RESUSCITATION OF HEMORRHAGED SEDATED MATURE MALE MINIATURE SWINE WITH 17β-ESTRADIOL (E2)

L.A. Baer[1], J.W. Burns[1], B.S. Jordan[1], J.L. Sondeen[1], M.A. Dubick[1], I.H. Chaudry[2], C.E. Wade[1]

[1]United States Army Institute of Surgical Research, Fort Sam Houston, TX USA, University of Alabama, Birmingham, AL, USA

The use of Army medical and/or other Army records in the preparation of the material is acknowledged, but it not to be construed as implying official Department of the Army approval of the conclusions presented.

Abstract

In environments with limited resuscitation fluids, maintaining life after hemorrhage is difficult. We hypothesized the use of E2 infusion would increase survival to 180 min. A miniature swine hemorrhage model was developed to test various resuscitation products. Anesthetized minipigs (43.4±1.3kg;14.2±0.4mths) were catheterized for hemorrhaging, infusions, sample collection and monitoring. Animals were placed in a sling with feet on the floor during recovery and sedated with midazolam to a brain bispectral index of 80-90. Swine were hemorrhaged 60% of estimated blood volume exponentially over 1 hr and assigned to no resuscitation (control;n=16), resuscitation with 1ml/kg/min of Hextend (Hex; n=7) to a systolic blood pressure (SBP) of 80±5 mm Hg, a 1 mg/kg or 10mg/kg (1.125mg/ml =45±1.2ml) bolus of E2 (n=6/group). Animals were observed for 180 min or death. Baseline data were not different between treatments. At the end of hemorrhage there were reductions in MAP and base excess (BE), and an increase in lactate (Lac). Survival time for controls was 60±11.7 min after hemorrhage with a 7% survival rate at 180 min. Hex animals required 669±138ml (38±8.2% replacement of the shed blood volume) to attain the target SBP and 100% survival. E2 nearly doubled survival time to 125±15.3 min ($p<0.05$) and survival rate by 25%. In a model of severe hemorrhage in male swine, that could be resuscitated with fluids, E2 tended to increase survival time, but not to 180 min. Vascular reactivity *in vitro* indicates hyporesponsiveness of pig carotid artery to E2-induced vasorelaxation compared to rat aorta, suggesting higher doses of E2 may be required.

Introduction

Serious injuries on the battlefield often result in critical loss of blood. Immediate resuscitation with whole blood is not an option. Other resuscitation fluids such as plasma, reconstituted lyophilized plasma, lactated Ringer's, saline or Hextend require large volumes, which may not be available. Small volume resuscitation products are being experimentally evaluated as pre-hospital treatment to increase survival time for evacuation, triage and treatment. 17β-estradiol (E2) has been suggested as a possible small volume resuscitation product.

A new hemorrhage model has been developed at the request of the Defense Advanced Research Projects Agency (DARPA) for testing new and novel resuscitation products for the Surviving Blood Loss (SBL) program. The model is a sedated, mature male miniature swine, 35-45 kg, socialized to human and laboratory activity and trained to lie quietly in a sling with feet on the floor. The swine were hemorrhaged 60% of estimated blood volume (EBV) exponentially over 1 hr with the anticipation of death within 3 hr after hemorrhage, without resuscitation. The SBL program will use this model to evaluate new, small volume resuscitation products to extend survival time after critical blood loss on the battlefield, or in the civilian environment, for evacuation, triage and supportive treatment.

The purpose of the present study was to establish a spontaneously breathing, sedated, mature male miniature swine hemorrhage model defined by the DARPA requirements and demonstrate that this model could be resuscitated using a standard of care fluid.

Methods

- Experimental procedures were approved by the Institutional Animal Care and Use Committee of the U.S. Army Institute of Surgical Research, Fort Sam Houston, TX.
- Healthy, mature, male miniature swine (Sinclair, Columbia, MO) with a mean weight of 43.4±1.3 kg; 14.2±0.4months were used.
- Swine were trained to all handling procedures (i.e. transport caging and sling) prior to conducting any protocols.

Surgical Protocol

- All animals were fasted 12 hrs prior to any procedures.
- Pre-treatment=0.05mg/kg Buprenorphine, followed by 5.0mg/kg Telezol 30 minutes after. Isoflurane introduced for intubation and catheter placement.
- External carotid artery, used to measure blood pressure telemetrically and lower femoral artery and vein used for hemorrhage procedure.
- After completion of cannulation, isoflurane was turned off and mechanical ventilation was continued until adequate spontaneous breathing occurred.
- Animals were placed in a sling, all feet touching the floor for the remainder of the protocol.
- ECG leads and Bispectral Index (BIS) were affixed directly to the animal and BIS was maintained between 80 and 90 with Midazolam infusion (0.6mg/kg/hr).

Hemorrhage Protocol

- After a 45min acclimation period, a baseline blood sample was taken and the hemorrhage protocol was started.
- Swine were exponentially hemorrhaged 60%of EBV over 60 min at 5 different rates (7.5,11.5,12,13,16 min) from the arterial catheter.
- Hemorrhage volume and rate were predetermined and computer controlled throughout the procedure (DREW Data Acquisition System).
- Immediately following hemorrhage, animals were either not resuscitated (Con; n=16), resuscitated with 1mL/kg/min Hextend (Hex; n=7), 1mg/kg or 10mg/kg; 1.125mg/mL (infused vol.=45 ±1.2mL) bolus of 17β-estradiol (E2; n=6/group). Because there was not a significant difference ($p>0.2$) in survival time between the E2 groups, all data was combined.
- At the completion of hemorrhage, blood samples were collected at pre-determined time intervals for up to 180 min or until death.

Results

Table 1. Physiological measurements at the end of hemorrhage.

	Baseline (n=26)	Control (n=16)	Hex (n=7)	E2 (n=12)
MAP (mmHg)	132±4.4	33±1.9	19±6.7*	45±5.4
BE (mmol/L)	6.5±0.5	-2.0±1.0	-2.2±1.1	-1.5±1.0
Lac (mmol/L)	1.0±0.1	8.3±0.9	7.3±0.6	6.8±0.6

	Wt. (kg)	Age (months)	Hem. Vol (ml)	Hem. Vol. (mL/kg)	Survival Time (min.)
Con	40.3±1.5	13.3±0.5	1565±53.7	38.8±0.1	64±11.5
Hex	43.7±1.7	14.8±0.9	1676±63.5	38.9±0.1	180±0.0
E2	47.1±1.4*	13.1±0.8	1821±52.0*	38.7±0.1	125±15.3

Volume replacement for Hex=669±138mL (38±8.2% of shed bld vol)

Mean ± SEM; * different from the control, $p<0.05$

Results (cont)

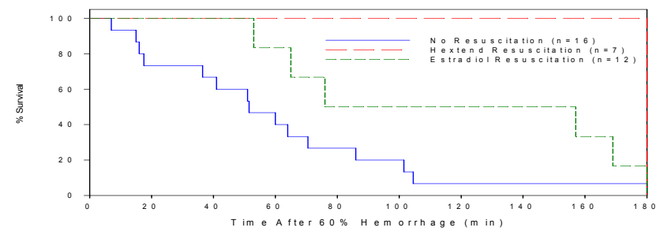

Figure 1. Survival rate after 60% hemorrhage. There was a significant increase in survival time ($p<0.05$)

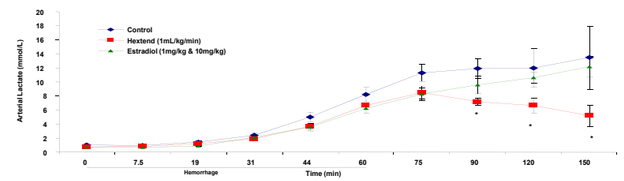

Figure 2. Arterial lactate from the start of hemorrhage through 150 min of Con, Hex & E2. Hex arterial lactate was significantly reduced at 90 min through 150 min. ($p<0.05$)

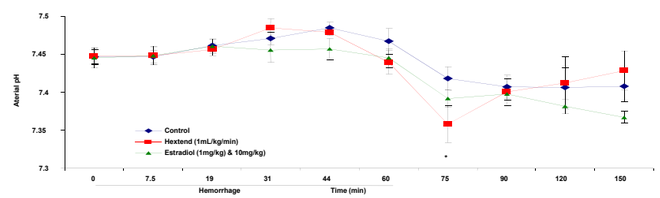

Figure 3. Arterial pH from the start of hemorrhage through 150 min of Con, Hex & E2. No significant differences were observed between treatments.

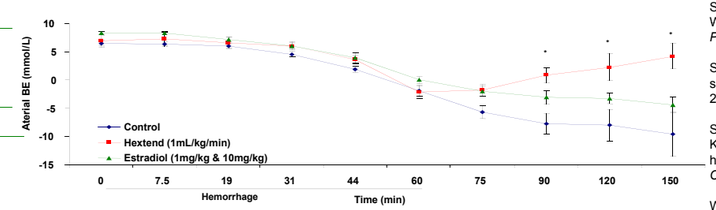

Figure 4. Arterial BE from the start of hemorrhage through 150 min of Con, Hex & E2. Hex was significantly increased from Con 90 min until 150 min.

Conclusions

- A conscious, sedated, mature male miniature swine hemorrhage model has been successfully developed and resuscitated with a standard of care fluid (Hextend). In addition, the model was used to evaluate E2 as a potential small volume resuscitation product.
- Survival time for the E2 group was not as successful as Hex, however, there was an increase in survival time as compared to controls.
- The response to lethal hemorrhage of the three groups was consistent with previous unanesthetized studies of hemorrhage with a decrease in blood BE and an increase in blood lactate, suggesting a conversion to anaerobic metabolism in many tissues.

In the model of severe hemorrhage in the male swine, E2 tended to increase survival time, however not to that of the standard of care. Vascular reactivity in vitro indicates hyporesponsiveness of pig carotid artery to E2-induced vasorelaxation compared to rat aorta, suggesting higher doses of E2 may be required.

References

Ba ZF, Lu A, Shimizu T, Szalay L, Schwacha MG, Rue LW, 3rd, Bland KI, and Chaudry IH. 17beta-Estradiol modulates vasoconstriction induced by endothelin-1 following trauma-hemorrhage. *Am J Physiol Heart Circ Physiol* 292: H245-250, 2007.

Hannon JP, Bossone CA, and Wade CE. Normal physiological values for conscious pigs used in biomedical research. *Lab Anim Sci* 40: 293-298, 1990.

Sondeen JL, Gonzaludo GA, Loveday JA, Deshon GE, Clifford CB, Hunt MM, Rodkey WG, and Wade CE. Renal responses to graded hemorrhage in conscious pig. *Am J Physiol* 259: R119-125, 1990.

Sondeen JL, Gonzaludo GA, Loveday JA, Rodkey WG, and Wade CE. Hypertonic saline/dextran improves renal function after hemorrhage in conscious swine. *Resuscitation* 20: 231-241, 1990.

Szalay L, Shimizu T, Suzuki T, Yu HP, Choudhry MA, Schwacha MG, Rue LW, 3rd, Bland KI, and Chaudry IH. Estradiol improves cardiac and hepatic function after trauma-hemorrhage: role of enhanced heat shock protein expression. *Am J Physiol Regul Integr Comp Physiol* 290: R812-818, 2006.

Wade CE and Hannon JP. Confounding factors in the hemorrhage of conscious swine: a retrospective study of physical restraint, splenectomy, and hyperthermia. *Circ Shock* 24: 175-182, 1988.

Supported by: DARPA & MRMC