Eryptosis induced by indoxyl sulfate is related to oxidative stress.

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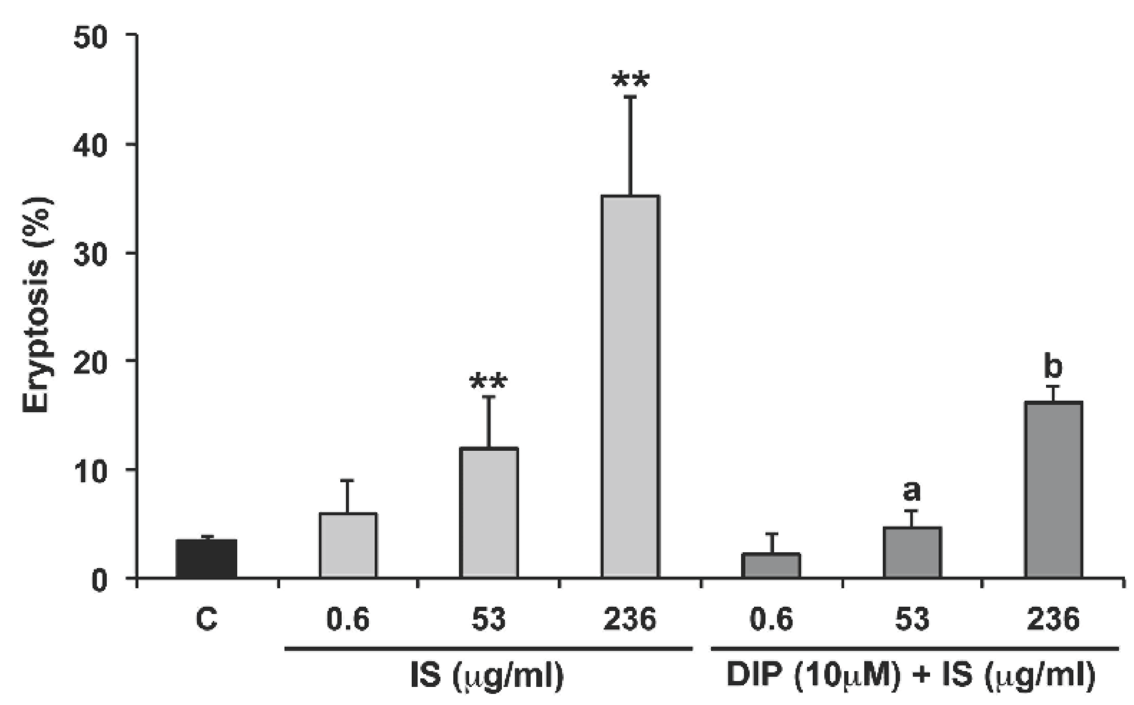
Introduction

Uremic toxins may play a critical role in chronic kidney disease (CKD)-related anemia (Lang et al., 2012) by inducing eryptosis thorough mechanisms not fully understood. Renal anemia is involved in the pathogenesis of increased oxidative stress in CKD patients (Klahr, 1997; Vaziri, 2004), due its association with the overproduction of reactive oxygen species (ROS) and reduction of antioxidant defenses (Dobashi et al., 2000). *N*-acetyl-L-cysteine (NAC) is a cysteine donor with antioxidant properties and diphenyleneiodonium (DIP) is an inhibitor of NADPH oxidase. The present study explored the potential of the uremic toxin indoxyl sulfate (IS) in promoting eryptosis and ROS production and the effect of NAC or DIP treatment.

Methods

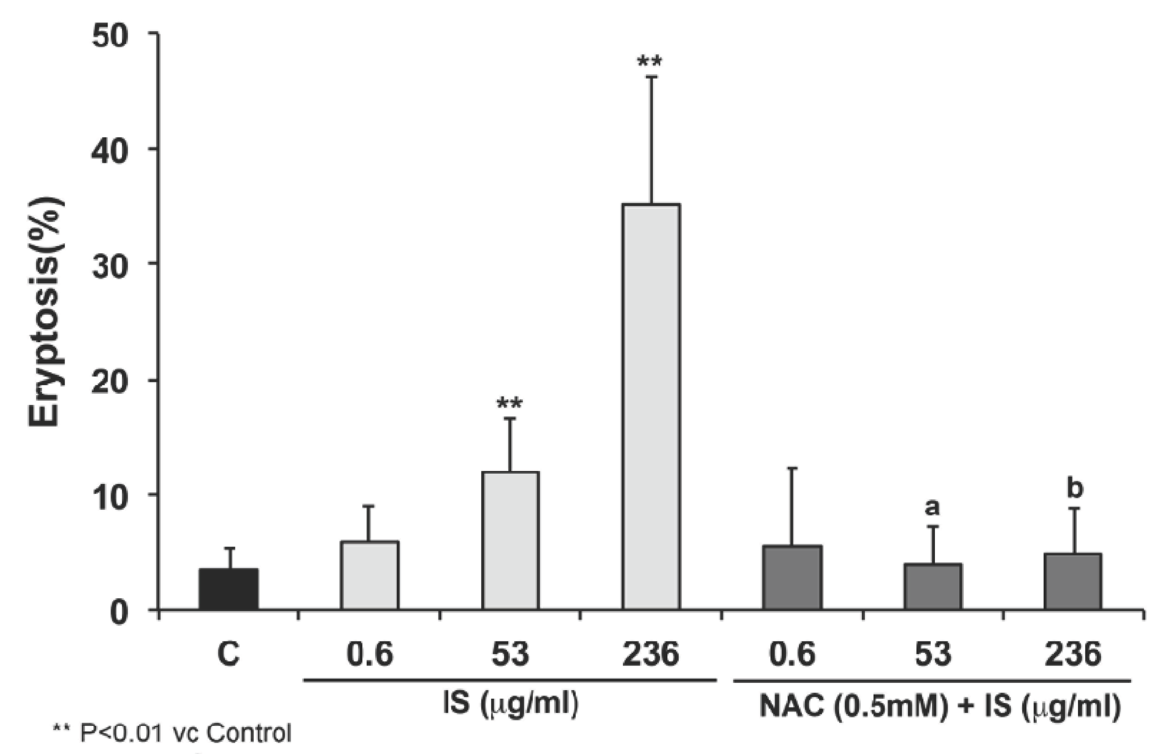
Erythrocytes obtained from healthy donors (n=6) were incubated for 24h with NAC (0.5mM) or DIP (10µM) before 24h incubation with different concentration of IS (0.6, 53 and 236 µg/ml). Eryptosis was evaluated by positivity of cells expressing phosphatidylserine (PS) by Annexin-V-PE. Intracellular ROS generation was evaluated by DCFH-DA oxidation and both were analyzed using flow cytometer.

Results



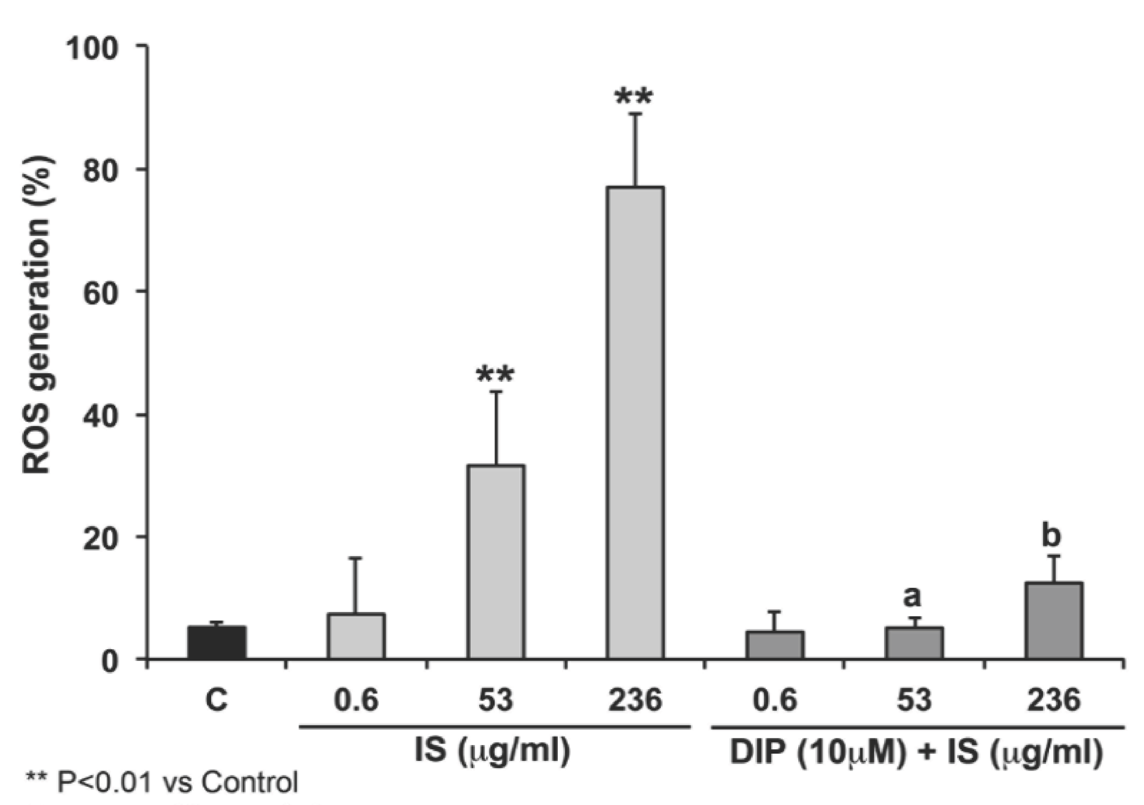
** P<0.01 vs Control
a P<0.05 vs IS 53µg/ml
b P<0.05 vs IS 236µg/ml

Eryptosis induced by IS and DIP inhibition. Erythrocytes were preincubated with DIP (10µM) for 24h and stimulated with IS (0.6, 53 and 236 µg/ml) for 24h and stained with Anexin-V-PE. IS (236 µg/ml) increased (35±11%) eryptosis compared to control erythrocytes (3.4±1.8%) while DIP atenuante the response (16.1±4.2%).



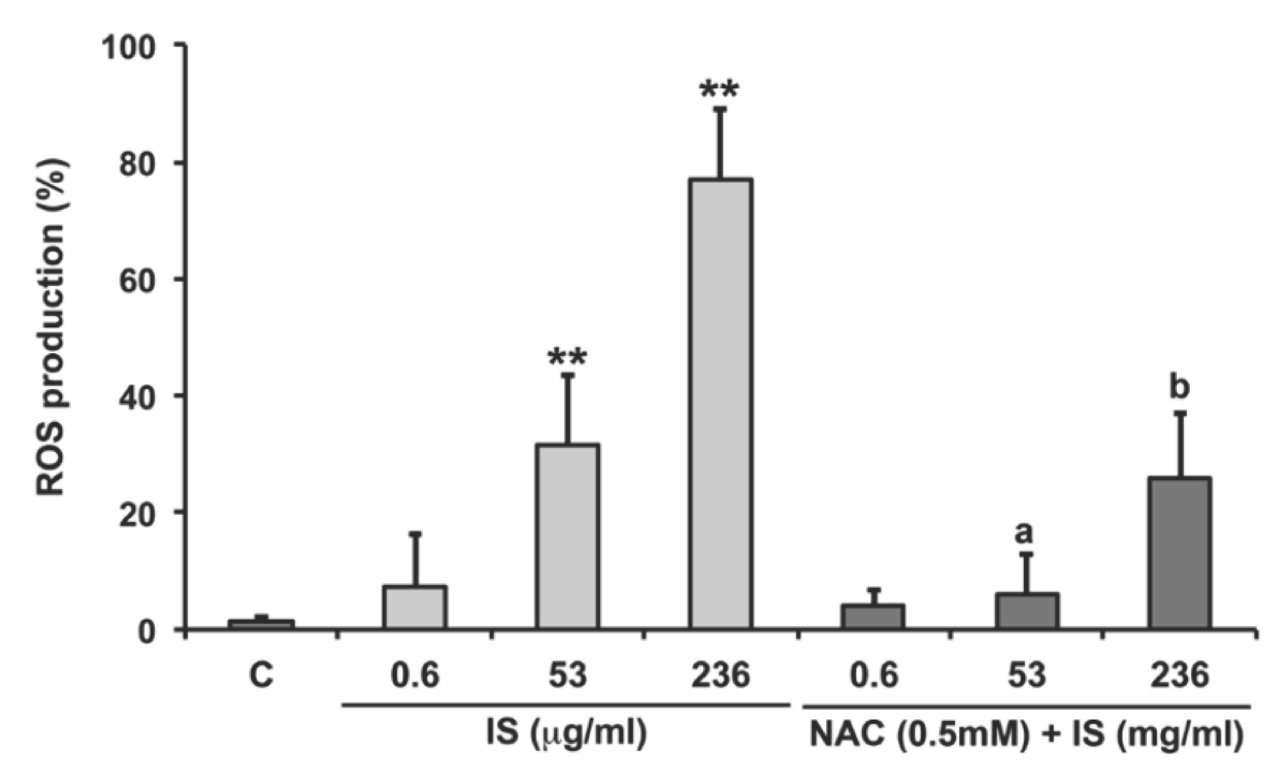
** P<0.01 vc Control
a P<0.05 vs IS 53µg/ml
b P<0.01 vs IS 236µg/ml

Eryptosis induced by IS and NAC inhibition. Erythrocytes were preincubated with NAC (0.5mM) for 24h and stimulated with IS (0.6, 53 and 236 µg/ml) for 24h and stained with Anexin-V-PE. IS (236 µg/ml) increased (35±11%) eryptosis compared to control erythrocytes (3.4±1.8%) while NAC decrease the toxin effect (5.9±3.4%).



** P<0.01 vs Control
a P<0.01 vs IS 53µg/ml
b P<0.01 vs 236µg/ml

ROS generation induced by IS and DIP inhibition. Erythrocytes were pre incubated with DIP (10µM) for 24h and stimulated with IS (0.6, 53 and 236 µg/ml) for 24h and incubated with DCFH-DA fluorescent probe. IS (236 µg/ml) induced (76±19.7%) ROS generation compared to control erythrocytes (1.2±0.7%) while DIP decrease the response (12±4.4%).



** P<0.01 vs Control
a P<0.05 vs IS 53µg/ml
b P<0.01 vs IS 236µg/ml

ROS generation induced by IS and NAC inhibition. Erythrocytes were pre incubated with NAC (0.5mM) for 24h and stimulated with IS (0.6, 53 and 236 µg/ml) for 24h and incubated with DCFH-DA fluorescent probe. IS (236 µg/ml) induced (76±19.7%) ROS generation compared to control erythrocytes (1.2±0.7%) while NAC inhibited the effect (25±11%).

Conclusion

Our results suggest that IS promotes both eryptosis and ROS generation, the latter being attenuated by NAC and DIP. These results indicate that the increase in IS-induced ROS generation may trigger phospholipid-exposure on erythrocytes surface, the early indicator of eryptosis.

References

Lang E, Qadri SM, Lang F. Killing me sofly - suicidal erythrocytes death. Int J Biochem Cell Biol., 44(8):1236-43, 2012.
Klahr S. Oxygen radical and renal diseases. Miner Electrolyte Metab., 23(3-6):140-3, 1997.
Vaziri ND. Oxidative Stress in uremia: nature, mechanisms and potential consequences. Semin Nephrol., 24(5):469-73, 2004.
Dobashi K, Ghosh B, Orak JK, Singh I, Singh AK. Kidney ischemia-reperfusion: modulation of antioxidant defenses.

CIÊNCIAS DA SAÚDE

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