Indoxyl sulfate increases vascular thrombosis induced by electric current and laser injury in animal models.

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Introduction and Objectives

Chronic kidney disease (CKD) patients are at high risk for thrombotic events including cerebrovascular disease, myocardial infarction, and pulmonary embolism [1]. Indoxyl sulfate (IS) is one of the most potent protein-bound uremic toxins that accumulates during CKD, and exerts aggressive and multidirectional effect on the body [2]. Despite IS is associated with an increased risk for cardiovascular disease [3], its impact on thrombotic events still remains not fully understood. The purpose of the study was to evaluate direct effect of IS on thrombus formation and clot firmness in rats and mice models.

Methods

We evaluated impact of three IS doses: 10, 30 and 100 mg/kg b.w. i.v. on arterial thrombus development induced by direct electric current in Wistar rat's model, and clot formation and stability using thromboelastometry (TEM). Moreover, we used confocal and widefield intravital microscopy to examine IS effect on thrombus formation after argon-ion laser-induced endothelial injury in real time in the mesenteric venules of a living mouse. Additionally, we assessed IS influence on blood morphology parameters, and evaluated plasma IS concentration by high-performance liquid chromatography.

Results and Conclusions

IS doses: 10, 30 and 100 mg/kg b.w. increased weight of arterial thrombus induced by direct electric current in dose-dependent manner ($p<0.001$). Furthermore, two highest IS doses increased laser-induced thrombus formation observed via confocal system in mice that was reflected by changes in fluorescence intensity and increase in total thrombus area ($p<0.01$). From data obtained due to TEM, only the highest IS dose decreased clotting time (CT) ($p<0.01$) and increased maximum clot firmness (MCF) ($p<0.05$). None of tested IS doses affected others TEM parameters like alpha angle and area under the curve, as well as blood morphology parameters.

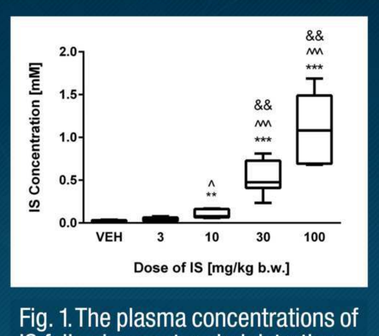

Fig. 1. The plasma concentrations of IS following acute administration.
VEH – control group; IS – indoxyl sulfate; **p < 0.01 compared to control; ***p < 0.001 compared to control; ^p < 0.05 compared to 3 mg/kg b.w. of IS; ^^^p < 0.001 compared to 3 mg/kg b.w. of IS; &&p < 0.01 compared to 10 mg/kg b.w. of IS

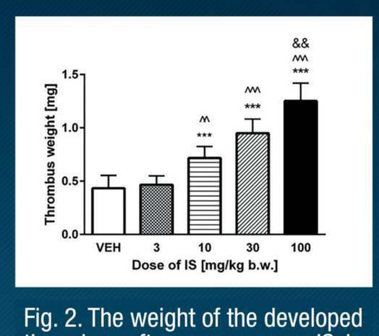

Fig. 2. The weight of the developed thrombus after exposure on IS in arterial thrombosis model.
VEH – control group; IS – indoxyl sulfate; ***p < 0.001 compared to control; ^^p < 0.01 compared to 3 mg/kg b.w. of IS; ^^^p < 0.001 compared to 3 mg/kg b.w. of IS; &&p < 0.01 compared to 10 mg/kg b.w. of IS

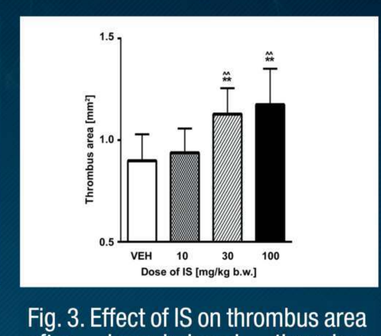

Fig. 3. Effect of IS on thrombus area after laser-induced thrombus formation in intravital mice model.
IS – indoxyl sulfate; VEH – control group; **p < 0.01 compared to control; ^^p < 0.01 compared to the dose of 10 mg/kg b.w. of IS

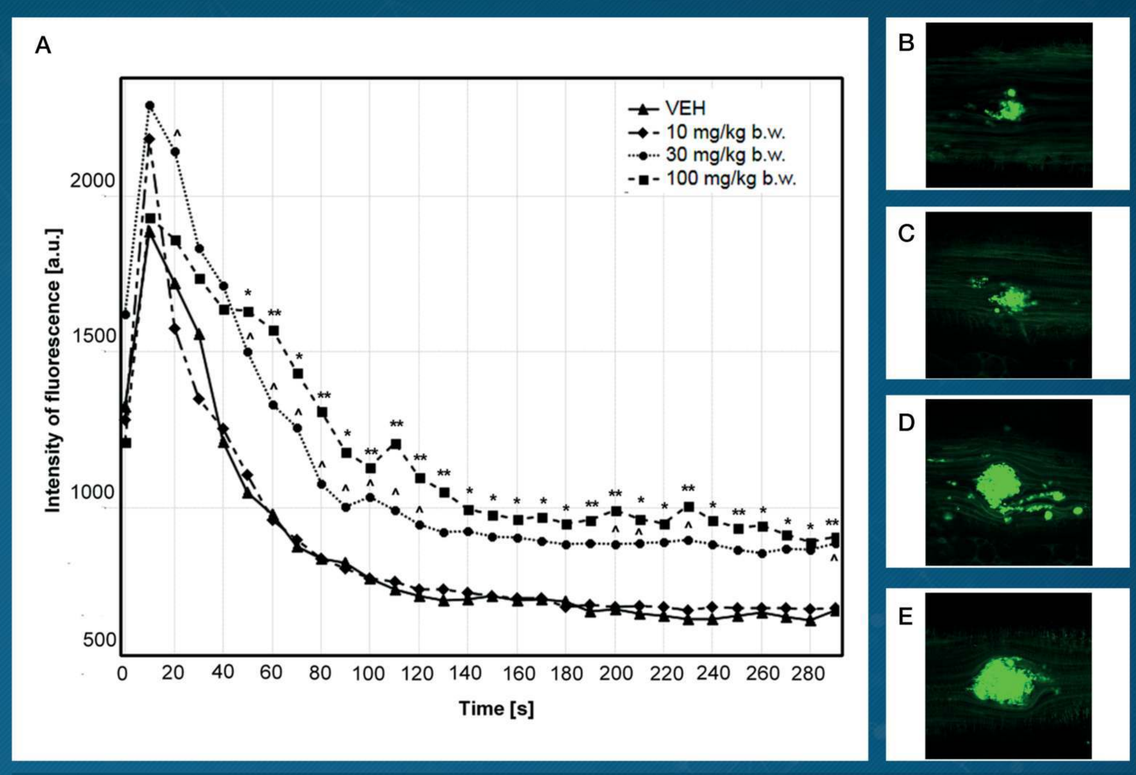

Fig. 4 Impact of IS on fluorescence intensity in intravital imaging of the thrombus development during 5 minutes evaluation after laser injury in mice model (A) and representative images of the developed thrombi in intravital model after administration of 0.9% natrium chloride (control group) or IS. B: VEH; C: 10 mg/kg b.w. of IS; D: 30 mg/kg b.w. of IS; E: 100 mg/kg b.w. of IS
IS – indoxyl sulfate; VEH – control group; *p < 0.05 compared to control; **p < 0.01 compared to control; ^p < 0.05 compared to the dose of 10 mg/kg b.w. of IS

	VEH	10[mg/kg b.w.]	30[mg/kg b.w.]	100[mg/kg b.w.]
Alpha [angle]	70 ± 3.6	67.7 ± 2.8 NS	68.4 ± 2.6 NS	71 ± 6.3 NS
AUC [a.u.]	6661 ± 337	6761 ± 202 NS	6764 ± 220 NS	7231 ± 642 *
CT [s]	382 (333 – 477)	391 (306 – 479) NS	392 (234 – 427) NS	323 (220 – 332) ** ## ^
MCF [mm]	66.1 ± 2.8	67.5 ± 3.3 NS	67.2 ± 2.2 NS	73.3 ± 6.3 * # ^
CFT [s]	129 ± 20.6	127 ± 20.5 NS	113 ± 12.7 NS	88.6 ± 30.6 *

Table 1. Parameters of dynamic of clot formation in whole blood after exposure to IS.
IS – indoxyl sulfate, VEH – control group, AUC – area under the curve, CT – clotting time, MCF – maximal clot firmness, CFT – clot formation time, NS – non-significant, *p < 0.05 compared to control, **p < 0.01 compared to control, #p < 0.05 compared to 10 mg. b.w. of IS, ##p < 0.01 compared to 10 mg/kg b.w. of IS, ^p < 0.05 compared to 30 mg/kg b.w. of IS

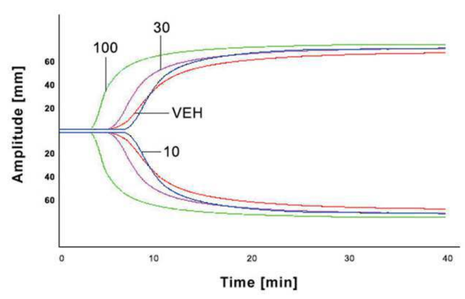

Fig. 5. Graphical presentation of IS impact on ROTEM analyses.
IS – indoxyl sulfate, VEH – control group, 10 – the dose of 10 mg/kg b.w. of IS; 30 – the dose of 30 mg/kg b.w. of IS; 100 – the dose of 100 mg/kg b.w. of IS

Obtained data indicate that IS affects hemostasis and contributes to more stable thrombus formation, and hypercoagulable state. On the basis of the results, we concluded that mentioned toxin is one of crucial uremic factors promoting thrombotic events in patients suffering from CKD.

This work was supported by funds from Polish National Science Centre 2015/17/N/NZ4/02334.

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Identifier/Topic: CDK - PATHOPYSIOLOGY & PROGRESSION I

54 ERA
288--SP
CDK - Pathophysiology & progression I
Malgorzata Karbowska
DOI: 10.3252/pso.eu.54ERA.2017
ePosters supported by F. Hoffmann- La Roche Ltd.
54th ERA-EDTA CONGRESS Madrid Spain June 3rd-6th 2017
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