CARBONIC ANHYDRASE IX AND COX-2 INHIBITORS AS A NEW CLASS OF ANTI-INFLAMMATORY DRUGS IN A MODEL OF PULMONARY FIBROSIS IN MICE

1)Lucarini L. 2)Lanzi C. 3)Durante M. 4)Turano F. 5)Carta F. 6)Supuran CT. 7)Masini E.

University of Florence

The carbonic anhydrase (CA) family includes 16 catalytically active zinc metallo-enzymes that catalyze the reversible interconversion of carbon dioxide and water to bicarbonate and protons. CA inhibitors, besides antitumour activity (Cianchi et al., 2010), exhibit anti-inflammatory effects in rats with permanent middle cerebral artery occlusion (Di Cesare Mannelli et al., 2016).

Fibrosis of lung tissue is a disease characterized by chronic inflammation that determines a pathological remodelling of lung parenchyma. The animal model obtained by intra-tracheal administration of bleomycin in C57BL/6 mice is one of the most validated murine model (Lucarini et al., 2016).

This study investigated the effects of a new class of drugs endowed with CAIX and COX-2 inhibition activity in the modulation of inflammation.

Initially, we tested the four compounds on cell culture, to assess their anti-inflammatory activity. RAW 264.7 macrophages were incubated for 18 hrs with LPS (1 μg/mL) and pre-treated or not with different concentrations of the studied drugs (10-7-10-4 M). The prostaglandin E2 (PGE2) production was quantified with a commercial ELISA kit. On human platelet-rich plasma (PRP), the inhibition of platelet aggregation, induced by 10 μM ADP after 5' incubation, was studied, in the presence or absence of the studied compounds. The concentration of TXA2 was measured as TXB2 production with an ELISA method on PRP after clotting.

We then selected the most active compound to test COX-2 inhibition and we administered it in a murine model of bleomycin-induced lung fibrosis. C57BL/6 mice were treated with bleomycin (0.05 IU) or saline intratracheally to induce lung fibrosis. Immediately after, mice were treated with vehicle, compound 1e (1 mg/kg b.wt.), ibuprofen (0,5 mg/kg b.wt.) or acetazolamide (0,5 mg/kg b.wt.) at equimolar doses, released by micro-osmotic pumps for 21 days.

The results of the first part show that PGE2 production is lowered in LPS-stimulated RAW 264.7 cells treated with CAIX/COX-2 inhibitors in a dose-response manner (Vehicle: 287.74±11.3 pg/mL; 1e 10-5M: 89.28 ±7.65 pg/mL; ibuprofen 10-5M: 116.5±7.81 pg/mL). CAIX/COX inhibitors do not modify the inhibition of platelet aggregation in comparison with the reference molecules (baseline: 100%; 1e 10-4M: 28.97%; ibuprofen 10-4M: 26.71%), nor the production of TXB2 in comparison with the reference molecules (baseline: 218.25±3.3 ng/mL; 1e 10-4M: 100.72±1.5 ng/mL; ibuprofen 10-4M: 97.32±4.3 ng/mL), suggesting a COX-2 inhibitory effect. On the murine model of bleomycin-induced lung fibrosis, we assayed airway resistance to inflation and lung samples were processed to measure 8OHdG, a marker of oxidative stress, COX-2 expression, pro-inflammatory cytokines production and apoptosis. Fibrosis and airway remodeling were evaluated by measuring TGF-β, the percentage of positive Goblet cells, and smooth muscle layer thickness

determination. Our preliminary results indicate that the compound 1e decreased inflammation and oxidative stress markers.

The results here reported demonstrate that these compounds, endowed with a dual COX-2 and CAIX inhibition, are interesting new anti-inflammatory drugs and have a beneficial effect in a model of lung fibrosis in mice, thus indicating that these compounds could be a novel therapeutic strategy for lung inflammatory diseases.

Cianchi et al. (2010). J Pharmacol Exp Ther. 334:710-719.

Di Cesare Mannelli et al. (2016). J Enzyme Inhib Med Chem. 31:894-899.

Lucarini et al. (2016). Pharmacol Res. 111:740-748.