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ATP Blocks Hydrogen Peroxide-induced Increase of $p21^{WAF1/Cip1}$ and $p27^{Kip1}$ Expression through PI3K and Akt Pathway in Renal Proximal Tubule Cells

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Reactive oxygen species (ROS) produced by various factors have been implicated in initiating, accompanying, or causing many diseases. Adenosine triphosphate (ATP) is an important extracellular signal in the regulation of many intracellular processes in normal tubular cells as well as in the pathogenesis of cell injury. This study investigated the effect of ATP on H_2O_2-induced increase of cyclin kinase inhibitors expression in primary cultured renal proximal tubule cells (PTCs). H_2O_2 inhibited cell proliferation in a concentration (>50 μM) and time-dependent manner (>2 hr), as determined by [^{3}H]-thymidine and BrdU incorporation, and by increase in the $p^{21WAF/Cip1}$ and $p27^{kip1}$ expression levels. In contrast, ATP increased the level of thymidine, BrdU incorporation (>10^{-5} M), and decreased the $p21^{WAF/Cip1}$ and $p27^{kip1}$ expression levels, suggesting that ATP has a protective effect against H2O2-induced oxidative damage. On the other hand, suramin (non-specific P2 purinoceptor antagonist), RB-2 (P2Y receptor antagonist), MRS 2159 (P2X1 receptor antagonist), and MRS 2179 (P2Y1 receptor antagonist) did block the reversing effect of ATP. In addition, AMP-CPP (P2X receptor agonist), or 2-methylthio ATP (P2Y receptor agonist) blocked H_2O_2-induced inhibition of cell proliferation, suggesting all these P2 purinoceptors may be potentially involved. ATP-induced stimulation of cell proliferation was blocked by LY294002, wortmannin [phosphoinositide 3-kinase (PI3K) blockers], and Akt inhibitor. These results suggest the involvement of P2 purinocceptors-mediated PI3K/Akt signal pathway in the protective effect of ATP against H_2O_2-induced oxidative damage. Indeed, pretreatment with PI3K blockers or Akt inhibitor did not protect H_2O_2-induced LPO production and inhibition of thymidine incorporation. In conclusion, ATP in part blocked H_2O_2-induced increase of $p21^{WAF1/Cip1}$ and $p27^{Kip1}$ expression through PI3K and Akt pathway in renal proximal tubule cells.