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Effects of butyltin exposures on MAP kinase dependent transcription regulators in human natural killer cells

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Abstract

Natural Killer (NK) cells are a major immune defense mechanism against cancer development and viral infection. The butyltins (BTs), tributyltin (TBT) and dibutyltin (DBT) have been widely used in industrial and other applications and significantly contaminate the environment. Both TBT and DBT have been detected in human blood. These compounds inhibit the lytic and binding function of human NK cells and thus could increase the incidence of cancer and viral infections. Butyltin (BT)-induced loss of NK function is accompanied by activation of mitogen activated protein kinases (MAPKs) and decreases in expression of cell-surface and cytolytic proteins. MAPKs activate components of the transcription regulator AP-1 and activate the transcription regulator Elk-1. Based on the fact that BTs activate MAPKs and alter protein expression, the current study examined the effect of BT exposures on the levels and phosphorylation states of the components of AP-1 and the phosphorylation state of Elk-1. Exposure to 300 nM TBT for 10 min increased the phosphorylation of c-Jun in NK cells. 1 h exposures to 300 nM and 200 nM TBT increased the phosphorylation and overall level of c-Jun. During a 300 nM treatment with TBT for 1 h the binding activity of AP-1 was significantly decreased. There were no significant alterations of AP-1 components or of Elk-1 with DBT exposures. Thus, it appears that TBT-induced alterations on phosphorylation, total levels and binding activity of c-Jun might contribute to, but are not fully responsible for, TBT-induced alterations of NK protein expression.

Keywords

Tributyltin; Dibutyltin; Fos; Jun; Elk-1

INTRODUCTION

Human Natural Killer (NK) cells function as part of the body's innate defense mechanism in destroying altered cells (Vivier et al., 2004). These large granular lymphocytes target virally infected cells, tumor cells and/or antibody coated cells (Lotzova, 1993; O'Shea and Ortaldo, 1992; Trinchieri, 1989). Their capacity to eliminate virally or oncogenically transformed cells, without need for sensitization to an antigen, makes them the dominant immune defense against tumor formation and the primary defense against viral infection (Lotzova, 1993; Vivier et al., 2004). The importance of their role in preventing tumor development has been shown by the fact that their absence leads to both development and metastasis of tumors (Kiessling and Haller, 1978; Hanna, 1980). The importance of NK cells in preventing viral infections has been

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noted by the fact that their absence leads to greatly increased incidences of viral infections in humans (Fleisher et al, 1982; Biron et al, 1989). It has been reported that a significantly reduced proportion of NK cell activating receptors were found on NK cells of individuals with persistent viral infection of hepatitis C (Nattermann et al., 2006).

Tributyltin (TBT) has been used as a fungicide, wood preservative, and as an antifoulant (Kimbrough, 1976; Roper, 1992; Yamada et al., 1993). Additional uses of this compound are, but not limited to, disinfection of circulating industrial cooling waters and slime control in paper mills (Roper, 1992; Yamada et al., 1998). Measurable levels of TBT have been found in household items like shower curtain liners and siliconized-paper and in food such as fish (Kannan et al., 1995a,b,c; Kannan and Falandyz, 1997;Takahashi et al., 1999; Yamada et al., 1993). Due to its contamination of the environment, TBT is also found in human blood at levels as high as 260 nM (Kannan et al., 1999; Whalen et al., 1999). TBT has been shown to decrease the cytotoxic function of human NK Cells (Whalen et al., 1999; Whalen et al., 2002a; Dudimah et al., 2007a). This suppression of NK function could lead to a higher incidence of tumors and viral infections. Dibutyltin (DBT) is used as a stabilizer in PVC pipes and plastic products such as plastic food wrap, food storage bags, and food containers (Roper,1992; Nakashima et al., 1990). Drinking water has been shown to contain butyltins (BTs) resulting from leaching inside PVC pipes (Sidiki et al., 1996). PVC pipes that stored beer, wine and fruit juices have also been reported to contain traces of BTs (Forsyth et al., 1992a,b). It has also been shown by us and others, that there are quantifiable levels of DBT in human blood (Kannan et al., 1999; Whalen et al., 1999). DBT like TBT has been shown to suppress the lytic function of human NK Cells (Whalen et al., 1999; Whalen et al., 2002a; Dudimah et al., 2007b).

In previous studies examining the effects of BTs on NK cells we have found that BT exposures decrease the lytic function (Whalen et al., 1999; Whalen et al., 2002a; Dudimah et al., 2007a,b), binding function (Whalen et al. , 2002b; Odman-Ghazi, 2003), lytic protein (granzyme and perforin) levels (Thomas et al., 2004; Catlin et al., 2005), and expression of certain cell surface proteins (Whalen et al. , 2002b; Odman-Ghazi, 2003) in NK cells. We have also shown that exposure to BTs caused activation of mitogen activated protein kinases (MAPKs) in NK cells within 10 minutes of exposure (Aluoch and Whalen, 2005; Aluoch et al, 2006; Aluoch et al., 2007: Odman-Ghazi et al., 2010). The MAPK, p44/42 has a role in the target-cell-stimulated release of the lytic proteins, perforin and granzyme (Trotta et al, 1998; Trotta et al., 2000) and is strongly activated by BTs (Aluoch and Whalen, 2005; Aluoch et al, 2006; Aluoch et al., 2007). MAPKs can also alter the functional status of the transcription regulators AP-1 and Elk-1 (Roux and Blenis, 2004). AP-1 is a transcription activator that is a dimer composed of Fos-Jun heterodimers or Jun-Jun homodimers (Neuberg et al., 1989). p44/42 can lead to activation of the Fos and Jun components of AP-1 while JNK activates the Jun component of AP-1 (Roux and Blenis, 2004). The promoters for granzyme B and perforin have been shown to be regulated by AP-1 (Hanson et al., 1993; Zhou and Meadows, 2003).

Our previous studies have shown BT-induced activation of MAPKs as well as BT-induced alteration of protein expression. The current study examined BT-induced effects on the components of AP-1 and the activation state of Elk-1. The phosphorylation state of Jun as well as the total levels of Jun and Fos were evaluated in BT-exposed and control NK cells. Additionally, the ability of AP-1 to bind to its DNA element was studied to determine if there was any alteration of the functional status of AP-1 in BT-exposed cells. BT-induced MAPK activation leading to alterations in levels and or phosphorylation state of AP-1 components could be at least in part responsible for the changes in protein expression that are seen with BT exposures

MATERIALS AND METHODS

Isolation of NK cells

Highly purified NK cells were prepared from peripheral blood (buffy coats) samples from healthy adult volunteer donors. Buffy coats were obtained from the American Red Cross (Portland,OR) and Key Biologic LLC (Memphis, TN). NK cells were purified using a rossetting procedure. Buffy coats were mixed with 0.6–0.8 mL of RosetteSep™ human NK cell enrichment antibody cocktail (Stem Cell Technologies, Vancover, British Colombia, Canada) per 45 mL of buffy coat. The mixture was incubated for 25 min at room temperature. Following the incubation, 6–8 mL of the mixture was layered onto 4 mL Ficoll-Hypaque (1.077 g/mL; Sigma, St. Louis, ML, USA) and centrifuged at 1200g for 30 min. The cell layer was collected and washed twice with PBS and stored in complete media (RPMI-1640 supplemented with 10% heat-inactivated BCS, 2mM L-glutamine and 50 U penicillin G with 50 μg streptomycin/ml) at 1×10^6 cells/mL. The final cell preparation was confirmed as a >95% $CD16^+$, 0% $CD3^+$ phenotype by fluorescence microscopy and flow cytometry (Whalen et al., 2002a).

Chemical Preparation

Tributyltin and dibutyltin were purchased from Aldrich Chemical Co. (Milwaukee, WI). Dimethylsulfoxide (DMSO) was obtained from Sigma Chemical Co. TBT was a liquid, dispersed initially in deionized water to give a 1mM solution. The concentration of TBT used in treating the cells ranged from 300 nM to 25 nM based on previous studies (Whalen et al., 1999; Whalen et al., 2002a; Dudimah et al., 2007a,b). The concentration of TBT used in the experiments is not far greater than the highest concentration that was detected in human blood (as high as 260 nM) (Kannan et al., 1999; Whalen et al., 1999). These blood concentrations appeared to be due to normal daily exposure due to foodstuffs and consumer products as the donors did not have occupational exposure (in those donors where occupation was known). DBT was a solid dissolved in DMSO to give a 658 mM solution The concentration of DBT used in treating the cells ranged from 10 μM to 500 nM based on previous studies (Kannan et al., 1999; Whalen et al., 1999).

Cell Treatment and Cell Lysate Preparation

For TBT exposures: NK cells were then exposed to (1) no TBT (control) for 10 min, 1 hour or 6 hours; (2) 300nM TBT for 10 min, 1 hour or 6 hours; (3) 200nM TBT for 10 min, 1 hour or 6 hours; (4) 100nM TBT for 10 min, 1 hour or 6 hours (5) 50nM TBT for 10 min, 1 hour or 6 hours; (6) 25nM TBT for 10 min, 1 hour or 6 hours. For DBT exposures were as described above (1–6) except that the concentration range was 10 μM to 0.5 μM. Following the above treatments, the cells were centrifuged and the cell pellets were lysed using 500 μL of lysis buffer (Active motif, Carlsbad, CA) per 10 million cells. The cell lysates were stored frozen at −80°C prior to being run on sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). Control cells were treated with cell culture media with an appropriate level of water or DMSO. Control and BT-exposed cells for a given experimental setup (described above) were from an individual donor.

Cell Viability

Cell viability was determined by trypan blue exclusion. Cell number and viability, assessed at the beginning and end of each exposure period, did not vary significantly among experimental conditions for any of the concentrations.

ELISA Transcription Factor Assay

Transcription factor binding assays were used to measure the function of AP-1 and Elk-1 (TransAm Transcription Factor Assay Kits, Active Motif, Carlsbad, CA). NK cell lysates exposed to BTs (as described above) were used for these studies. The assay measures the ability of AP-1 or Elk-1 in cell lysates to bind their consensus sequences. This was monitored by using a 96-well microtiter plate, where each well is coated with an oligonucleotide of the AP-1 (or Elk-1) consensus sequence. The positive control well for transcription factor binding contained a K-562 nuclear extract diluted in complete lysis buffer. Samples were then loaded onto the appropriately coated plate in a total volume of 50 μL of complete binding buffer (Active Motif). The plate was then sealed and allowed to incubate for 1 hour at room temperature with gentle rocking. Following the incubation period, the wells were then rinsed three times with 200 μL of wash buffer (Active Motif). In order to detect AP-1 binding , phospho-c-Jun or Fos primary antibodies were applied in a volume of 100 μL (Elk-1 antibody was used for the Elk-1 assay kit). The plate was covered, incubated at room temperature for 1 hour. Following the incubation the plate was washed three times as described above. A horse-radish-peroxidase-conjugated secondary antibody was applied in 100 μL of binding buffer and incubated for 1 hour. Following this incubation the plate was washed four times with wash buffer and 100 μL of developing solution was added (Active Motif) The absorbance was read at 450 nm.

Western Blot

Cell lysates were run on 10% SDS-PAGE and transferred to PVDF (polyvinylidene difluoride) membrane. The PVDF was immunoblotted with β-actin (Sigma Aldrich), phospho-c-Jun (S63), c-Jun, Fos and phospho-Elk-1(S383) antibodies. Phospho-c-Jun, c-Jun, Fos and phospho-Elk-1 were all acquired from Cell Signaling Technologies, Inc. (Beverly, MA) A conjugated horseradish peroxidase secondary antibody (GE Healthcare, Piscataway, NJ) was then applied in preparation for visualization. Antibody binding was detected using an ECL chemiluminescent detection system (GE Healthcare, Piscataway, NJ) and the blot was imaged on a Kodak Imaging System (Kodak, Rochester, NY). The density of each protein band was determined by densitometric analysis using the Kodak Image Station analysis software. The settings on the image station were optimized to detect the largest possible signal range and to prevent saturation of the system. A given experimental set-up (as described in the cell lysate section) always had its own internal control. Thus, differences and changes in protein expression were determined relative to the internal control. The internal control was lysate from cells from the same donor that were treated with cell culture media containing no compound (see cell treatment and cell lysates preparation section). This determination provided relative quantitation by evaluating whether a given treatment changed expression of phospho-c-Jun, c-Jun, Fos and phospho-Elk-1 relative to untreated cells. β-actin levels were determined for each condition to verify that equal amounts of protein were loaded. In addition, the density of each protein band was normalized to β-actin to correct for small differences in protein loading among the lanes. This method allowed quantification of protein per band followed by fold increases above control calculations.

Statistical analysis

Statistical analysis for the data was carried out using Student's *t*-test (ELISA assay). Analysis of variance (ANOVA) followed by pair-wise comparison of data was carried out for Western blots analyses. A minimum of three separate determinations were carried out for each experimental set up ($n \geq 3$) and statistical significance was noted at $p < 0.05$.

RESULTS

Effect of exposures to 300-25 nM TBT on phospho-c-Jun, c-Jun, Fos and phospho-Elk-1

A 10 minute exposure to 300 nM TBT caused a 52% increase ($p<0.01$) in the phosphorylated form of c-Jun (Fig.1A), but no significant change in total Jun (Fig. 1B) or Fos (Fig. 1C) ($P>0.05$). There were also no significant changes seen in the phosphorylation state nor the overall level of Elk-1 as indicated in (Fig. 1D) ($P>0.05$). There were no significant changes in phosphorylation state or levels of proteins at any of the other concentrations tested in the 10 min exposures. Figure 1E show the results from a representative experiment.

When pure NK cells were exposed to 300-25 nM TBT for 1 h, there was a 1.52-fold increase in the levels of phospho-c-Jun at the 300 nM concentration ($p<0.03$). Additionally, 200 nM TBT, caused a 1.77 fold increase in phosphorylation of c-Jun ($p<0.02$). These changes are reflected in Fig. 2A. There were also statistically significant changes in the overall levels of c-Jun (Fig 2B). 300 nM caused an increase in the level of c-Jun of 56% ($p<0.04$). When NK cells were exposed to 200 nM for 1 h, an increase in the level of c-Jun of 98% was seen ($p<0.04$). Exposures to 100 and 25 nM TBT also produced significant changes in the level of Fos as shown in Fig. 2C. A 1.48 fold increase above the control was noted for exposure to 100 nM TBT and a 1.47 fold change resulted from a 25 nM TBT exposure to this transcription factor. There were no changes observed in phospho-Elk-1(Fig. 2D), Figure 2E shows the results of a representative experiment at the 1 h exposure.

NK cells exposed to 300-25 nM TBT for 6 h exhibited no significant changes in any of the AP-1 components or in Elk-1 phosphorylation. Figure 3 shows the results of a representative experiment at the 6 h exposure.

Effect of exposure to 10-0.5 μM DBT on phospho-c-Jun, c-Jun, Fos and phospho-Elk-1

After a 10 minute exposure to 10, 5, 2.5, 1, and 0.5 μM DBT, there were no statistically significant changes in the activation states or total levels of the AP-1 components or Elk-1 ($p>0.05$). Figure 4A–D show the combined results of 10 minute exposures to DBT on c-Jun phosphorylation, total c-Jun, Fos, and phosho-Elk-1 from at least 3 separate experiments. Figure 4E shows the results from a representative experiment at the 10 min exposure.

NK cells exposed to 10-0.5 μM DBT for 1 h showed no significant changes in the status of the AP-1 components or Elk-1 ($p>0.05$, data not shown). The results were similar to those seen after a 10 minute exposure.

Following 6 h exposures to 10-0.5 μM DBT, there were also no significant alterations of phospho-c-Jun, c-Jun, Fos, or phospho-Elk-1 in NK cells. As with the 1 h exposure, the results were similar to those seen at 10 min (data not shown).

Effect of TBT exposures on the ability of AP-1 to bind to its DNA element

Based on the changes seen in c-Jun levels and phosphorylation state as well as the changes seen in Fos levels with 10 min and 1 h exposures to TBT, NK cells were exposed to 300, 200, 100, 50, and25 nM TBT for 10 min and 1 h and the ability of AP-1 from these cell to bind to its DNA element was tested using the TransAM transcription factor assay kit. Following a 1 h exposure to 300 nM TBT there was significant decrease (18%) in the binding activity of AP-1 ($p<0.01$) compared to the control (Figure 5). There were no significant changes seen by a 10 min exposure to TBT.

DISCUSSION

It has been shown that exposure to BTs can decrease the ability of human NK cells to destroy their target cells (Whalen et al., 1999; Whalen et al., 2002a; Dudimah et al., 2007a,b). Both TBT and DBT significantly inhibit the lytic function of NK cells with accompanying loss of cell surface and cytolytic protein expression (Thomas et al., 2004; Catlin et al., 2005; Whalen et al. , 2002b; Odman-Ghazi, 2003). The predominant decrease in cell surface proteins were in CD16 and CD56 which are both important in the binding of NK cells to their targets (Mandelboim et al., 1999; Nitta et al., 1989). Decreases in cytolytic proteins and their mRNAs were also seen with both TBT and DBT (Thomas et al., 2004; Catlin et al., 2005). BT exposures have also been shown to cause a rapid activation of several MAPKs, most especially, p44/42 (Aluoch and Whalen, 2005; Aluoch et al, 2006; Aluoch et al., 2007; Odman-Ghazi, et al., 20101). MAPK activation leads to changes in the activation state of the transcription regulators AP-1 and Elk-1 (Roux and Blenis, 2004). Thus, exposures of NK cells to BTs may alter the activation states as well as the level of these immediate early genes which could then alter transcription of genes regulated by AP-1 or Elk-1. The promoters for the cytolytic proteins, granzyme B and perforin, appear to be regulated in part by AP-1 (Zhou and Meadows, 2003; Hanson et al., 1993). It is also possible that there may be AP-1 or Elk-1 regulation of several of the cell surface markers whose levels are changed upon BT exposures. As mentioned above, decreases in granzyme and perforin levels and the levels of several key cell-surface proteins accompany the loss of lytic function in NK cells exposed to BTs. Thus, it is important to examine the effects of BT exposures on transcription regulators such as AP-1 and Elk-1, whose function can be affected by activation of MAPKs, and that have the potential to regulate proteins involved in NK lytic function.

We addressed the effects of BT exposure on the transcription factors c-Jun, Fos and Elk-1. We also investigated the effects of BT exposure on the binding activity of AP-1. We saw changes in the c-Jun and Fos activation states and levels in response to TBT exposures, which led to the examination of the ability of AP-1 to bind to the DNA element sequence. A 10 min exposure of NK cells to 300 nM TBT resulted in a significant increase in the phosphorylated form of c-Jun. However, this exposure did not alter the total levels of the transcription proteins c-Jun or Fos. When NK cells were exposed to 300 nM and 200 nM TBT for 1 h, there were increases in phospho-c-Jun as well as in total c-Jun. It is important to note that the concentrations where these changes in c-Jun were seen have been found in a human blood sample (Kannan et al., 1999). Thus, alterations of AP-1 components leading to functionally relevant decreases in protein expression appear to be another consequence of TBT exposures in NK cells. There were no statistically significant alterations in the functional status of Elk-1 in TBT-exposed NK cells, as monitored by phosphorylation state. A 1 h exposure to 300 nM TBT led to a decrease in the ability of AP-1 to bind to it DNA element sequence. Thus, TBT which causes very strong activation of the MAPK p44/42 at 300 and 200 nM within 1 h of exposure ((Aluoch and Whalen, 2005; Aluoch et al, 2006; Aluoch et al., 2007) was also able to cause alterations in the phosphorylation state and levels of c-Jun under these same conditions. Interestingly, the only significant change in AP-1 functioning seen with TBT exposures was a decrease in AP-1 binding function. While this may seem contradictory to the fact that there were increases in the levels and phosphorylation of c-Jun under these conditions, it is consistent with the fact that with greater levels of c-Jun the likelihood of forming Jun-Jun homodimers would be increased. It is known that Jun-Jun homodimers bind to the AP-1 DNA element less well (about 30 fold lower affinity) (Neuberg et al., 1989). Thus, the decreased binding of AP-1 seen after a 1 h exposure to TBT is consistent with fewer Jun-Fos homodimers causing decreased AP-1 binding to its element. As mentioned above NK cytolytic protein levels appear to be at least in part regulated by AP-1 (Zhou and Meadows, 2003; Hanson et al., 1993) and thus, the decrease in their expression with TBT exposures (Thomas et al., 2004) is consistent with the decreased AP-1 function noted in this study.

DBT did not cause any significant alterations in AP-1 components or in Elk-1. This may be in part due to the fact that the activation of MAPKs especially p44/42, by DBT is less robust than that seen with TBT (Odman Ghazi et al., submitted).

The data presented in this study support the hypothesis that BTs, specifically TBT, induced changes in the activation state/levels and binding activity of the transcription regulator AP-1. The results of this study provide important details regarding the regulatory effects BTs have on transcription. These results indicate that (1) following a brief 10 min treatment with TBT, the phosphorylation state of c-Jun is altered (2) alterations are seen in the phosphorylation state of c-Jun and the total levels of c-Jun at 10 min and 1 h exposures with TBT (3) the reduction in AP-1 binding to its DNA element may be a result of the TBT-induced effects on c-Jun activation and total levels. (3) DBT exposures did not cause changes in the phosphorylation state of the transcription factors or their total levels in NK cells. (4) The effects of TBT on AP-1 were no longer seen after 6 hours.

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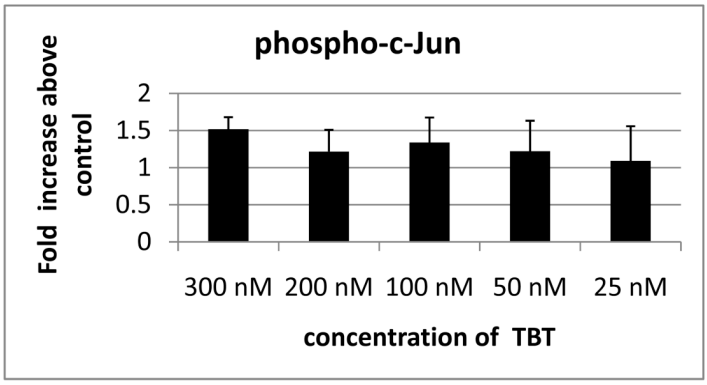

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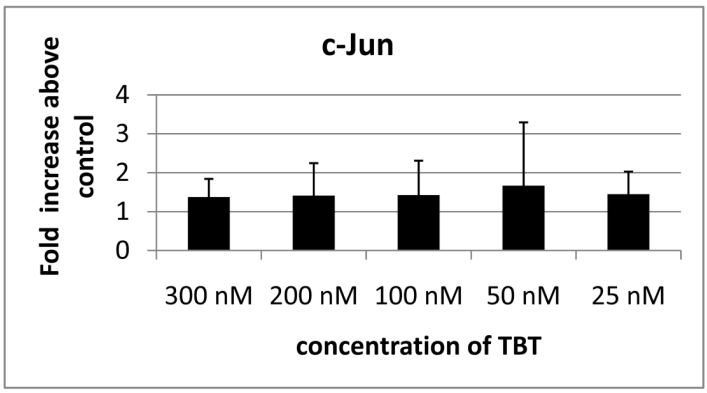

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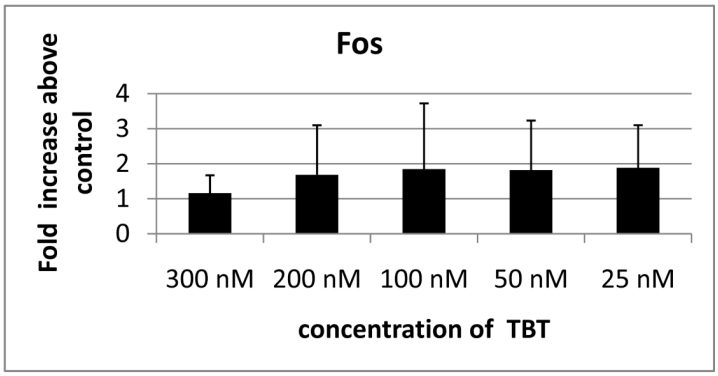
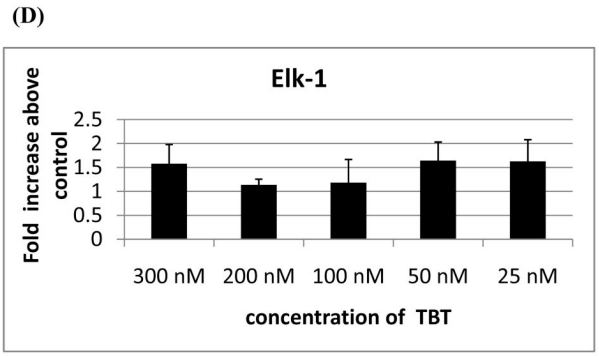




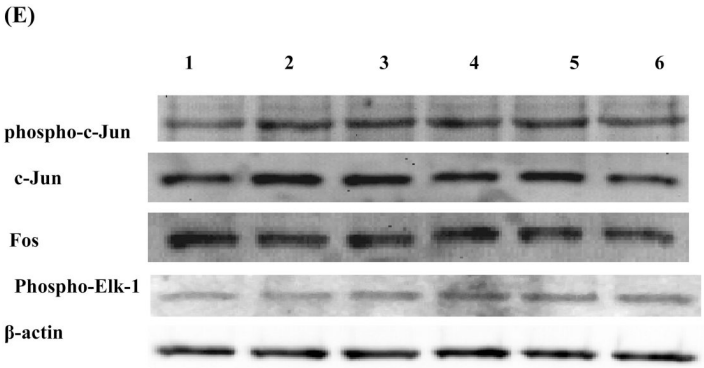
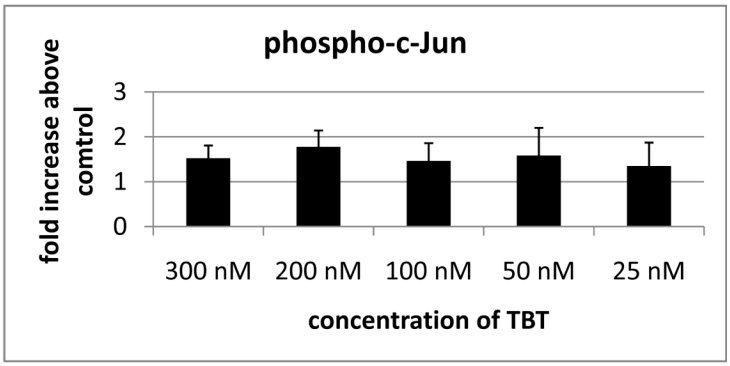


Figure 1.

Effect of 10 min exposures to 300-25 nM TBT on levels of phospho-c-Jun, c-Jun, Fos, and phospho-Elk-1 in pure NK cells: (A) phospho-c-Jun; (B) c-Jun; (C) Fos; (D) phospho-Elk-1 normalized to control. The band densities for both the control and TBT-treated cells were first divided by β-actin density to correct minor loading differences. The treatment was then normalized to control. The same procedure was followed for all the experiments. Values are mean ± S.D. from at least three separate experiments using different donors. Statistically significant differences ($p<0.05$) as compared to the control are indicated by an asterisk. (E) Representative experiments for each of the proteins. (1) Control Cells, (2) NK cells exposed to 300 nM TBT for 10 min, (3) NK cells exposed to 200 nM TBT for 10 min, (4) NK cells exposed to 100 nM TBT for 10 min, (5) NK cells exposed to 50 nM TBT for 10 min, (6) NK cells exposed to 25 nM TBT for 10 min.

(A)



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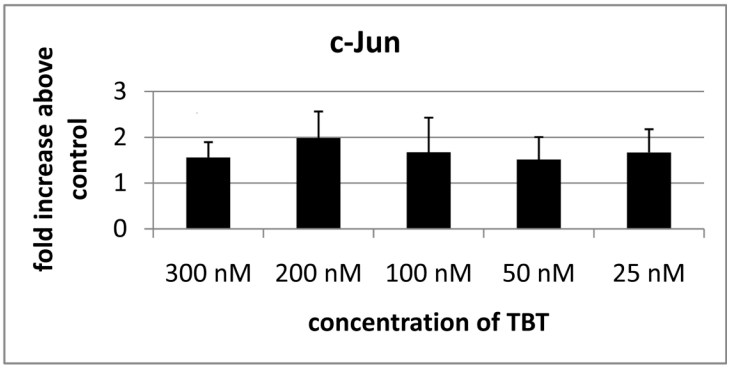

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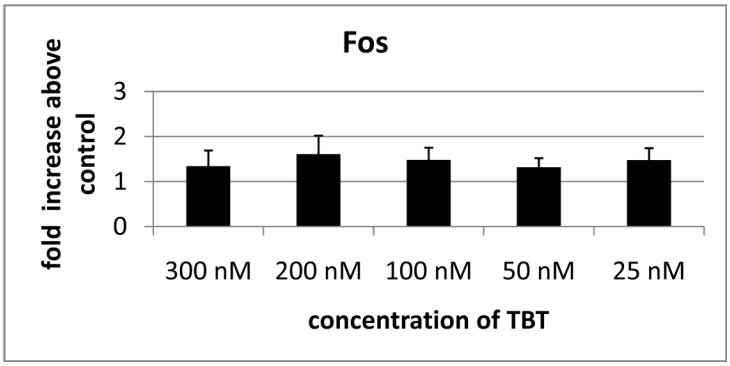

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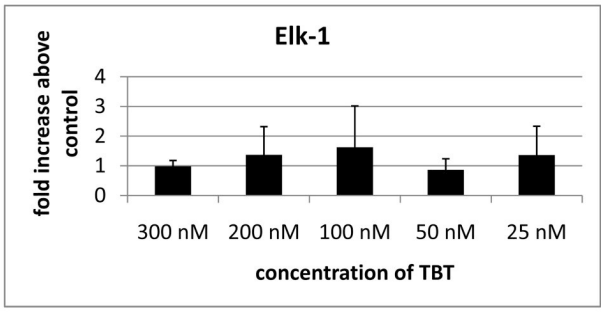

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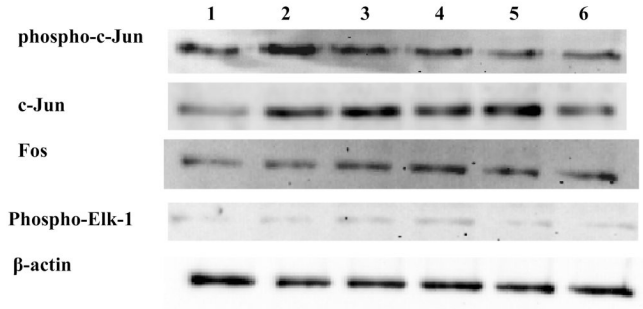


Figure 2.

Effect of 1 h exposures to 300-25 nM TBT on levels of phospho-c-Jun, c-Jun, Fos, and phospho-Elk-1 in pure NK cells: (A) phospho-c-Jun; (B) c-Jun; (C) Fos; (D) phospho-Elk-1 normalized to control as described in Figure 1. Values are mean ± S.D. from at least three separate experiments using different donors. Statistically significant differences ($p<0.05$) as compared to the control are indicated by an asterisk. (E) Representative experiments for each of the proteins. (1) Control Cells, (2) NK cells exposed to 300 nM TBT for 1 h, (3) NK cells exposed to 200 nM TBT for 1 h, (4) NK cells exposed to 100 nM TBT for 1 h, (5) NK cells exposed to 50 nM TBT for 1 h, (6) NK cells exposed to 25 nM TBT for 1 h.

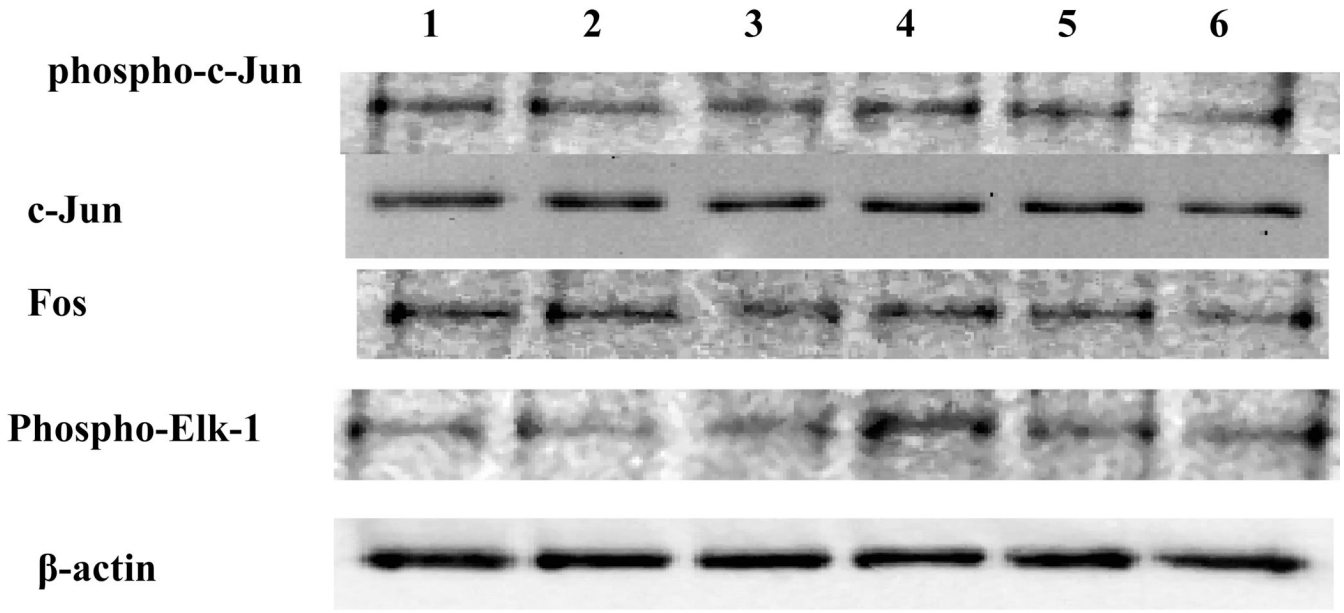
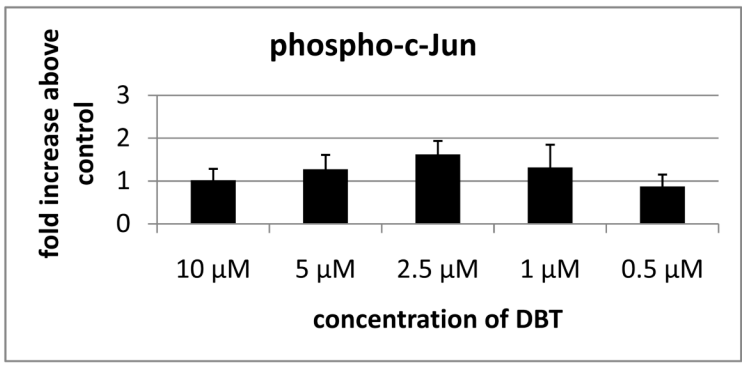


Figure 3.
Effect of a 6 h exposure to 300-25 nM TBT on levels of phospho-c-Jun, c-Jun, Fos, and phospho-Elk-1 in pure NK cells. Representative experiment of phospho-c-Jun. (1) Control Cells, (2) NK cells exposed to 300 nM TBT for 6 h, (3) NK cells exposed to 200 nM TBT for 6 h, (4) NK cells exposed to 100 nM TBT for 6 h, (5) NK cells exposed to 50 nM TBT for 6 h, (6) NK cells exposed to 25 nM TBT for 6 h.

(A)



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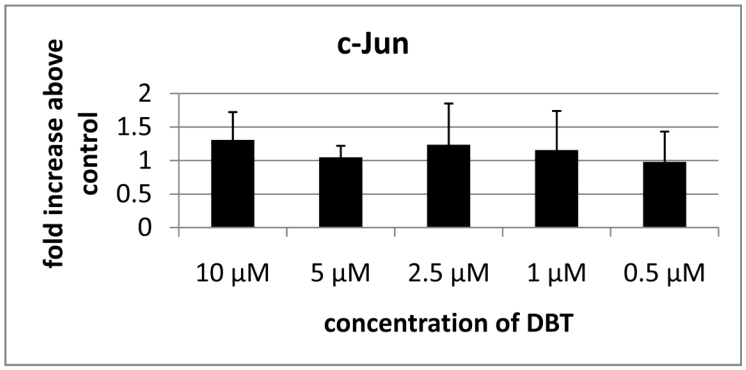

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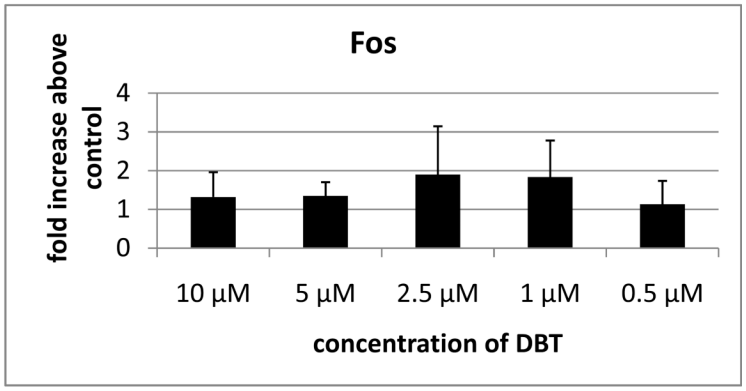

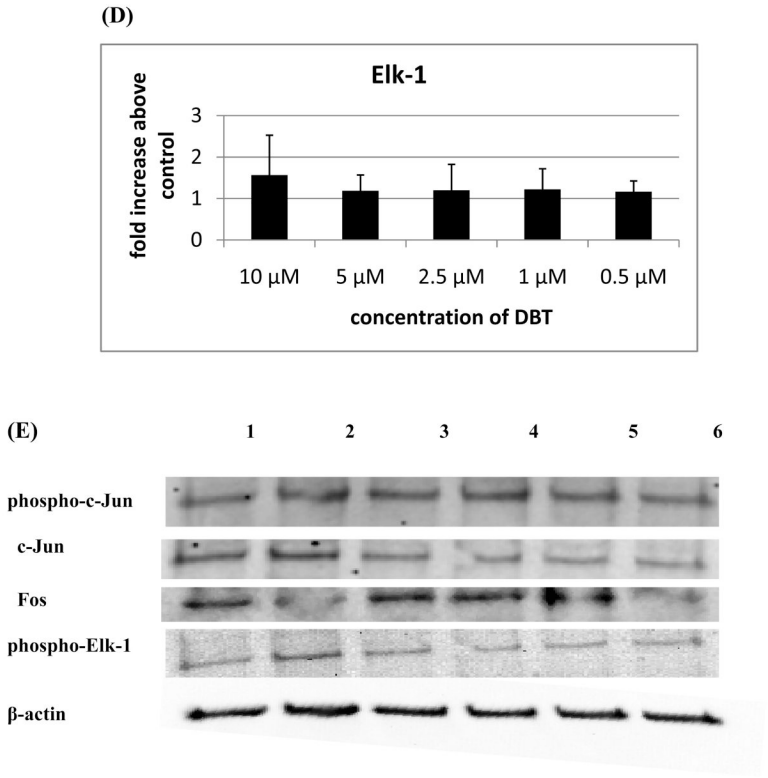

Figure 4.

Effect of 10 min exposures to 10-0.5 μM DBT on levels of phospho-c-Jun, c-Jun, Fos, and phospho-Elk-1 in pure NK cells: (A) phospho-c-Jun; (B) c-Jun; (C) Fos; (D) phospho-Elk-1 normalized to control as described in Figure 1. Values are mean ± S.D. from at least three separate experiments using different donors. Statistically significant differences ($p<0.05$) as compared to the control are indicated by an asterisk. (E) Representative experiments for each of the proteins. (1) Control Cells, (2) NK cells exposed to 10 μM DBT for 10 min, (3) NK cells exposed to 5 μM DBT for 10 min, (4) NK cells exposed to 2.5 μM DBT for 10 min, (5) NK cells exposed to 1 μM DBT for 10 min, (6) NK cells exposed to 0.5 μM DBT for 10 min.

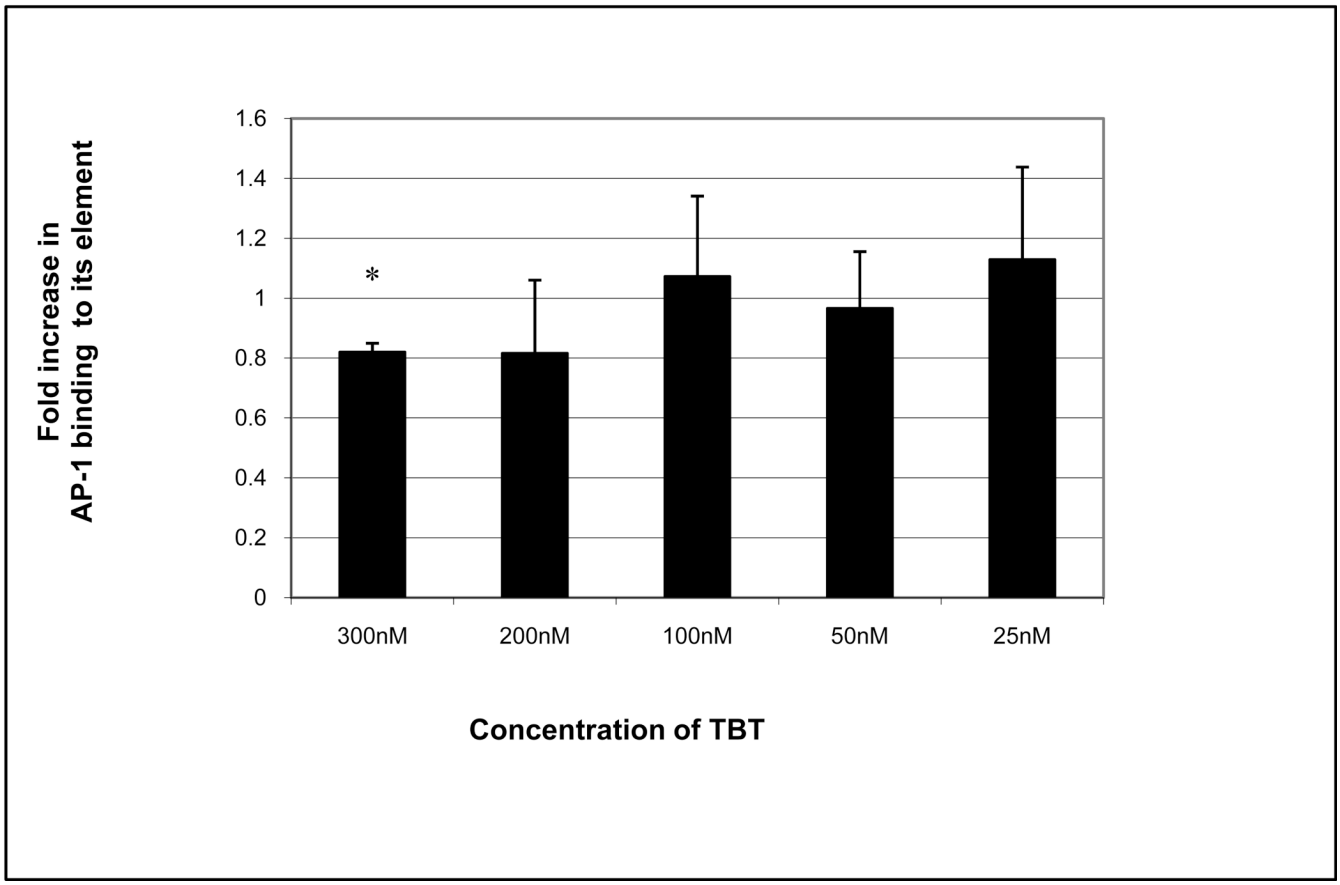

Figure 5.
Effect of a 1 h exposure to 25– 300 nM TBT on the binding activity of Fos (A) Levels of Fos normalized to control in pure NK cells exposed for 1 h. Values are mean ± S.D. from three separate experiments using different donors (triplicate determinations for each experiment). Statistically significant differences ($p<0.05$) as compared to the control are indicated by an asterisk.