Cold atmospheric plasma for mild blood coagulation in visceral surgery

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Introduction

In surgery, blood vessel injury is inevitably and therefore an **efficient haemostasis is essential** to minimize blood loss. By **necrotizing** healthy tissue, thermal coagulation is a frequently used but also a harsh method to limit bleeding. Moreover, increasing **prescriptions of new oral anticoagulants (e.g. Rivaroxaban, Clopidogrel)** compromise efficient bleeding management of emergency patients.

A dielectric barrier discharge plasma has previously been suggested to be haemostatically effective. We would like to extend on this using a **cold atmospheric plasma (CAP)** generated by kINPen med.

Fig. 1: The kINPen med served as a plasma source. It generates an atmospheric pressure non thermal jet-plasma and was fed with Argon at three standard-litre per minute.

Résumé

CAP generated by kINPen med leads to **significant coagulation** *in vitro* and *in vivo*.

A **degradation of fibrinogen** to fibrin could not be verified. However, a slight tendency in **consumption of calcium** was shown, which could be the result of an activated plasmatic or cellular coagulation.

Furthermore, a **significant activation of isolated platelets** was obvious after treatment with CAP.

In our *in vivo* model it was revealed that CAP leads to a **significantly better coagulation management** in native and Rivaroxaban mice. CAP was not able to stop bleeding in Clopidogrel mice.

It can be concluded, that **platelets are of most importance** for CAP blood coagulation.

Furthermore, **CAP was not significantly inferior to thermal coagulation** in native mice.

Moreover, **CAP did not result in necrosis** compared to thermal coagulation.

Materials & Methods

in vitro:

Blood of C57BL/6N mice (12 weeks, f.) was treated for 2 minutes (min) in a 12 well plate (whole blood and platelets isolated in $PBS^{Ca2+/Mg2+}$). For treatment **cold atmospheric plasma (CAP)** generated by the kINPen med was used in a standardised manner. **Argon-flow (Ar)** and **no treatment (none)** served as control. Point-of-care-testing (ABL 90) and ELISA conduced to supernatant analysis. Cellular effects were measured by flow cytometry.

in vivo:

C57BL/6N mice (12 weeks, f.) were treated with **Clopidogrel, Rivaroxaban or water 50, 26 and 2 hours prior to surgery**. Mice underwent laparotomy. A standardised resection of the left lateral liver lobe was performed to achieve a bleeding area of 1 cm length. The area was treated for 1 minute with **CAP, Ar or a thermal coagulator (thermal)**. Blood was absorbed by cotton bud and dissolved in acetic acid. **Absorbance of haemoglobin (405 nm)** was measured to quantify bleeding during treatment and for 30 minutes afterwards.

Statistical analysis was performed using the Mann-Whitney U-Test for intergroup comparison (ns = not significant; * = $p < 0,05$; ** = $p < 0,01$; *** = $p < 0,001$; **** = $p < 0,0001$). Data is stated as mean plus standard deviation. In Fig. 8 a) to c) standard error is given to provide clarity.

Results

in vitro

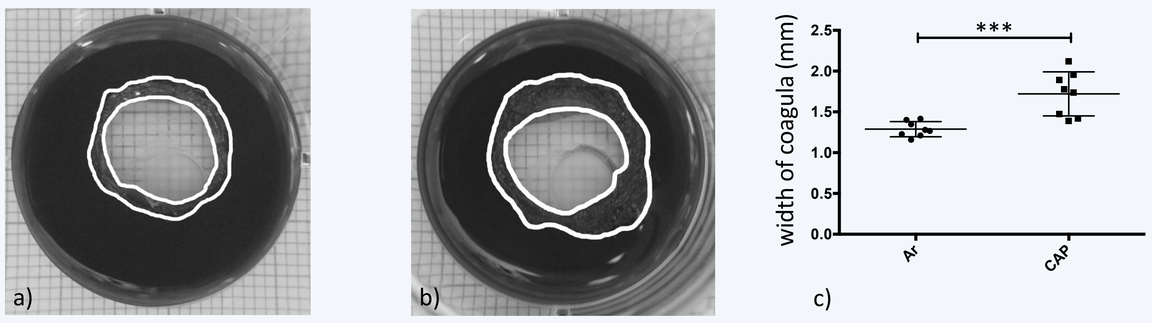

Fig. 2: CAP coagulated murine whole blood *in vitro* to a significantly greater extend compared to Ar. Blood after 2 minute treatment using **a) Ar** and **b) CAP. c) Width of blood coagula** (n = 8) was measured.

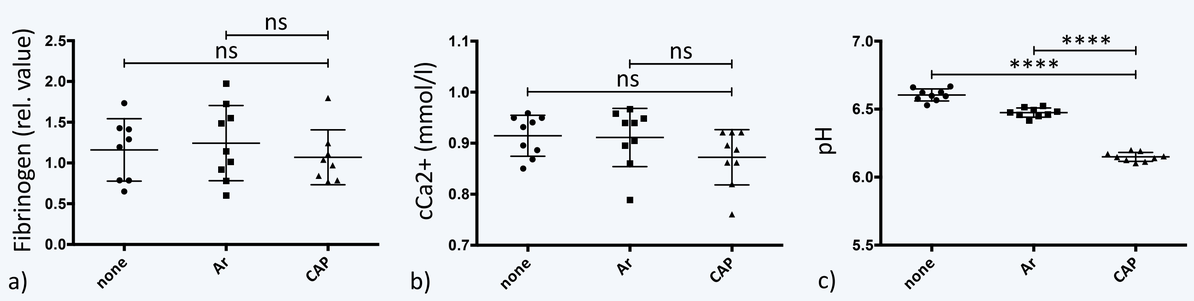

Fig. 3: Analysis of plasmatic coagulation factors in supernatant of murine whole blood after 2 minute treatment**. a) CAP did not change fibrinogen concentration** and therefore a degradation to fibrin was not obvious. **b) Concentration of calcium (cCa2+)** showed slight tendency to decrease after CAP treatment. **c) A significant decrease in pH due to CAP** was observed.

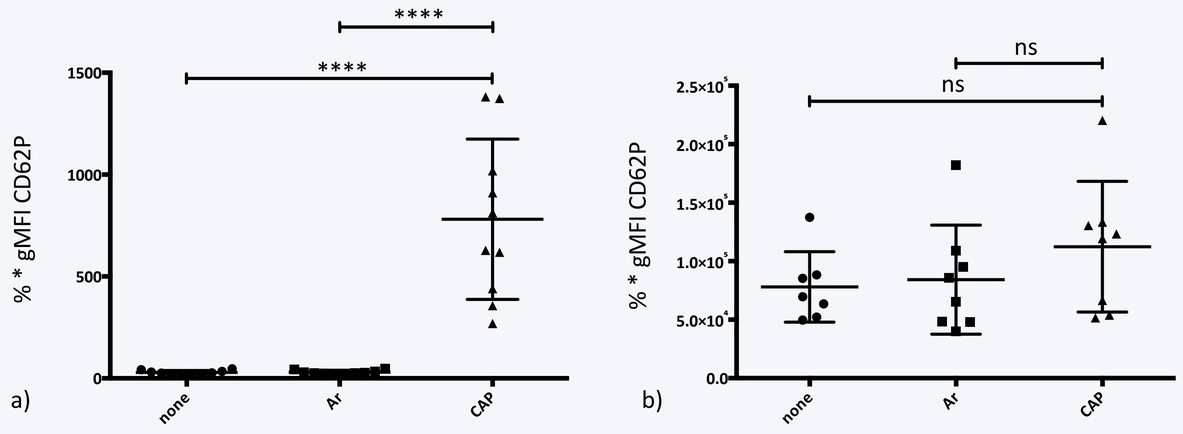

Fig. 4: Activation of platelets after 2 minute treatment. a) Isolated platelets showed significant increase of activation after treatment with CAP. **b) FACS analysis of treated whole blood** showed just a slight tendency of platelet activation.

in vivo

Fig. 5: liver incision of native mouse. 10 min after **a)** Ar **b)** thermal **c)** CAP treatment.

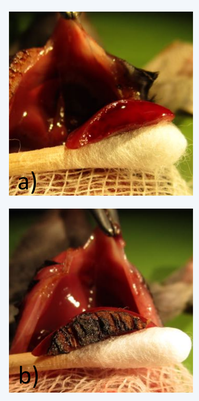

Fig. 6: liver incision of Rivaroxaban mouse. 10 min after **a)** Ar **b)** thermal **c)** CAP treatment.

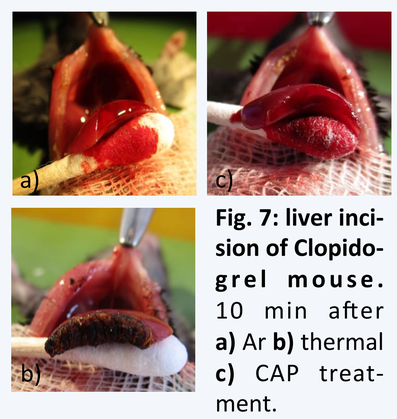

Fig. 7: liver incision of Clopidogrel mouse. 10 min after **a)** Ar **b)** thermal **c)** CAP treatment.

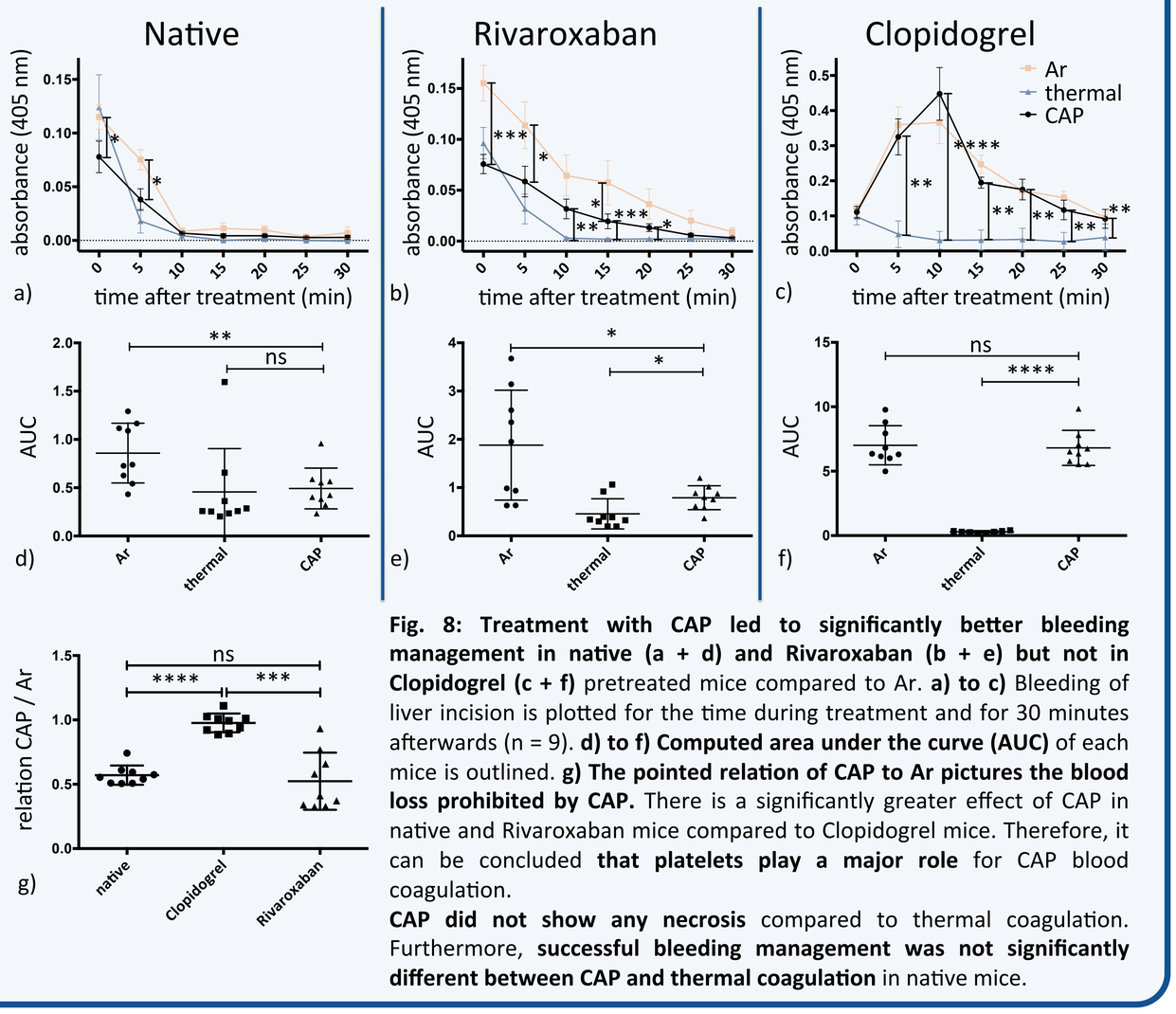

Fig. 8: Treatment with CAP led to significantly better bleeding management in native (a + d) and Rivaroxaban (b + e) but not in Clopidogrel (c + f) pretreated mice compared to Ar. **a) to c)** Bleeding of liver incision is plotted for the time during treatment and for 30 minutes afterwards (n = 9). **d) to f) Computed area under the curve (AUC)** of each mice is outlined. **g) The pointed relation of CAP to Ar pictures the blood loss prohibited by CAP.** There is a significantly greater effect of CAP in native and Rivaroxaban mice compared to Clopidogrel mice. Therefore, it can be concluded **that platelets play a major role** for CAP blood coagulation.

CAP did not show any necrosis compared to thermal coagulation. Furthermore, **successful bleeding management was not significantly different between CAP and thermal coagulation** in native mice.