MOLECULAR MECHANISMS UNDERLYING THE ASSOCIATION BETWEEN HYDROCHLOROTHIAZIDE USE AND THE RISK OF NON-MELANOMA SKIN CANCERS

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Background: Pharmaco-epidemiological studies reported a dose-dependent increased risk of non-melanoma skin cancers (NMSC) with exposure to cumulative doses of hydrochlorothiazide (HCTZ), one of the most widely prescribed diuretic drug over the world. On the basis of these findings, the EMA Pharmacovigilance Risk Assessment Committee (PRAC), recently recommended to amend HCTZ product information with special warnings and precautions. Although the exact mechanism underlying this association is unclear, photosensitizing actions of HCTZ could act as possible mechanism for NMSC development. UVA is the major portion of UV light reaching the earth surface and it is a potent environmental risk factor in skin cancer pathogenesis. UVA-mediated cellular damage occurs primarily through the release of reactive oxygen species (ROS) which can cause oxidative damage to proteins, lipids and nucleic acids. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the main mutagenic DNA oxidative modification and has been found in several cancers including those of the skin.

Aim: Our aims were to investigate whether HCTZ may enhance the damaging effects of UVA radiation on human keratinocytes, after acute and chronic exposure and to get insight into the molecular mechanisms involved.

Methods: Acute exposure: normal human keratinocytes (HaCaT) were treated with three concentrations of HCTZ (70, 140 and 375ng/ml), corresponding to the values of C(max) following the administration in humans of 12.5, 25and 75mg doses, respectively. HaCaT were exposed for two hours to HCTZ before UVA-irradiation with 10 J/cm^2, mimicking a summertime recreational sun exposure of 40 minutes. After light irradiation, cells were incubated under standard conditions for 1hour and collected for analysis. Chronic exposure: HaCaT, chronically treated with HCTZ (70 ng/ml), were exposed to UVA irradiations (10 J/cm^2) twice a week for a total of 9weeks, to mimic long term or professional exposure.

Results: Under acute exposure conditions, therapeutically relevant concentrations of HCTZ did not decreased HaCaT viability neither alone nor in the presence of UVA irradiation but amplified UVA induced ROS production up to 60%, in a concentration dependent manner. In addition, HCTZ augmented UVA-induced 8-OHdG ($p<0.05$); this effect was associated to a defective repair of oxidative DNA damage due to a concentration dependent reduced activity of the major base excision repair (BER) enzyme OGG1, which recognizes and specifically repairs 8-OHdG; maximum inhibition (44%) of OGG1activity was noted at the concentration of 375ng/ml ($p<0.05$). HCTZ also enhanced oxidative protein damage at 140 ($p<0.05$) and 375ng/ml ($p<0.01$) compared to UVA alone. Furthermore, acute effects of HCTZ included increased nuclear anomalies (apoptotic bodies and micronuclei) and decreased the percentage of mitotic figures compared to UVA alone.

Conclusions and discussion: We demonstrated that short term exposure to HCTZ enhanced UVA-induced oxidative DNA and protein damage as well as nuclear anomalies and reduced the activity of OGG1in human keratinocytes.
As these experimental conditions simulate those in the skin of patients taking HCTZ, our preliminary findings suggest a mechanism whereby UVA in sunlight may contribute to skin carcinogenesis in these patients. Results from the ongoing chronic, combined exposure to HCTZ and UVA will further shed light into the mechanisms of enhanced non-melanoma skin cancer risk in patients treated with HCTZ and what are the progressive changes occurring in the biology of these skin cells.