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Haemostatic efficacy of an ethyl-2-cyanoacrylate-based aerosol in combination with tourniquet application in a large wound model with an arterial injury

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Summary

Background: Tourniquet application is the standard method for the control of severe trauma haemorrhage. However, it may result in severe ischaemic injuries when used for a long time. In this study, we developed a modified ethyl-2-cyanoacrylate-based aerosol (ECA) aerosol spray and determined its efficacy for short-time control of bleeding of large wounds in pigs when used in combination with tourniquet application.
Methods: A large wound model with a femoral arterial injury was made in the middle of either thigh of the pig. Thirty white female hybrid pigs were divided evenly and randomly into three groups, including tourniquet application only group (group A), tourniquet–ECA group (group B, a combination of ECA with tourniquet application) and tourniquet–ECA with wound cleaning group (group C, a combination of ECA with tourniquet application plus wound cleaning).
Results: The success rates of haemostasis were 0%, 30%and 90% in groups A, B and C, respectively ($P < 0.05$). The incidence of haematoma and the membrane forming time were 100% and 20%, and 5.9 ± 1.0 min and 8.3 ± 1.1 min, respectively, in groups

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B and C (both $P < 0.05$). The haemostatic time and the thickness of membrane were 11.9 ± 1.8 min and 10.2 ± 1.4 min, and 0.68 ± 0.29 mm and 0.79 ± 0.25 mm, respectively, in the two groups ($P > 0.05$, both).
Conclusion: The ECA spray achieves haemostasis within a very short time when it is used in combination with tourniquet application in a large wound model with an arterial injury. It may effectively prevent the wound from bleeding without the need for any long-term pressure bandage to wrap the wound, and it is easy to be disposed in debridement. Therefore, it may serve as an optimal choice for the first aid of large wounds with an arterial injury.

Introduction

Severe limb wounds occur in both battlefields and civilian trauma. One of the difficulties in the treatment of the wound is attributable to the large amount and speed of blood loss from the wound. Uncontrollable bleeding, especially from wounds with major arterial injuries account for approximately 50% and 31% of the total mortality in the battlefield and civilian trauma, respectively.[2,20,22,25]

The traditional first aid haemostatic methods include the application of tourniquets, local pressure, electrocautery, haemostatic forceps clipping, and suture ligation.[15,26] Although many new topical haemostatic agents, such as poly-*N*-acetyl glucosamine-derived rapid deployment haemostat bandage and fibrin bandage, have been developed for the treatment of wound bleeding, tourniquets are still used as the standard care for the control of severe haemorrhage due to their convenience and proven efficacy. However, tourniquet application has well-known limitations; it can be used only for temporary haemostasis because long-term persistent application unavoidably results in severe ischaemic injuries of the vessels and nerves distal to the wound, while a periodical release of the tourniquet may bring a large volume of blood loss.

Our hypothesis is that a combination of tourniquet application with a haemostatic bandage may shorten the application time of the tourniquet, and hence minimise the ischaemic injury of the wounded limb. The newly developed haemostatic bandages, including poly-*N*-acetyl glucosamine-derived Rapid Deployment Haemostat (RDH) bandage,[5] TOMETA KUN compression system,[19] Nycomed product decreased fibrin bandage,[17,18] dry fibrin sealant dressings (DFSD)[8,9] and a granular mineral haemostatic agent (QuikClot)[28]added bandage,[10,17] all belong to pressure bandages and thus should be wrapped firmly around the wound to achieve a haemostatic effect. However, this still causes ischaemia in the distal nerves and vessels of the wounded limb. Meanwhile, heavy blood loss may occur during the haemostatic procedure. Several products, such as those with the granular mineral haemostatic agent (QuikClot), are even accompanied with reactive heat which may harm the wounded tissues.[28] Therefore, the haemostatic effect of these bandages is not satisfactory in controlling severe limb bleeding when they are used in combination with a tourniquet.

Alkyl-2-cyanoacrylate was first used as a medical adhesive by Coover in 1959.[6] In the past several decades, up to 10 types of cyanoacrylate adhesives have been developed for clinical use. However, only ethyl-, butyl-, and octyl-cyanoacrylate adhesives are currently widely applied in clinical practice, as some cyanoacrylate adhesives cause toxicity and irritation to the tissues. Of those three types of cyanoacrylate adhesives, ethyl-2-cyanoacrylate has been reported to possess the most adhesive bonding strength,[11,13,16] and was used in the present study in combination with tourniquet application for the control of severe wound bleeding. Since ethyl-2-cyanoacrylate is highly brittle and its pliability is not satisfactory, formula modifications were required to overcome these weaknesses.

The aim of the present study was to develop a modified ethyl-2-cyanoacrylate-based aerosol (ECA) spray, and to determine its efficacy for rapid control of bleeding in pigs with large wounds when ECA was used in combination with tourniquet application. We observed that ECA, in combination with tourniquet application, achieves a satisfactory effect for the control of arterial bleeding in pigs with large wounds.

Materials and methods

Materials

The ECA aerosol spray agent was developed by our laboratory. Compared to our previous publicated recipe, an intensifier, a plasticiser and an environmentally friendly propellant were added to this ECA preparation.[23] The compounded ECA spray agent

was poured into a 15 ml spray bottle. The formula of this ECA spray has been granted an invention patent in China (Patent number CN200310103799.9).

Animals

Thirty white female hybrid pigs aged 3—6 months weighing 23—27 kg were purchased from the Experimental Animal Center of Southern Medical University (Guangzhou, China). All animal experiments in this study were carried out with the approval of the Animal Care Committee of Southern Medical University, in accordance with the Chinese Council on Animal Care Guidelines. Animals were housed individually in large animal runs. The room was maintained at a temperature of 20—26 °C with a relative humidity of 40—70%, and on a 12-h light/dark cycle. Animals were fed with a standard diet twice daily, and water was available *ad libitum*.

Surgical procedures

Anaesthesia

Animals were anaesthetised by intravenous injection of 3% pentobarbital sodium at a dosage of 50 mg/kg. The anaesthetised pigs were then restrained supine on a standard pig operating board. Blood pressure (BP) was monitored through the left common carotid catheter connected to a pressure transducer (Version 3.0, the Pclab Biological Signal Collection and Manage System, Beijing Microsignalstar Technology Development Co., Ltd., China).

Establishment of the bleeding model

A circular incision of 10 cm in diameter was made in the middle of either thigh along the body surface projection of femoral artery. A segment of femoral artery with 10 mm in length and 3.5—4.0 mm in diameter was surgically isolated after the removal of the local skin, subcutaneous tissue, fascia and part of the muscle tissues. The position of the selected thigh was adjusted to make the surface of the wound horizontal. Two incisions each 10 mm in length were made parallel to the groin ligament at the inner and outer sides of the groin. A subcutaneous tunnel through the dorsal area of the thigh was made to connect the two incisions. A rubber tourniquet was passed through the subcutaneous tunnel to avoid possible slippage when tightened. The femoral artery was divided 6 mm distal to the bifurcation to produce free bleeding.

Trial grouping and haemostatic treatments

A total of 30 pigs were divided evenly and randomly into three groups. Pigs in group A received tourniquet application only. Pigs in group B were treated with a combination of ECA and tourniquet application, and pigs in group C were treated with a combination of ECA and tourniquet application plus wound cleaning.

The animals in all three groups received tourniquet application immediately after the free bleeding, and the tourniquet was removed 10 min later. The pigs in groups B and C received ECA spraying twice (5 s each) in addition to the tourniquet application. The spray was applied from a distance of 10 cm to the surface of the wound. In group B, the ECA spray was applied directly, while in group C the blood on and around the wound was removed by dry sterilised gauzes to make the wound relatively dry before spray. The blood removal procedure and the spray were completed within 30 s after the free bleeding.

Evaluation of haemostasis

The time duration between the time when ECA was sprayed onto the wound and the time when complete haemostasis was achieved was defined as the haemostatic time (HT). Any re-bleeding that occurred immediately after the releasing of the tourniquet was defined as failure. In addition, for wounds with initial nets haemostasis, 10 of extreme back and forth movements were performed in both hip and knee joints. In pigs with haematoma, the size of haematoma was measured before and/or after the joint movements. The occurrence of any of the following events, including ECA membrane breaking, re-bleeding, and the enlargement of previously formed haematoma, which were observed within 10 min or 10 min after the joint movements were also defined as failure. Therefore, successful haemostasis was defined as no re-bleeding after 10 min of tourniquet application, and no enlargement of previously formed haematoma after joint movements.

ECA membrane evaluation

The forming time of the membrane (FTM), which was defined as the duration between the time when ECA reached the wound and the time when it was completely solidified to form a membrane, was recorded. Five minutes after the spray, dry filter papers were repeatedly placed on the surface of ECA membrane with a 30 s interval, and the complete solidification was considered to be achieved when the paper remained dry. The ECA membrane was uncovered after the evaluation of haemostasis was completed. The thickness of membrane (TM) was measured by a sliding Vernier caliper (Zhe-zhi 01010259, Hangzhou tool general factory, Hangzhou, China). In each of the four quadrants of the membrane, two randomly selected points were used

for the thickness measurement. The mean value of the total eight points was used as TM for the asymmetric membrane. The ECA membrane was then folded by 180° to evaluate the flexibility, and whether or not the membrane was broken during the folding was recorded.

Surgical treatment after haemostasis evaluation

A arterial ligations were performed to the animals whose bleeding failed to be stopped in the trial. The wounds in animals that achieved haemostasis were re-opened, and the openings of ruptured arteries were examined to determine whether there were formed clots, and whether the re-bleeding would occur when the clots were removed by eye forceps.

Statistical analysis

Statistical analysis was performed using SPSS 10.0 software (SPSS/PC Inc., IL, USA). The success rates of haemostasis were represented as percentage (%), while the remaining data were expressed as mean value ± standard deviation (S.D.). Chi-square test was used for the analysis of the difference in haemostasis rates between any two groups, while the other analyses were performed by ANOVA. A P value of <0.05 was considered statistically significant.

Results

Blood pressure of animals

During the whole procedure, blood pressure of all animals was maintained stable. The mean arterial pressure (MAP) of each pig was always higher than 60 mmHg. No significant difference in the MAP was found among the three groups.

Haemostatic outcomes

None of the pigs in group A achieved haemostasis, while successful haemostasis was achieved in no less than 30% of the animals that were treated with tourniquet–ECA combination ($P < 0.05$). The best success rate of haemostasis was found in group C, in which pigs were treated with tourniquet–ECA and wound cleaning before ECA was sprayed (Table 1). Haematoma occurred in all animals in group B and in two animals in group C after the tourniquet release ($P < 0.05$). However, seven animals in group B and one of the two in group C showed rapid haematoma enlargement after the tourniquet release which resulted in the breaking of the ECA membrane, and thus lead to haemostatic failure (Table 1). The measurement of the haematoma size could only be performed on animals whose haemorrhage was successfully controlled, i.e. all 3 of the 10 animals in group B and one of the two in group C (Table 1). The sizes of the haematoma were from 4 cm × 3 cm × 1 cm to 12 cm × 3 cm × 5 cm, however, there was no significant difference between groups B and C. After the movements of joints, no obvious haematoma enlargement was found in both groups. Moreover, HT in the animals with successful haemostasis was not significantly different between groups B and C (Table 2).

Table 1 Performance of the three different haemostatic methods

Group	Occurrence of haematoma, n (%)	Successful haemostasis, n (%)
A (n = 10)	0 (0)	0 (0)
B (n = 10)	10 (100)*	3 (30)*
C (n = 10)	2 (20)	9 (90)

* $P < 0.05$, compared with group C.

ECA membrane evaluation

All the ECA membranes could be peeled off completely. Few membranes remained entirely full. Although most membranes fractured, they were still relatively integrated and few pieces were generated that required removal. Removal of these pieces could cause small wound oozing. Breaking was found in six membranes in group B and one in group C when folded by 180°. No significant difference was found in TM between groups B and C, while the FTM in group C was longer than that in group B ($P < 0.05$) (Table 2).

Table 2 Haemostatic related parameters in haemostatic cases

Group	Haematoma size (cm^3)[a]	FTM (min)	HT (min)	TM (mm)
B (n = 3)	120 ± 34	5.9 ± 1.0	11.9 ± 1.8	0.68 ± 0.29
C (n = 9)	58	8.3 ± 1.1*	10.2 ± 1.4	0.79 ± 0.25

FTM: forming time of membrane; HT: haemostatic time; TM: thickness of membrane. Data were presented as mean ± S.D.

[a] n = 3 in group B and n = 1 in group C.

* $P < 0.05$, compared with group B.

Examination of the opening of ruptured artery

The openings of the arteries were completely clogged by clots in all the animals with successful haemostasis, and no re-bleeding occurred when the clots were removed.

Discussion

The major factors that should be considered for the establishment of a limb wound animal model include animal types, traumatic factors, location of injured limbs, and the sizes of wounds.[3,4,14,24] Bleeding from an incomplete arterial rupture with the opening equal to or greater than 1/2 the diameter is more severe than that from a complete rupture, because the latter may result in retraction of the distal and proximal ends, and the blood vessels would cramp and the clots may form at the rupture site. However, in the cases of incomplete arterial rupture, the elasticity of vessels does not cause retraction, and may even enlarge the rupture. In this situation, bleeding will not stop by itself except that the artery blood pressure is decreased to an exceptionally low level.

The present study was designed for first aid or urgent treatment of large wounds with a large artery rupture that frequently occur in battlefields and civilian trauma, especially those caused by modern firearms. For this purpose, the experimental limb site of the animal model could easily be used to create a large wound, and should have an artery that can be easily cut in half using common surgical equipment. Therefore, the medial thigh of a pig is recommended as a suitable choice for this purpose.[1,10,17] In the present study, the size of the rounded wound model was about 12 cm in diameter, conforming to the criteria of a large wound.[7,12,21,27]

In the pre-experimental trial, we attempted to use ECA spray only for haemostasis of a large wound model with an arterial injury, but we observed that the haemorrhage could not be stopped. Therefore, we believe that temporary haemostasis needs to be achieved by tourniquet application before ECA spray.

In the present study, we observed that the HT in groups B and C were only 11.9 ± 1.8 min and 10.2 ± 1.4 min, which might minimise the potential ischaemic injuries of the vessels and nerves distal to the wound, and, in turn, reduce potential blood loss. Since no significant difference was found in the HT and TM between groups B and C in the present study, it seems that FTM may be a key factor for the haemostatic effectiveness of ECA. We found that although the removal of blood in the wound prolonged the FTM from 5.9 ± 1.0 min to 8.3 ± 1.1 min, it significantly increased the haemostasis rate from 30% to 90% (Table 1). A reasonable explanation for this phenomenon could be deduced from the characteristics of ECA. As soon as the ethyl-2-cyanoacrylate monomers reach the wound surface, they react with each other with the aid of the catalyser (i.e. the water inside the soft tissue) and rapidly become a liquid polymer membrane, which sticks tightly to the wound. Then the monomers which arrive later are solidified by absorbing tiny amount of water in the air. As a result, the membrane quickly becomes thick and strong. The residual blood on the wound surface would accelerate the formation of the ECA membrane but it significantly affects the solidification and quality of the membrane because it contains a large amount of water, and thus the membrane cannot stick firmly to the wound surface, resulting in a reduced haemostatic effect.

The incidence of haematoma may increase the surface tension of the membrane, which may reduce its crushing strength. When the arterial flow resumes, the blood pressure in the wound might be higher than the intensity of the ECA membrane which will cause re-bleeding. In the present study, we found that clearing the blood on the wound surface was an effective method to avoid the formation of haematoma, which reduced the incidence of haematoma from 100% to 20%.

Due to various sizes and shapes of wounds, the limited sizes of standard haemostatic bandages may not meet the haemostatic needs for large and irregular wounds, which is one of the major limitations of traditional haemostatic dressing. In contrast, the ECA spray is convenient to carry and suitable for various irregular wounds, and can achieve haemostatis in a very short time when it is used in combination with a tourniquet. It can effectively prevent the wound from bleeding without the need for any pressure bandage to wrap the wounds. In addition, it can be easily removed at debridement, and should be a good choice for the first aid of large wounds with an arterial injury.

However, it should be mentioned that the pig model established in the present study only mimicked, to some degree, the wounds from some modern firearms, and consequently, the conclusion drawn from this study might not completely represent the actual haemostatic effect of ECA in large wounds with a big arterial rupture generated by firearms in reality. Its actual effect needs further clinical confirmation.

In conclusion, ECA spray achieves haemostasis within a very short time when it is used in combina-

tion with a tourniquet in a large wound model with an arterial injury, and thus may be an optimal choice for the first aid of such wounds. However, further studies are required to investigate the biological properties of ECA, including bacteriostasis.

Conflict of interest statement

All authors have declaimed that there is no conflict of interest involved in this manuscript.

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