Effects of a recombinant FVIIa analogue, NN1731, on blood loss and survival after liver trauma in the pig


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Background. We considered whether haemorrhage after a liver trauma would be reduced by early administration of a pro-haemostatic agent and evaluated the effect of i.v. vs i.m. administration of the coagulation factor VIIa analogue NN1731 on haemorrhage after a liver trauma in the pig.

Methods. The pharmacokinetics of i.v. and i.m. NN1731 was evaluated in eight minipigs, and the effects of dose and administration route of NN1731 (i.v. 180 μg kg⁻¹, n=6; i.m. 540 μg kg⁻¹, n=4, or 2000 μg kg⁻¹, n=6) vs vehicle (n=16) were studied on a liver laceration injury in pigs. To simulate a pre-hospital setting, the administration of NN1731 was delayed by 1 min for i.m. administration and 7 min for i.v. administration, at which time fluid resuscitation also began.

Results. In the minipigs, NN1731 exposure was similar after i.v. 180 μg kg⁻¹ and i.m. 540 μg kg⁻¹, with a bioavailability of ~35%. The injury and blood loss at 7 min was comparable between the four groups of pigs; however, after 60 min, the blood loss was lower in the i.v. treated animals: 1.3 (0.3) (i.v.) vs 2.2 (0.8) litres (i.m.540, i.m.2000, and vehicle) (P<0.001). Also, the survival time was increased: 117 (14) (i.v.) vs 84 (28) min (i.m.540, i.m.2000, and vehicle) (P<0.001).

Conclusions. After a liver trauma in the pig, i.v. administration of NN1731 reduced the bleeding and increased the survival time. In contrast, i.m. administration had no effect, presumably because reduced muscle perfusion during haemorrhage reduced the uptake of NN1731.

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Haemorrhage may be fatal for both injured civilians and military personnel.1–3 In civilians, haemorrhage accounts for ~35% of trauma mortality,2 and ~85% of the combat mortality rate within 2 h of the trauma.4,5 To avoid haemorrhagic shock, the intravascular volume has to be maintained; thus, it will be advantageous to administer a pro-haemostatic agent to reduce haemorrhage. We evaluated the effect of the recombinant coagulation factor VIIa (rFVIIa) analogue NN1731 (Novo Nordisk A/S, Måløv, Denmark) on haemorrhage and survival time after a liver trauma in the pig. NN1731 is an rFVIIa analogue with enhanced thrombin generating capacity localized on the surface of activated platelets, but with similar tissue factor-dependent activity.6,7 At pharmacological doses, rFVIIa mediates enhanced thrombin generation on the activated platelet surface, leading to formation of a stable fibrin plug.8,9 rFVIIa is effective in controlling bleeding in patients with inhibitor-complicated haemophilia and other rare congenital bleeding disorders.10 Similarly, the pro-haemostatic effect of NN1731 is considered to be localized at the site of injury without causing systemic activation of the coagulation system and it is more efficacious and fast-acting than rFVIIa in animal bleeding models11–13 and currently tested in clinical trials.14 I.V. administration of a systemic pro-haemostatic agent is favoured after a severe blood loss, but i.m. administration may also be an option in the case of difficult venous access or because trained personnel are not available. Furthermore, i.m. injection could be administered faster and at any body site, a potential benefit for military first responders, allowing them to focus on tasks and casualties.

The haemostatic effect of rFVIIa has been evaluated in liver injury models in pigs,15–17 demonstrating mixed
results on the efficacy of rFVIIa in severe haemorrhagic and coagulopathic conditions, whereas the use of a more potent agent has the potential for better bleeding control after treatment. Here, the effects of early i.v. or i.m. administration of NN1731 on haemorrhage and survival time were recorded after a liver trauma in the pig and compared with results obtained for vehicle-treated control animals.

Methods

Pharmacokinetic evaluation
See Supplementary material.

Haemostatic evaluation
The experiment was approved by The Danish Ministry of Justice. The animals were provided by a commercial breeder and housed for 1 week before experimentation during which they were fed daily and had free access to water. Food was withheld for 12 h before experimentation.

Haemostatic efficacy of NN1731 was evaluated on 34 domestic pigs at an age of 3–4 months [34 (4) kg; mean (sd)] with an estimated blood volume of 2.5 (0.2) litres [0.16×kg^0.78]. Two blinded and randomized experimental protocols were followed: a first blinded and randomized study assessed the effect of i.v. administration of 180 µg kg^-1 NN1731 (n=6) vs vehicle (n=5) and a second blinded and randomized study addressed the effect of i.m. administration of 540 µg kg^-1 NN1731 (i.m.; n=6) vs vehicle (n=5). The 540 µg kg^-1 dose for i.m. administration was selected on the basis of the preliminary pharmacokinetics study of NN1731. In a third non-blinded study, six pigs received i.m. administration of 2000 µg kg^-1 NN1731 (i.m.; 2000) and five pigs received a similar volume vehicle. This dose was chosen as a proof-of-concept.

Anaesthesia and instrumentation
Anaesthesia was induced with midazolam (0.5 mg kg^-1, i.m.) and Zoletil 50 vet (5 mg kg^-1, i.m.). Adequate anaesthesia was ascertained by absent reflexes, pinching snout and ear. The animals were orally intubated and ventilated with oxygen 2.5 litre and atmospheric air 0.5 litre adjusted to an arterial oxygen tension (P_aO_2) of 13 kPa and a carbon dioxide tension (P_aCO_2) of 5.3 kPa. Anaesthesia was maintained with propofol (15 mg kg^-1 h^-1, i.v.) and fentanyl (0.05 mg kg^-1 h^-1, i.v.) until the animal exsanguinated or was killed 2 h after injury.

After induction of anaesthesia, Baby-feeding tubes (Unoplast, Rødovre, Denmark) were placed in the right external jugular vein for fluid administration and in the left femoral artery for recording mean arterial pressure (MAP) and blood sampling. Another catheter (93A-783H-7.5F, Baxter Healthcare Corporation, Edwards Critical Care Division, Irvine, CA, USA) was positioned in the pulmonary artery by incision via the left external jugular vein for central venous pressure (CVP), pulmonary arterial mean pressure, and blood sampling via the pulmonary port. ECG electrodes were secured after shaving and cleaning the skin.

Cardiovascular monitoring and blood variables
Arterial and venous pressures were measured using Uniflow transducers (Baxter Healthcare Corporation), whereas heart rate (HR) was calculated from the ECG by a Hewlett Packard monitoring system (Agilent Component Monitoring System, Vicare Medical, Birkersd, Denmark). Cardiac output (CO) was determined as an average from triplicate injections of 10 ml of ice-cooled isotonic glucose solution. The CO and blood temperature were displayed (SAT-2TM, Baxter Healthcare Corporation) and noted at baseline and every 10th min after the trauma. Oxygen delivery (DO_2) and uptake (VO_2) were calculated.

Arterial and venous blood (1–2 ml; PICO50, Radiometer, Copenhagen, Denmark) were sampled at baseline and every 10th min after the trauma and immediately analysed for oxygen saturation (S_aO_2 and S_vO_2), P_aCO_2, P_aO_2, haemoglobin, ionized calcium, bicarbonate, pH, and standard base excess using AB605 and OSM 3 Hemoximeter apparatus (Radiometer). At baseline and 0, 5, 15, 30, 45, 60, 90, and 120 min after the trauma, venous blood (~3 ml) was sampled in tubes containing EDTA (K3E, BD Vacutainer, Becton & Dickinson, UK) and analysed for platelet count 2–3 h after sampling (XE-2100, Sysmex Corporation, Kobe, Japan).

Trauma
The animals were laparotomized along the abdominal midline, positioned on the right side, and covered with a blanket. The liver was mobilized with the left lateral lobe positioned extra-abdominally and packed in a sterile plastic bag without compromising perfusion of the liver. After a 30 min resting period, baseline values were obtained and after 10 min, a liver injury was inflicted on the left lateral lobe to equal a grade IV liver trauma (93A-783H-7.5F, Baxter Healthcare Corporation, Edwards Critical Care Division, Irvine, CA, USA) was positioned in the pulmonary artery by incision via the left external jugular vein for central venous pressure (CVP), pulmonary arterial mean pressure, and blood sampling via the pulmonary port. ECG electrodes were secured after shaving and cleaning the skin.

Blood loss
After the liver trauma, blood was collected by suction from the bag surrounding the liver without disrupting formed blood clots, into a series of containers hanging from a custom-built force transducer. The transducer signal was amplified (Scout55, Hottinger Baldwin
Messtechnik GmbH, Germany) and recorded on a computer.

**Haemostatic agent**

Plasma was analysed for FVIIa-like clot activity using a modified version of an FVIIa-specific coagulation assay.\(^{22}\) Clotting time was measured in test samples diluted in 0.1 M NaCl, 0.05 M Tris–HCl, 1% BSA, pH 7.4 (TBS/BSA, all from Sigma-Aldrich, Steinheim, Germany) with 25 \(\mu l\) human FVII-deficient plasma (Helena BioSciences, Sunderland, UK), 50 \(\mu l\) diluted rabbit brain cephalin (Hepstest, St Louis, MO, USA), and 50 \(\mu l\) 30 nM truncated (1–209) recombinant soluble tissue factor (Novo Nordisk A/S) with 4.2 mM calcium (Merck, Damstadt, Germany) using an ACL-9000 automated coagulation instrument (Instrumentation Laboratories Scandinavia, Allerød, Denmark).

**Treatment**

To simulate pre-hospital treatment, it was considered that i.v. administration could be established after 7 min (according to the time claimed to be the average arrival time of a trauma team in the Copenhagen area), whereas it was considered that i.m. administration could be performed already after 1 min by a person on the scene of the injury or, possibly, even by the injured person. Administration of NN1731, or similar volume of carrier solution, was i.v. at a dose of 180 \(\mu g\) kg\(^{-1}\) (~4 ml, 1.65 mg ml\(^{-1}\)), whereas i.m. administration was in a dose of 540 (~3 ml, 5.85 mg ml\(^{-1}\)) or 2000 \(\mu g\) kg\(^{-1}\) (2 × ~6 ml, 5.85 mg ml\(^{-1}\)), that is, 3–or 11-times the i.v. dose, respectively, considering that i.m. administration reduces the bioavailability. I.M. administration was performed with one bolus in the quadriceps muscle (540 \(\mu g\) kg\(^{-1}\)) or, because of the larger volume, with a bolus in each trapezius muscle (2 × 1000 \(\mu g\) kg\(^{-1}\)). Changes in blood flow of the injected muscle were monitored with a laser Doppler flowmeter (BLF 21D; Transonic Systems Inc., Ithaca, NY, USA) on its surface after a skin incision. For studies 1 and 2, the investigators were blinded with regard to the agent used, since the test substance was injected by a veterinary nurse not directly involved in the study.

**Volume administration**

Volume administration simulated that of a trauma patient and was therefore delayed by 7 min and accounted for in three consecutive phases to simulate that the trauma team would be likely to establish, with increasing i.v. access and potentially larger cannulae.\(^{18-23}\) Initial support of the blood volume was with lactated Ringer’s solution (Fresenius Kabi, Sweden): 0.7 ml kg\(^{-1}\) min\(^{-1}\) for 10 min (phase 1: P1) and 1.65 ml kg\(^{-1}\) min\(^{-1}\) for 20 min (P2) to allow for clot formation. Thereafter, volume administration was with hydroxyethyl starch 130/0.4 (HES; 60 mg ml\(^{-1}\); Voluven\textsuperscript{®}, Fresenius Kabi) according to blood loss (ratio 1:1) and aimed to stabilize the intravascular volume (P3). Infusion of HES continued until termination of the experiment, that is, until death or 2 h after the treatment with NN1731 or vehicle, allowing time for definitive surgery to be initiated. All fluids were preheated to 35–40°C before i.v. infusion.

**Blood samples**

To evaluate NN1731 activity, venous blood (4.5 ml) was sampled in BioPool\textsuperscript{®} Stabilyte\textsuperscript{TM} tubes (0.5 ml 0.5 M citrate buffer, pH 4.3; Trinity Biotech plc, Ireland) for analysis of FVIIa-like clot activity at baseline and 5, 15, 30, 60, 90, and 120 min after administration of the drug. The blood was centrifuged (4000g, 20°C, 5 min) after which the supernatant was stored at ~80°C until analysis of FVIIa-like clot assay.\(^{22}\) Recombinant FVIIa was used for creation of a standard curve. In brief, citrated plasma samples were mixed with truncated (1–209) recombinant human-soluble tissue factor and phospholipids and the reaction was triggered by re-calcification. In all cases, the baseline level before NN1731 administration was below the detection limit of 5 × 10\(^{-3}\) \(\mu g\) ml\(^{-1}\).

**Data collection and statistical analysis**

Death was considered when similar MAP, PAMP, and CVP were apparent. Arterial and venous pressures, ECG, and cumulative blood loss were recorded using WinDaq\textsuperscript{TM} Pro\textsuperscript{+} software version 2.41 and data acquisition (DI-720; DataQ Instruments, OH, USA). Statistical analysis was performed by one- or two-way ANOVA followed by a Student–Newman–Keul post hoc test to identify deviations (SigmaStat, version 3.0). If a normality test failed, a non-parametric test was applied (Kruskal–Wallis one-way ANOVA or Friedman repeated-measures ANOVA). The limit for statistical significance was chosen as \(P<0.05\), and data are presented as mean (SD). The data were normalized to the average survival time for the respective group, while taking account for the three phases of fluid administration. The three subcontrol groups had similar bleeding rates and survival times and were therefore treated as one sample.

**Results**

**Pharmacokinetic evaluation**

See Supplementary material.

**Haemostatic evaluation**

In all pigs, as intended, \(P_{aO_2}\) and \(S_bO_2\) were >13 kPa and >98%, respectively, and \(P_{aco_2}\) was between 5.2 and 5.9 kPa. For all groups, pH decreased from 7.5 to 7.4 during
the experiment, whereas blood temperature was 37–38°C until it decreased ~15 min before death.

Data from two pigs in the i.m. 540 group were excluded from the data set because of irregular baseline values: high HR (>100 beats min⁻¹), low CO (<2 litre min⁻¹), low MAP (20–55 mm Hg), high pulmonary arterial mean pressure (25–45 mm Hg), and a low platelet count (<150×10⁹ litre⁻¹).

**Vehicle and i.m. administration**

The cross-section area of the incision surface and the number of severed vessels were similar for the two i.m. groups and the control group (Table 1). At 7 min after the trauma, the blood loss and a decrease in MAP, CVP, CO, and DO₂ were similar for the three groups; with the exception of CO and DO₂ which decreased more for the i.m. 2000 group compared with the control group ($P < 0.05$). The bleeding rate was similar for the three groups and almost linear during the first 50 min, but hereafter the bleeding rate increased exponentially (Fig. 1A). The blood loss after the 60th min was 2.4 (0.8), 2.6 (0.7), and 2.5 (0.2) litres for the control, i.m. 540, and i.m. 2000 groups, respectively. The administration of fluid volumes was, therefore, also similar. The survival time was the same for the three groups and none of the pigs survived to 2 h post-treatment (Fig. 1B). MAP did not recover to baseline values and CVP remained reduced during P1 and P2, but increased to above baseline level during P3 (Fig. 2). The HR was unchanged after the trauma and until death, but CO recovered briefly to the baseline level during P3. The DO₂ continued to decrease until death, whereas VO₂ was unchanged at 150–200 ml min⁻¹ for 50 min after the trauma, but it decreased hereafter.

Muscle blood flow for the i.m. 540 group and for the sub-control group paired with the i.m. 540 group was reduced by 60% at 7 min after the trauma and remained at that level until death. We were unsuccessful in obtaining a reliable muscle blood flow for the i.m. 2000 group and the corresponding sub-control group. For the i.m. groups, the FVIIa-like clot activity increased to 0.9 (1.2) and 1.0 (0.8) μg ml⁻¹ 5 min after administration of NN1731 (Fig. 3).

For blood biochemistry, see Supplementary Figure 4.

### Table 1

<table>
<thead>
<tr>
<th>NN1731 (i.v.) 180 μg kg⁻¹</th>
<th>NN1731 (i.m.) 540 μg kg⁻¹</th>
<th>NN1731 (i.m.) 2000 μg kg⁻¹</th>
<th>Control</th>
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<tr>
<td>n</td>
<td>6</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>36 (4)</td>
<td>33 (3)</td>
<td>35 (2)</td>
</tr>
<tr>
<td>Estimated blood volume (litre)</td>
<td>2.6 (0.2)</td>
<td>2.5 (0.2)</td>
<td>2.6 (0.1)</td>
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<tr>
<td>Cross-section area of trauma (mm²)</td>
<td>839 (120)</td>
<td>1068 (55)</td>
<td>1085 (91)</td>
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<tr>
<td>Number of severed vessels</td>
<td>2.5 (0.8)</td>
<td>3.0 (1.2)</td>
<td>2.5 (0.5)</td>
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<tr>
<td>60 min, lactated Ringer's solution (litre)</td>
<td>1.4 (0.1)</td>
<td>1.3 (0.1)</td>
<td>1.4 (0.1)</td>
</tr>
<tr>
<td>60 min, hydroxyethyl starch 130/0.4 (litre)</td>
<td>1.2 (0.7)</td>
<td>1.1 (0.3)</td>
<td>1.0 (0.4)</td>
</tr>
<tr>
<td>Survival time (min)</td>
<td>117 (14)†</td>
<td>74 (11)</td>
<td>83 (17)</td>
</tr>
<tr>
<td>Surviving 2 h post-treatment</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

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**Fig 1** Blood loss (a) and Kaplan–Meier survival plot (b) for pigs after a grade IV liver trauma and i.v. administration of NN1731 180 μg kg⁻¹ [n=6; 36 (1) kg], i.m. administration of 540 [n=4; 33 (1) kg] or 2000 μg kg⁻¹ [n=6; 35 (1) kg], or control [n=16; 33 (1) kg]. I.V. administration of NN1731 and initiation of fluid administration were 7 min after the trauma, while i.m. administration of NN1731 was 1 min after the trauma. Dashed lines (a) beginning of the different fluid administration phases: P1, 0.7 ml kg⁻¹ min⁻¹; P2, 1.65 ml kg⁻¹ min⁻¹; and P3, according to blood loss (ratio 1:1). Fluid administered was with lactated Ringer in P1–2 and HES 130/0.4 in P3. Data for P3 are normalized to experimentation time and presented as mean (sd). **Different from the control group ($P < 0.01$).
I.V. administration

The cross-section area of the incision surface and the number of severed vessels were similar to those of the i.m. and control groups (Table 1). Also, the blood loss and the decrease in MAP, CVP, CO, and DO$_2$ at 7 min after the trauma were similar to those in the control group. The blood loss was almost linear until the 70th min; hereafter, the bleeding rate increased exponentially. After 30 min, the bleeding rate started to diverge from that of the i.m. and control groups, and after 60 min, the blood loss was $\sim$50% of that of the i.m. and control groups ($P<0.001$; Fig. 1A). The administration volume of lactated Ringer’s solution was similar to that of the i.m. and control groups, but the volume of HES administered at 60 min was less compared with the other groups ($P<0.01$). The survival time was $\sim$30 min longer than for the i.m. and control groups and two of the six pigs survived the 2 h post-treatment threshold ($P<0.001$ compared with control; Fig. 1B). The MAP was maintained at a higher level compared with the i.m. and control groups (Fig. 2). During P1 and P2, CVP remained reduced, but increased to above baseline level during P3; this increase occurred at a slower rate than for the i.m. and control groups ($P<0.01$). The HR was unchanged after the trauma and was maintained.
for a longer period than for the i.m. and control groups ($P<0.001$). During P1 and P2, CO remained reduced, but during P3, it increased to $\sim6$ litre min$^{-1}$ to decrease again (to $<2$ litre min$^{-1}$) before death. The DO$_2$ and VO$_2$ were maintained for a longer period than for the i.m. and control groups.

The FVIIa-like clot activity increased to 11.6 (2.0) $\mu$g ml$^{-1}$ 5 min after the administration of NN1731, but was 5.0 (1.5) $\mu$g ml$^{-1}$ after 15 min and continued to decrease thereafter (Fig. 3). For blood biochemistry, see Supplementary Figure 4.

**Discussion**

The main finding of this study is that i.v. administration of NN1731 (180 $\mu$g kg$^{-1}$), a recombinant FVIIa analogue, reduces the bleeding rate after a liver trauma in the pig, thereby prolonging survival time compared with vehicle-treated control animals. In contrast, early and large (3- and 11-times the i.v. dose) i.m. administration of NN1731 did not affect the bleeding rate or the survival time, presumably because of reduced bioavailability during haemorrhage. Furthermore, arterial pressure was maintained at a higher level and oxygen uptake was supported for $\sim30$ min after i.v. administration compared with the i.m. and control pigs.

A major dilemma for the pre-hospital critical care team is fluid resuscitation during haemorrhage. Fluid administration has to be balanced, so it adequately replenishes the intravascular system without provoking uncontrolled haemorrhage or coagulopathy and administration of colloids should be avoided until bleeding has ceased.$^{18}$ $^{24}$–$^{26}$

The total volume of HES in P3 reached 3–4 litres which might have been excessive and mitigate the effect of the haemostatic agent towards the end of the experiment. Thus, the administration of HES in the present study may have aggressively diluted the effect of all coagulation agents, thereby accelerating bleeding and exsanguination. The pro-haemostatic effect of NN1731 reduced haemorrhage in mice and rats and improved coagulation competence in whole blood from both haemophilia patients and healthy volunteers.$^{11}$–$^{13}$ $^{27}$–$^{29}$ These studies pointed towards a higher haemostatic potential of NN1731 compared with rFVIIa.$^{11}$–$^{13}$ $^{27}$–$^{29}$ However, the prohaemostatic effect of rFVIIa has been evaluated with trauma models in pigs.$^{15}$–$^{17}$ but has not been directly compared with NN1731 in the present trauma model. Further experiments are needed to directly compare rFVIIa and NN1731 in trauma models on large mammals.

The pharmacokinetics of NN1731 in non-traumatized minipigs indicated a bioavailability of $\sim36$% and, therefore, an i.m. administration of 540 $\mu$g kg$^{-1}$ should provide an overall exposure similar to that following i.v. administration of 180 $\mu$g kg$^{-1}$. We considered that i.m. administration of 2000 $\mu$g kg$^{-1}$ would allow for an even larger exposure of NN1731 with a peak FVII-like clot activity similar to that following i.v. administration of 180 $\mu$g kg$^{-1}$.

The four groups of pigs were comparable at baseline with the exception of venous oxygen saturation, which was somewhat higher in the i.m. groups compared with the i.v. and control groups, although it is unlikely that this difference influenced bleeding rate and survival time. The trauma created a similar cross-section trauma surface area in the three treated groups, compared with the control group, and the number of severed vessels (1–3 mm diameter) was similar in the four groups; even though the area for the i.m. groups was larger than that of the i.v. group. Furthermore, the blood loss at 7 min and the associated reduction in MAP and CVP were similar between the four groups. Thus, we consider that both the pigs and the trauma allowed for direct comparison of the four groups of pigs.

The i.v. group had a reduced bleeding rate compared with the two i.m. groups and the control group, even though the i.m. groups received 3- and 11-times higher dose of NN1731 compared with the i.v. group. The difference in the effect of NN1731 between i.v. and i.m. administration was caused by a lower bioavailability of NN1731 after i.m. administration and we tried to compensate by increasing the i.m. dose. The FVIIa-like clot activity 5 min after NN1731 administration was $\sim1$ $\mu$g ml$^{-1}$ for both i.m. groups, but it was $\sim12$ $\mu$g ml$^{-1}$ for the i.v. group and, although the FVIIa-like clot activity after i.v. administration decreased rapidly, it was higher than after i.m. administration for $\sim60$ min. Also, the FVIIa-like clot activity was comparable between the i.m. groups, despite a 3.5-fold difference in the dose of NN1731 administered in these groups, which may be because of differences in blood flow of the quadriceps and trapezius muscles. Although the large dose was administered by two injections ($2\times\sim6$ ml for i.m.$^{2000}$ vs $\sim4$ ml for i.m.$^{540}$), it...
cannot be ruled out that the difference in injection volumes influenced uptake. In non-traumatized minipigs, i.m. administration of 540 μg kg⁻¹ resulted in a peak FVIIa-like clot activity of ~3.5 μg ml⁻¹ after 10–15 min which suggests that the ~60% reduction of muscle blood flow during haemorrhage is an important factor for the poor uptake of NN1731 into the vascular system. We only obtained reliable blood flow measurements from the quadriceps muscle, but it is recognized that haemorrhage may reduce muscle blood flow.³⁰

The amount of ionized calcium available for the coagulation cascade was similar during early administration of lactate Ringer’s solution and was at all times >0.9 mmol litre⁻¹ in the four groups, such that it is unlikely that ionized calcium was a determining factor of the difference in blood loss.³¹ We acknowledge that the i.v. group had a somewhat higher baseline platelet count than the other groups that may influence bleeding rate,³² but analysis of survival time vs baseline platelet count of the control and i.m. groups indicates that survival time was independent of baseline platelet count (data not shown). For all four groups, blood temperature was >34°C and pH was >7.2 throughout the experiment and they were, therefore, not factors in the development of uncontrolled haemorrhage.³¹

Since bleeding from the liver is mainly venous, the reduced bleeding rate of the i.v. group manifested, although MAP was slightly higher. Although CVP was unchanged by the administration of lactated Ringer’s solution, it increased during administration of HES and that may have facilitated the increase in bleeding rate along with haemodilution. The haemoglobin levels were similar at baseline, but the lower bleeding rate in the animals treated i.v. with NN1731 resulted in a smaller decrease in this group than in the other groups. Although oxygen uptake was maintained for 80 min in the i.v. group, this was only the case for 50 min in the other groups. Standard base excess is an indicator of cellular metabolic state and, thus, linked to its oxygenation, and standard base excess was also maintained for longer in the i.v. group. Bleeding rate increases as haemodilution progress and during administration of HES, a negative feedback was established because of the 1:1 resuscitation regimen. For all groups, excessive haemorrhage developed at a haemoglobin level of 2–3 mmol litre⁻¹, but because of the reduced bleeding rate and, therefore, slower rate of haemodilution, this level was reached at a later time for the i.v. group.

Conclusion

When administered i.v., the rFVIIa analogue, NN1731, reduced the bleeding rate and prolonged survival time after severe liver trauma in pigs and, as a consequence of the reduced bleeding rate, oxygen uptake was maintained for a longer period. In contrast, i.m. administration of the drug did not have these effects, presumably due to low bioavailability during uncontrolled haemorrhage.

Supplementary material

Supplementary material is available at British Journal of Anaesthesia online.

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References

3 Champion HR, Bellamy RF, Roberts CP, Leppaniemi A. A profile of combat injury. J Trauma 2003; 54: S13–9


27 Sørensen B, Persson E, Ingerslev J. Factor VIIa analogue (V158D/E296V/M298Q-FVIIa) normalises clot formation in whole blood from patients with severe haemophilia A. Br J Haematol 2007; 137: 158–65

28 Brophy DF, Martin EJ, Nolte ME, Kuhn JG, Carr ME Jr. Effect of recombinant factor VIIa variant (NN1731) on platelet function, clot structure and force onset time in whole blood from healthy volunteers and haemophilia patients. Haemophilia 2007; 13: 533–41


30 Tripathi A, Nadel ER. Forearm skin and muscle vasoconstriction during lower body negative pressure. J Appl Physiol 1986; 60: 1535–41
