

# A potential mechanism of propofol-induced pain on injection based on studies using nafamostat mesilate

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To elucidate the mechanism of propofol-induced pain on injection, we performed several studies using nafamostat mesilate, a kallikrein inhibitor, or lidocaine. As both pretreatment and low-dose mixing with nafamostat produced the same effects on pain reduction, we used the latter method in the following experiments. Low-dose mixing had the same effect on injection pain as mixing with lidocaine. The extent of pain was assessed by measuring bradykinin concentrations by mixing with blood. Propofol and its lipid solvent mixed with blood produced approximately two-fold generation of bradykinin compared with the saline control, and this was inhibited completely by nafamostat and lidocaine. Injection of the lipid solvent before propofol significantly aggravated pain compared with prior injection of saline, although the lipid solvent injected twice caused no change in pain. These results suggest that the lipid solvent for propofol activates the plasma kallikrein–kinin system and produces bradykinin which modifies the injected local vein. This modification of the peripheral vein may increase the contact between the aqueous phase propofol and the free nerve endings of the vessel, resulting in aggravation of propofol-induced pain.

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Propofol is used widely for induction and maintenance of anaesthesia, but can often cause local pain when administered into peripheral veins. Several methods have been described to reduce this pain, of which the most effective and common are use of a larger vein and mixing with lidocaine,<sup>1–4</sup> although complete inhibition has not been achieved. In addition to these methods, we have demonstrated recently the preventive effect of pretreatment with nafamostat mesilate (6-amino-2-naphthyl p-guanidinobenzoate dimethanesulphonate, FUT-175; Torii Pharmaceutical Co., Tokyo, Japan) (FUT) on propofol-induced pain.<sup>5</sup> As this drug acts via a kallikrein inhibitor,<sup>6–8</sup> we hypothesized that the cause of pain is activation of the plasma kallikrein–kinin system by contact with propofol.

In this study, we tested this hypothesis and present other experiments designed to elucidate further the mechanisms responsible for propofol-induced pain.

## Patients and methods

The studies were approved by the Institutional Committee, and all adult elective surgical patients, ASA I or II, gave informed consent. Patients were premedicated with atropine

0.005–0.008 mg kg<sup>–1</sup> and butorphanol 0.5–1.0 mg i.m., 30 min before entering the operating room where a 20-gauge i.v. catheter was inserted in the forearm. FUT was prepared by diluting a 10-mg vial with 5% glucose 10 ml (1 mg ml<sup>–1</sup> of FUT solution). Then, 0.1 ml of this solution, containing FUT 100 µg, was diluted with 10 ml of 5% glucose (10 µg ml<sup>–1</sup> of FUT solution). These two solutions were stored at 4°C and used within 48 h. Data are presented as mean (range or SD) or number of patients.

### *Comparison of propofol-induced pain between pretreatment and mixing with FUT, and blood FUT concentrations after injection of propofol mixed with FUT*

We studied 150 patients, allocated randomly to one of three groups of 50 patients each: pretreatment with FUT in the same vein (pretreatment), pretreatment with FUT in a remote vein (remote pretreatment) and mixing of propofol with FUT (mixing). The pretreatment group received FUT 0.02 mg kg<sup>–1</sup> by injection of the 1-mg ml<sup>–1</sup> FUT solution, followed 1 min later by 1% propofol at room temperature (24–26°C) injected into the same vein at a rate of

200 mg min<sup>-1</sup>. The remote pretreatment group received the same dose of FUT injected into a peripheral vein on the other side of the forearm, followed 1 min later by injection of propofol, as above. For the mixing group, 1 ml of the 10- $\mu$ g ml<sup>-1</sup> FUT solution was mixed with 20 ml of 1% propofol at room temperature before induction of anaesthesia. The mixing group then received this propofol-FUT mixture at a rate of 200 mg min<sup>-1</sup>. The injected dose of propofol was titrated against patient response. During induction, patients were asked repeatedly to report and grade any discomfort or pain of highest score as: none=0; discomfort=1; mild pain=2; moderate pain=3; and severe pain=4.

Another 10 patients undergoing major surgery and requiring direct intra-arterial monitoring of arterial pressure were recruited. Before induction of anaesthesia, a 22-gauge arterial catheter was inserted into the radial artery under local anaesthesia. Subsequently, propofol 2.5 mg kg<sup>-1</sup> mixed with FUT in the same preparation as above was administered i.v. for 30 s. At 1 and 2 min after the start of injection, 1 ml of arterial blood was collected from the catheter and mixed with 1% 0.5-N formic acid in ethanol. FUT concentrations in blood were assayed using high-pressure liquid chromatography.<sup>9</sup>

#### *Comparison of propofol-induced pain when mixing propofol with lidocaine or FUT*

We studied 300 patients, allocated randomly to one of three groups of 100 patients each: control, propofol mixed with lidocaine (lidocaine) and propofol mixed with FUT (FUT). The control group received 1% propofol at room temperature at a rate of 200 mg min<sup>-1</sup>. The lidocaine group received propofol mixed with lidocaine at room temperature, in which 20 ml of 1% propofol were mixed with 2 ml of 2% lidocaine before induction of anaesthesia, at the same rate. The FUT group received propofol mixed with FUT at room temperature, as for the same preparation in the first study, at the same rate. The injected dose of propofol was titrated against patient response. During induction, pain was classified and evaluated as in the first study.

#### *Assessment of bradykinin generation*

We studied 10 ASA I patients. Six plastic syringes were prepared at room temperature containing saline 1.5 ml (saline), 10% lipid solvent 1.5 ml (Intralipid, Kabi Pharmacia AB, Stockholm, Sweden) (lipid), 1% propofol 1.5 ml (propofol), propofol 1.5 ml mixed with lidocaine as in the second study (lidocaine mixing), propofol 1.5 ml mixed with FUT as in the first study (FUT mixing) and 1% propofol 1.5 ml (FUT pretreatment). After induction of anaesthesia using thiamylal, isoflurane and nitrous oxide, a 22-gauge arterial catheter was inserted into the radial artery. Subsequently, arterial blood 3.5 ml was aspirated from the catheter over 10 s using the saline, lipid, propofol, lidocaine mixing and FUT mixing syringes in turn. Finally, FUT

0.02 mg kg<sup>-1</sup>, using the 1-mg ml<sup>-1</sup> FUT solution, was administered i.v., followed 1 min later by aspiration of arterial blood 3.5 ml by the FUT pretreatment syringe in the same way as above. Each sample was shaken gently for 20 s and mixed immediately with edetic acid, aprotinin and trypsin inhibitor, followed by centrifugation at 4°C. The supernatant was used for measurement of concentrations of bradykinin. Bradykinin was assayed using a radioimmunoassay kit (SRL Co., Tokyo, Japan).<sup>10</sup>

#### *Comparison of propofol-induced pain after pretreatment with saline or lipid solvent, and effect of lipid solvent injected twice before propofol injection*

We studied 100 patients, allocated randomly to one of two groups of 50 patients each: the saline group received saline 0.15 ml kg<sup>-1</sup> at room temperature at a rate of 20 ml min<sup>-1</sup>, followed 10 s later by 1% propofol 1.5 mg kg<sup>-1</sup> at room temperature injected at a rate of 200 mg min<sup>-1</sup>; the lipid group received 10% lipid solvent (Intralipid) 0.15 ml kg<sup>-1</sup> at room temperature at a rate of 20 ml min<sup>-1</sup>, followed 10 s later by injection of propofol as above. During the first and second injections, pain was classified and evaluated as in the first study.

Another 50 consecutive patients received two injections of 10% lipid solvent (Intralipid) 0.15 ml kg<sup>-1</sup> at room temperature at a rate of 20 ml min<sup>-1</sup>, 10 s apart, followed 10 s later by 1% propofol 1.5 mg kg<sup>-1</sup> at room temperature injected at a rate of 200 mg min<sup>-1</sup>. During each injection, pain was classified and evaluated as in the first study.

#### *Effect of temperature on propofol-induced pain*

We studied 200 patients, allocated randomly to one of four groups of 50 patients each: 1% propofol at room temperature (control), 1% propofol at 37°C (warm), 1% propofol at 4°C (cool) and propofol at room temperature mixed with FUT (FUT). Room temperature was maintained at 25°C. For preparation of warm and cool propofol, a 20-ml ampoule of 1% propofol was stored in a 37°C warmer box or 4°C refrigerator for several hours, and removed immediately before use. The control, warm and cool groups received propofol at a rate of 200 mg min<sup>-1</sup>. The FUT group received propofol mixed with FUT prepared as in the first study at the same rate. The injected dose of propofol was titrated against patient response. During induction, pain was classified and evaluated as in the first study.

#### *Effect of propofol-generated bradykinin on the circulation*

We studied 12 ASA II patients with hypertension receiving a calcium channel blocker (amlodipine, *n*=6) or an angiotensin converting enzyme inhibitor (captopril, *n*=6). Patients receiving other medications were excluded. All patients were treated with these drugs until the morning of the day of surgery. Before induction of anaesthesia, a 22-

**Table 1** Comparison of propofol-induced pain between pretreatment and mixing with FUT (number of patients). Pain scores are: 0=none, 1=discomfort, 2=mild pain, 3=moderate pain and 4=severe pain

Group	Pain score				
	0	1	2	3	4
Pretreatment ( <i>n</i> =50)	27	12	9	2	0
Remote pretreatment ( <i>n</i> =50)	24	13	11	0	2
Mixing ( <i>n</i> =50)	26	14	6	3	1

gauge arterial catheter was inserted into the radial artery under local anaesthesia, and arterial blood 10 ml was collected from the catheter and mixed immediately with edetic acid, aprotinin and trypsin inhibitor, followed by centrifugation at 4°C. Plasma was used for measurement of bradykinin. Subsequently, patients received 1% propofol 3.5 mg kg<sup>-1</sup> at room temperature as a bolus dose at a rate of 2.5 mg kg<sup>-1</sup> min<sup>-1</sup>, and then as a continuous infusion at a rate of 10 mg kg<sup>-1</sup> h<sup>-1</sup> for 9 min. During these procedures, arterial blood 10 ml was collected from the catheter 1 and 5 min after the start of injection of propofol, followed by the same procedure as above for measurement of bradykinin. Plasma bradykinin was assayed using a radioimmunoassay kit (SRL Co., Tokyo, Japan). Also, systolic and diastolic arterial pressures and heart rates were monitored and recorded every minute.

## Results

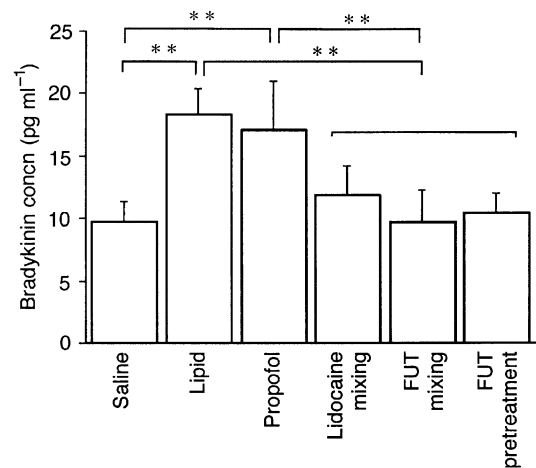
### *Comparison of propofol-induced pain between pretreatment and mixing with FUT, and blood FUT concentrations after injection of propofol mixed with FUT*

The pretreatment, remote pretreatment and mixing groups were similar in age (mean 53 (range 16–93) yr, 53 (19–75) yr and 52 (25–78) yr, respectively), weight (56 (SD 10) kg, 59 (11) kg and 58 (11) kg, respectively), height (155 (8) cm, 158 (9) cm and 158 (8) cm, respectively), male/female ratio (17/33, 19/31 and 20/30) and ASA I/II ratio (30/20, 29/21 and 33/17, respectively). There was no difference in injected dose of propofol (80 (21) mg, 87 (18) mg and 88 (18) mg, respectively), as determined by one-way analysis of variance (ANOVA). There was no significant difference in pain scores (Kruskal–Wallis test) (Table 1).

For the additional 10 patients, blood FUT concentrations were less than 6 nmol litre<sup>-1</sup> in all samples.

### *Comparison of propofol-induced pain when mixing propofol with lidocaine or FUT*

The control, lidocaine and FUT groups were similar in age (mean 48 (range 19–78) yr, 53 (19–80) yr and 53 (16–81) yr, respectively), weight (57 (SD 9) kg, 57 (10) kg and 58 (11) kg, respectively), height (157 (9) cm, 157 (9) cm and 156 (8) cm, respectively), male/female ratio (35/65, 32/68 and 34/66, respectively) and ASA I/II ratio (72/28, 65/35

**Fig 1** Generation of bradykinin in the saline, lipid, propofol, lidocaine mixing, FUT mixing and FUT pretreatment samples (see text for details). Data are mean (SD). \*\**P*<0.01.**Table 2** Comparison of propofol-induced pain when mixing propofol with lidocaine or FUT (number of patients). Pain scores are: 0=none, 1=discomfort, 2=mild pain, 3=moderate pain and 4=severe pain. \*\**P*<0.01

Group	Pain score				
	0	1	2	3	4
Control ( <i>n</i> =100)	25	10	26	16	23
Lidocaine ( <i>n</i> =100)	39	25	17	15	4
FUT ( <i>n</i> =100)	53	20	14	11	2

and 66/34, respectively). Injected doses of propofol were 83 (18) mg, 87 (19) mg and 88 (18) mg, respectively (*P*>0.05, one-way ANOVA). There was a significant difference in pain scores (*P*<0.01, Kruskal–Wallis test) (Table 2). The control group showed a significantly higher incidence of propofol-induced pain than the lidocaine and FUT groups (*P*<0.01, Mann–Whitney *U* test with Bonferroni correction). The lidocaine and FUT groups showed a similar incidence of pain (*P*>0.05).

### *Assessment of bradykinin generation*

Age, weight, height and male/female ratio were mean 28 (range 22–39) yr, 58 (SD 11) kg, 164 (10) cm and 5/5, respectively. Bradykinin concentrations in the saline, lipid, propofol, lidocaine mixing, FUT mixing and FUT pretreatment samples are shown in Figure 1. There was a significant difference between samples (*P*<0.01, one-way ANOVA). The lipid and propofol samples showed significantly higher bradykinin concentrations than the other samples (*P*<0.01, Scheffé's *F* test).

### *Comparison of propofol-induced pain after pretreatment with saline or lipid solvent, and effect of lipid solvent injected twice before propofol injection*

The saline and lipid groups were similar in age (mean 46 (range 17–80) yr and 50 (18–85) yr, respectively), weight

**Table 3** Comparison of propofol-induced pain after pretreatment with saline or lipid solvent (number of patients). Pain scores are: 0=none, 1=discomfort, 2=mild pain, 3=moderate pain and 4=severe pain. \*\* $P<0.01$ 

Group	Pain score				
	0	1	2	3	4
First injection					
Saline ( $n=50$ )	50	0	0	0	0
Lipid ( $n=50$ )	30	15	3	2	0
Second injection					
Saline ( $n=50$ )	9	11	12	12	6
Lipid ( $n=50$ )	7	6	5	12	20

**Table 4** Effect of lipid solvent injected twice before propofol injection (number of patients) ( $n=50$ ). Pain scores are: 0=none, 1=discomfort, 2=mild pain, 3=moderate pain and 4=severe pain. \*\* $P<0.01$ 

Group	Pain score				
	0	1	2	3	4
First injection (lipid solvent)	35	13	2	0	0
Second injection (lipid solvent)	33	15	2	0	0
Third injection (propofol)	1	6	5	22	16

(56 (SD 8) kg and 56 (9) kg, respectively), height (158 (7) cm and 159 (8) cm, respectively), male/female ratio (14/36 and 21/29, respectively) and ASA I/II ratio (35/15 and 37/13, respectively). During the first injection, all patients in the saline group reported no pain, while some patients in the lipid group reported mild or moderate pain (Table 3). This difference was statistically significant ( $P<0.01$ , Mann–Whitney  $U$  test). When propofol was subsequently injected, the lipid group had a significantly higher incidence of pain than the saline group ( $P<0.01$ ).

The age, weight, height, male/female ratio and ASA I/II ratio of the further 50 patients were 47 (23–76) yr, 57 (10) kg, 159 (8) cm, 15/35 and 33/17, respectively. There was a significant change in pain scores ( $P<0.01$ , Friedman test) (Table 4). Although there was no significant change between the first and second injections of lipid solvent ( $P>0.05$ , Wilcoxon signed rank test with Bonferroni correction), there was a significant change between both the first lipid solvent and the third propofol injection and the second lipid solvent and third propofol injection ( $P<0.01$ ).

#### Effect of temperature on propofol-induced pain

The control, warm, cool and FUT groups were similar in age (mean 46 (range 19–76) yr, 51 (17–77) yr, 51 (16–76) yr and 51 (16–78) yr, respectively), weight (57 (SD 8) kg, 56 (10) kg, 58 (9) kg and 58 (11) kg, respectively), height (158 (8) cm, 156 (8) cm, 157 (10) cm and 158 (8) cm, respectively), male/female ratio (18/32, 17/33, 18/32 and 21/29, respectively), ASA I/II ratio (41/9, 34/16, 35/15 and 34/16, respectively) and injected dose of propofol (86 (11) mg, 87 (15) mg, 89 (17) mg and 90 (21) mg, respectively). There was a significant difference in pain

**Table 5** Effect of temperature on propofol-induced pain (number of patients). Pain scores are: 0=none, 1=discomfort, 2=mild pain, 3=moderate pain and 4=severe pain. \* $P<0.05$ , \*\* $P<0.01$ 

Group	Pain score				
	0	1	2	3	4
Control ( $n=50$ )	6	7	14	11	12
Warm ( $n=50$ )	5	9	13	14	9
Cool ( $n=50$ )	18	8	12	5	7
FUT ( $n=50$ )	26	14	7	3	0

scores ( $P<0.01$ , Kruskal–Wallis test) (Table 5). There was no significant difference between the control and warm groups ( $P>0.05$ , Mann–Whitney  $U$  test with Bonferroni correction), but these groups showed a significantly higher incidence of pain than the cool and FUT groups ( $P<0.01$ – $0.05$ ). Furthermore, the cool group showed a significantly higher incidence of pain than the FUT group ( $P<0.05$ ).

#### Effect of propofol-generated bradykinin on the circulation

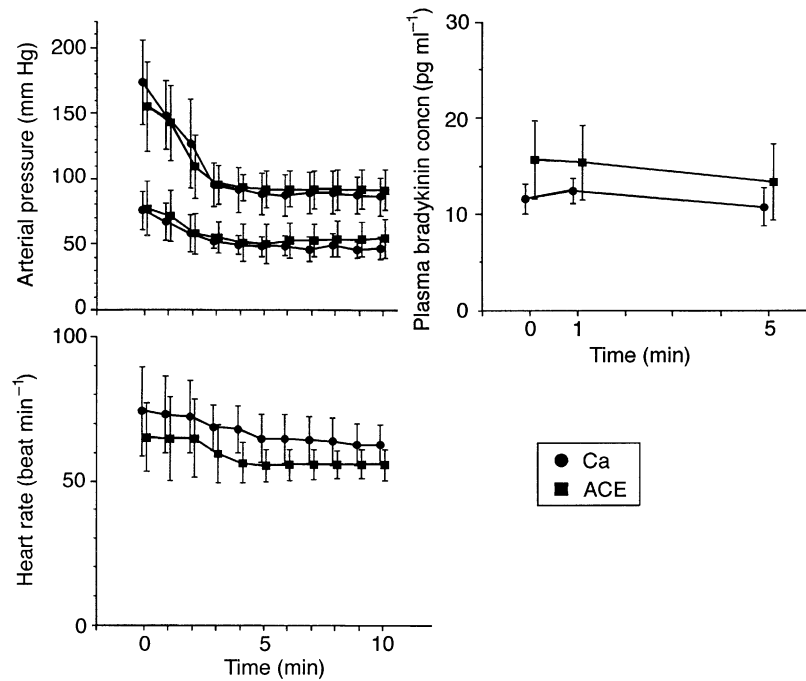
The calcium channel blocker and angiotensin converting enzyme inhibitor groups were similar in age (mean 66 (range 56–75) yr and 63 (45–75) yr, respectively), weight (60 (SD 10) kg and 54 (8) kg, respectively), height (153 (5) cm and 153 (5) cm, respectively) and male/female ratio (4/2 and 3/3, respectively). There was no significant difference in the time course of systolic and diastolic arterial pressures, heart rate or plasma bradykinin concentrations between patients receiving a calcium channel blocker compared with those receiving an angiotensin converting enzyme inhibitor ( $P>0.05$ , repeated measure ANOVA) (Fig. 2). Also, there were no significant changes in plasma bradykinin concentrations in either group ( $P>0.05$ , repeated measures one-way ANOVA).

## Discussion

Until now, the mechanism of propofol-induced pain has been unclear. Scott, Saunders and Norman<sup>1</sup> speculated that pain is caused by activation of the kallikrein–kinin system in plasma by contact with propofol, consequently generating kinins, probably bradykinin. Our previous report<sup>5</sup> showed that blood FUT concentrations were approximately 100 nmol litre<sup>-1</sup>, 1 min after administration of FUT 0.02 mg kg<sup>-1</sup> i.v., and propofol-induced pain was reduced significantly at this time. As this concentration is sufficient to inhibit plasma kallikrein activity,<sup>6–8</sup> these results are consistent with the hypothesis that propofol activates the plasma kallikrein–kinin system.

FUT is a synthetic serine protease inhibitor used clinically in Japan for treating patients with disseminated intravascular coagulation and acute pancreatitis, and as an anticoagulant during various extracorporeal circulation procedures.<sup>11</sup> I.v. FUT is given as 10 mg over 2 h twice daily for acute pancreatitis, 0.1–0.2 mg kg<sup>-1</sup> h<sup>-1</sup> continuously for dissemin-





**Fig 2** Time course of systolic and diastolic arterial pressures, heart rate and plasma bradykinin concentrations after injection of propofol in patients receiving a calcium channel blocker (Ca) or angiotensin converting enzyme inhibitor (ACE). Data are mean (SD).

ated intravascular coagulation and 20–40 mg h<sup>-1</sup> as an anticoagulant.<sup>11</sup> These doses were determined from studies showing that 50% inhibition of trypsin and thrombin activity are achieved at 10 and 100 nmol litre<sup>-1</sup>,<sup>6</sup> and that prolongation of thrombin time, prothrombin time and activated partial thromboplastin time are achieved at 10, 100 and 1 µmol litre<sup>-1</sup>, respectively.<sup>7, 12</sup> Blood FUT concentrations in healthy volunteers after injection of 10, 20 and 40 mg over 90 min are 20–40, 60–100 and 130–170 nmol litre<sup>-1</sup>.<sup>13</sup> As FUT is hydrolysed rapidly by blood esterases, its biological half-time is approximately 8 min.<sup>13</sup> When FUT is administered as a bolus injection, highest concentrations are achieved immediately after injection and decrease subsequently, as demonstrated in our previous report.<sup>5</sup> As determined by the inhibitory effects on the kallikrein–kinin system, 50% inhibition of plasma kallikrein and activated coagulation factor XII are achieved at 1–100 and 100 nmol litre<sup>-1</sup>, respectively.<sup>6–8</sup>

Results from our previous study<sup>5</sup> suggested that the effect of pretreatment with FUT on propofol-induced pain may be attributed to systemic inhibition of kallikrein activity. As FUT also has a vasodilatory action on the injected vessel,<sup>14, 15</sup> another mechanism may be vasodilatation, reducing propofol-induced pain in the same way as using a larger vein.<sup>1</sup> In contrast, a mixture of 1 ml of 5% glucose containing FUT 10 µg (molecular weight 539.58) with 20 ml of 1% propofol provides approximately 1000 nmol litre<sup>-1</sup> of FUT. If this solution is injected i.v. and mixed with circulating blood in the local vein, the FUT concentration in that local vein during injection would decrease but not to less than 100 nmol litre<sup>-1</sup>. Thus the mixed solution would

also be expected to reduce propofol-induced pain. Furthermore, if the solution produces the same effect as pretreatment with FUT, then the systemic pharmacological effects of FUT could be minimized because the dose of FUT injected is reduced by at least 99% compared with pretreatment.

To clarify these possibilities, a comparison was made of pain during pretreatment with FUT on the same vein or on a remote vein, and mixing propofol with FUT in the first study. All of these procedures had the same effect on propofol-induced pain. These results demonstrate that pain reduction is achieved equally by pretreatment with FUT, either on the same vein or on a remote vein, which can be attributed to systemic inhibition of kallikrein activity, and by administering FUT at the same time as propofol which can be attributed to local inhibition of kallikrein activity. Blood FUT concentrations after injection of propofol 2.5 mg kg<sup>-1</sup> mixed with FUT were less than 6 nmol litre<sup>-1</sup>. This concentration of FUT has no systemic pharmacological effects. There is still the possibility that FUT may induce anaphylactic reactions, but only one report<sup>16</sup> has described such a reaction induced by FUT. However, previous anaphylactic reactions to this class of drug, which includes gabexate mesilate and aprotinin, in addition to FUT, may be considered a contraindication. Furthermore, use of FUT in children and pregnant women is not established, and there is one report of its transfer into mother's milk in rats.<sup>17</sup> Excluding these situations, propofol mixed with FUT, as described in this report, may be a safer alternative to pretreatment with FUT.

We subsequently performed a comparison of pain reduc-

tion when propofol was mixed with either lidocaine or FUT. The results showed that lidocaine and FUT had a similar effect. This finding suggests a potential use for FUT on propofol-induced pain as an alternative to lidocaine. In Japan, an ampoule of 2% lidocaine 5 ml and a vial of FUT 10 mg cost 97 yen and 1966 yen, respectively, which implies approximately 39 yen for lidocaine and 2 yen for FUT per person. There is an obvious economic advantage in using FUT. However, a definite conclusion comparing the effect of lidocaine and FUT on propofol-induced pain cannot be made until a randomized, double-blind study has been performed.

Activation of the kallikrein-kinin system in plasma involves activation of coagulation factor XII which converts prekallikrein to kallikrein, which in turn cleaves high-molecular weight kininogen to release bradykinin. Bradykinin is broken down rapidly by kininases; of these, kininase II is identical to angiotensin converting enzyme.<sup>18</sup> Bradykinin concentration can be measured accurately by radioimmunoassay and can be used to assess activation of the kallikrein-kinin system. In the third study, therefore, bradykinin concentrations were measured and regarded as an index of kallikrein-kinin activity, and a measure of bradykinin generation. In addition to the variables tested in the first and second studies, a lipid solvent sample was included because the lipid emulsion vehicle of propofol could potentially affect the kallikrein-kinin system. Because of our clinical impression that propofol-induced pain often occurs in the latter half of the injection period, we aspirated blood into each tested solution at a ratio of 3.5:1.5 over a period of 10 s, followed by 20 s of shaking. The results demonstrated that bradykinin generation by propofol was attributable to the lipid solvent, and this generation was inhibited completely by FUT. Interestingly, inhibition of bradykinin generation was also achieved by lidocaine to the same extent as FUT. Bradykinin generation is commonly noted when plasma contacts negatively charged substances<sup>19</sup> and we assume that the lipid solvent carries a weak negative charge. Although the inhibitory effect of FUT on bradykinin generation is caused by suppression of kallikrein activity, the mechanism of inhibition by lidocaine is unknown. Further study to elucidate this mechanism is needed.

Our *in vitro* study suggests that the lipid solvent causes generation of bradykinin, but propofol causes more pain on injection than lipid solvent alone. Thus bradykinin is not the only factor inducing pain on injection. We considered the possibility that although bradykinin is a pain-producing substance, it does not cause injection pain directly on the vein. Several reports have documented the high level of bradykinin production after use of a negatively charged white cell-reduction filter during platelet transfusion.<sup>19-21</sup> However, we have found no reports of pain using this type of filter, and there have been no reports concerning pain on injection. While bradykinin dilates the vessel and causes hyperpermeability,<sup>22,23</sup> resulting in hypotension,<sup>24</sup> the actual substance inducing pain on injection has been reported to

be propofol itself.<sup>25</sup> This is suggested by the trend for a higher incidence of pain on injection with 2% propofol compared with 1% propofol.<sup>26,27</sup> Also, the reduced pain on injection of propofol diluted with lipid solvent<sup>25,28</sup> is attributed to decreased concentrations of aqueous phase propofol. Based on these reports, we considered a hypothesis in which the lipid solvent for propofol produces bradykinin which acts on the local vein to make it dilate and become permeable. In this bradykinin-modified vein, the aqueous phase propofol contacts more free nerve endings outside the endothelial layer of the vessel.

As documented in this study, complete elimination of propofol-induced pain cannot be achieved, even if generation of bradykinin is repressed completely. Even if the local vein is not altered, the aqueous phase propofol can make contact with some free nerve endings, which is probably dependent on the individual variability of the vein. In addition to this base condition, bradykinin produced by propofol results in increased contact between the aqueous phase propofol and free nerve endings by its vasodilatory and hyperpermeability effects, thus aggravating injection pain.

To test this hypothesis, we compared pain on injection after administration of saline or lipid solvent, 10 s before propofol injection, because the lipid solvent produces the same levels of bradykinin as propofol, and bradykinin has a biological half-time of 15 s.<sup>24</sup> We found a higher incidence of propofol-induced pain after prior injection of lipid solvent than saline. Although injection of lipid solvent resulted in some pain on injection, we considered that this could not be the reason for the much greater difference in propofol-induced pain. Furthermore, the additional study examining the effect of lipid solvent injected twice before propofol injection demonstrated no change in lipid solvent-induced pain and subsequently high propofol-induced pain. If bradykinin is the only factor inducing pain on injection, this result would not be appropriate. Thus both these results support our hypothesis that the lipid solvent for propofol activates the plasma kallikrein-kinin system and produces bradykinin which subsequently modifies the local vein, increases contact between the aqueous phase propofol and the free nerve endings and aggravates pain on injection.

The above studies suggest a potential mechanism for propofol-induced pain. As the plasma kallikrein-kinin system is an enzymatic cascade, the degree of activation may be affected by temperature. Several reports have shown significantly reduced pain on injection after using cooled propofol.<sup>29,30</sup> Our results comparing the effect of the temperature of propofol on pain on injection were consistent with this. However, the effect of cooled propofol was smaller than that of propofol mixed with FUT. Although cooled propofol reduced pain on injection, FUT or lidocaine were more effective.

Whether bradykinin produced by propofol affects the circulation is important. As bradykinin is metabolized rapidly, the effect may be small in healthy patients. However,

patients receiving angiotensin converting enzyme inhibitors, whose capacity for metabolism of bradykinin is limited, could be adversely affected.<sup>31,32</sup> Consequently, we compared changes in heart rate and arterial pressure and plasma bradykinin concentrations in patients receiving this class of drug or those receiving a calcium channel blocker during injection of propofol at the highest clinical dose. The results showed that changes in heart rate and arterial pressure were similar in both groups and that bradykinin did not accumulate. This finding suggests that propofol-generated bradykinin had no adverse circulatory effect, probably because of preservation of metabolism of bradykinin, even in patients receiving an angiotensin converting enzyme inhibitor. Therefore, we believe that the effects of FUT on inhibition of kallikrein activity are limited to reduction of propofol-induced pain.

In summary, our studies have revealed a potential mechanism for propofol-induced pain in which the lipid solvent for propofol activates the plasma kallikrein-kinin system and produces bradykinin which modifies the local vein by its vasodilatory and hyperpermeability actions. This modification in the local vein increases contact between the aqueous phase propofol and free nerve endings, resulting in aggravation of pain on injection. FUT and lidocaine reduced propofol-induced pain because they inhibit generation of bradykinin, although complete reduction of pain cannot be expected.

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