Caffeine inhibits ATM-dependent phosphorylation of p53 in gamma-irradiated leukaemic MOLT-4 cells.

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Objective: Activation of ATM-kinase (ATM) is involved in cell cycle arrest and reparation of ionizing radiation (IR)-induced DNA damage. Caffeine, a non-specific inhibitor of ATM, shortens IR-induced G2 arrest. It was shown to inhibit ATM/Chk-2/p53 signalling pathway and to diminish DNA reparation of IR-induced double strand breaks. In this work we studied effect of ATM inhibition on activation of other proteins involved in induction of apoptosis and cell cycle control in human T-lymphocyte leukaemic MOLT-4 cells (p53 wild type). We evaluated expression of tumour-suppressor p53 (itself and phosphorylated at Ser-15 and Ser-392) and p21 (a cell cycle regulator). Methods: We added 2mM caffeine 30 min prior irradiation (^{60}Co) and washed out after 24 h. The cells were irradiated by the doses of 1 or 3 Gy, lysed and the proteins were separated by SDS-PAGE and detected by Western-blotting. Apoptosis (annexin V detection) and DNA content were determined by flow-cytometry. Results: Protein p53 was up-regulated 2 h after irradiation by the doses of 1 and 3 Gy with maximum after 16 h. After 24 h its amount decreased. Both doses of IR induced phosphorylation at both Ser-15 and Ser-392 after 2 h with maximum after 4 h. Adding caffeine significantly inhibited both p53 phosphorylations. Similarly, p21 was up-regulated 4 h after irradiation by the doses of 1 and 3 Gy with maximum after 24 h. Caffeine caused a substantial decrease of p21 in combination with both doses of IR. Three days after irradiation caffeine significantly potentiated induction of apoptosis. Conclusion: Caffeine significantly inhibited ATM/Chk-2/p53 signalling pathway in MOLT-4 cells, which via decreased expression of p21 led to inhibition of cyclin-dependent kinases. This resulted in shortened cell cycle arrest (necessary for effective DNA reparation) and induction of apoptosis. Therefore we conclude that ATM activity inhibitors such as caffeine have a radio-sensitising effect and could be exploited in radio-therapy as radio-sensibilisators. Acknowledgement: The authors acknowledge the financial support of Ministry of Education of Czech Republic (project no. MSM 0021620820) and Ministry of Defence of Czech Republic (project no. MO0FVZ0000501).