and secondary fibrinolysis occurred

on day 8. In conclusion, 1.5 mg of Fondaparinux may not be sufficient for the prophylaxis of silent DVT, but it was found to be useful for that of fatal pulmonary embolism. Consequently, monitoring anti-Xa activity may be unnecessary for the administration of Fondaparinux at such doses.

Introduction

Orthopedic surgery is associated with a very high rate of postoperative venous thromboembolism (VTE) [1, 2], the incidence of venographically proven VTE ranges from 45 to 57% after total hip replacement (THR) surgery in the absence of thrombo-prophylaxis, and 40 to 84% after total knee replacement (TKR) surgery [1]. Multiple studies [3-7] have established the superior efficacy of low-molecular-weight heparin (LMWH) over unfractionated heparin (UFH) or warfarin for VTE prophylaxis in orthopedic surgery patients, with relative risk reductions ranging from 44% to 70%, depending on the type of surgery. The incidence of symptomatic postoperative breakthrough VTE is considerably lower (1% to 4%) [3-6] and studies have demonstrated that 40% to 90% of such episodes manifest as proximal deep venous thrombosis (DVT) [3, 4], which is associated with a high risk of pulmonary embolism (PE)[7].

Fondaparinux is the first selective factor Xa inhibitor approved for use in thrombo-prophylaxis after orthopedic surgery [8-10] and studies comparing Fondaparinux to LMWH showed very efficient in thrombo-prophylaxis in patients after orthopedic surgery [9, 10]. Fondaparinux is frequently administered at a dose of 1.5 mg instead of 2.5 mg of Fondaparinux carried

out in Japan to avoid serious bleeding. No method has so far been clinically established to monitor for drugs because a sufficient prolongation of activated partial thromboplastin time (APTT) cannot be observed in patients treated with Fondaparinux or LMWH. Anti-Xa activity has been measured as UFH or LMWH activity [11, 12].

This study measured the anti-Xa activity of Fondaparinux in 98 orthopedic patients after THR or TKR and examined the relationship between anti-Xa activity and various factors.

Materials and Methods

Ninety-eight orthopedic patients treated with 1.5 mg of Fondaparinux (GlaxoSmithKline, Tokyo, Japan) and intermittent pneumatic compression for prophylaxis of DVT from February 1, 2010, to December 31, 2010 were registered in this study (**Table 1**). Anti-Xa activity, fibrin and fibrinogen degradation products (FDP), D-dimer, soluble fibrin (SF) and antithrombin (AT) activity were measured in 73 patients after THR and 23 patients after TKR on day 1, day 4, day 8, and day 15 of the administration of Fondaparinux. The patients received 1.5 mg of Fondaparinux by

hypodermic injection once a day during 14 days beginning 24 hours after extubation of epidural anesthesia. The anti-Xa activity was monitored 3 hours after injection of Fondaparinux. The study protocol was approved by the Human Ethics Review Committee of the Mie University School of Medicine and a signed consent form was obtained from each subject. This study was faithfully carried out in accordance with the Declaration of Helsinki.

The anti-Xa activity of Fondaparinux was measured using Testzym® Heparin S (Sekisui Medical Co. Ltd. Japan) and a Coagrex®800 (an instrument from Sysmex Co. Ltd.). Testzym® Heparin S consists of bovine Xa (71 nkat/vial), AT (10 IU/vial), Chromogenic substrate (S-2222: Benz-Ile-Glu-Gly-Arg-pNA・HCl 25mg), pooled lyophilized normal plasma, and buffer (pH8.4)[11, 12]. A standard curve was made up for the lyophilized normal plasma using various concentrations of Fondaparinux.

The reagents and objects were loaded into the Coagrex 800, and the anti-Xa activity of Fondaparinux was automatically measured. A 135 μ L aliquot of Xa was added to 8 μ L of plasma (with diluent solution added in advance), and 75 μ L of substrate was added. The rate at which the p-NA

was released was measured photometrically at 405nm. The anti-Xa activity of Fondaparinux was then calculated using the standard curve.

Plasma levels of FDP, D-dimer and SF were measured by the latex agglutination method using Nanopia FDP, Nanopia D-dimer and Nanopia SF (Sekisui Medical), respectively [13]. The plasma levels of AT were measured by chromogenic substrate using a Testzym S ATⅢ kit (Sekisui Medical).

The diagnosis of DVT was carried out by echography before the operation, on day 4 and day 14.

Statistical analysis

The data are expressed as the medians (25%-75% tile) or (95%CI). The differences between the groups were examined using the Mann-Whitney U test. A *P* -value of less than 0.05 was considered to be statistically significant. The correlations between 2 variables were tested by Pearson's correlation analysis. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo).

Results

The median (95% CI) of anti-Xa activity was 0.02 (0.0 – 0.16) mg/L, 0.30 (0.19 – 0.54) mg/L, 0.40 (0.23 – 0.70) mg/L, 0.47 (0.26 – 0.73) mg/L and 0.22 (0.02 – 0.51) mg/L in before, and on day 1, day 4, day 8 and day 15 of the administration of Fondaparinux, respectively (**Figure 1**). There was a wide range of anti-Xa activity but there were no patients with less than 0.15 mg/L or more than 0.90 mg/L. The anti-Xa activity from day 1 to day 8 was significantly high in comparison to day 0 (before treatment) ($p< 0.001$), and gradually increased during this period.

Table 2 shows the relationships between anti-Xa activity and various factors. The correlation coefficient (r value) was high with AT before the injection, with weight, height, body surface area (SBA), AT, creatinine and estimated glomerular filtration rate (eGFR) at day 1, with weight, SBA and AT at day 4, and with weight, height, body mass index (BMI), SBA and AT at day8. There were 18 patients with a reduction of more than 2 g/dl of hemoglobin from the beginning of Fondaparinux injection to day 15, but no fatal bleeding. There was no significant difference in anti-Xa activity between the patients with and without a reduction of more than 2 g/dl of hemoglobin during the above period.

Sixteen patients developed DVT, despite prophylaxis with Fondaparinux, but there was no incidence of fatal PE. Only one case developed proximal DVT. **Figure 2** shows that there was no significant difference in anti-Xa activity between patients with and without DVT.

The plasma levels of FDP, D-dimer and SF were markedly high before the injection of Fondaparinux, and those were significantly lower at day 1 and day 4 in comparison to those before the injection (**Figure 3**). The plasma levels of SF were significantly lower at day 8 and day 15 in comparison to those before the injection but the levels of FDP and D-dimer were not lower. Plasma SF levels were also high at day 1, 4 and 8 in comparison to those in healthy volunteers (less than 5.5 μ g/ml).

Discussion

In the artificial joint replacement of our hospital, there were more than 15% of patients with reduction of more than 2 g/dl of hemoglobin from the beginning of the 2.5 mg Fondaparinux injection until day15. Therefore, the patients with artificial joint replacement were treated with 1.5 mg instead of 2.5 mg Fondaparinux in this hospital. Indeed, 1.5 mg Fondaparinux is often used in Japanese patients with a low weight, renal failure or who pose

a high risk. Table 1 shows that most of those patients were females, old patients and low weight individuals.

Sixteen of 98 cases receiving prophylaxis with 1.5 mg Fondaparinux developed DVT, but 15 cases were distal DVT, which has a low risk for PE. Several previous studies [3-6] of orthopedic surgery patients found that the rates of symptomatic VTE after similar durations of LMWH prophylaxis ranged from 1% to 4%. There was only one case of proximal DVT in the current cohort, thus suggesting that the injection of 1.5 mg Fondaparinux is useful for the prophylaxis of proximal DVT following THR or TKR. Clinical examination for DVT in the context of orthopedic surgery has poor predictive value [14]; therefore patients with symptoms caused by the surgery itself (e.g, pain, lower leg swelling) may have DVT diagnosed by venous ultrasound and, thereby the DVT is misclassified as symptomatic.

There was a wide range of anti-Xa activity in the patients treated with Fondaparinux, suggesting that high dose administration of Fondaparinux should be monitored by the anti-Xa activity. However, it might not be necessary to monitor anti-Xa activity following the injection of 1.5 mg Fondaparinux. Indeed, there was no significant difference in the anti-Xa

activity between patients with and without DVT, and the highest anti-Xa activity was less than 1mg/L. The plasma levels of FDP, D-dimer and SF were markedly high from day 1 to day 8. D-dimer remained elevated long after the onset of DVT but that of SF was short [15]. These finding suggested that the generation of thrombin continued until day 8. While, a re-elevation of FDP and D-dimer indicated that secondary fibrinolysis may occur from day 8 to day 15. The dose of Fondaparinux might be not sufficient.

The anti-Xa activity was significantly correlated with weight, height, BMI, SBA and AT. Obesity with BMI > 25 also increases the risk of postoperative symptomatic VTE, which is likely related to an insufficient dosage of LMWH and the ineffectiveness of mechanical prophylaxis such as pneumatic compression [16]. The current study did not find independent associations between traditional VTE risk factors and breakthrough VTE. There is a relationship between the concentration of Fondaparinux and renal function, but only a slight correlation was observed in this study. The AT activity was low after the operation (“before” the injection and on “day1” of the injection) and it significantly correlated with the anti-Xa

activity, thus suggesting that the patients with a reduced AT activity may therefore have a low anti-Xa activity.

Studies [17] of symptomatic DVT after orthopedic surgery show that the proportion proximal DVTs ranges from 50 to 90% in THR patients and from 40 to 50% in TKR patients; however, only about 1.0% of the DVTs diagnosed in the current patients were proximal, and there were no pulmonary emboli. Most postoperative DVTs begin in the deep veins of the calf. Isolated distal DVT has a negligible rate of PE; however, one in six asymptomatic distal DVTs, and up to one in three symptomatic distal DVTs will extend to involve the proximal veins without treatment [7].

In conclusion, the administration of 1.5 mg Fondaparinux was useful for the prevention of fatal PE, but this amount of Fondaparinux might not be sufficient for the prophylaxis of the silent DVT. The monitoring of the anti-Xa activity may not be necessary for this amount of Fondaparinux.

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Conflict of interest statement

All authors disclose no financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

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Figure legends

Figure 1 Anti-Xa activity in patients treated with Fondaparinux.

The blood was sampled 3 hours after injection of Fondaparinux.

***; P< 0.001

Figure 2 Anti-Xa activity in patients treated with Fondaparinux with or without DVT.

The blood was sampled 3 hours after injection of Fondaparinux.

Figure 3-A FDP levels in patients treated with Fondaparinux.

***; P< 0.001, *; p< 0.05

Figure 3-B D-dimer levels in patients treated with Fondaparinux.

***; P< 0.001, **; p< 0.01

Figure 3-C SF levels in patients treated with Fondaparinux.

***; P< 0.001, **; p< 0.01

Table 1 Patients' Characteristics

	Median (25% - 75%)
Age (years old)	68.0 (61.0 – 75.0)
Female：male	75：23
THA:TKA	73：25
Weight (kg)	57.1 (50.1 - 66.9)
Height (cm)	153.0 (147.5 - 158.5)
Body mass index (kg/m^2)	24.2 (21.9 - 27.0)
Body surface area (cm^2)	1.52 (1.44 - 1.68)
Creatinine (mg/ml)	0.67 (0.56 - 0.80)
eGFR	73.0 (59.2 - 85.2)
Hemoglobin (Hb, pre; g/dl)	12.2 (11.4 - 12.8)
Reduction of Hb from the beginning of Fondaparinux injection to day 15 (g/dl)	1.20 (0.68 - 1.60)
Antithrombin (pre; %)	81.5 (72.5 - 88.8)

Table 2 Relationships between the anti-Xa activity and various factors

	age	weight	height	BMI	SBA	FDP	D-dimer	SF	AT	Creatinine	eGFR	Hb
Before	-0.088 (NS)	-0.040 (NS)	0.046 (NS)	-0.090 (NS)	-0.008 (NS)	0.099 (NS)	0.150 (NS)	-0.077 (NS)	0.247 (p<0.05)	-0.186 (NS)	0.185 (NS)	0.083 (NS)
Day 1	-0.175 (NS)	-0.275 (p<0.01)	-0.223 (p<0.05)	-0.166 (NS)	-0.284 (p<0.01)	0.068 (NS)	0.057 (NS)	0.005 (NS)	0.342 (p<0.01)	-0.222 (p<0.05)	0.252 (p<0.05)	-0.107 (NS)
Day 4	-0.019 (NS)	-0.237 (p< 0.05)	-0.142 (NS)	-0.168 (NS)	-0.223 (p<0.05)	-0.017 (NS)	-0.014 (NS)	-0.726 (NS)	0.350 (p<0.01)	0.163 (NS)	-0.247 (NS)	-0.421 (NS)
Day 8	-0.074 (NS)	-0.524 (p<0.001)	-0.237 (p<0.05)	-0.429 (p<0.001)	-0.477 (p<0.001)	0.005 (NS)	-0.042 (NS)	0.064 (NS)	0.446 (p<0.001)	0.076 (NS)	-0.116 (NS)	0.083 (NS)

Data show the correlation coefficient

BMI; body mass index, SBA; body surface area, FDP; fibrin and fibrinogen degradation products, SF; soluble fibrin, AT; antithrombin, eGFR; estimated glomerular filtration rate, Hb; hemoglobin

Table 3 Effects of Fondaparinux on the fibrin related markers and AT activity

	FDP(μ g/ml)	D-dimer (μ g/ml)	SF (μ g/ml)	AT (%)
Before	16.3 (10.1 - 30.3)	8.3 (4.6 - 17.3)	18.4 (10.7 - 42.2)	81.5 (72.5 - 88.8)
Day1	12.7 (9.5 - 17.9)*	6.1 (4.1 - 9.4) **	12.5 (7.7- 19.2) **	83.0 (75.3 - 90.7)
Day4	12.0 (9.7 - 14.4)***	5.4 (4.3 - 7.2) ***	11.8 (8.2- 17.1) ***	92.8 (84.2 - 103.7)***
Day8	14.9 (11.2 - 19.7)	8.2 (5.6 - 10.4)	7.0 (4.6 - 11.2) ***	99.9 (92.1 - 113.2)***
Day15	15.3 (11.0 - 20.3)	7.8 (5.6 - 11.6)	4.500 (2.9 - 8.0) ***	94.5 (88.1- 104.6) ***

Data express the median (25-75% tile)

*, **, ***; $p< 0.05$, $p< 0.01$ and $p< 0.001$ in comparison to “before”.

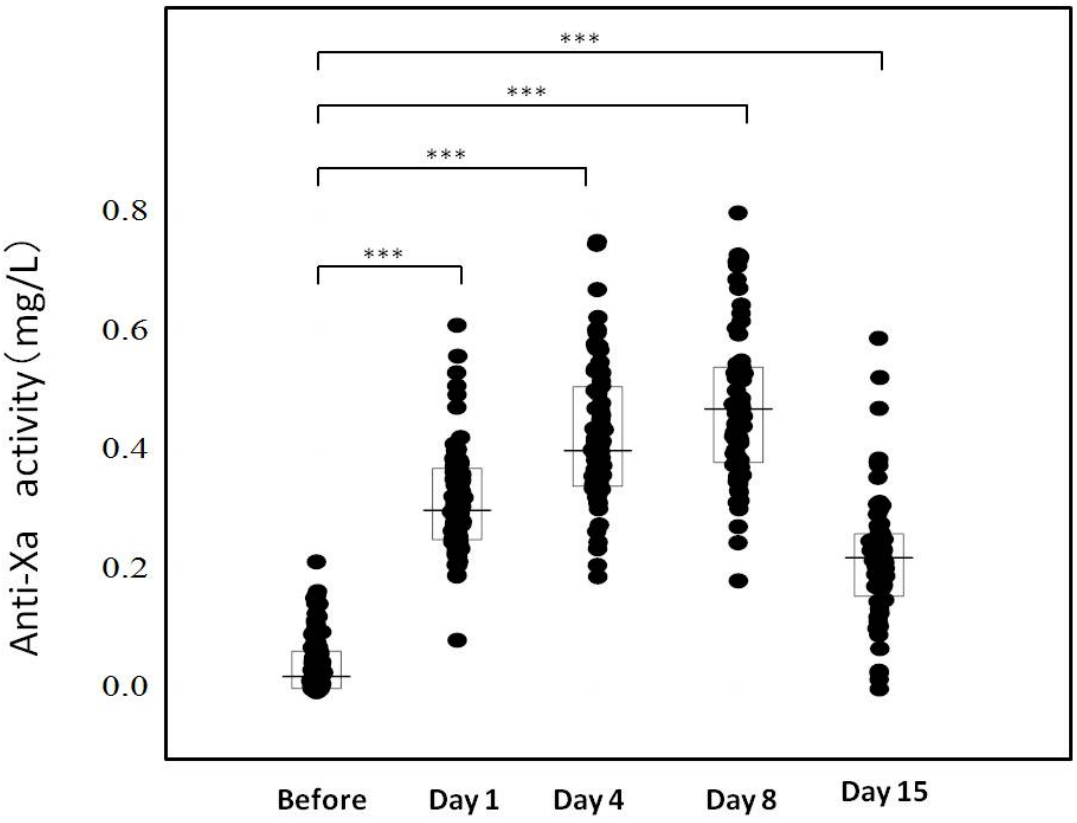

Figure 1 Anti-Xa activity in patients treated with Fondaparinux.

The blood was sampled 3 hours after injection of Fondaparinux.

***; P< 0.001

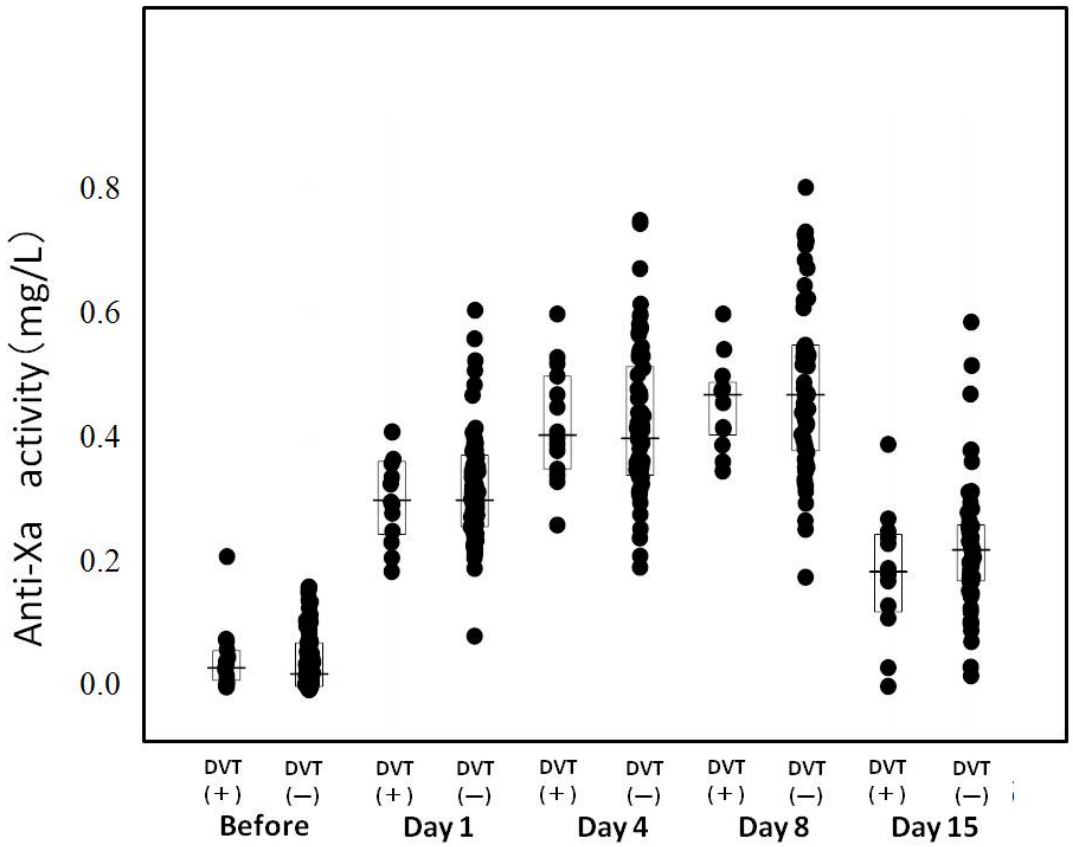

Figure 2 Anti-Xa activity in patients treated with Fondaparinux with or without DVT.

The blood was sampled 3 hours after injection of Fondaparinux.

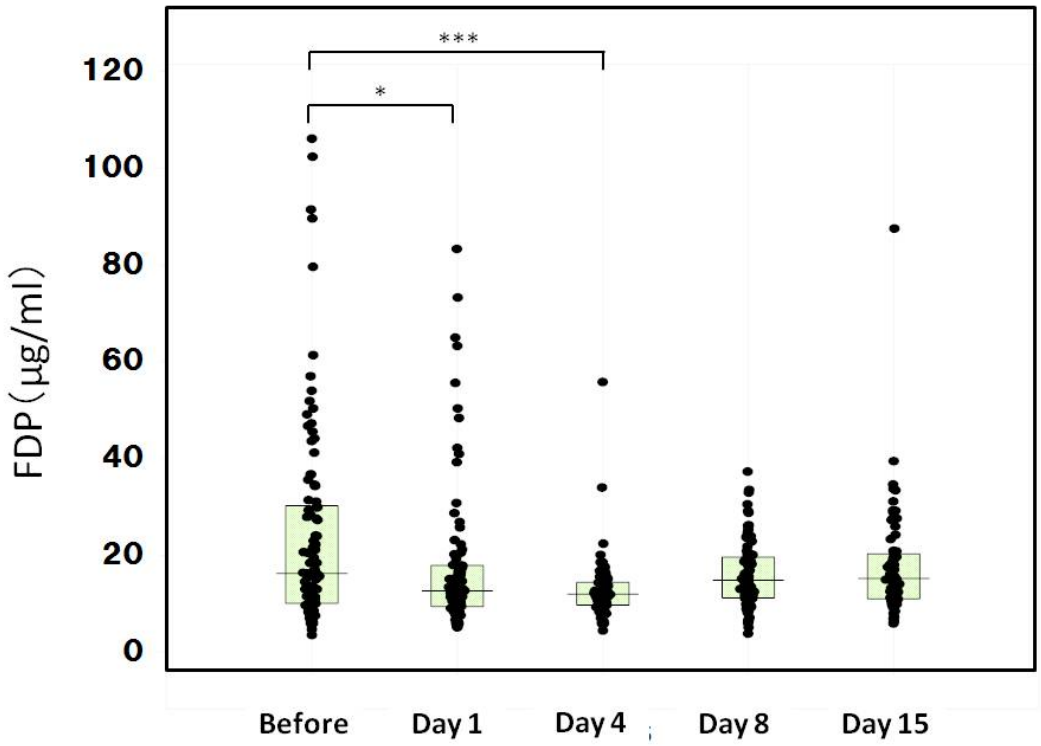

Figure 3-A FDP levels in patients treated with Fondaparinux.

***; P< 0.001, *; p< 0.05

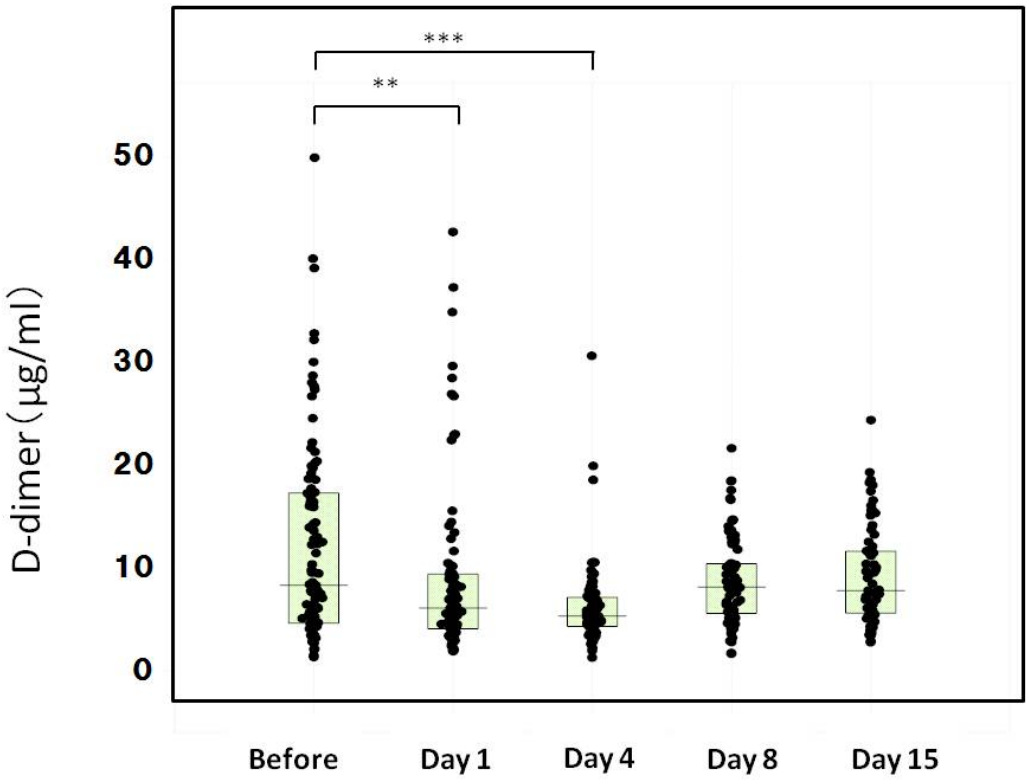

Figure 3-B D-dimer levels in patients treated with Fondaparinux.

***; P< 0.001, **; p< 0.01

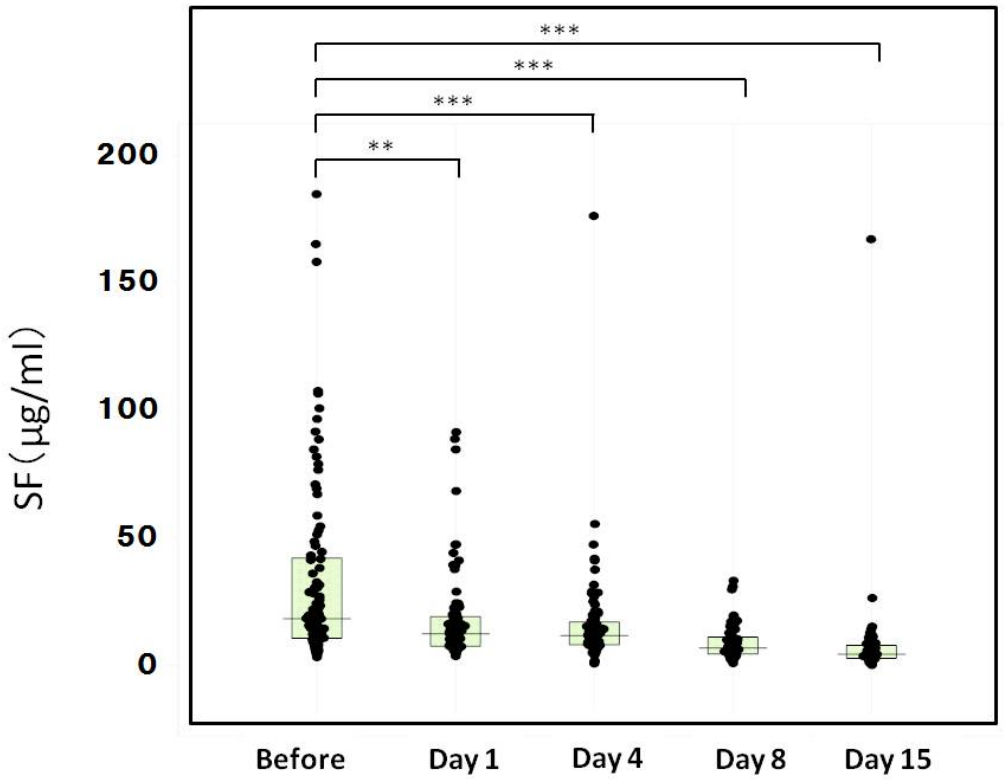

Figure 3-C SF levels in patients treated with Fondaparinux.

***; P< 0.001, **; p< 0.01