Journal of Plastic, Reconstructive & Aesthetic Surgery (2014) 67, 707—711

JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

Protective effects of antithrombin on free groin flaps after secondary venous stasis in the rat model

J. Wallmichrath [a,*], R. Knab [b], R.G.H. Baumeister [c], E. Volkmer [a], R.E. Giunta [a], A. Frick [a]

[a] *Hand Surgery, Plastic Surgery and Aesthetic Surgery, University Hospital of the Ludwig Maximilians University, Munich, Germany*
[b] *Department of Surgery, Institute for Surgical Research, Walter-Brendel-Centre for Experimental Medicine, Ludwig Maximilians University of Munich, Campus Grosshadern, Munich, Germany*
[c] *Department of Surgery, Surgical Clinic Munich Bogenhausen, Munich, Germany*

Received 8 April 2013; accepted 28 January 2014

KEYWORDS
Antithrombin (AT);
Antithrombin III (AT-III);
Free flap;
Groin flap;
Microsurgery;
Intravital microscopy (IVM);
Ischaemia;
Reperfusion (I/R);
Rat model

Summary *Background:* The anticoagulant activity of heparin is well established and led to its widespread clinical use for the prophylaxis and treatment of venous thrombosis in microsurgery. Heparin accelerates antithrombin (AT)-mediated inhibition of clotting and fibrinolytic proteinases.
Aim: The aim of the study is to determine whether the focussed delivery of AT by rinsing of free adipocutaneous groin flaps shows protective effects on flap survival, following a fatal secondary venous stasis in the rat model. Further, intravital video microscopy (IVM) is used to detect substance-specific alterations in microvascular perfusion with special focus on regional differences between central and peripheral flap regions.
Methods: Free microvascular groin flaps ($n = 22$) were transplanted to the neck in adult Sprague-Dawley rats. The flap pedicle was re-explored and the distal stump of the flap artery was catheterised 20 h later. Animals in group I ($n = 11$) were treated with 1 ml of Ringer's solution administered over 10 min via intraarterial infusion. Those in group II ($n = 11$) received 1 ml of AT (50 IU/kg). Afterwards, the flap vein was clamped for 35 min. The skin of the flaps and the native contralateral groin was examined by IVM using the plasma-marker fluorescein isothiocyanate (FITC)—dextran and carboxyfluorescein diacetate succinimidyl ester (CFDA-SE)-labelled thrombocytes. After 14 days, the viability of the flaps was evaluated.
Results: The treatment with AT significantly increased the functional capillary density (FCD) of the flaps. After 14 days, flap necrosis occurred in nine animals of group I and three animals of group II, respectively. No partial flap loss was detected.

* Corresponding author. Hand Surgery, Plastic Surgery and Aesthetic Surgery, University Hospital of the Ludwig Maximilians University, Marchioninistrasse 15, D-81377 Munich, Germany. Tel.: +49 89 7095 3502; fax: +49 89 7095 6505.
E-mail addresses: Jens.Wallmichrath@med.uni-muenchen.de, wallmichrath@gmx.de (J. Wallmichrath).

http://dx.doi.org/10.1016/j.bjps.2014.01.046

Conclusions: The focussed delivery of AT resulted in significantly improved flap salvage. The results may reinforce the clinical custom of AT substitution in the setting of major surgical procedures such as elaborate microsurgical reconstructions, at least in cases with diminished AT levels.

Introduction

The anticoagulant activity of heparin is well established and led to its widespread clinical use for the prophylaxis and treatment of venous thrombosis in microsurgery.[1,2] The activity of heparin is due predominantly to the ability of the polysaccharide to accelerate greatly (approximately 2000-fold), the rate by which antithrombin (AT) inactivates clotting proteinases.[3] The half-life of thrombin at optimal plasma concentrations of AT and heparin is only approximately 140 ms.[4] AT levels are reduced by, for example, liver disease, trauma or (at least high dosage) treatment with heparin.[5] Thus, substitution of AT in the setting of major surgical procedures such as elaborate microsurgical reconstructions is of importance. This study focusses on the question whether the natural anticoagulant activity can be increased effectively by administration of AT in a free-flap animal model. AT, also termed AT-III, is a small glycoprotein (serine protease inhibitor) that inactivates several enzymes of the coagulation system.[6] The physiological target proteinases of AT are those of the intrinsic coagulation system, primarily Factor IIa, IXa, Xa, XIa, XIIa and thrombin, but the inhibitor also inactivates certain non-coagulation serine proteinases, such as plasmin, kallikrein, the complement proteinase, Cls and trypsin.[4] The goal of this study was to determine whether the focussed delivery of AT by rinsing of the flap tissue shows protective effects on free groin flap survival, following fatal secondary venous stasis in the rat model. Besides clinical assessment, intravital video microscopy (IVM) was applied to detect drug-dependent alterations in microvascular perfusion with special focus on regional differences between central and peripheral flap regions.

Materials and methods

Free microvascular adipocutaneous groin flaps ($n = 22$) were transplanted to the neck in adult Sprague-Dawley rats. Prior to re-exploration, the animals were randomly allocated in equal shares across the two treatment groups: group I ($n = 11$; lactated Ringer's solution (RL)) and group II ($n = 11$; AT III, 50 IU/kg). The preparation of the test solution was performed by a surgical assistant. The blinded test substance (1 ml) was administered by the microsurgeon. Both the investigator and the assessor collecting the outcome data were blinded throughout the experiment.

Free-flap transplantation

Experiments were performed according to the guiding principles for research involving animals and the German legislation on protection of animals. Approval was obtained by the local governmental animal care committee. Male Sprague-Dawley rats weighing 300 g were purchased from Charles River (Sulzfeld, Germany) and housed at the Institute for Surgical Research (Walter Brendel Zentrum, Ludwig Maximilians University, Munich, Germany). Animals were allowed to accommodate to the standard conditions of the animal facility with free access to rat chow and water for at least 1 week. Solid food was withheld from the rats 12 h prior to the surgery. All surgical procedures were performed by a single surgeon experienced in microsurgery. The rats were anaesthetised by intraperitoneal injection of 0.15 mg/kg Medetomidin™ (Pfizer GmbH, Karlsruhe, Germany), 0.01 mg/kg Fentanyl™ (CuraMED Pharma GmbH, Karlsruhe, Germany) and 2 mg/kg Midazolam™ (Hoffmann-La-Roche AG, Grenzach-Wyhlen, Germany) and supplemented as required. The animals were placed on a heated operating table in a supine position with the extended hind leg. The region of the right groin was shaved and the flap borders were outlined on the skin with a diameter of 3 cm centred in the right groin. The inguinal vessels were exposed after skin incision and the superficial iliac vessels were ligated using Vicryl™ 5/0 (polyglactin 910; Ethicon, Germany). The microvascular preparation was started distally at the femoral vessels and was continued proximally towards the groin. The femoral nerve was isolated and excluded from the flap. Finally, the distal ends of the femoral vessels were ligated 6 mm distally to the branch point of the flap vessels using Vicryl™ 5/0 (Polyglactin 910; Ethicon, Germany). Following preparation of the recipient site, the vascular pedicle was transected distally to the branching point of the profunda system and the stumps of the femoral vessels were ligated. The flap border was outlined in the centre of the neck and the skin was excised. The carotid artery and the internal jugular vein were transected and irrigated using Heparin solution (100 IU/ml) and clamped temporarily with single Acland vessel clamps Type B-3 (Art. No. 00400V and 00400A, respectively; S&T AG, Neuhausen, Switzerland). The free groin flap was transferred to the neck and the vessels were anastomosed to the flap pedicle in an end-to-end fashion using a single-stitch technique (Ethilon™ 9/0 for the arterial and 8/0 for the venous anastomosis; Ethicon, Germany). The Acland clamps were removed after completion of the anastomoses to start reperfusion of the flap. Finally, the flaps were sutured in place using single stitches of Ethilon™ 3/0 and the donor site was closed with a buried single suture using Ethilon™ 3/0 (Ethicon, Germany). Anaesthesia was terminated by intraperitoneal injection of 0.75 mg/kg atipamezole hydrochloride (Pfizer GmbH, Karlsruhe, Germany), 0.20 mg/kg Flumazenil™ (Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany) and 0.12 mg/kg Naloxon™ (Ratiopharm GmbH, Ulm, Germany). The animals were