EVALUATION OF THE ANTIPYROPTOTIC ACTIVITY OF NEW INFLAMMASOME INHIBITORS

Valentina Boscaro, Simone Gastaldi, Massimo Bertinaria, Margherita Gallicchio

Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Torino, Italia

BACKGROUND: The inflammasome, an intracellular multiprotein complex responsible for the coordination of the innate immune response, plays a fundamental role in defending the body from potential threats. The activity of the inflammasome relies on the activation of caspase 1, a proteolytic enzyme that induces the cleavage and the release of interleukin 1β (IL-1 β) and IL18 and causes the cell death through pyropotosis. Numerous inflammasome variants have been identified, among which the absent inflammasome in melanoma 2 (AIM2), the NLRC4, NLRP1 and NLPR12 inflammasome and the extensively studied NLPR3 inflammasome. The fine regulation of inflammasome makes it a central player in the pathophysiology of numerous autoimmune and inflammatory diseases such as type 2 diabetes, gout, obesity, atherosclerosis, cryopyrinopathies, chronic inflammatory bowel diseases but also Alzheimer's and Parkinson's disease [1]. The aim of the present study was to evaluate new inhibitors of inflammasome NLRP3, synthesized by the SynBioMed group of the Department of Drug Science and Technology; the novel series of compounds was designed by taking as a template the compound INF39, which has demonstrated good pharmacological and toxicological properties [2].

METHODS: THP-1 cell line, human monocytes derived from an acute monocytic leukemia patient, was used to study if these compounds, used at a concentration of 10μM, were able to reduce inflammasome activation induced by treating the cells with LPS first, and then with ATP (5mM) or MSU (200μg/ml). Levels of lactate dehydrogenase (LDH), a cytosolic protein released in the extracellular space during pyroptosis, were evaluated using the Non-Radioactive CytoTox96® Cytotoxicity assay, while IL-1β concentrations were quantified through an enzyme-linked immunosorbent assay. MCC950, an established NLPR3 inhibitor [3], was used as control. Finally, the cytotoxicity of these inhibitors was evaluated after 72h of treatment through the MTT assay.

RESULTS: Compounds tested are not cytotoxic at 10μM concentration used in pyroptosis assays. The maximum inhibition of LDH release following ATP stimulation is about 45%; the same compounds are able to reduce IL-1β release by about 20-30%.

CONCLUSIONS: Future studies are required in order to perform a more accurate characterization of the anti-pyropototic activity of the novel series of compounds and to modulate their structures to increase their ability to inhibit NLPR3 inflammasome. 1) Awad et al. Pharmacol Ther 2018, 187:133-49
2) Cocco et al. J Med Chem 2014, 57:10366-82
3) Coll et al. Nat Med 2015, 21(3):248-55