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A phase III clinical trial of a mixture agent of plasma-derived factor VIIa and factor X (MC710) in haemophilia patients with inhibitors

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Abstract

Introduction: MC710, a 1:10 protein weight ratio mixture of plasma-derived activated factor VII (FVIIa) and factor X (FX), is a novel bypassing agent for haemostasis in haemophilia patients with inhibitors. We evaluated the haemostatic efficacy and safety of one to two administrations of MC710 in 21 joint, muscle, and subcutaneous bleeding episodes in 14 male patients, in a multi-center, open-label, non-randomized clinical trial.

Methods: Subjects were intravenously administered one or two doses of 60 or 120 μg kg^{-1} MC710 (as FVIIa) once or twice (to a maximum of 180 μg kg^{-1}) over up to five bleeding episodes per subject. The haemostatic efficacy of MC710 was determined for each episode by investigator evaluation using changes in visual analogue scale (VAS) for pain relief, and/or knee joint or muscle circumference for swelling reduction, and range of motion (ROM) for improvement of joint mobility.

Results: In 21 treatments for bleeding episodes, 19 were rated "excellent" or "effective" 8 h after the last treatment. VAS significantly decreased over time, and ROM significantly improved over time compared with the values before treatment. One mild adverse reaction, decreased blood potassium, and two serious adverse events, both knee joint bleeding, were observed within one week after first administration, with no significant effect on safety. Furthermore, diagnostic markers did not show any signs of disseminated intravascular coagulation (DIC).

Conclusion: These results show that MC710 has sufficient haemostatic efficacy and safety, and can be used as a potential bypassing agent to control bleeding in haemophilia patients with inhibitors.

Keywords (6)

bypassing agents, activated factor VII, factor X, haemophilia patient with inhibitors, haemostatic efficacy, safety

Introduction

Replacement therapy for deficient factors using factor VIII (FVIII) and factor IX (FIX) products is the most efficient haemostatic therapy for haemophilia patients. However, some patients develop FVIII and FIX neutralizing antibodies (inhibitors) after repeated infusions, and making it difficult to continue haemostatic therapy **[1,2]**. For the treatment of haemophilia patients with inhibitors, bypassing agents, such as recombinant activated factor VII (rFVIIa; NovoSeven®, Novo Nordisk A/S, Bagsværd, Denmark) and activated prothrombin complex concentrates (APCC; FEIBA®, Baxter International Inc., Deerfield, IL, USA), are generally used in acute bleeding and during surgeries **[3-5]**. These bypass FVIII and FIX, which are blocked by inhibitors, in the coagulation cascade **[6, 7]**. However, if the haemostatic effect of one bypassing agent is insufficient, the patients require another product or a combination of the two bypassing agents **[8-10]**.

MC710 is a new formulation intended for bypass therapy. It contains highly purified coagulation factors, FVIIa and FX, from donated blood plasma in a 1 to 10 protein weight ratio **[11]**. To minimize the risk of virus infection, there are three virus removal/inactivation steps in the MC710 production process: virus removal membrane filtration (average pore size of 15 nm), solvent/detergent treatment, and dry heating at 65°C for 96 h **[12]**. MC710 promotes FVIIa-mediated FX activation due to an enhanced enzyme/substrate (FVIIa/FX) reaction in the extrinsic pathway of blood coagulation, leading to increased thrombin generation and fibrin clot formation to stop bleeding **[13]**. Further, FX has a longer half-life than FVIIa, and blood FX levels will remain high after MC710 administration, even after FVIIa levels have decreased. This could contribute to a sustained haemostatic effect by utilizing endogenous FVIIa, which is present in plasma at a concentration of approximately 3.1 ng mL^{-1} in healthy adults **[14]**.

We previously reported results from a phase I clinical pharmacological study of MC710 in haemophilia patients with inhibitors in a non-bleeding state **[15, 16]**, and a phase II dose-exploratory study in haemophilia patients with inhibitors during joint bleeding **[17]**. In the phase I study, pharmacokinetic (PK) and pharmacodynamic (PD) parameters were examined to assess MC710 dose response in a dose range of 20 to 120 μg kg^{-1} (FVIIa is used to describe MC710 dosage) **[15]**. In addition, PD parameters, including activated partial thromboplastin time (APTT), clot waveform analysis (CWA), and thrombin

generation test (TGT), were analyzed to compare MC710 efficacy with that of the positive controls rFVIIa and APCC **[16]**. Furthermore, the acceptable safety profile of MC710 administered at doses up to 120 μg kg^{-1} demonstrated the tolerability of that dose. In the phase II study, MC710 was administered at a single dose of 60 or 120 μg kg^{-1} to patients with mild to moderate joint bleeding. The results demonstrated sufficient efficacy (haemostatic effect) and safety, and the clinical dose range was determined to be 60 to 120 μg kg^{-1} **[17]**. With the administration of 60-120 μg kg^{-1} MC710, FVIIa and FX levels in plasma should reach 1.5-2.0 μg mL^{-1} (30-40 nM) and 20-35 μg mL^{-1} (400-700 nM), respectively **[16]**. Under these conditions, FX is increased 2.5-4.3 times the K*m* values (160 nM **[13]**) against FVIIa, and the enzyme reaction rate of FVIIa accelerates and approaches V_{max}.

In this phase III study, we evaluated the haemostatic efficacy and safety of 60 or 120 μg kg^{-1} of MC710 in the treatment of different types of haemorrhages (joint, intramuscular, subcutaneous, and nasal) in single or double administration (doses totaling up to 180 μg kg^{-1}) in haemophilia patients with inhibitors.

Materials and methods

1) Subjects and trial design

The trial was a multi-center, open-label, non-randomized study conducted in male Japanese haemophilia patients with inhibitors. In this trial, a patient was defined as having inhibitors, even if the inhibitor titer was undetectable, if they had been previously diagnosed as a haemophilia patient with inhibitors, and they currently required bypassing agents for the treatment of haemostasis. The institutional review board of each participating institute approved the trial and informed consent procedures. All subjects voluntarily provided written informed consent. Consent was also obtained from legal guardians for subjects who were minors. The key inclusion and exclusion criteria for the subjects are shown in **Table 1**.

The subjects were administered 60 or 120 μg kg^{-1} MC710 intravenously, depending on symptoms, within 5 h after the onset of bleeding, and were administered a second dose after 8 to 12 h, if required. For the second dose, subjects were administered 60 or 120 μg kg^{-1} MC710 so that the total dose did not exceed 180 μg kg^{-1}. The dosage regime of MC710 was established based on the results from a preclinical study

(thrombogenic test using monkeys) **[13]** and phase I/II clinical studies **[15-17]**. At least 20 bleeding episodes were planned to be treated during the study, and each subject was allowed to be treated for up to 5 episodes. Only subjects who had been treated for a first episode in the clinic were allowed to perform home treatment from the second episode.

The bleeding episodes evaluated in this study were intra-articular (shoulder, elbow, wrist, knee, or ankle) bleeding and intramuscular (upper arm, forearm, thigh, or lower thigh) bleeding, subcutaneous haematoma, and epistaxis (heavy bleeding requiring treatment with a bypassing agent). The severity of each episode was rated as either mild, moderate, or severe by the investigator.

2) Haemostatic efficacy

The haemostatic efficacy of MC710 was evaluated by the investigator after each treatment using the same criteria as in the phase II trial, including changes in the VAS, knee joint or muscle circumference, and ROM of the bleeding joint to indicate pain relief, swelling reduction, and improvement of joint mobility, respectively. The VAS was a 10 cm line with choices ranging from "no pain" (0 cm) to "the worst pain ever experienced" (10 cm) **[18]**. Knee joint or muscle circumference and ROM were measured with a tape measure and goniometer, respectively. Haemostatic efficacy measurements (VAS, knee joint or muscle circumference, and ROM) were taken at three time points: before treatment, 8 h after treatment, and 24 h after treatment for the single administration, and before treatment, 8 h after the first dose, and 8 h after the second dose for the double administration.

Using these data, the haemostatic efficacy for the treatment of each bleeding episode was assessed according to the investigator efficacy rating previously described **[17]**.

1. Excellent – complete pain relief and clear improvement in joint bleeding signs (swelling and/or mobility)
2. Effective –
    i. Complete pain relief and no changes in joint bleeding signs
    ii. Definite pain relief and slight improvement in joint bleeding signs
    iii. Slight pain relief and improvement in joint bleeding

3. Partially effective – slight pain relief and slight improvement in joint bleeding signs
4. Ineffective – no improvement or worsening of symptoms

The primary endpoint was defined as haemostatic efficacy 8 h after the last MC710 administration for both the single and double dose treatments.

3) Safety assessment

Subjective symptoms and objective findings, including recording of vital signs and laboratory tests before and after MC710 administration, were used to assess safety. Platelet counts, fibrinogen, D-dimer, thrombin-antithrombin complex (TAT), and prothrombin fragment F_{1+2} (F1+2) were measured to evaluate DIC before and 24 h after the first dose, and before and 10 min after treatment when a second dose was administered. The observation period for adverse events was one week after the first administration. Virological and serological tests were conducted to detect the production of new viral antigens (hepatitis B surface) or antibodies (hepatitis B surface, hepatitis B core, hepatitis C virus), and anti-FVII, anti-FX, or anti-mouse IgG antibodies 12 weeks after the first treatment.

Results

1) Subjects and treatment

Nineteen subjects [9 haemophilia A (HA) patients with inhibitors and 10 haemophilia B (HB) patients with inhibitors] were enrolled in this trial. MC710 was administered to 14 subjects in 21 bleeding episodes. All treatments for bleeding episodes were carried out in a clinic, not at home. The FVIII inhibitor titer of the 8 HA patients was in the range of 3.1–281 BU mL^{-1}, and the FIX inhibitor titer of the 6 HB patients was in the range of <0.5–119 BU mL^{-1} **(Table 2)**.

2) Haemostatic efficacy

In this study, 60 or 120 μg kg^{-1} of MC710 was administered for 21 bleeding episodes, occurring in 14 subjects **(Table 3)**. Two subjects were treated for multiple episodes (4 in subject No. 13 and 5 in subject No. 14), and the other 12 subjects were treated for a single episode.

There were 16 joint, 3 intramuscular, and 2 subcutaneous bleeding cases, and no epistaxis cases. Seven episodes were mild, 13 moderate, and 1 severe.

The treatment regimens were a single 60 μg kg^{-1} dose for 3 episodes, a single 120 μg kg^{-1} dose for 7 episodes, double 60 μg kg^{-1} doses for 1 episode, and a single 120 μg kg^{-1} dose followed by a 60 μg kg^{-1} dose for 10 episodes.

The efficacy rate, defined as the proportion of episodes for which MC710 was excellent or effective 8 h after the last administration, was 90.5% (19 of 21 episodes) in the primary endpoint evaluation **(Table 4)**. The efficacy rate was 85.7% (18 of 21 episodes) 8 h after the first administration, and 90.5% (19 of 21 episodes) 24 h after the first administration and 8 h after the second administration, demonstrating a strong and long-lasting haemostatic effect up to 16-24 h after administration **(Table 4)**.

The mean VAS score significantly decreased over time, while the mean ROM score significantly improved over time compared with the scores given before the first administration **(Figs. 1a and 1b)**.

The mean knee joint or muscle circumference decreased significantly 8 h after the first treatment compared with that before the first administration ($p < 0.05$, paired t-test), however, in one of the subjects it increased 8 h after the second MC710 administration without significant improvement compared with the baseline values **(Fig. 1c)**. The increase in mean knee joint circumference was observed in a single episode (No. 9-1), rated as "ineffective", where knee joint circumference increased from 36.0 cm (before treatment) to 41.0 cm (8 h after the second MC710 administration).

3) Safety

One mild adverse reaction, decreased blood potassium, was observed in subject No. 6 24 h after administering 120 μg kg^{-1} MC710, but it spontaneously resolved without treatment. Two serious adverse events (SAEs), both knee joint bleeding, were observed in the same bleeding episode (No. 13-4) at days 5 and 6 after administration of 120 μg kg^{-1} MC710. Although hospitalization was required for these SAEs, there was no causal relationship with MC710 treatment, and the symptoms were resolved at days 9 and 10 after onset. No other SAEs were observed within one week after MC710 administration, and no subject discontinued the trial due to adverse events. In cases where MC710 was administered twice, the

platelet count and fibrinogen did not change significantly up to 24 h after the first administration **(Figs. 2a and 2b)**. On the other hand, D-dimer increased approximately two-fold 24 h after the first administration, compared to before treatment **(Fig. 2c)**. Although TAT and F1+2 levels increased beyond the normal upper range of the healthy population before the second MC710 administration and continued to increase 10 min after, they normalized 24 h after the first administration (data not shown). Other laboratory tests and clinical symptoms observations did not indicate changes after MC710 administration. In addition, the results of virological and serological tests confirmed that subjects did not develop new viral antigens or produce new antibodies following MC710 administration.

Discussion

In this trial, MC710 was administered at 60 or 120 μg kg^{-1} once or twice (up to a total dose of 180 μg kg^{-1}) to treat mild, moderate, or severe bleeding in haemophilia patients with inhibitors. The efficacy rate of MC710 was 90.5% (19 of 21 episodes) 8 h after the last administration, and the effect was maintained for up to 24 h after the first administration **(Table 4)**. These results indicate sufficient haemostatic efficacy of one or two administrations of MC710 for different types of bleeding in haemophilia patients with inhibitors. Moreover, it was suggested that MC710 may have haemostatic efficacy in severe bleeding episodes. The haemostatic potential of MC710 may also be long lasting, up to 16-24 h after administration.

One (No. 9-1) of the two episodes rated as "ineffective" was severe bleeding in the knee joint. The subject showed clear improvement of swelling (a 1.5 cm reduction in the knee joint circumference) 8 h after the first MC710 administration and the efficacy was rated as "partially effective”. When the patient had previously presented with a similar bleeding episode, rFVIIa and APCC were administered and arthrocentesis was performed, and the bleeding stopped 10 days after onset. In this trial, the episode was initially treated with MC710, and the bleeding eventually stopped after rFVIIa treatment without arthrocentesis 4 days after onset. Based on these findings, we speculate that MC710 has potential for haemostatic efficacy even in severe haemorrhage. The other episode (No. 5-1) rated as "ineffective" was moderate bleeding in the elbow joint. In this episode, there was almost no improvement in VAS and

ROM 8 h after the second administration compared with before the first administration. One day following second administration, another bypassing agent (rFVIIa) was administrated to the patient. The investigator reported that the subject had shown unstable therapeutic responses even with commercial bypassing agents.

In this trial, a non-serious adverse reaction and two SAEs were observed within one week after the first dose of MC710, but none of these events had a clinically significant effect on safety. Furthermore, the level of D-dimer increased after MC710 administration, but DIC induction is unlikely because the platelet count and fibrinogen level did not decrease in association with D-dimer.

Based on these observations, the treatment regimens used in the trial (with a total dose not exceeding 180 μg kg^{-1}) are acceptably safe for the treatment of bleeding in haemophilia patients with inhibitors. When a second 60 μg kg^{-1} dose was administered 8 h after the first 120 μg kg^{-1} dose, APTT was 42.6 ± 4.2 s 10 min after the second administration, which was close to the upper normal limit in the healthy population (36.0 s). This is similar to the APTT level (40.7 ± 2.4 s) observed 10 min after a single 120 μg kg^{-1} MC710 dose in the phase II study **[17]**. Therefore, by administering a second 60 μg kg^{-1} dose 8 h after the first 120 μg kg^{-1} dose , APTT levels can be adjusted to a level similar to that achieved by a single 120 μg kg^{-1} dose, thereby stimulating the coagulation reaction again.

MC710 has haemostatic efficacy with one or two administrations at 8 h intervals for different types of bleeding, and has acceptable safety with a total dose up to 180 μg/kg for the treatment regimen. MC710 is thus expected to be a safe and efficacious novel bypassing agent for controlling bleeding in haemophilia patients with inhibitors, and a viable alternative to other commercially available bypassing agents. In this trial, the evaluation of haemostatic efficacy and safety of MC710 was limited due to the small number of subjects and bleeding episodes. It is necessary to accumulate evidence via post marketing surveillance to verify the haemostatic efficacy and safety of MC710.

Addendum

Akira Shirahata developed the clinical trial protocol, evaluated efficacy and safety data, and discussed clinical events in the role of medical expert.
Hidehiko Saito, Katsuyuki Fukutake, Junki Takamatsu, and Midori Shima acted as coordinating investigators, ensuring that investigators at different institutions had a common understanding of the protocol and implementation of the trial, as well as giving general advice on the conduct of the trial.
The investigators had responsibility for all medical judgments associated with this trial at their respective institutions and conducted the trial in accordance with the protocol including the selection of subjects, obtaining of informed consent, provision of data and information, reporting of adverse events, documentation of case reports, and storage of essential documents.
Yasuo Ohashi, acting as the statistical advisor, gave advice and instruction on statistical analysis methods for the trial.
KAKETSUKEN (The Chemo-Sero-Therapeutic Research Institute) managed the trial, including development and amendment of the protocol, data management, statistical analysis, quality control and assurance, and data preservation.
Mitsubishi Chemical Medience Corporation collected the samples taken at the trial sites, conducted the tests, maintained measurement results, and guaranteed the reliability of the results of the analyses.
Shin Nippon Biomedical Laboratories, Ltd. (Kagoshima, Japan) was responsible for the estimation of PK parameters and conducted the statistical analysis.

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Disclosures

Akira Shirahata received a fee from *KAKETSUKEN* for the implementation of the trial. The other authors have no conflict of interest to declare.

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Table 1. Key inclusion and exclusion criteria

(a) Key inclusion criteria
Patients: male congenital haemophilia A or B patients with inhibitors
Age: ≥ 12 years and ≤ 65 years
(b) Key exclusion criteria
Patients with the following condition
・hypercoagulability
・history of DIC
・development of AIDS
・hypersensitivity to NovoSeven, FEIBA, and other plasma products
・decompensated liver cirrhosis
・heart failure, angina or pathologic arrhythmia such as atrial fibrillation
・ongoing immune tolerance induction (ITI) treatment

Table 2. Subject characteristics

Subject No.	Haemophilia type	Age (years old)	Body weight (kg)	Inhibitor titer (BU mL^{-1}) *	
				FVIII inhibitor	FIX inhibitor
1	A	41	62.0	3.2	-
2	B	32	59.7	-	< 0.5 **
3	B	19	71.5	-	31.2
4	B	14	52.0	-	119
5	A	17	80.4	281	-
6	A	29	75.0	106	-
7	A	31	74.4	10.5	-
8	A	26	82.4	46.0	-
9	B	20	49.0	-	25.7
10	A	14	41.2	3.1	-
11	A	29	60.0	4.3	-
12	B	21	56.8	-	3.0
13	A	12	35.3	169	-
14	B	22	89.7	-	1.5
Mean ± SD	-	23.4 ± 8.3	63.5 ± 16.1	77.9 ± 101.8	30.2 ± 45.5

* Inhibitor titer was measured using the Bethesda assay and the values at the time of enrolment in this study are shown.

** Although the inhibitor titer of subject No.2 was below the detection limit, the subject has been previously diagnosed as a haemophilia patient with inhibitors and needs bypassing agents for controlling haemorrhage. Values less than 0.5 were rounded to 0.5.

Table 3. Bleeding episode, MC710 treatment, and haemostatic efficacy

Episode No.*	Bleeding site (L, left; R, right)		Bleeding severity	Time from bleeding to 1st administration (h)	MC710 1st administration (μg kg^{-1})	MC710 2nd administration (μg kg^{-1})	Haemostatic efficacy		
							8 h after 1st administration	24 h after 1st administration or 8 h after 2nd administration	8 h after last administration *(primary endpoint)*
1-1	muscle	L. forearm	mild	3.82	120	60	effective	effective	effective
2-1	joint	R. knee	moderate	4.97	60	60	effective	effective	effective
3-1	joint	R. elbow	moderate	1.75	120	-	excellent	excellent	excellent
4-1	subcutaneous	R. inguinal	mild	4.38	120	-	effective	excellent	effective
5-1	joint	R. elbow	moderate	4.62	120	60	ineffective	ineffective	ineffective
6-1	joint	L. elbow	moderate	4.13	120	-	excellent	excellent	excellent
7-1	joint	L. ankle	mild	2.33	120	-	effective	effective	effective
8-1	muscle	R. thigh	moderate	4.88	120	60	effective	effective	effective
9-1	joint	R. knee	severe	2.83	120	60	partially effective	ineffective	ineffective
10-1	subcutaneous	L. hip	mild	2.43	120	-	effective	excellent	effective
11-1	joint	L. elbow	moderate	3.53	120	60	partially effective	effective	effective
12-1	muscle	R. thigh	mild	3.38	120	-	effective	effective	effective
13-1	joint	L. knee	mild	4.65	60	-	effective	excellent	effective
13-2	joint	L. knee	moderate	3.75	60	-	effective	effective	effective
13-3	joint	L. knee	moderate	4.70	60	-	effective	effective	effective
13-4	joint	R. knee	moderate	4.25	120	-	effective	effective	effective
14-1	joint	R. knee	mild	3.75	120	60	effective	effective	effective
14-2	joint	L. ankle	moderate	2.47	120	60	effective	effective	effective
14-3	joint	R. ankle	moderate	2.58	120	60	effective	effective	effective
14-4	joint	R. ankle	moderate	2.23	120	60	effective	excellent	excellent
14-5	joint	L. ankle	moderate	1.32	120	60	effective	effective	effective

* Episode No. is denoted as “subject No. – episode time”.

Table 4. Time-course of MC710 haemostatic efficacy

Evaluation time point	Bleeding episodes	Haemostatic efficacy				Efficacy rate	
		excellent	effective	partially effective	ineffective	excellent + effective	[two-tailed 95% CI]
8 h after 1st administration	21	2	16	2	1	18 (85.7%)	[63.7 - 97.0%]
24 h after 1st administration or 8 h after 2nd administration	21	6	13	0	2	19 (90.5%)	[69.6 - 98.8%]
8 h after last administration *(primary endpoint)*	21	3	16	0	2	19 (90.5%)	[69.6 - 98.8%]

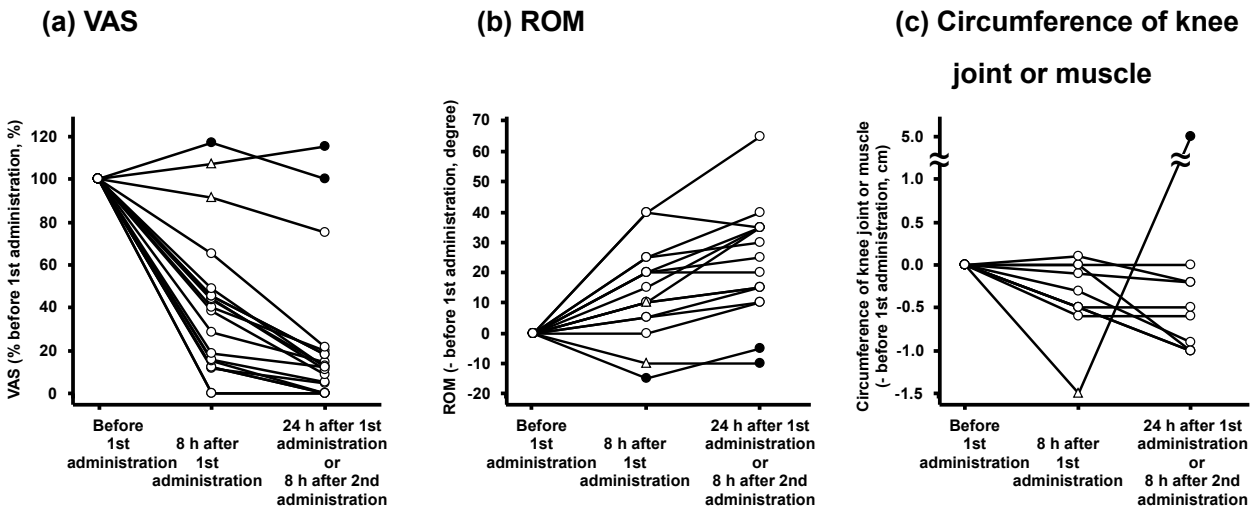

Figure 1. Changes in haemostasis parameters

(a) VAS, (b) ROM, and (c) circumference of knee joint or muscle were measured respectively at three evaluation points (before first administration, 8 h after first administration, and 24 h after first administration or 8 h after second administration). VAS was shown by the ratio before first administration. ROM and circumference of knee joint or muscle were shown by the difference between before the first administration and the other evaluation points. Symbols show the haemostatic efficacy of MC710 at each evaluation point. Excellent or effective is indicated by an open circle (○), partially effective by an open triangle (△), and ineffective by a closed circle (●).

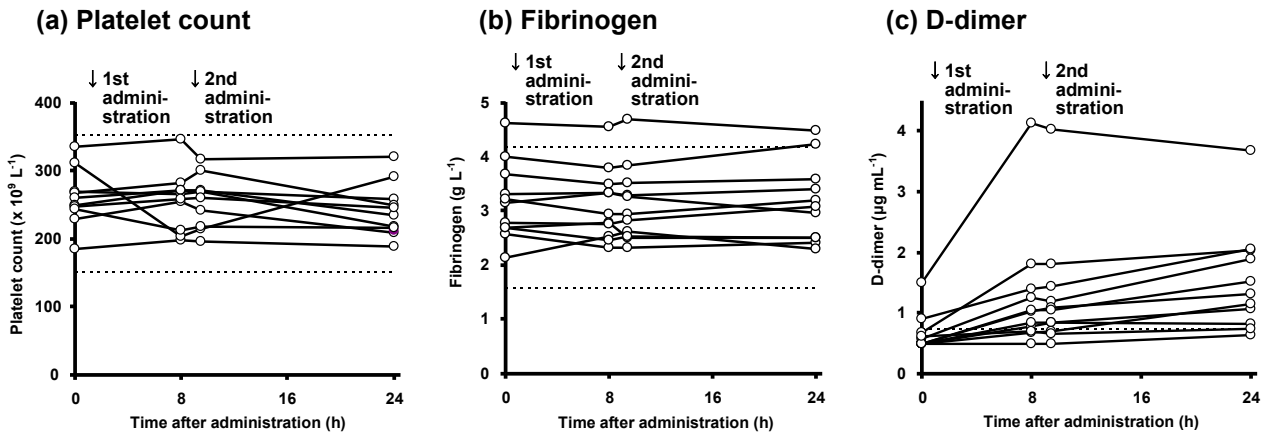

Figure 2. Changes in DIC parameters

Time-course of DIC parameters such as (a) platelet count, (b) fibrinogen, and (c) D-dimer were measured respectively in subjects who were administrated a second dose of MC710. The normal ranges of healthy control (---) for platelet count were defined as 350 (upper limit) and 150 (lower limit) x 10^9 L^{-1}, for fibrinogen as 4.15 (upper limit) and 1.55 (lower limit) g L^{-1}, and for D-dimer as 0.72 (upper limit) μg mL^{-1}.