Role of poly(ADP-ribose) polymerase-1 (PARP-1) and poly(ADP-ribose) glycohydrolase (PARG) in the regulation of cell death and in the expression of inflammatory mediators

Katalin Erdélyi

Supervisor:
Dr. László Virág

University of Debrecen, Medical and Health Science Center
Department of Medical Chemistry

Doctoral School of Molecular Medicine
Program: I.

SUMMARY

Poly(ADP-ribosyl)ation, catalysed by poly(ADP-ribose) polymerases (PARP-s), is a reversible post-translational modification of glutamate and aspartate residues of nuclear proteins and represents an immediate eukaryotic cellular response to DNA damage as induced by ionizing radiation, alkylating agents and oxidants. The ADP-ribose polymer formed by sequential attachment of ADP-ribosyl moieties form NAD^+ can reach high complexity with chain lengths of up to 200 units and multiple branching points. The major enzyme catalyzing poly(ADP-ribose) catabolism is poly(ADP-ribose) glycohydrolase (PARG), splitting the polymer's unique ribose-ribose linkages generating free ADP-ribose units.

Here we have investigated the possible role of poly(ADP-ribosyl)ation in cytokine-stimulated A549 cells. Cells were pretreated with a potent PARP-1 inhibitor PJ34 or the PARG inhibitor gallotannin (GT). Cytokines induced the expression of several chemokines and cytokines through NF-κB and AP-1 activation in A549 cells which was strongly suppressed by GT and weakly inhibited by PJ34. GT decreased NF-κB transactivation by inhibiting its nuclear translocation. This effects was due to the inhibition of IκB phosphorylation. PJ34 did not affect the nuclear translocation of the transcription factor, however it inhibited its DNA binding. GT also inhibited the DNA binding of the transcription factor, AP-1 even in the absence of cytokines. However, GT did not inhibit the activation of the AP-1 subunits. Moreover, we observed an elevated phosphorylation of c-Jun. GT alone induced phosphorylation of JNK, p38MAPK and ERK and their targets. This might be due to the inhibition of protein phosphatases (PP1 and PP2A). The cytokine exposure stimulated no detectable poly(ADP-ribose) synthesis in either the absence or presence of GT. Lack of poly(ADP-ribose) accumulation in GT-treated cells suggests that no major alteration of poly(ADP-ribose) metabolism occur in A549 cells in response to GT treatment.

Since GT didn't prove to be a suitable tool for the investigation of PARG, we established A549 cell lines with stable suppression of the PARG and PARP-1 genes. These knockdown cells were protected from necrosis (propidium iodide uptake) induced by severe oxidative stress, however, they were more sensitive to apoptosis (as measured by caspase activation and DNA fragmentation) induced by mild oxidative stress. We identified inefficient DNA repair as the underlying mechanism of this latter effect. The similar responses of the PARG and PARP-1 knockdown cells indicate that PARG knockdown may result in indirect inhibition of PARP-1 via inhibitory auto-poly(ADP-ribosyl)ation.

Key words: poly(ADP-ribosyl)ation, chemokine expression, gallotannin, DNA-damage, hydrogen peroxide, apoptosis, necrosis.

Kulcsszavak: poli-ADP-riboziláció, kemokin expresszió, gallotannin, DNS-károsodás, hydrogen peroxide, apoptózis, nekrózis.