ANTICANCER EFFECTS OF NOVEL LDH-A AND GLUT-1 INHIBITORS ON HUMAN PANCREATIC ADENOCARCINOMA CELLS ASPC-1.

1)Citi V. 2)Granchi C. 3)Piragine E. 4)Testai L. 5)Breschi MC. 6)Minutolo F. 7)Calderone V. 8)Martelli A.

University of Pisa - Department of Pharmacy

Type 1 glucose transporter (GLUT-1) and the glycolysis enzyme lactate dehydrogenase A (LDH-A) are both overexpressed in cancer cells and thus they represent promising pharmacological target against cancer. In particular, pancreatic cancer cells exhibit an increased rate of glycolysis and are intrinsically more sensitive to therapeutic strategies based on the inhibition of the glycolytic pathway [1]. In this work, the anticancer effects of GLUT-1 and LDH-A inhibitors (PGL-17 and 161 respectively) have been evaluated on human pancreatic adenocarcinoma cells (AsPc-1). In particular, the anti-proliferative effect has been evaluated after 72h of treatment with PGL-17 and 161 at different concentrations using the tetrazolium salt WST-1 which is cleaved to a soluble formazan by viable cells. Both the tested compounds completely abolished cell viability with similar growth inhibitory indexes (GI50) of about 40μM. Furthermore, in order to further investigate their mechanism of toxicity towards cancer cells, the concentration which evoked half inhibition of cell proliferation has been selected. Cell cycle analysis has been performed with a microcytofluorimeter: both PGL-17 and 161 resulted to be cell cycle blockers, although with different features. Indeed, PGL-17 induced a G2/M and S phase arrest with reduction of G0/G1 phase; instead, 161 significantly increased the number of cells in G0/G1 phase. This underlines that the cell proliferation inhibitory effects of the two compounds act in those cells with rate of replication. Since cancer cells can acquire reduction in apoptosis or apoptosis resistance, promising anticancer molecules should also increase apoptotic events. This feature is likely to ensure lower toxicity for normal cells, if compared with drugs inducing non-specific damage [2]. Thus, in order to clarify a possible involvement of PGL-17 and 161 in the apoptotic cascade, depolarization of mitochondrial potential, marker of early apoptosis, and the activity of caspase3-7, sign of a more advanced stage of apoptosis, have been evaluated. Both the compounds significantly increased the number of cells exhibiting depolarization of mitochondrial potential after 72h. In contrast, only the LDH-A inhibitor 161 significantly increased the activity of caspase3-7. PGL-17 didn't affect the caspase 3-7 activity, probably because a 72h-treatment may be not sufficient to evoke a late stage of apoptosis. Finally, in order to understand a possible mechanism of action of PGL-17 and 161, phosphorylation status in MAPK pathway has been evaluated. AsPc-1 has been reported in literature to be characterized by the activating mutation p.G12D on KRAS gene, resulting in a hyper phosphorylation of the downstream kinases [3]. As expected, the AsPc-1 basal level of p-ERK1/2(Thr202/Tyr204) resulted to be dramatically higher. Interestingly, both PGL-17 and 161 induced a significant reduction of p-ERK1/2(Thr202/Tyr204) activation suggesting an involvement of MAPK pathway in the anti-proliferative effect on AsPc-1. In conclusion, these data suggest that AsPc-1 are sensitive to glycolytic inhibition and abolishment in glucose support. Thus the novel tested compounds PGL-17 and 161, inhibitors of GLUT-1 and LDH-A respectively, represent a promising strategy in the treatment of pancreatic cancer.

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