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EXPERIMENTAL PAPER

Efficacy of FDA-approved hemostatic drugs to improve survival and reduce bleeding in rat models of uncontrolled hemorrhage☆

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Summary Several FDA-approved intravenous drugs are used to reduce surgical bleeding. This series of studies tested whether these drugs (aprotinin, desmopressin, tranexamic acid, ε-aminocaproic acid) could reduce bleeding due to traumatic injuries in two models of uncontrolled hemorrhage in rats. In the first phase of each study, a nonlethal tail bleeding model was used that incorporated limited fluid resuscitation (lactate Ringer's solution). Four doses of vehicle or the test substance were given successively with bleeding time and blood loss measured after each dose. In the second phase of each study, a lethal liver injury was produced by excising a section of the median lobe (approximately 0.8% of body weight) and an infusion of either vehicle or the test substance was immediately begun. This model included aggressive fluid resuscitation and a severe dilutional coagulopathy. Blood loss, survival time and mortality rate were recorded. Three studies were performed, testing each of the drugs singly and in combination. None of the drugs significantly reduced either bleeding time or blood loss in the tail bleeding model, nor were blood loss, survival time or mortality rate altered in the liver injury model. Taken together, these results suggest that these FDA-approved drugs, when used either singly or in combination, are not efficacious in these models of traumatic uncontrolled hemorrhage.

Introduction

The concept of using intravenous drugs to enhance or augment the body's innate clotting mechanisms during situations in which blood loss is expected is not new. Indeed, drugs have been used in the treatment of bleeding complications for over 30 years.[1]

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For example, the drugs aprotinin, desmopressin (DDAVP), epsilon-amino caproic acid (EACA) and tranexamic acid (TXA) have been used to reduce bleeding complications and blood loss in a variety of clinical situations including cardiac surgery, hepatic surgery, orthopedic surgery and in patients with bleeding disorders.[1–3] Three of these drugs (aprotinin, EACA and TXA) are FDA-approved for use in perioperative hemostasis, while the fourth (desmopressin) has an approved indication for use in bleeding in patients with hemophilia or von Willebrand's Disease. Although many case reports documenting successful use of these drugs appear in the literature, it should be noted that the few randomized clinical trials investigating surgical use of these drugs have produced contradictory results.[3–5]

Hemorrhage is a leading cause of death from both battlefield and civilian trauma.[6,7] Many of these deaths are from noncompressible hemorrhage (i.e., that which is not accessible for manual pressure), for which there is currently no efficacious pre-surgical treatment. Finding an intravenous treatment that could assist endogenous clotting mechanisms is therefore a major mission for military researchers. The possibility that hemostatic agents might be useful in reducing blood loss following traumatic injury has received new impetus from the recent use of rFVIIa in both animal models of traumatic injury [8–14] and in human patients who have suffered traumatic injury.[15–17] Although rFVIIa holds promise for such indications, it is also very expensive and is not yet approved for a trauma indication. Because of this, the possibility that the much cheaper drugs aprotinin, DDAVP, EACA and TXA might be effective in reducing traumatic bleeding was investigated using two models of uncontrolled hemorrhage in rats. This drug screening program was performed as a series of three studies, which investigated the efficacy of each of these drugs, used singly or in combination, to reduce blood loss in traumatic hemorrhage.

Materials and methods

General

A series of three studies was performed, each incorporating two separate experiments. In all experiments, animals were assigned randomly to treatment and investigators were blinded to treatment. The first experiment within each study incorporated a tail bleeding model with limited fluid resuscitation. This nonlethal model was considered the least hemostatically challenging of the two experiments. Because repeated bleeding time (BT) measurements were possible in this model, a dose escalation approach was possible, with the primary outcome measure being BT. The second experiment in each study employed a model of severe liver injury and aggressive fluid resuscitation. This was the more hemostatically challenging of the two and was selected to incorporate a rapidly developing dilutional coagulopathy. The primary outcome measures for this experiment were blood loss and survival. The specific experimental treatments and procedures are described below.

Animals

All experiments and animal care procedures were approved by the Institutional Animal Care and Use Committee, and the animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 86-23, revised 1996). The animals were maintained in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Male Sprague—Dawley rats (total $n = 222$) were housed individually in standard plastic cages with water and food available ad libitum. Rats weighed between 400 and 475 g at the time of experiments. A 12-h light/12-h dark cycle (light on at 06:00) was used, and the room temperature was maintained at 22—24 °C.

Instrumentation

Anesthesia was induced by placing the rat in a sealed clear plastic box ventilated with 5% isoflurane in 100% oxygen. After induction, rats were removed from the box and placed in dorsal recumbency on a water-perfused heating pad. Anesthesia was maintained with 1—3% isoflurane in 100% oxygen via a nose mask throughout all surgical and experimental procedures. A temperature probe (Physitemp Instruments, Inc., Clifton, NJ) was inserted approximately 5 cm past the anus for measurement of colonic temperature, which was maintained at 37.5 ± 0.5 °C throughout experimentation. For tail bleeding experiments, an additional temperature probe was placed subcutaneously 5 cm from the base of the tail to measure tail temperature. Arterial and venous catheters (PE-50) were placed via a femoral cutdown. The arterial catheter was used to monitor arterial blood pressure and for the withdrawal of blood samples, while the venous catheter was used for administration of hemostatic agents and fluid infusion. Mean arterial blood pressure (MAP), systolic and diastolic blood pressures, and heart rate were recorded at 10 s intervals