URINARY IODINE CONCENTRATIONS IN MOTHERS AND THEIR TERM NEWBORNS

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Introduction

The development of fetal thyroid gland and its function in fetus and newborn are influenced by maternal pathologies, especially maternal iodine supplementation and maternal disorders of thyroid gland. WHO/ICCIDD recommend daily iodine supplementation of 150 μg in healthy nonpregnant women and of 250 μg in pregnant and lactating women. Pregnant and lactating women are considered a risk group although the Czech Republic ranks among countries with sufficient iodine supplementation. Iodine supplementation of population is mainly detected by urinary iodine concentrations (UIC). The epidemiologic normal range for pregnant women is 150-249 μg/l. In case of maternal iodine deficiency, the fetus is more susceptible to other factors influencing fetal thyroid gland development and function and its neurologic development during all fetal growth phases.

Aims

The aim of this study was to establish urinary iodine concentrations in mothers and their term newborns and to detect possible influencies of maternal iodine supplementation on newborn UIC.

Methods

In the presented study we examined 50 mothers without thyroid disorder and their 50 healthy term newborns. The urine samples were gathered on Day 0 (before delivery) from mothers and on Day 3 from mothers and newborns. All the samples were frozen and stored at the Department of Clinical Immunology and Allergology in Hradec Králové. The sample examination was performed at the Endocrinologic Institute in Prague and the method used was modified Sandell-Kolthoff´s reaction. All the newborns had the obligatory TSH screening

performed from dry-drop sample by fluoroimmunoanalysis. This screening was done at a certified laboratory at Faculty Hospital in Královské Vinohrady, Prague.

Results

46 % of mothers declared regular iodine supplementation in the form of multivitamins or iodide. According to the UIC levels detected, 78 % of mothers before delivery suffered from mild to moderate iodine deficiency (median 92,35 μg/l) and 78 % of mothers on day 3 scored mild iodine deficiency (median 61,9 μg/l). These results did not depend on their iodine supplementation as other studies from the Czech Republic concluded too. The newborn UIC levels on day 3 were below 100 μg/l (median 99,2 μg/l) in 54 % of cases. UIC levels in the newborns of mothers without regular iodine supplementation were lower than the UIC levels in the newborns of mothers with regular iodine supplementation ($p=0,168$). All the newborns had normal TSH levels.

Discussion

Examined population of mothers was not iodine sufficient and mild to moderate iodine deficiency was found. Higher UIC levels in newborns of mothers with regular iodine supplementation were not statistically important. In general, maternal UIC levels were not influenced by the declared iodine supplementation and this finding is in agreement with other studies from the Czech Republic.

Conclusion

Thyroid hormones are essential for normal development of fetus and newborn. Sufficient iodine supplementation during the whole pregnancy prevents maternal and fetal complications. Lower UIC levels in our newborns of the mothers without regular iodine supplementation, even though the resulting data do not carry statistical significance, should help us emphasize the importance of regular iodine supplementation for all pregnant women as a prevention of possible developmental impairment in their children.

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EFFECT OF INCB018424, A JAK1 AND JAK2 INHIBITOR, ON SENESCENCE PHENOTYPE IN OLD MICE

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Introduction & Aims

Aging is a major risk factor for many chronic diseases, particularly atherosclerosis, cancer, diabetes mellitus, dementia, and more. These are then responsible for the majority of morbidity in developed countries.

Senescence, i.e. aging at the cellular level, was originally defined as a result of shortening of the telomeres at the end of chromosomes due to repeated cell division. Other factors independent on cell replication, e.g. oxidative stress, may also cause senescence. Senescence is associated with inflammation and with senescence-associated secretory phenotype (SASP), when senescent cells produce various cytokines in order to attract immune cells and in order to get removed[2,3].

The aim of our study was to determine if INCB018424, a selective inhibitor of Janus kinases JAK1 and JAK2[4], has an effect on senescent cell accumulation and/or SASP in adipose tissue of chronologically old mice.

Methods

26-month-old BL75/6 male mice were treated with INCB018424 or vehicle for two months. Mice were treated daily with a single dose (60 mg/kg/ body weight) of INCB018424 mixed with food. Epidydimal, inguinal, mesenterial, perirenal, and subscapular adipose tissues were collected after treatment. The fat tissues were fixed and stained for senescence-associated beta-galactosidase (SA-beta-gal), a marker of cellular senescence, and counterstained with DAPI, a nuclear stain. Images of histological samples were taken and the percentage of SA-beta-gal positive cells estimated using computer-aided image analysis. RNA was isolated from fat depots. SASP components and senescence markers were assayed using qRT-PCR.

Results

No difference was observed in SA-beta-gal positivity in epidydimal depots. In all other depots, there was a non-significant trend to lower SA-beta-gal positivity in INCB018424 treated animals when compared to controls. However, expression of following senescence markers and SASP components were downregulated in the fat tissue of treated mice compared to controls: p16, p21, IL6, Emr1, MMP3, MMP9, MMP12, CEBPα, ZFN423, Hmgb1. Expression of other genes (PAI, TNF-α, FABP4, PPAR-γ, MCP1) was not different.

Discussion & Conclusions

In previous study, health span was increased by removing senescent cells in progeroid mice[1]. INCB018424 ameliorated some symptoms of geriatric frailty in patients with myelofibrosis[4] and seems to be a promising agent in preventing tissue dysfunction associated with inflammation.

In the present study, we observed that INCB018424 did not change senescent cells abundance, but significantly reduced senescence-associated inflammmation in adipose tissue in chronologically old mice. Thus, INCB018424 might prevent age-associated adipose tissue dysfunction.

Based on results above, targeting the JAK1 and JAK2 with INCB018424 seems to be promising way of ameliorating SASP. More studies are necessary to determine if inhibiting the JAK1 and JAK2 kinases can promote health span.

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Radio-sensitization of human leukemic cells HL-60 by ATR-kinase inhibitor (VE-821): Phosphoproteomic analysis

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Introduction

One of the treatment modalities in oncology is radio-therapy. It often combines chemical agents increasing sensitivity towards ionising radiation (IR). IR induces the most deleterious lesions of DNA, double strand breaks (DSB), and their repair is regulated by ataxia telangiectasia-mutated kinase (ATM), DNA-dependent protein kinase (DNA-PK), and ATM and Rad3-related kinase (ATR), whose inhibition has been reported to increase radio-sensitivity (Tichy et al. 2010).

We have previously compared the effect of inhibitor of ATM (KU55933) and ATR kinases (VE-821) on the radio-sensitization of human promyelocyte leukaemia cells (HL-60), lacking functional protein p53. The inhibition of ATR by VE-821 resulted in a more pronounced radio-sensitizing effect in HL-60 cells compared to the inhibition of ATM. In contrast to KU55933, VE-821-treatment prevented HL-60 cells from undergoing G2 cell-cycle arrest (Vavrova et al. 2013).

Aims

Our goal was to describe the changes of phosphoproteome in radio-sensitized tumour cells, since in the DNA damage response (as well as in the other molecular processes) it is phosphorylation that frequently initiates and propagates signal transduction pathways (Johnson 2009). We aimed to characterise the effect of specific inhibition of ATR by VE-821 and to report the mechanisms and signalling pathways involved in the processes triggered by IR in a leukemic cell line that served as a model of p53-negative cells.

An inevitable part of a mass spectrometric analysis of phosphorylation is the enrichment of phosphorylated peptides from the mixture with their non-phosphorylated counterparts. It compensates their low abundance, insufficient ionization, and suppression effects of non-phosphorylated peptides (Tichy et al. 2011). Hence, we initially optimized the metal oxide affinity chromatography (MOAC) enrichment using titanium dioxide and applied it in our experiments (Salovska et al. 2013).

Methods

HL-60 cells were cultured either in SILAC light (Arg0/Lys0) or SILAC heavy (Arg6/Lys6) supplemented IMDM medium for at least 6 doublings. Thirty minutes prior irradiation by the dose of 6 Gy, VE-821 was added to the heavy labeled cells in concentration of 10 μM (control cells were treated with DMSO at the same time-point). The cells were harvested 1 hour after irradiation and the proteins were extracted. After digestion with trypsin, peptides were separated into 22 fractions using HILIC chromatography. Each fraction was enriched for phosphopeptides using TiO_2 chromatography. The MS analysis was performed

on nanoRPLC-ESI-MS/MS system using Q Exactive mass spectrometer (Thermo Fisher). Raw files were processed using MaxQuant software with Andromeda search engine. Gene ontology and Reactome pathways over-representation analysis was done using ConsensusPathDBs web tool. Sequence logos were created using IceLOGO. 1D enrichment of consensus kinase motifs was performed using Perseus and kinases were predicted using Networkin 2.0 database (supported by PhosphositeAnalyzer). Regulated subnetworks were extracted using Subextractor algorithm.

Results and Discussion

TiO_2 chromatography with HILIC prefractionation and nanoRPLC-ESI-MS/MS analysis revealed 6927 class I phosphorylation sites on 2430 proteins; among them 893 were differentially up- or down-regulated by phosphorylation (FDR 5%). Most of the regulated proteins were localized in the nuclear compartments. Nevertheless, a considerable fraction of regulated proteins was localized outside the nucleus, implying that ATR inhibition also affects various non-nuclear functions.

The proteins regulated by ATR inhibition were mostly involved in biological processes like metabolism of nucleic acids, cell-cycle phase transition, mitosis or DNA damage response. ATR inhibition resulted in the increase of activity of cyclin-dependent kinases (indicating abrogation of cell-cycle checkpoints through the ATR inhibition-mediated decrease of CHK1 activity) and decreased activity of ATM/ATR group, which confirmed biological relevance of our data.

In addition, the increased activity of PLK1 kinase - an essential component of G2/M transition processes - was also proved in our study. The key role of ATR in regulation of PLK1 after UV-irradiation has been described recently (Qin et al., 2013) However, it is now for the first time that ATR regulation of PLK1 after IR-induced DNA damage has been reported. Interestingly, the activity of NEK2 kinase (involved in centrosome separation) and CK2A1 kinase (involved in G2 arrest in response to spindle damage) was also shown to be decreased in our study. Since NEK2 is known to be PLK1-dependent (Zhang et al., 2005), its activity might be regulated by another yet unknown mechanism.

The network analysis using Subextractor algorithm revealed regulated phosphoproteins involved in multiple types of DNA repair (single/double strand break repair and base excision repair) emphasizing the importance of ATR kinase in these molecular mechanisms. Several E3 ubiquitin ligases are known to be phosphorylated upon DNA damage. Our data indicate that ATR kinase might be also involved in their phosphorylation.

Conclusion

Our phosphoproteomic study provided for the first time a complex insight into the mechanisms of inhibition of DNA repair enzyme, ATR kinase. We described phosphorylation processes triggered by radiation-induced DNA damage in radio-sensitized cancer cells and we proved that the novel potent and selective inhibitor VE-821 could be developed into a future drug for eradication of p53-negative tumour cells.

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α-TOMATINE DOES NOT INDUCE APOPTOSIS IN HUMAN BREAST ADENOCARCINOMA CELL LINE MCF-7

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Introduction

α-Tomatine (TOM) is a steroidal glycoalkaloid occuring mainly in tomatoes (*Solanum lycopersicon L.*). It is composed of the steroidal aglycone tomatidine and a tetetrasaccharide chain (containing xylose, galactose and two glucoses). This compound has antiviral, antibiotic and anti-inflammatory activity, affects immune response and inhibits acetylcholinesterase activity. Further, it forms insoluble complexes with cholesterol resulted in decrease in the plasma cholesterol levels and disruptions mammalian biomembranes containing cholesterol [Rev. in 1]. In the recent years, the mechanism of action of TOM on cancer cells has been studied. It is cytotoxic to different human and mouse cancer cells *in vitro*. The main reported cytotoxic effect of TOM is the induction of apoptosis or the caspase-independent cell death. TOM inhibits the migration and invasion of cancer cells by inactivating FAK/PI3K/Akt and Erk signalling pathways connected with a decrease in the binding activity of NF-κB [2-12]. The results of *in vivo* attempts showed, TOM inhibited tumour growth at doses of 1 mg/kg in tumor-bearing mice [2,10,11,13].

Aims

The aim of this study was to assess the anticancer effect of α-tomatine on human breast adenocarcinoma cell line MCF-7 after α-tomatine treatment focused on the induction of apoptosis *in vitro*.

Methods

Human breast adenocarcinoma cell line MCF-7 (ATCC) was maintained at standard conditions [12]. The comet assay was used to assess DNA damage [8]. The levels of proteins were determined by western blot assay [8]. Cell viability was assessed using WST-1 (Roche) reagent and absorbance was measured in Tecan SpectraFluor Plus spectrometer (Tecan Austria GmbH). Cell proliferation was monitored using the xCELLigence RTCA MP instrument (Roche). LDH was measured spectrophotometrically after sonication of the cancer cells. In order to detect caspases activity, Caspase-Glo® Assays (Promega Corporations) were used according to the manufacturer's instructions. Flow cytometric assay was carried out using flow cytometer Dako CyAn (Beckman Coulter). Electron transmission microscopy was carried out on a Tesla BS-500 transmission electron microscope (Tesla). ATP content was measured by an ATP bioluminescent assay kit (Sigma-Aldrich).

Results

TOM at concentrations of 6 and 9 µM caused the significant decrease in viability and proliferation of MCF-7 cells after 24, 48 and 72 hours of exposure ($p \leq 0.05$), but after 48 and 72 h of incubation cell proliferation and viability began to increase. Using LC-MS, we found that this increase in the cell growth is not due to biotransformation of TOM by MCF-7 cells, but due to the binding of TOM with cholesterol present in incubation media. The EC_{50} value after 72 hours of TOM treatment was 7.17 µM. When the LDH release was measured, the results were similar (6 µM TOM was cytotoxic after 4-72 h, 9 µM TOM after 2-72 h) ($p \leq 0.05$) which suggests membrane destabilization of MCF-7 cells. These findings correspond with the amount of check-point kinase 1 that increases with increasing concentrations (1, 3 and 6 µM) of TOM after 4 hours of exposure. Then we tried to find out whether TOM causes DNA damage in treated cells. TOM at concentrations of 1 – 9 µM did not cause single- nor double-strand breaks of DNA in MCF-7 cells after 4 hours of exposure ($p \leq 0.001$) detectable by comet assay and these results were supported by determining check-point kinase 2 non-activation after 4 h of TOM treatment (1, 3 and 6 µM). Although no DNA damage was proved, we tried to find whether apoptosis is induced by another triggering mechanism. TOM at concentrations of 1, 3 and 6 µM caused no changes in the levels of proteins p53, p53 phosphorylated on serine 15 and $p21^{WAF1/CIP1}$ after 4 and 24 h of incubation in MCF-7 cells. Moreover, no increase in the activity of caspase-8 and -9 and no specific apoptotic sub-G1 peak after TOM treatment (1, 3 and 6 µM after 24 and 72 h) in MCF-7 cells were observed ($p \leq 0.001$). To confirm our theory that TOM does not induce apoptosis in MCF-7 cells, we measured the loss of ATP in TOM-treated MCF-7 cells (3, 6 and 9 µM). The concentration of 9 µM of TOM significant decreased the content of ATP in MCF-7 cells in all treatment intervals between 4 and 72 h and 6 µM of TOM decreased the content of ATP in all treatment intervals between 6 and 72 h ($p \leq 0.05$). Finally, the transmission electron microscopy of MCF-7 cells treated with 6 µM TOM was performed. The treated cells showed no typical morphologic signs of apoptosis, like cell shrinkage, nuclear fragmentation, chromatin condensation, membranes blebbing or formation of apoptotic bodies, but rather unspecific changes such as membrane disintegration.

Discussion

In recent years, TOM has shown the cytotoxic effect to different cancer cell lines at low concentrations (approximately 2 µM) within 48 h of incubation [2-11]. We proved the cytotoxic effect of TOM at slightly higher concentrations than in previous works. The concentrations of TOM 6 and 9 µM caused a decrease in proliferation and viability of MCF-7 cells after 24-72 h of incubation, but after 24 h of incubation, the cells recovery occurred. We demonstrated that this is a result of the binding of TOM with cholesterol. Although this interaction has been known for a long time [Rev. in 1], it was not taken into consideration in the previous works. The inhibition of proliferation and viability corresponded with the increase and activation of check-point kinase 1 that is essential for cell viability and proliferation and maintaining DNA integrity [14]. Further we focused on studies on the mechanism of action of TOM on MCF-7 cells because of the existing results differ significantly. In MCF-7 cells, TOM induced no DNA damage after 4 h of treatment and no changes in the levels of proteins p53, p53 phosphorylated on serine 15 and $p21^{WAF1/CIP1}$. Contrary to previous works [6,7], no increase of apoptotic cells in sub-G1 phase was observed in MCF-7 cells. In PC-3 cells, caspase-3, -8 and -9 were activated after TOM treatment [6]. In leukemic cell lines, however, the activity of caspase-3, -6, -7, -8 and -9 was unchanged [2,8]. In MCF-7 cells, the activity of caspase-8 and -9 was also unchanged. The induction of

apoptosis is process highly dependent on ATP levels [15]. In MCF-7 cell, we demonstrated the high decrease in ATP content occurred after TOM treatment and cells showed no morphological signs of apoptosis in transmission electron microscopy.

Conclusion/Summary

Our results show antiproliferative effect of TOM on human breast adenocarcinoma cell line MCF-7 after 72 h of incubation. TOM does neither induce DNA damage, activation of caspases nor changes in the levels of proteins p53 and p21 in MCF-7 cells, but it causes the decrease in cellular ATP. These findings together with the morphological changes observed in MCF-7 cells suggest that the decrease in cell viability is not due to apoptosis induction.

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