Eur J Vasc Endovasc Surg (2015) 49, 426–431

Detergent Sclerosants are Deactivated and Consumed by Circulating Blood Cells

D.E. Connor [a,b,c], **O. Cooley-Andrade** [a,b], **W.X. Goh** [a,b], **D.D.F. Ma** [b,c], **K. Parsi** [a,b,*]

[a] Dermatology, Phlebology and Fluid Mechanics Research Laboratory, St Vincent's Centre for Applied Medical Research, Sydney, Australia
[b] The University of New South Wales, Sydney, Australia
[c] Haematology Research Laboratory, St Vincent's Centre for Applied Medical Research, Sydney, Australia

WHAT THIS PAPER ADDS

This work demonstrates that in addition to the neutralizing effects of plasma proteins on sclerosant activity, circulating blood cells also consume and deactivate detergent sclerosants. This study reinforces our previous clinical recommendations that discourage extensive mixing of detergent sclerosants with intravascular blood.

Objective: To investigate the deactivating effects of circulating blood cells on the lytic activity of detergent sclerosants.

Methods: Samples of whole blood (WB), platelet-rich plasma (PRP), and isolated leukocytes were incubated with various concentrations of sodium tetradecyl sulfate (STS) or polidocanol (POL) and added to human umbilical vein endothelial cells (HUVECs), which were then counted using a fluorescent plate reader. Full blood counting was performed using a hematology analyzer. Platelet lysis and microparticle formation was assessed using lactadherin binding in flow cytometry.

Results: Detergent sclerosant activity was decreased in WB when compared with plasma and saline controls. The sclerosant lytic activity on endothelial cells was increased 23-fold for STS and 59-fold for POL in saline controls compared with WB. At high concentrations, sclerosants lysed erythrocytes, leukocytes, and platelets. Platelets were more sensitive to the lytic activity of sclerosants than other cell types. Neutrophils were the most susceptible of all leukocytes to the lytic activity of sclerosants. The presence of erythrocytes and leukocytes in samples decreased the lytic activity of sclerosants. Sclerosants at all concentrations induced erythrocyte-derived microparticle formation.

Conclusions: Detergent sclerosants are consumed and deactivated by circulating blood cells. This deactivating effect is above and beyond the neutralizing effects of plasma proteins and contributes to the overall neutralizing effect of blood. Different blood cell types exhibited varying levels of vulnerability to the lytic activity of sclerosants with platelets being the most and erythrocytes the least vulnerable (platelets > leukocytes > erythrocytes).

Article history: Received 30 June 2014, Accepted 22 December 2014, Available online 14 February 2015

Keywords: Detergent sclerosants, Neutralization, Cell lysis, Cell-derived microparticles

INTRODUCTION

Detergent sclerosants such as sodium tetradecyl sulfate (STS) and polidocanol (POL) are injected in varicose veins or vascular malformations with the intention of stimulating endothelial lysis and vessel destruction. The intravascular administration and in particular injection into large veins or vascular lesions results in mixing of sclerosants with blood, a reduction in the lytic activity and clinical potency.[1]

Historically, the neutralizing effect of blood was attributed to dilution. This group successfully challenged this notion and demonstrated that dilution only plays a small part in the reduction of the lytic activity of sclerosants and that binding to plasma proteins and in particular albumin has a more significant impact.[2] Subsequently, we demonstrated that both sclerosants, and in particular POL, are significantly more active when diluted in saline than in albumin or in whole blood (WB). These findings were confirmed later by Watkins,[3] who, using a titration based method, demonstrated that approximately 0.5 mL of blood is sufficient to completely deactivate 1 mL of 3% STS. This method was limited to STS and hence unable to detect the active concentration of POL following mixing with blood. Furthermore, as the main target of detergent sclerosants is the endothelial layer of the target veins, a more appropriate

* Corresponding author. K. Parsi, Dermatology, Phlebology and Fluid Mechanics Research Laboratory, St Vincent's Centre for Applied Medical Research, Level 8, 405 Liverpool St, Darlinghurst, NSW 2010, Australia.
E-mail address: kparsi@stvincents.com.au (K. Parsi).

1078-5884/

http://dx.doi.org/10.1016/j.ejvs.2014.12.029

measure of sclerosant activity is the ability to induce endothelial lysis. In the present study, it was postulated that circulating blood cells deactivate sclerosants independent of the role played by plasma proteins. The mechanism to involve direct consumption of the active detergent in the process of inducing the lysis of erythrocytes, leukocytes, and platelets and the subsequent release of microparticles from these cell types was hypothesized.

MATERIALS AND METHODS

Materials

STS was obtained as Fibrovein (3%) from Australian Medical and Scientific Ltd. (Chatswood, NSW, Australia). POL was obtained as Aethoxysklerol (3%) from Chemische Fabrik Kreussler & Co (Wiesbaden, Germany). Flow cytometry antibodies, Annexin V and TRUCount tubes were obtained from Becton Dickinson (Franklin Lakes, NJ, USA). Bovine serum albumin (BSA) was obtained from Sigma Aldrich (St. Louis, MO, USA). Heparin was obtained from Pfizer, Pharmacia and Upjohn (New York, NY, USA). Endothelial cell growth supplement (ECGS) and bovine serum albumin (BSA) were obtained from Sigma Aldrich. Calcein-AM, human umbilical vein endothelial cells (HUVECs), Medium 199, and all other cell culture reagents were obtained from Life Technologies (Carlsbad, CA, USA) and grown on Corning culture ware (Tewksbury, MA, USA).

Cell culture

HUVECs were cultured in media containing 20% fetal bovine serum, 100 μg/mL heparin, 10 mM L-glutamine, 10 μL/mL penicillin/streptomycin, 25 μL/mL HEPES, and 30 μg/mL ECGS made up in Medium 199. Cells were grown in T75 culture flasks.

Sample collection and preparation

Following informed consent, samples were collected from normal healthy donors taking no medications or supplements. WB was collected into 2.7-mL Vacutainer tubes containing sodium citrate as the anticoagulant. Platelet-rich plasma (PRP) was obtained after centrifugation of WB for 10 minutes at 150*g*. Platelet-poor plasma (PPP) was obtained after centrifugation of WB for 20 minutes at 1,500*g*. All experiments were performed with $n \geq 3$, where n refers to the number of repetitions of experiments using different samples from the same donor.

HUVEC lysis assay

HUVECs (6×10^6 cells) were incubated with 1 μM calcein for 15 minutes. Excess calcein was removed by centrifugation at 400*g* for 5 minutes replaced with 10 mL of PBS. A 50-μL sample of the cells was then transferred to 96-well, black-wall plates that had been coated with poly-L-Lysine for 30 minutes. The cells were allowed to attach for 1 hour before washing with PBS. In a separate 96-well plate, varying concentrations (0—0.6%) of detergent sclerosants were added to each blood sample type and 50 μL of this mixture was immediately transferred to the cultured HUVECs. Samples were incubated for 15 minutes at 37 °C, following which cells were rinsed with PBS. The plate was then reanalyzed using the fluorescent plate reader and the decrease in fluorescence converted into a percentage lysis value. A standard curve was generated for each milieu (saline, plasma, and WB) containing serial dilutions of endothelial cells in a fixed volume of each milieu.

Sclerosant incubation

For all other remaining tests, all samples (WB, PRP, erythrocyte suspensions, and platelet suspensions) were incubated with varying concentrations of sclerosants (described in Figs. 1—4) for 15 minutes at room temperature before analysis.

Blood cell counting

Cell counting, including differential cell counting, was performed using an automated hematology analyzer (Cell-Dyn, Abbott Diagnostics, IL, USA) in 1 mL of WB samples diluted with sclerosant.

Flow cytometric platelet and microparticle counting

Platelet and platelet microparticle (PMP) counting was performed using flow cytometry, as previously described.[4] PMP counting was performed on an LSR-II flow cytometer (Becton Dickinson) using FACSDiva software.

Statistical analysis

Statistical analysis was performed using a paired *t* test using Prism software (v6.03, GraphPad Software Inc., CA, USA).

RESULTS

Neutralization of sclerosant activity by blood components

The incubation of STS and POL with endothelial cells resulted in lysis (Fig. 1), but this activity was influenced by the diluent (saline, plasma, and WB). The concentration required to achieve 50% endothelial lysis in saline was extrapolated to be 0.09375%, and in WB was 0.22% (for STS) and 0.55% (for POL). The sclerosant lytic activity on endothelial cells was therefore increased 23-fold for STS and 59-fold for POL in saline compared with WB.

We found both sclerosants achieved complete lysis of target endothelial cells at 0.6% in WB. Preliminary experiments were performed in concentrations up to 1.5% for each sclerosant (results not shown). At these higher concentrations residual sclerosant molecules would be present and available for lytic activity, despite the lack of any target cells.

Lysis of whole blood components

Incubation of STS and POL with WB resulted in cell lysis (Fig. 2). Incubation with the same concentration of sclerosants at 0.3% resulted in platelet lysis (100%), erythrocyte lysis (25%), and leukocyte lysis (91% for STS, 32% for POL).