

Soluble Guanylyl Cyclase Expression (sGC) in Platelets in Experimental Sepsis: Effect of the sGC Activator BAY 60-2770

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Abstract

Soluble guanylyl cyclase (sGC) acts as the principal intracellular receptor for nitric oxide (NO), facilitating the generation of cyclic GMP. Soluble guanylyl cyclase is a heterodimer complex consisting of two subunits, α and β , each of which contains three domains, namely one domain N-terminal regulator, a region of dimerization and a C-terminal domain that is responsible for substrate recognition and activity catalytic of GCs, which is responsible for the conversion of GTP to cGMP. NO-independent sGC stimulators/activators have emerged as valuable tools to elucidate the physiopathology of the NO-cGMP signaling pathway in pathophysiological conditions. Stimulators of sGC (BAY 41-2272) depends on presence of the reduced haem (Fe^{2+}) prosthetic moiety within sGC, whereas sGC activators (BAY 58-2667 and BAY 60-2770) are able to activate the enzyme when the heme is oxidized (Fe^{3+}). The NO-sGC-cGMP signaling is impaired in conditions when oxidized heme (Fe^{3+}). Lipopolysaccharide (LPS) is widely used to mimic sepsis conditions. Platelets obtained from LPS-treated rats display high intracellular levels of reactive-oxygen species (ROS), which may lead to sGC degradation. Therefore, this project aimed to evaluate the protein expressions of α and β -subunits of sGC in washed platelets from LPS-treated rats. The effects of BAY 41-2272 and BAY 60-2770 in ADP- and thrombin-induced platelet aggregation from control and LPS will be also investigated.

Key words: soluble guanylyl cyclase, sepsis, oxidative stress,

Introduction

The nitric oxide (NO)-independent soluble guanylyl cyclase (sGC) activator BAY 60-2770 reactivates the heme group of the enzyme in vessels and platelets in pathological conditions. This study was undertaken to investigate the effects of the NO-independent sGC activator BAY 60-2770 in washed platelets from rats treated with LPS (*Escherichia coli*, L4130) to mimic sepsis. The hypothesis that sGC oxidation potentiates the antiplatelet activities of BAY 60-2770 has been tested.

Results and Discussion

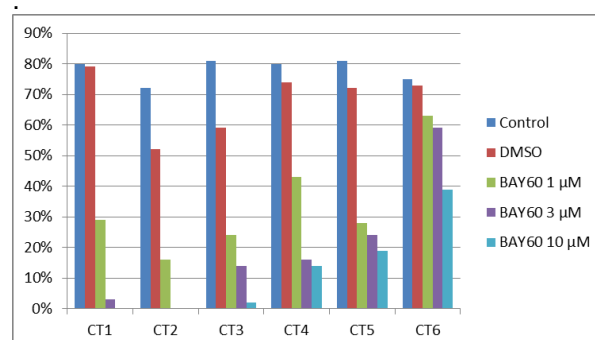
Platelet aggregation was performed with an optical aggregometer (Chrono-log) at 37°C with 400 μL of washed platelets placed in glass cuvettes. The maximal aggregation (%) was calculated using the Aggrolink Software (Chrono-log). Krebs solution without vehicle provided a signal representing 0% aggregation.

BAY 60-2770 or its vehicle dimethylsulfoxide (DMSO, 50%) was added to washed platelets 3 min prior to activation with ADP (20 μM).

Incubation with BAY 60-2770 (1 to 10 μM) produced a significant inhibition of ADP (20 μM)-induced platelet aggregation in control rats (Figure 1).

Figure 1. Inhibitory responses produced by BAY 60-2770 (1, 3 and 10 μM) in ADP-induced washed platelet aggregation from control rats. Maximal

aggregation (%) was calculated using the Aggrolink Software (Chrono-log).



Dimethyl sulfoxide (DMSO): vehicle for BAY 60-2770

Conclusions

BAY 60-2770 inhibits ADP-induced washed platelet aggregation.

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¹ Mendes-Silverio CB, Leiria LOS, Morganti RP, Anhê GF, Marcondes S, et al. (2012) Activation of Haem-Oxidized Soluble Guanylyl Cyclase with BAY 60-2770 in Human Platelets Lead to Overstimulation of the Cyclic GMP Signaling Pathway. PLoS ONE 7(11): e47223. doi:10.1371/journal.pone.0047223

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