N-acetyl-S-(p-chlorophenylcarbamoyl)cysteine, a novel inhibitor of thioredoxin reductase, induces mitochondrial-mediated apoptosis and suppresses migration in melanoma cells

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BACKGROUND: Malignant melanoma is aggressive, therapy-resistant. Its incidence has risen dramatically worldwide in white populations. Accumulated evidence indicates that thioredoxin reductase (TrxR) is overexpressed in many human malignant cells including melanoma and plays important roles in the development and progression of cancer. TrxR has become a promising target for anticancer drug development. However, by 2015, only two anticancer compounds targeting TrxR were under clinical trials: Motexafin gadolinium (MGd, phase III) and Ethaselen (Phase I). In this study, N-acetyl-S-(p-chlorophenylcarbamoyl)cysteine (NACC) was found to be an irreversible inhibitor of TrxR with novel mechanism of action and inhibited growth of melanoma cells.

HYPOTHESIS: Inhibition of TrxR in the melanoma cells triggers apoptosis and suppresses cell migration. NACC induces apoptosis through reactive oxygen species (ROS)-dependent and mitochondrial-mediated pathways. TrxR is a potential therapeutic target in treating melanoma.

METHODS: The mechanism of inhibition against TrxR by NACC was investigated using substrate protection, dialysis and LC-MS/MS. The caspase activities were determined by using a spectrophotometer. Protein expression was examined by Western Blot. The ROS levels and mitochondrial permeability transition pore (PTP) were investigated by fluorescent microscopy. Mitochondrial membrane potential disruption was monitored by flow cytometry. The migration of UACC-62 cells in vitro was investigated in real time using the xCelligence system.

RESULTS: NACC inhibits purified rat liver TrxR1 in a time and concentration dependent manner. The K_i and k_{inact} of NACC against TrxR1 were determined to be 80 μM and 0.178 min^{-1}, respectively. The inhibition occurred only in the presence of NADPH and persisted after extensive dialysis. The tandem mass spectrometric analysis demonstrated that the selenocysteine rather than cysteine residue at the active site was p-chlorophenyl carbamoylated by NACC. In consequence of TrxR activity inhibition by NACC, apoptosis was induced in the UACC-62 cells. Significant elevation of ROS level was observed. The flow cytometric analysis demonstrated that NACC induced significant mitochondrial membrane potential disruption. Increased activities of caspase-3 and caspase-9 but not caspase-8 were observed in the cell lysates. The Western blot analysis showed that the pro-apoptotic protein Bax was up-regulated and the anti-apoptotic protein Mcl-1 was down-regulated and cytochrome c (Cyto c) was released to cytosol. However, opening of PTP which could be involved in Cyto c leakage from mitochondria was found to be unaffected by NACC. Taken together, all the results demonstrated that NACC inhibited TrxR and triggered apoptosis in the UACC-62 cells via ROS-mediated mitochondrial-dependent pathway. The results generated by the xCelligence system implied that NACC are capable of inhibiting migration of melanoma cells in vitro. The IC_{50} value of NACC on migration in UACC-62 cells in 96-h treatment was determined to be 4.93 ± 2.64 μM. In conclusion, NACC is a potential anti-melanoma agent which not only triggers apoptosis in melanoma cells, but also suppresses the cell migration. Inhibition of TrxR can be a promising therapeutic strategy in the treatment of melanoma.