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EFFECTS OF DIBUTYLTIN EXPOSURES ON TRANSLATION REGULATORY FACTOR S6 IN HUMAN IMMUNE CELLS

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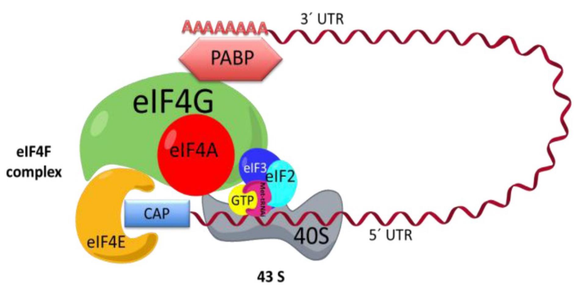
Abstract

Dibutyltin is an organotin contaminating the environment through its use as a stabilizer in polyvinylchloride, PVC plastics. DBT has been found in drinking water and beverages, such as beer and wine, due to leaching from the PVC plastics used during the distribution of these drinks. Along with PVC plastics, DBT has been used as a deworming agent in poultry, infiltrating additional food products and increasing the exposure of the toxin to humans. Due to its multiple uses, it has entered the food chain and has been detected in human blood at levels as high as 0.3µM. Inflammatory cytokines are important mediators of the response to injury or infection. However, if their levels are increased in the absence of a needed immune response, chronic inflammation can occur. Chronic inflammation is associated with a number of pathologies including, rheumatoid arthritis, Crohn's disease, atherosclerosis, and cancer. DBT can increase the synthesis of pro-inflammatory cytokines such as interferon gamma (IFNγ), tumor necrosis factor alpha (TNFα), interleukin 1 beta (IL-1β), and interleukin 6 (IL-6) in human immune cells. DBT appears to use the ERK 1/2 and/or p38 MAPK pathways to stimulate pro-inflammatory cytokine production by immune cells. MAPK pathways have the capacity to regulate translation including processes leading to the phosphorylation (activation) of the S6 ribosomal subunit. The current study examines the levels and phosphorylation state of S6 after 1-hour and 6-hour exposures to DBT in peripheral blood mononuclear cells (PBMCs). The results indicated that, within 1 hour of exposure, DBT (at several concentrations) elevated levels of phospho (P)-S6 and S6. At 6 hours of exposure, DBT caused increased levels of S6, along with significant increases at higher concentrations for P-S6. These results suggest that DBT may be elevating the synthesis of key pro-inflammatory cytokines in immune cells by its ability to activate translation.

Introduction

- DBT can alter the secretion as well as cellular production of both IL-1β and IL-6 in human immune cells.
- Increased levels of the mRNA for IL-1β and IL-6 suggest that certain concentrations of DBT increase transcription of the genes for these critical cytokines in immune cells.
- DBT uses the p38 ERK 1/2 MAPK pathway to alter production of IL-1β and IL-6 in human immune cells.
- The ERK1/2 pathway has been noted to catalyze the phosphorylation (activation) of eukaryotic initiation factor 4E (eIF4E), which is a key player of binding the 5' cap of mRNAs and stimulating their translation.
- The phosphorylation of eukaryotic initiation factor 4B (eIF4B) on the Ser 406 and 422 sites leads to the activation of eIF4A and thus the translational process.
- Ribosomal protein S6 is a subunit of 40S ribosomal unit necessary for translation.
- This study investigates whether DBT is able to activate the translational regulatory protein S6



Initiation Complex

Objective

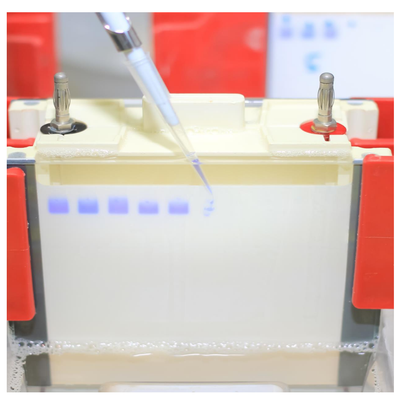
- Examine the activation state and protein levels of P-S6 and S6 after 1 hour and 6 hours of exposure to 5-0.05 µM DBT

Experimental Method

- PBMCs were isolated from Leukocyte filters (PALL-RCPL or RC2D) obtained from Red Cross Blood Bank Facility (Nashville, TN).
- PBMCs (concentration of 4.5-6 million cells/mL) were treated with 5-0.05 µM DBT or control for 6 h or 24h.
- Cell lysates were run on 10% SDS-PAGE and transferred to a PVDF membrane. The PVDF was immunoblotted with specific primary antibodies.
- Antibodies were visualized using a ECL chemiluminescent detection system and UVP Imaging Software. The density of each protein band was determined by densitometric analysis using the UVP analysis software.
- β-actin levels were determined for each condition to verify that equal amounts of protein were loaded. The density of each protein band was normalized to β-actin to correct for slight differences in protein loading among the lanes.

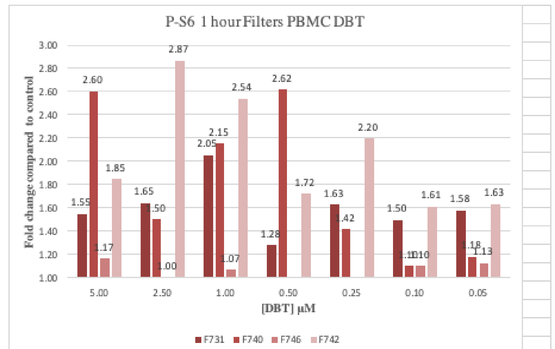
Dibutyltin dichloride

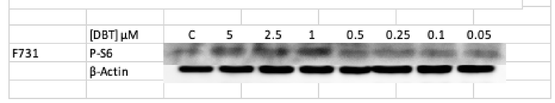
Blood Sample after Centrifugation

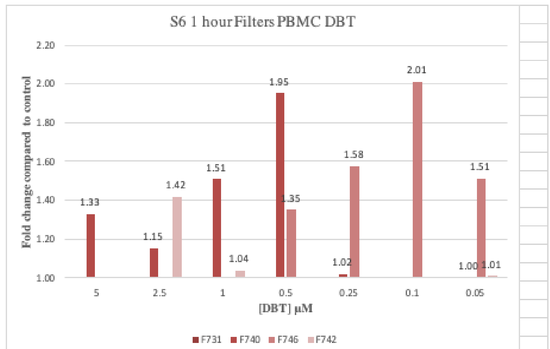


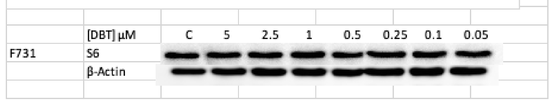
SDS-PAGE Protein Loading

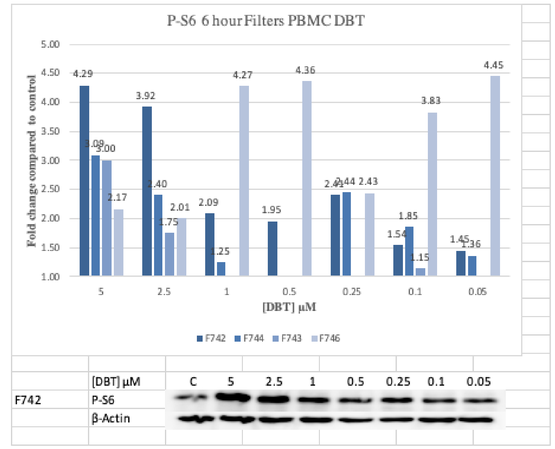
Results

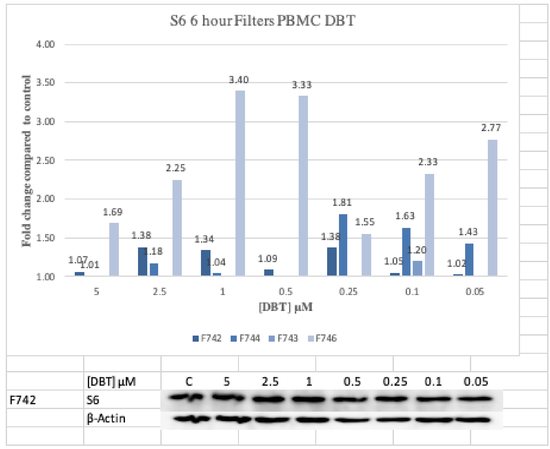












Conclusions

- At 1 hour of exposure to DBT, P-S6 displayed an increase in protein levels at each concentration
- At 1 hour of exposure to DBT, S6 showed an increase at most concentrations,
- At 6 hours of exposure to DBT, P-S6 illustrates a drastic increase for higher concentrations of DBT, especially at 5 and 2.5 µM
- For total S6 at 6 hours of exposure to DBT, there was a slight increase of the protein levels at each concentration
- Generally, DBT increased the protein levels of P-S6 and S6 at most concentrations after 1 h and 6 h exposure
- These data indicate that DBT exposure stimulates activation of a key translational protein
- DBT-induced stimulation of translation may account for the increases in IL-1β and IL-6 seen in DBT exposed immune cells

References

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