**Antifibrinolytics**

**Table A: Antifibrinolytics: location, institution, ethics, animals, numbers**

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| **Study ID** | **Location** | **Institution** | **Ethics statement** | **Animal** | **Number animals** |
| 18 | Switzerland | University of Bern | No | Albino rats | 370 |
| 19 | USA | Department of Medicine, Downstate Medical Center, Brooklyn, New York | No | Hybrid New Zealand and California rabbits | 37 |
| 20 | Sweden | University of Goteburg | No | Sprague-Dawley rats | 88 |
| 21 | USA | Division of Gastroenterology, University of Alabama at Birmingham, Alabama | No | Wistar rats | 27 |
| 22 | Sweden | Departments of Plastic and Reconstructive Surgery & Experimental Research, Malmo General Hospital, Malmo | No | Rabbits | 12 |
| 23 | USA | Du Pont Merck Pharmaceutical Company, Cardiovascular Sciences, Wilmington | No | New Zealand White rabbits | 60 |
| 24 | France | Departement d'Anesthesie, Hopital Pitie, Paris; INSERM U 353, Institut des Vaisseaux et du Sang, Paris; Departement d'Anatomo-Pathologie, Hopital Broussais, Paris; Laboratoire d'Explorations fonctionnelles, Hopital Lariboisiere, Paris; I.N.R.A, Jouy-en-Josas, France | Guidelines cited, licenses cited | Large white pigs | 12 |
| 25 | USA | University of Mississippi Medical Centre, Jackson, Mississippi | Guidelines cited | Yorkshire cross pigs | 62 |
| **8 studies** | **4 USA, 2 Sweden**  **1 France, 1 Switzerland** | **4 universities, 2 medical centres / hospitals, 1 pharmaceutical company, 1 hospital/ independent institute collaboration** | **6 no ethics statement; 1 guidelines cited; 1 guidelines and licenses cited** | **Rats 3 studies, rabbits 3 studies, pigs 2 studies** | **668**  **Average no. animals used per study 83** |

**Table B: Antifibrinolytics: animal model, anaesthesia, how and when killed, what animals experienced**

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| **Study ID** | **Animal model** | **Anaesthesia for experimental procedures** (excluding anaesthesia prior to death) | **How killed** | **When killed (endpoint)** | **What did animals experience?** |
| 18 | Rat model of blood loss induced by tail cutting | Ethyl urethane anaesthesia intraperitoneally | Not reported | Not reported, but animals alive for at least six days | Tail cutting conducted under anaesthesia  IV injections continued for 6 days post operatively |
| 19 | Rabbit model of blood loss induced by renal injury | Intravenous pentobarbital | Not reported | Between 5 and 10 days after experiment | Renal injury conducted under anaesthesia  10 control (but injured) rabbits had urine collected for 7-10 days.  20 injured and treated rabbits had urine collected for 5-7 days.  Postoperatively rabbits were housed individually in metabolic cages |
| 20 | Rat model of gastric ulcer and gastric bleeding induced by hydrochloric acid | ‘General anaesthesia’ | Not reported | 6 hours after ligature | Cells injected into rats, presumably while awake.  Operation conducted under anaesthesia but *unclear whether anaesthesia continued for the 6 hrs until they were killed (non-recovery?)* |
| 21 | Rat model of gastric bleeding induced by infusing saline directly onto a wound of the mucous membrane | Pentobarbital sodium intraperitoneally; supplementary doses given subsequently *as necessary.* | By ‘air emboli’ | At the end of the experiment | NON-RECOVERY  Operation / experiment conducted under anaesthesia.  Not sure how long operation continued for. |
| 22 | Rabbit model of severe micro-arterial trauma | Sodium pentobarbital, maintained by repeat injections. Lidocaine as intradermal injection in groin and subcutaneously as nerve block at base of ears. | Not reported | Not reported | Operations lasted about 50 mins for both ears, and conducted under anaesthesia.  Assessment continued for 2 hours after. *Unsure if rabbits aware during these hours.* |
| 23 | Rabbit model of haemorrhage induced by ear cutting | Ketamine (i.m.) and xylazine (i.m.). Additional anaesthetic administered as needed. | Not reported | Not reported | Ear cutting conducted under anaesthesia  Treatments administered IV 30 or 45 mins after cuts; unsure if still anaesthetised.  Blood sampling continued for 2hrs |
| 24 | Pig femoral artery model to assess the thrombogenicity of aprotinin | Ketamine chloride (im), maintained by inhalation of mixture of halothane, nitrous oxide and oxygen. | Not reported | Not reported | Operation conducted under anaesthesia but unsure how long operation took.  Pigs’ ears were cut and bleeding times measured before, during and after anaesthesia, up to 24 hrs.  Blood samples taken during anaesthesia and up to 2hrs after treatment. |
| 25 | Pig model of blood loss induced by liver injury and /or phlebotomy | im injections of acetylpromazine and ketamine. Vecuronium bromide, fentanyl and nembutal given every 30 mins for paralysis, pain relief, and anaesthesia respectively | Not reported | Animal death or four hours | Liver injuries inflicted under anaesthesia but animals also restrained, paralysed and given pain relief. Unclear why restraints and paralytics used. Level of awareness?  *Not sure how long anaesthesia continued for but procedures not terminated until 4 hrs.* Those pigs that died did so by 2hrs and 5 mins. (non-recovery?) |
| **8 studies** | **Range of different models of blood loss** | **Anaesthesia reported 8; mention repeat anaesthesia given as needed 5** | **7 not reported;**  **1 by air emboli** | **4 reported experimental endpoint (non-recovery 1; 6 hours 1; 5-10 days 1; 4 hours 1)**  **3 not reported**  **1 did not report experimental endpoint but noted animals alive at day 6.** | **Study 25 used restraints and paralytics – level of awareness?** |

**Table C: Antifibrinolytics: unexpected deaths and events, painkillers, paralytics, welfare**

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| **Study ID** | **Unexpected deaths** | **Unexpected events** | **Painkillers** | **Paralytic** | **Welfare** |
| 18 | Not reported | Not reported | Not reported | Not reported | Nothing reported |
| 19 | All rabbits survived until killed | Not reported | Not reported | Not reported | Rabbits were fed standard chow and permitted water ad libitum. Each rabbit was individually housed in a metabolic cage for urine collection. |
| 20 | Not reported | Not reported | Not reported | Not reported | Rats were fasted for 48 hours before experiments in metabolic cages to avoid coprography. All rats had free access to water. |
| 21 | Not reported | Not reported | Not reported | Not reported | Nothing reported |
| 22 | Not reported | Not reported | Not reported | Not reported | Nothing reported |
| 23 | Not reported | Not reported | Not reported | Not reported | Nothing reported |
| 24 | Not reported | Not reported | Not reported | Not reported | Nothing reported |
| 25 | Not reported | Not reported | Fentanyl | Vecuronium bromide - and animals restrained. | Pigs were quarantined with free access to food and water for two days prior to experiments |
| **8 studies** | **7 not reported**  **1 report** | **0 unexpected events** | **1/8 painkillers** | **1/8 paralytic agent reported** | **5 not reported**  **3/8 reported animal welfare considerations (including 2/8 used metabolic cages)** |

**Table D: Antifibrinolytics: Procedures**

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| **Study ID** | **Procedures** |
| 18 | The tail was introduced into a 2mm hole in a metal plate until it reached the end. It was then cut with a 'nail blade'. Blood from the wound was collected in glass tubes every thirty seconds. This procedure was done once to each rat. Blood was also collected from the bifurcation of the aorta using a needle mounted on a siliconised syringe. Some animals were injected i.v. with different kinds of platelet extract on a daily basis for six days. Some animals were injected with an antifibrinolytic agent (Trasylol) i.v. for six days. Others had a combination of both (i.v.). Animals appear to have been followed up at 30 mins, 2, 4 and 6 days after the 'first injection'. Unclear whether the injections given before or after the tail cut? |
| 19 | Both kidneys were exposed through a midline abdominal incision. First, seven rabbits in an initial group were subjected to the following injuries: (i) two rabbits were stabbed once with a needle in the cortex of each kidney creating 10mm wounds; (ii) two rabbits were stabbed 6 times in each kidney creating 10mm wounds; (iii) one rabbit had a 5X3mm wedge cut from both kidneys; (iv) two rabbits had bilateral punch biopsies removing a 6-10-mm core of tissue. For all rabbits bleeding had diminished 48hrs after injury. Secondly, 30 rabbits were subjected to severe renal injury by crushing 40-50% of each kidney within the jaws of a sponge-holding forceps. Kidneys so injured bled externally for 5-7 minutes, sustaining an estimated blood loss of 35 ml. The thirty rabbits were divided in two groups. In 10 rabbits (controls), the abdomen was closed and the degree of hematuria in each daily urine collection was estimated. 7-10 days after the renal trauma all control rabbits were killed. The remaining 20 rabbits were given i.v. bolus injections of an antifibrinolytic agent (EACA) 5 mins before renal trauma and 1 hr and 24 hr after abdominal closure. Urine was collected daily using metabolic cages, individually. They were killed 5-7 days post injury. |
| 20 | Cells from rat donor blood were injected i.v. into the experimental rats. An operation was then conducted to ligate (tie up) the pylorus (which connects the stomach to the duodenum). Hydrochloric acid solution was then poured into the rats' stomachs through an oro-gastric tube. Rats were put in groups that had either tranexamic acid, cimetidine or saline. Tranexamic acid or cimetidine were administered i.v. at the time of the ligation and then every second hour until death. Rats were killed 6 hours after the ligature and their stomachs were removed. All rats had developed extensive ulcerations and their stomachs were distended with fluid. In a 'separate study', rats were given pyloric ligation without acid and were then given either tranexamic acid, cimetidine or saline. |
| 21 | Tracheostomy was performed, and a catheter inserted to maintain an open airway. Body temperature was monitored. A jugular vein and a carotid artery were cannulated for drug administration and for withdrawing blood and monitoring blood pressure, respectively. A midline laparotomy was performed, and the stomach lifted out. The stomach was then opened and the gastric contents removed. The edges of the stomach were pinned out and fastened with a plastic ring forming the wall of the chamber. The exposed mucosa in the bottom of the chamber was washed several times with saline. Tranexamic acid or vehicle was infused with an infusion pump, commencing immediately after establishment of the iv line, before laparotomy. Gastric bleeding was induced by taking mucosal biopsy specimens from the acid-secreting part of the stomach, using a needle, one from one side of the stomach and an hour later another from the opposite side. The mucosal lesions were superfused with pre-warmed saline. Renewed bleeding was provoked after 30 min by aspirating the clot from the biopsy lesion. Other clots that might have formed were removed at the same time. Infusion and sampling continued for an hour after the second biopsy. |
| 22 | A unilateral groin incision was made and a plastic catheter placed in the aorta via the femoral artery. Segments of the central arteries of both ears were prepared. Animals were maintained at a temperature of 39.5°C. Lidocaine was applied to prepared vessels *as required*. Radioactive platelets were infused into the aorta. Tranexamic acid (6 rabbits) or saline (6 rabbits) was administered as a single intra-aortic bolus injection. Five minutes later longitudinal arteriotomies (opening or cutting the wall of the artery) (7 mm) and intimectomies (5 mm) were performed. The operations took about 25 mins per ear. Vessels were covered with gauze pads and examined once per minute by removing the gauze. The time taken until bleeding ceased completely was recorded. Radioactivity in labelled platelets accumulating at trauma sites was recorded for 2 hours after reperfusion. Vessel patency was assessed. |
| 23 | Rabbits were placed on heated pads to maintain core temperature. A catheter was placed in the right jugular vein for continuous infusion of r-tPA. The ear was immersed in a saline bath and maintained at 37°C. In Protocol I the r-tPA infusion began 10 min prior to incision to induce blood loss. Cuts (1.5 mm) were made in both marginal ear veins of one ear using a needle and the ear was placed in the saline bath. A single bolus intravenous injection (iv) of rPAI-1 or EACA was administered 45 min after the cuts into the opposite ear. In Protocol II, the RtPA infusion began 15 min prior to incisions and cuts were larger (3mm), through both marginal veins of one ear, which was immersed in a saline bath immediately after cuts made. rPAI-1 or EACA was administered 30 min after incisions. Samples from the bath were collected throughout 120 mins. Control rabbits received only the r-tPA infusion and did not have treatment. |
| 24 | Aim was to consider the potential thrombogenicity of aprotinin. As soon as the animal was anaesthetised sufficiently it was tracheotomised and ventilated. The left carotid artery was cannulated to withdraw blood samples. Arterial flow was monitored with a Doppler. Under anaesthesia experiments were performed on the carotid and femoral arteries, involving injury to the arteries to produce stenosis (narrowing of the arteries). The prothrombotic (blood clot forming) properties of aprotonin or saline infused via the left jugular vein were assessed. We are not told how long the operation continued for. *At the end of the experiment the surgical incisions were closed and the animals allowed to recover.* To assess bleeding times pigs’ ears were cleaned and shaved on the external side. An incision was made with a surgical blade and the ear placed in a beaker containing warmed saline solution. Bleeding times were measured before general anaesthesia, during anaesthesia and 10, 30, 1, 2, and 24 h after the end of treatment. Blood samples were taken during anaesthesia, 10 and 30 mins and one and two hours after the end of bolus administration. |
| 25 | An IV catheter was placed in the ear vein. The animal was restrained supine with four quarter restraints. An open tracheostomy was performed for insertion of an endotracheal tube and mechanical ventilation. Animals were surgically prepared and monitored. They were given either Aprotonin or Lactated Ringers (LR) as a bolus infusion over 30 mins prior to liver crush injury. After the injury pigs were given an unknown infusion (either Aprotonin or LR). The liver crush injury was inflicted by applying a clamp three times to each of the four lobes of the liver and the abdomen was then closed. There was an 'induced shock treatment group' in which pigs had blood taken (phlebotomy) through the femoral arterial line until pressure dropped to a certain point, after which they received either aprotonin (administered iv via the ear) or LR (through femoral vein) until original pressure was achieved. Some of the induced shock treatment group also had liver crush injury. LR boluses were given every 15 mins. *The experimental endpoint was either animal death or four hours.* Four experimental control pigs had neither injury nor induced shock and survived to four hours. Pigs that had injury without induced shock survived to four hours. Pigs that had induced shock but no injury survived to four hours. Those that had induced shock and injury and placebo died by 125 mins - aprotonin increased survival time to 205 mins. |

**Table E: Antifibrinoloytics: Models**

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| **Summary of animal model**  This model induces blood loss by various methods and then tests anti-fibrinolytic agents that aim to increase clotting/ reduce bleeding. The studies were disparate in design.  **Induced bleeding/ trauma and follow up 2–6 h post-operatively**   * Rats and rabbits had ears cut/ artery trauma inflicted under anaesthesia, plus at least 2hrs post-operative assessment. Unclear if/ when killed. (studies 22,23) * Pigs had livers injured by crushing and /or induced shock under anaesthesia. They were restrained, paralysed and given painkillers. Some died before 4hrs, rest killed at 4h. (study 25) * Rats housed in metabolic cage pre-operatively. Gastric bleeding induced by acid under anaesthesia, killed at 6 hrs (study 20)   **Induced trauma and follow up 24 h post-operatively**   * Pigs had operation under anaesthesia to produce narrowing of the artery. Ear cutting, some without anaesthesia. Assessed up to 24 hrs post-operatively. Unclear if/ when killed. (study 24)   **Induced bleeding/ trauma and follow-up 5-10 days post-operatively**   * Rats had tails cut under anaesthesia. Post-operative i.v. administration of test agents for 6 days. Unclear if/ when killed. (study 18) * Rabbits had renal injuries inflicted under anaesthesia. Individually housed in a metabolic cage post-operatively. Urine collected daily for 5-10 days. Killed between 5-10 days. (study 19) |