ABSTRACT

IN VITRO RADIOBIOLOGICAL EFFECTS OF ^{131}I ON THYROID CELLS: TOWARDS A MECHANISTIC UNDERSTANDING OF THYROID STUNNING

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An ongoing controversy in nuclear medicine is a phenomenon known as *thyroid stunning* observed during ^{131}I therapy of thyroid cancer. Specifically, this involves an unexpected reduction of the ^{131}I uptake in the tumor or thyroid remnant after administration of the therapeutic ^{131}I dose. The preceding ^{131}I test dose given for diagnostic scanning is inferred a causal role. However, previously no explanation to the stunning mechanism has been offered. In papers I-II of this thesis, ^{131}I-induced thyroid stunning was investigated for the first time *in vitro* in cultured thyroid cells. Growth-arrested (G0) cells forming a tight and polarized epithelium on filter in bicameral chambers were continuously exposed to ^{131}I for 48 hours after which iodide transport from the basal to the apical chamber compartment was evaluated using ^{125}I$^-$ as a tracer. ^{131}I dose-dependently inhibited ^{125}I$^-$ transport (by 50-90% at 3-80 Gy) 24 hours and onwards (for at least three days) after irradiation was stopped. Kinetic studies further showed that the stunning phase was preceded by accelerated ^{125}I$^-$ transport. TSH receptor signalling and secretion of thyroglobulin participating in thyroid hormone production did not change after irradiation. Stable iodide did not reproduce the radiation effect. Also, the cell number was not affected. Together, these findings indicate that ^{131}I induces biphasic changes of thyroidal iodide transport without simultaneously affecting pro-hormone biosynthesis and thyroid regulation by TSH. Ionizing radiation from ^{131}I may thus negatively affect iodide transporter(s) specifically. In papers III-IV, a possible correlation between ^{131}I-induced thyroid stunning and radiation induced DNA damage signalling to the ataxia teleangiectasia mutated (ATM) kinase was investigated. Using genotoxic agents to induce preferentially DNA double strand breaks (DSB) (by calicheamicin 1γ), DNA strand cross-linking (by cisplatin), and radiomimetic DNA lesions i.e. DSB in minority (by bleomycin), ^{125}I$^-$ transport was found to be significantly inhibited, although most effectively by calicheamicin 1γ, at sublethal drug concentrations. This was preceded by ATM-dependent phosphorylation of the H2AX histone variant, indicating nuclear foci of DNA-DSBs, after all treatments including cisplatin. Formation of H2AX foci was much less abundant in cells receiving ^{131}I at 1-10 Gy for 4-48 hours. However, the same absorbed doses of ^{131}I induced ATM-mediated global activation of Chk2, a key DNA damage checkpoint kinase, and transient cell cycle arrest of subconfluent cells. After recovery of cell cycle progression many of the irradiated cells formed micronuclei indicating chromosomal missegregation during mitosis. In contrast, ^{131}I did not induce micronucleation in quiescent cells, although a delayed G0/M transition timing with the stunning phase was observed with cell cycle entry was evoked by epidermal growth factor stimulation. Collectively, this correlates ^{131}I-induced inhibition of iodide transport to DNA damage, presumably DSBs. Nevertheless, proliferating thyroid cells are more radiosensitive than quiescent cells in terms of risk of developing genomic instability. In conclusion, thyroid stunning is a real phenomenon that may compromise the outcome of ^{131}I therapy.

Keywords: thyroid, radioiodide, stunning, ^{131}I, stress response, DNA damage, Chk2, H2AX, micronuclei, cell cycle