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EFFECTS OF A SERIES OF TRIORGANOTINS ON ATP LEVELS IN HUMAN NATURAL KILLER CELLS

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Abstract

Natural killer (NK) cells are our initial immune defense against viral infections and cancer development. Thus, agents that are able to interfere with their function increase the risk of cancer and/or infection. A series of triorganotins, (trimethyltin (TMT), dimethylphenyltin (DMPT), methyldiphenyltin (MDPT), and triphenyltin (TPT)) have been shown to decrease the lytic function of human NK cells. TPT and MDPT were much more effective than DMPT or TMT at reducing lytic function. This study investigates the role that decreased ATP levels may play in decreases in the lytic function of NK cells induced by these OTs. A 24 h exposure to as high as 10 μM TMT caused no decrease in ATP levels even though this level of TMT caused a greater than 75% loss of lytic function. TPT at 200 nM caused a decrease in ATP levels of about 20% while decreasing lytic function by greater than 85%. There was no association between ATP levels and lytic function for any of the compounds when NK cells were exposed for 1h or 24 h. However, after a 48 h exposure to both DMPT and TPT decreased lytic function was associated with decreased ATP levels. There was an association between decreased lytic function and decreased ATP levels after a 6 day exposure to each of the four compounds. These studies indicate that the loss of lytic function seen after 1 h and 24 h exposures to this series of organotins cannot be accounted for by decreases in ATP. However, after longer exposures loss of lytic function may be in part be attributable to inadequate ATP levels.

INTRODUCTION

Organotins (OTs) are used in a variety of industrial and agricultural applications (Laughlin and Linden, 1985; Kannan and Lee, 1996; Federal Register, 2000; USDA, 2000; USDA 2001). OT contamination has been reported in water, sediment and fish from both freshwater and marine environments in the United States, Europe and Japan (Alzieu et al., 1991; Fent and Hunn., 1991; Kannan et al., 1995a,b,c; Tolosa et al., 1992; Ueno et al., 1999; Borghi and Porte,

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2002). Environmental contamination resulting from extensive use of OTs has been of great concern due to the potential for deleterious effects on non-target organisms (Kimbrough, 1976; Kannan et al., 1997; Maguire, 2000; Nielsen and Strand, 2002).

Organotins include methyltins (MTs), butyltins (BTs) and phenyltins (PTs). Tributyltin (TBT) has been used in a variety of anti-microbial applications (Kimbrough, 1976; Roper, 1992). TBT has been found in fish (Kannan et al., 1995a, b, c), various household products such as siliconized-paper baking parchments (Yamada et al., 1993), and in human blood (Kannan et al., 1999; Whalen et al., 1999). Triphenyltin (TPT) is used as a fungicide on major food and food-stock crops (Kannan and Lee, 1996; Federal Register, 2000; USDA, 2000; USDA, 2001). It has also been used in antifouling paints and is found in sea water (Shim et al., 1998). Trimethyltin (TMT) is present in some PVC products at levels of 8.5–24.9 µg/g (Ohno et al., 2002). This is due to its formation as a byproduct in plastic production. It is also a byproduct in the production of certain wood and textile preservatives (Chang and Dyer, 1983; Whittington et al., 1989). Measurable levels of TMT (as high as 0.13 µg/L, average value 0.042 µg/L) have been found in urine samples from humans who had not experienced an acute exposure (Braman and Tompkins, 1979). Human exposure to OTs might come from occupational exposure and/or consumption of contaminated food (Kimbrough, 1976; Borghi and Porte, 2002).

Both TBT and TPT are immunotoxic (Snoeij et al., 1985; Snoeij et al., 1986a; Snoeij et al., 1987; Whalen et al., 1999, Whalen et al, 2000). Rats fed TPT showed diminished thymus dependent immune responses, such as delayed-type hypersensitivity reactions (Snoeij et al., 1987). Triphenyltin hydroxide produced tumors in rats and mice (Roper,1992; Snoeij et al., 1987). TMT appears to be primarily neurotoxic (Brown et al., 1979; Jenkins and Barone, 2004; Ross et al., 1981; Snoeij et al., 1985). In a recent study we demonstrated that TMT is immunotoxic to human NK cells (Gomez et al. 2006) at levels similar to those that caused neurotoxicity (Jenkins and Barone, 2004). TMT has also been shown to immunotoxic in rats (Hioe and Jones, 1984)

Human natural killer (NK) lymphocytes play a central role in immune defense against virus infection and formation of primary tumors (Lotzova, 1993). They are responsible for limiting the spread of blood-borne metastases as well as limiting the development of primary tumors (Kiessling et al., 1978; Hanna et al., 1980). A greatly increased incidence of viral infections has been reported in individuals lacking the NK subset of lymphocytes (Fleisher et al., 1982; Biron et al., 1989). NK cells are defined by the absence of the T cell receptor/CD3 complex, the presence of CD56 and/or CD16 on the cell surface, and the presence of cytoplasmic granules containing cytolytic proteins such as granzyme B and perforin (Lotzova, 1993). Thus, any agent that interferes with the ability of NK cells to lyse their targets could increase the risk of tumor incidence and/or viral infections.

In a recent study we examined the effects of TMT, dimethylphenyltin (DMPT), methyldiphenyltin (MDPT), and TPT on the tumor-cell destroying function of human NK cells (Gomez et al., 2006). This series of compounds allowed us to monitor the capacity of OT compounds to decrease NK cell lytic function as their lipophilicity increases from TMT to TPT (Jensen et al., 1991). The ability of the OTs to diminish the lytic function of human NK cells increased in the following sequence, TMT, DMPT, MDPT, TPT. Thus, increasing lipophilicity of the OTs appears to increase their immunotoxicity. This suggests that access to the cell interior and interference with intracellular molecular functions (Aluoch and Whalen 2005) may be required for the OT-induced loss of function.

In the current study we examine the capacity of this same series of OT to interfere with the production of ATP which is essential for many cellular functions. There have been studies

indicating that TBT interferes with mitochondrial production of ATP (Snoeij, et al., 1986b; Marinovich et al., 1990; Aldridge, 1976; von Ballmoos et al., 2004). The role of the organic groups attached to the tin atom in this interaction has not been explored. The current study will address whether this series of OTs is able to decrease ATP levels in NK cells and the extent to which any decrease is ATP may be associated with the loss of lytic function.

MATERIALS AND METHODS

Isolation of NK cells

Blood samples (buffy coats) were obtained from American Red Cross (AMC), Portland, OR, which obtained informed consent. We were approved for receipt and use of blood products by the Institutional Review Board (IRB) of the AMC as well as by the IRB of our University. The donor population had an age range of about 31–86 years old and was about 40% female and 60% male. Highly purified NK cells were obtained using a rosetting procedure. Buffy coats were mixed with 1 mL of RosetteSep™ Human NK cell enrichment antibody cocktail (StemCell Technologies, Vancouver, British Columbia, Canada) per 30 mL of buffy coat. The mixture was incubated for 40 min at room temperature with periodic mixing. Following the incubation 4 mL of the mixture was layered onto 4 mL of Ficoll-Hypaque (1.077 g/mL) (Sigma), and centrifuged at 1200g for 20 min. The cell layer was collected and washed twice with phosphate buffered saline (PBS; 10 mM phosphate (pH 7.2)/2.7 mM KCl/140 mM NaCl) and stored in complete media (RPMI-1640 supplemented with 10% heat-activated BCS, 2 mM L-glutamine and 50 U penicillin G with 50 μg streptomycin/ml) at 1 million cells/mL. The yield of NK cells per buffy coat averaged 15–20 million cells. The resulting cell preparation was >95% CD16^{+}, CD56^{+} 0% CD3^{+} by fluorescence microscopy and flow cytometry (Whalen and Loganathan, 2001, Whalen et al., 2002).

Chemical Preparation

TMT and TPT were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). DMPT and MDPT were synthesized as previously described (Apodaca et al., 2001; Kapoor et al., 2005). All were triorganotin chlorides. Stock solutions of each compound were made in dimethylsulfoxide (DMSO) (Sigma-Aldrich). Stock solutions of the compounds were diluted in gelatin media (0.5% gelatin replaced the calf serum in complete media) to achieve the desired concentrations. The final concentration of DMSO did not exceed 0.01%.

Cell Treatments

Purified NK cells were separated by centrifugation from complete media (defined above) and transferred to complete media containing 0.5% gelatin in place of the 10% bovine serum. This was done in order to avoid binding of the hydrophobic compounds to serum albumin, which could interfere with their delivery to the cells. NK cells were then exposed to the compounds for 1h, 24 h, 48h or 6 days. The concentration ranges examined for each of the compounds were: TMT, 20 μM-500 nM; DMPT, 10 μM-100 nM; MDPT, 5μM-25 nM; and TPT, 1 μM -25 nM.

Cell Viability

Cell viability was determined by trypan blue exclusion. Cell numbers and viability were assessed at the beginning and end of each exposure period. The viability of treated cells was compared to that of control cells (cells from the same NK cell preparation that were exposed to the same conditions as the treated cells without any compound being added) at each concentration and length of exposure (Whalen et al., 2003).

ATP assay

ATP levels were measured as described by Dudimah et al., 2006 a,b. Following the cell exposure to the various concentrations of TMT, DMPT, MDPT, and TPT; 250,000 NK cells (167μL of the cell suspension) were removed, diluted by addition of 300 μL of PBS, and centrifuged for ninety seconds to pellet the cells. All media was removed and the cell pellet was resuspended in 375 μL of distilled water. 150μL of this suspension was added to 300 μL of somatic cell ATP releasing reagent (Sigma-Aldrich). To measure ATP, 100 μL of the lysed cell suspension was added to 100 μL of a Luciferin/Luciferase mixture. The light emission was measured using the Kodak Imaging system (Kodak, Rochester NY). ATP levels were determined from a standard curve. Statistical analysis of the data was carried out utilizing ANOVA and followed by pairwise comparison using Student's t test. Additionally, Type I statistical error was also accounted for by using a sequential Bonferroni tests (Holm, 1979).

RESULTS

Viability of NK cells exposed to varying concentrations of OTs

The effects of exposures to varying concentrations of TMT, DMPT, MDPT and TPT for varying lengths of time on the viability of NK cells as compared to control cells was monitored after every incubation (Table 1). Only those concentrations of compound that did not significantly decrease viability (after a given length of exposure 24 h, 48 h, 6 days) were studied for effects on the ability to decrease ATP levels in NK cells.

ATP levels in NK cells exposed to TMT for 1 h, 24 h, 48 h, and 6 days

Highly purified NK cells were exposed to TMT: 20-5 μM for 1 h; 10-1μM for 24 h and 48 h; and 5-0.5μM for 6 days (Figure 1). Following the exposure period the TBT-containing medium was removed and the cells were assayed for ATP levels as described above. Exposure of NK cells to TMT for 1 h or 24 h caused no significant decreases ($p>0.05$) in ATP levels at any concentration. A 48 h exposure of NK cells to 10 μM TMT caused a $22\pm13\%$ decrease in ATP levels ($p<0.001$). A 6 d exposure to both 5 and 2.5 μM TMT caused significant decreases in ATP levels ($85\pm16\%$, $p<0.0001$; 63 ± 19, $p<0.0001$, respectively).

ATP levels in NK cells exposed to DMPT for 1 h, 24 h, 48 h, and 6 days

Exposure of NK cells to DMPT for 1 h caused no significant decreases ($p>0.05$) in ATP levels at any concentration (10 - 1 μM) (Figure 2). The same was true after a 24 h exposure to 10-0.2μM DMPT. Exposure to 5 and 2.5 μM DMPT for 48 h decreased levels of ATP in NK cells by $46\pm18\%$ and $40\pm16\%$, respectively (Figure 2, $p<0.001$). A 6 d exposure to 2.5 and 1 μM DMPT decreased ATP levels by $97\pm3\%$ and $72\pm14\%$, respectively ($p<0.0001$).

ATP levels in purified NK cells exposed to MDPT for 1 h, 24 h, 48 h, and 6 days

In contrast to TMT and DMPT, exposure of NK cells to MDPT for 1 h caused a small but significant decreases in ATP levels at 5 μM ($16\pm6\%$, $p<0.0001$) (Figure 3). Exposure to MDPT for 24 h caused no significant decreases in ATP levels at any of the concentrations tested (Figure 3). A 6 d exposure to 100 nM MDPT decreased ATP levels by $44\pm13\%$ ($p<0.0001$) (Figure 3).

ATP levels in purified NK cells exposed to TPT for 1 h, 24 h, 48 h, and 6 days

Exposure to 200 nM TPT for 24 h caused a small ($20\pm10\%$) decrease in the levels of ATP in NK cells (Figure 4, $p<0.001$). After a 48 h exposure to 200 nM TPT there was a $66\pm22\%$ decrease in ATP levels (<0.0001, Figure 4). A 6 d exposure to TPT caused substantial decreases in ATP levels at all of the concentrations tested. At 200, 100, 50, and 25 nM TPT the decrease were $99\pm2\%$, $79\pm24\%$, $45\pm23\%$, and $31\pm18\%$ ($p<0.0001$, Figure 4).

Effect of Glucose addition on the ATP levels of NK cells exposed to TMT, DMPT, MDPT, and TPT

Substantial decreases in ATP levels were not seen until the 48 h time point with any of the compounds. This suggested that, although the OTs might be interfering with mitochondrial ATP production, ATP production via the glycolytic pathway might be able to sustain ATP levels until glucose supplies began to decline. To examine this possibility we tested whether glucose supplementation of the culture medium affected the OT-induced decreases in ATP levels. Glucose was added at a final concentration of 2 g/L to control and OT-exposed NK cells after 24 h to cells receiving a 48 h exposure to OTs and after 24 h and 5 days for those receiving a 6 day exposure. Cells from the same donor were exposed to OTs without glucose supplementation. Figure 5 shows that there was no alteration of the capacity of any OT to decrease ATP levels when glucose was added to the cell culture.

DISCUSSION

The current study examined the effects of a series of OTs that differ in their lipophilicity (TMT, DMPT, MDPT, and TPT) on the ATP levels of human NK cells. We have recently shown that each of these compounds was able to decrease the lytic function of NK cells in exposures ranging from 1 h to 6 days (Gomez et al., 2006). In addition to determining the effects of TMT, DMPT, MDPT, and TPT exposures on ATP levels in NK cells we examined whether glucose depletion might in part be responsible for any declines in ATP.

When NK cells were exposed to TMT for 24 h, the level of ATP in the cells was unaffected. Recently, we have shown that exposure of NK cells to 10 and 5 μM TMT for 24 h decreases lytic function (Gomez et al. 2006). NK cells exposed to TMT for 48 h (10 μM) and 6 days (2.5 μM and 1 μM) did show decreased ATP levels. There were no significant relationship between ATP levels and lytic function after 24 or 48 h exposures to TMT (Figure 6B and 6C). However, after 6 days of exposure to TMT there was a strong association between the levels of ATP and the loss of lytic function, R^2=0.97 Figure 6D).

The replacement of one methyl group in TMT with a phenyl group, to produce DMPT, caused an increase in the capacity to decrease ATP levels in exposed NK cells. Like TMT, DMPT had no effect on ATP levels in NK cells exposed for 24 h and did decrease ATP levels after 48 h and 6 day exposures. However, the concentrations of DMPT required to cause a significant decrease in ATP were lower (5 μM and 2.5 μM DMPT caused 46% and 40% decreases after 48 h, respectively). There was no relationship between ATP levels and lytic function in NK cells exposed to DMPT for 1 h or 24 h (Figure 7A and 7B). However, there was a significant association between ATP levels and lytic function after 48 h (unlike TMT) (Figure 7C) as well as after 6 days (Figure 7D).

The replacement of two of the methyl groups in TMT by phenyl groups to produce MDPT had a very significant impact on the capacity of the compound to interfere with NK cell lytic function (Gomez et al., 2006). For instance, a 24 h exposure to 200 nM MDPT caused about the same decrease in lytic function (greater than 80%) as 5 μM DMPT. However, those concentrations that decreased lytic function after 24 h had no affect on ATP levels (Figure 8B). Thus, like DMPT there was no relationship between loss of lytic function and ATP levels in NK cells exposed to MDPT for 1 h or 24 h (Figure 8A and 8B). While DMPT began to show an association between ATP levels and lytic function after 48 h of exposure (R^2= 0.96), MDPT showed no association (Figure 8C). An association between ATP levels and lytic function in MDPT-exposed NK cells seem to be developing after 6 days (Figure 8D).

When all three organic groups in the OT were phenyl groups the compound became more effective at decreasing NK lytic function following a 1 h exposure. TPT's ability to decrease

lysis after 24 h, 48 h, and 6 d exposures was similar to that of MDPT (Gomez et al. 2006). However, unlike MDPT, exposure of NK cells to TPT caused significant decreases in ATP levels at concentrations that decreased lytic function after 48 h (Figure 9C). This was similar to what was seen with DMPT. After a 6 day exposure to TPT there was a strong association between lytic function and ATP levels (R2=0.997, Figure 9D).

TMT is much less lipophilic than TPT. TMT has a measured $logP_{O/w}$ of -1.2, while that of TPT is 1.5 (Jensen et al., 1991). Increased lipophilicity would allow the compound to cross the cell membrane more readily. Once in the interior of the cell the compound could interfere with molecular processes required for NK lytic function (Aluoch and Whalen 2005). The fact the most lipophilic compound tested, TPT, was also the most rapid and effective at decreasing ATP levels (Figure 4) and lytic function is consistent with the suggestion that access to the cell interior (and specific molecular components) is essential to the loss of function. Thus, the increase in capacity to block NK lytic function as the OTs progressed from TMT to TPT may at least in part be due the increase in the lipophilic nature of the compounds.

Studying this series of compounds indicated that there is no significant relationship between ATP levels and decreases in lytic function after 1 h or 24 h exposures of NK cells to the compounds. However, a 48 h exposure to DMPT and TPT and a 6 day exposure to each of the compounds showed a correlation between ATP levels and lytic function (Figures 5–8). These findings are consistent with the results seen with butyltins, DBT and TBT (Dudimah et al., 2006a, b). TBT has been shown to inhibit the function of the mitrochondrial ATP synthase (von Ballmoos et al., 2004; Matsuno-Yagi and Hatefi, 1993). Thus, sustained decreases in ATP levels seen with TBT exposures may be partially due to the ability to inhibit ATP synthase. The OTs examined in this study appear, thus far, not to have been evaluated for their ability to interfere with ATP synthase, however the current results suggest that they may be able to do so. But, the fact that glucose supplementation cannot reverse the decreases in ATP that were seen with these OTs indicates that they may be interfering with glycolytic ATP production (in some manner) as well. The current results suggest that after longer duration exposures (longer than 24 h) to OTs there may be an OT-induced decrease in mitochondrial and glycolytic ATP production which is at least in part responsible for loss of lytic function. Previous studies examining TBT- and DBT -induced decreases in the levels of ATP in NK cells have shown that if the cells were exposed to the compounds for 1h followed by removal of the compound-containing medium for at least 24 h prior to assaying for ATP levels that there was essentially the same decrease in ATP as was seen if the compound had remained in the medium for 24 h (Dudimah et al., 2006a,b). This indicated that there may be a very tight association of the compounds with a protein(s) involved in ATP synthesis such as ATP synthase. Others have shown that TBT binds to ATP synthase in bacteria (von Ballmoos et al., 2004). It is quite possible that the OTs tested in this study may also bind quite tightly to some component(s) such as ATP synthase. Thus, it might be expected that if NK cells were exposed to the OTs used in this study for 1h followed by 24 h or more in OT-free media that decreases in ATP levels would be similar to those seen when the OT remained in the medium (as was seen with TBT and DBT). This is an area for future study.

TMT and MDPT did not show correlations between ATP levels and lytic activity after 48 h. MDPT also showed a weaker correlation after the 6 day exposure than any other OT. Thus, properties of the organic groups attached to the tin atom beyond lipophilic nature, such as molecular geometry, may influence the capacity of the compound to diminish the lytic function of NK cells. Additionally, the current results indicate that loss of lytic function does not require decreased levels of ATP (with any of the OTs tested). However, decreased ATP levels may be a contributing factor in sustained loss of lytic function.

In summary the results of these studies indicate: 1.) The capacity of the TMT, DMPT, MDPT and TPT to decrease ATP levels in exposed NK cells was related to the lipophilic nature of the compound increasing in the order TMT<DMPT<MDPT<TPT. 2.) The decreases in ATP levels could not be diminished by addition of glucose, indicating that there was no significant glycolytic production of ATP in NK cells exposed to the compounds. 3.) There was no correlation between loss of lytic function and decreases in ATP levels in NK cells exposed to the compounds for 1 h, 24 h or 48 h (TMT and MDPT). 4.) There was a strong correlation between ATP levels and lytic function in NK cells exposed to the compound for 6 days.

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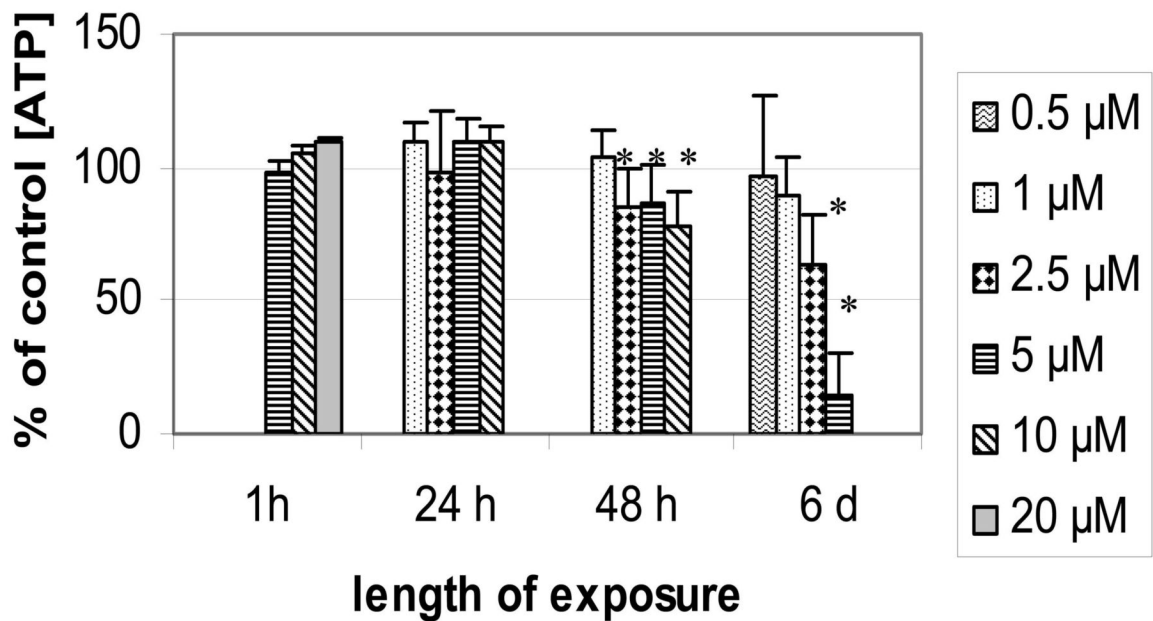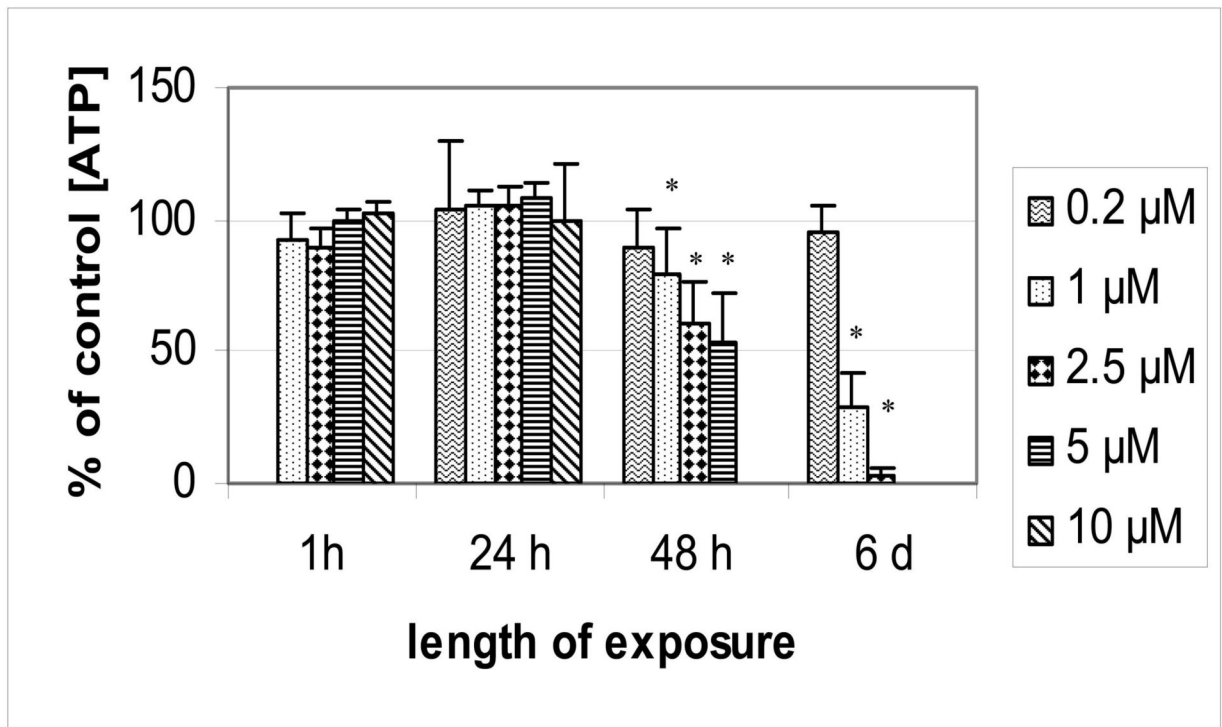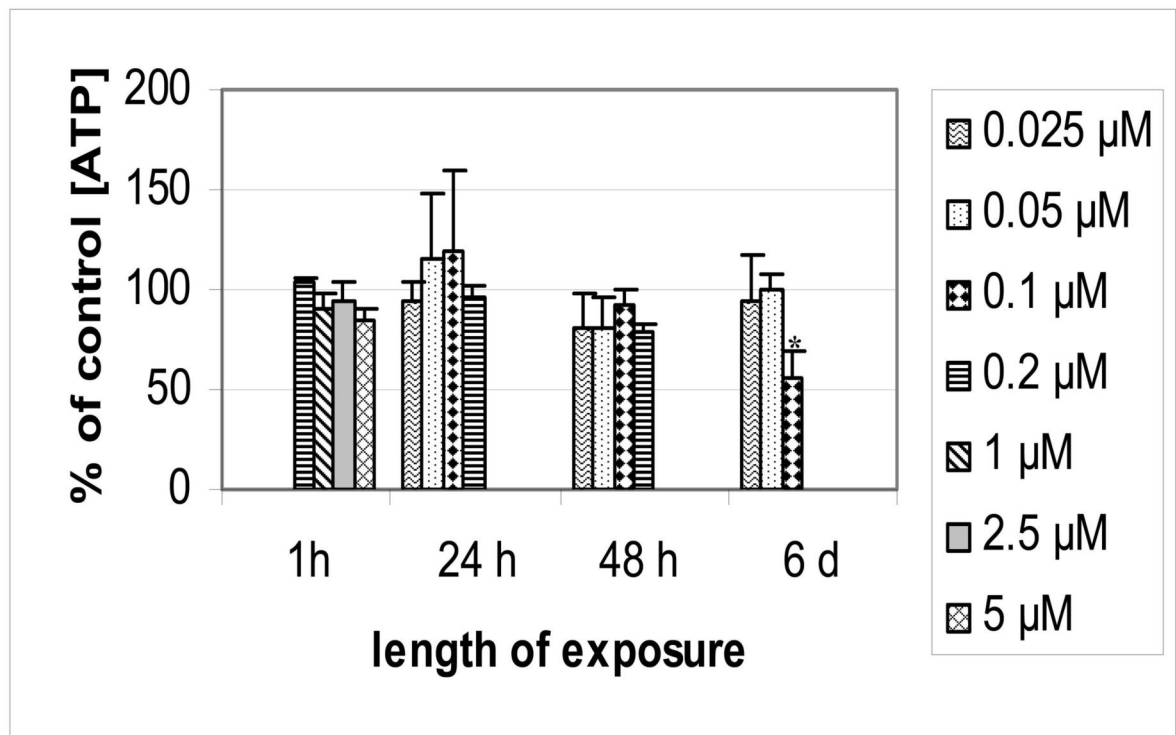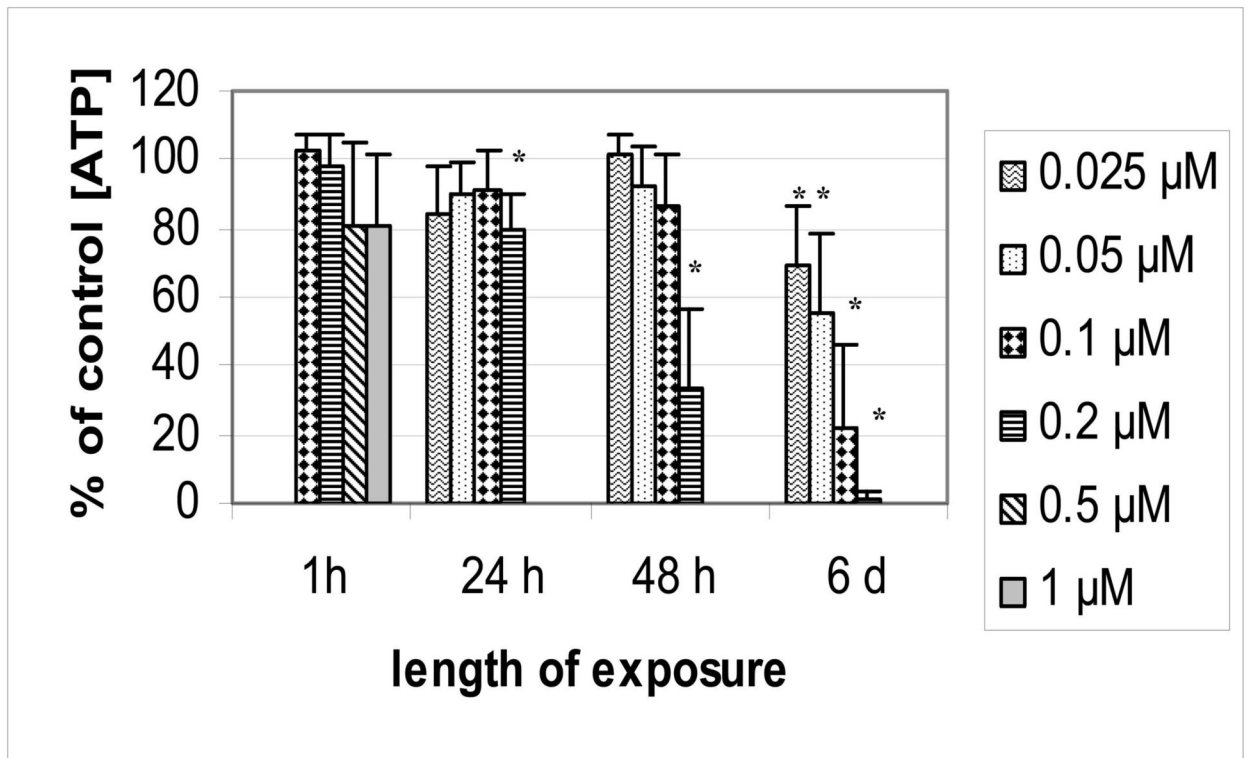
Figure 1.
Effect of exposures to TMT on ATP levels in human NK cells: 1 h exposure to 5–20 μM TMT; 24 h exposure to 1 –10 μM TMT; 48 h exposure to 1–10 μM TMT; 6 day exposure to 0.5–5μM TMT. Data were combined from replicate experiments using cells from different donors. To combine results from separate experiments the levels of lysis were normalized as percentage of the lytic function of the control cells in a given experiment. Results were the average ± S.D. of 3 determinations for 1h; and 9 or more determinations for all other time points. * indicates statistically significant difference from control (p<0.01)
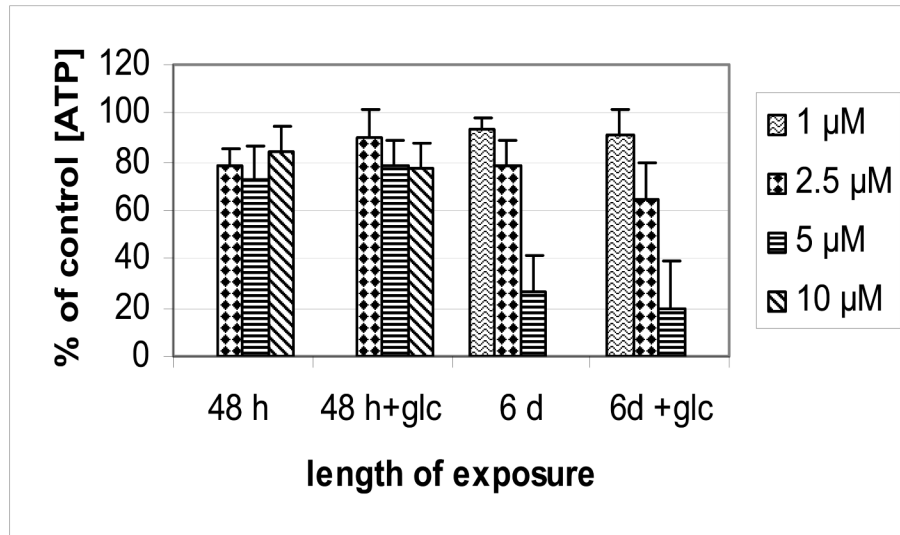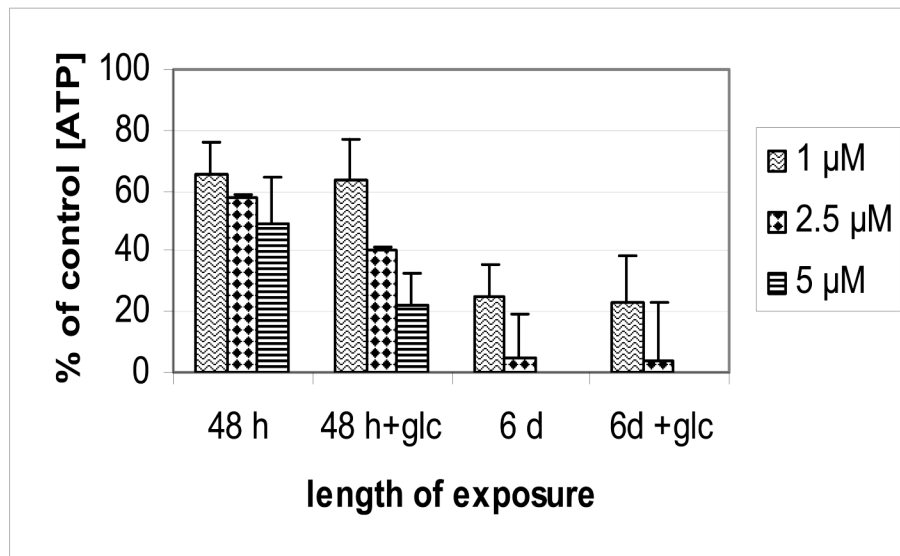
Figure 2.
Effect of exposures to DMPT on ATP levels in human NK cells: 1 h exposure to 1–10 μM
DMPT; 24 h exposure to 200 nM -10 μM DMPT; 48 h exposure to 200 nM -5 μM DMPT; 6
day exposure to 200 nM -2.5 μM DMPT. Data were combined as described in Figure 1. n=6
for 1h, n=9 for all other time points. * indicates statistically significant difference from control
(p<0.01)

Figure 3.
Effect of exposures to MDPT on ATP levels in human NK cells: 1 h exposure to 200 nM -5 μM MDPT; 24 h exposure to 25 – 200 nM MDPT; 48 h exposure to 25–200 nM MDPT; 6 day exposure to 25–100 nM MDPT. Data were combined as described in Figure 1. n=9 for all time points. * indicates statistically significant difference from control (p<0.01)
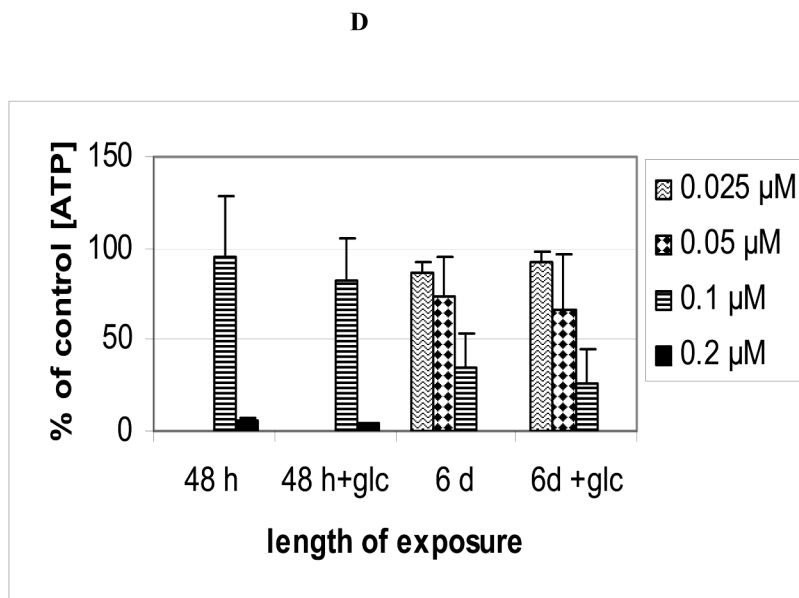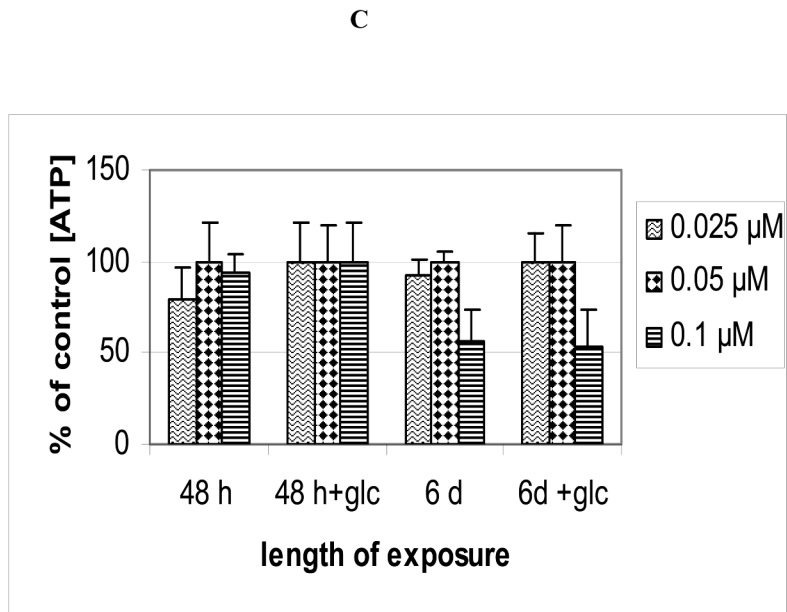
Figure 4.
Effect of exposures to TPT on ATP levels in human NK cells: 1 h exposure to 100 nM -1μM TPT; 24 h exposure to 25 – 200 nM TPT; 48 h exposure to 25–200 nM TPT; 6 day exposure to 25–200 nM TPT. Data were combined as described in Figure 1. n≥9 for all time points. * indicates statistically significant difference from control (p<0.01)

A

B

C

D

Figure 5.
Effects of glucose supplementation on ATP levels in NK cells exposed to: A.) TMT; B.) DMPT; C.) MDPT; and D.) TPT, for 48 h and 6 days. . n=9 for all time points.
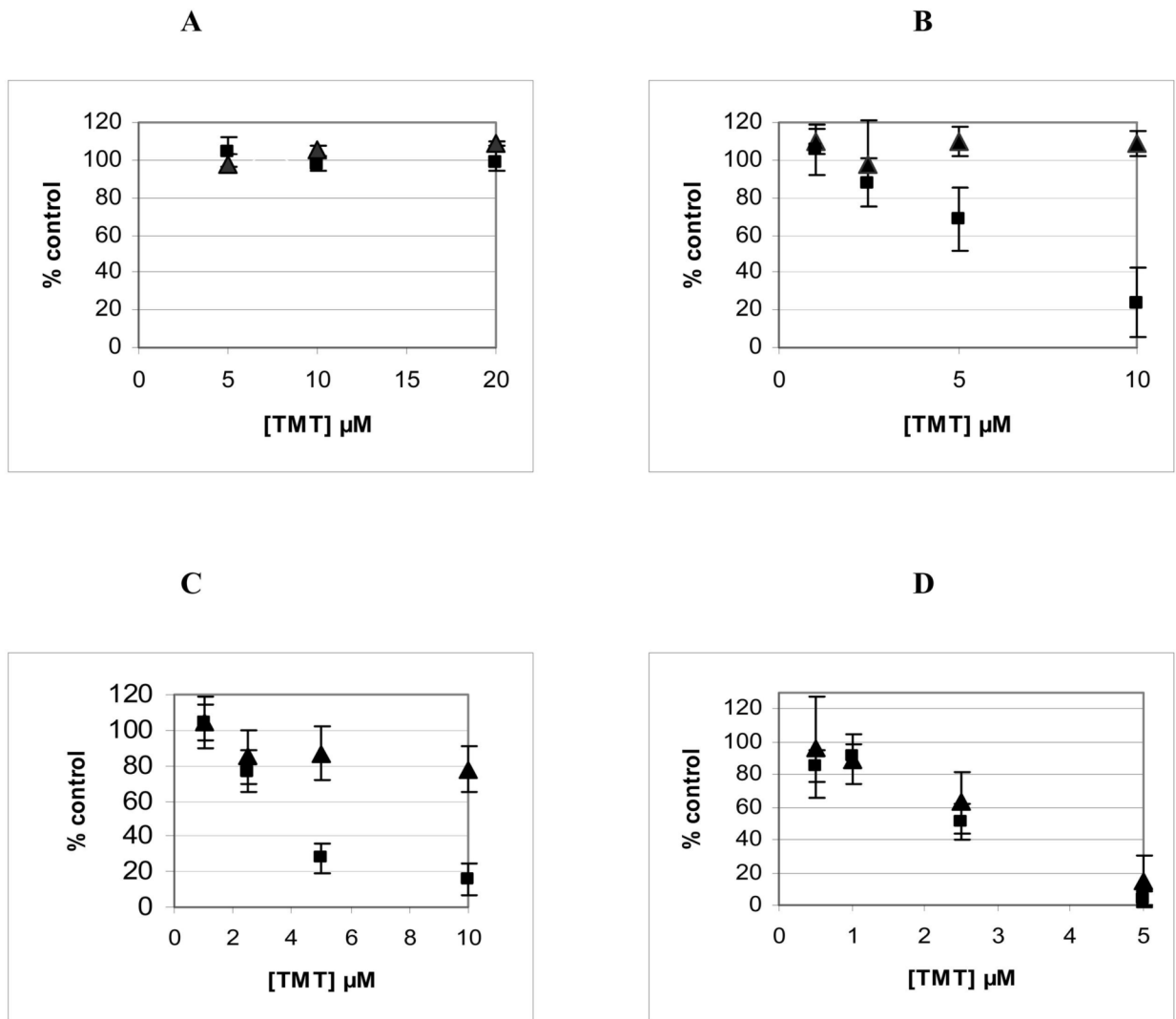
A

B

C

D

Figure 6.
Effects of exposures to TMT on ATP levels (▲) and lytic function* (■) plotted on the same graph. A). 1 h exposure to TMT, R^2=0.75; B). 24 h exposure to TMT, R^2=0.06; C). 48 h exposure to DBT, R^2=0.53; D). 6 d exposure to TMT, R^2=0.97. *Lytic function data from Gomez et al. 2006.
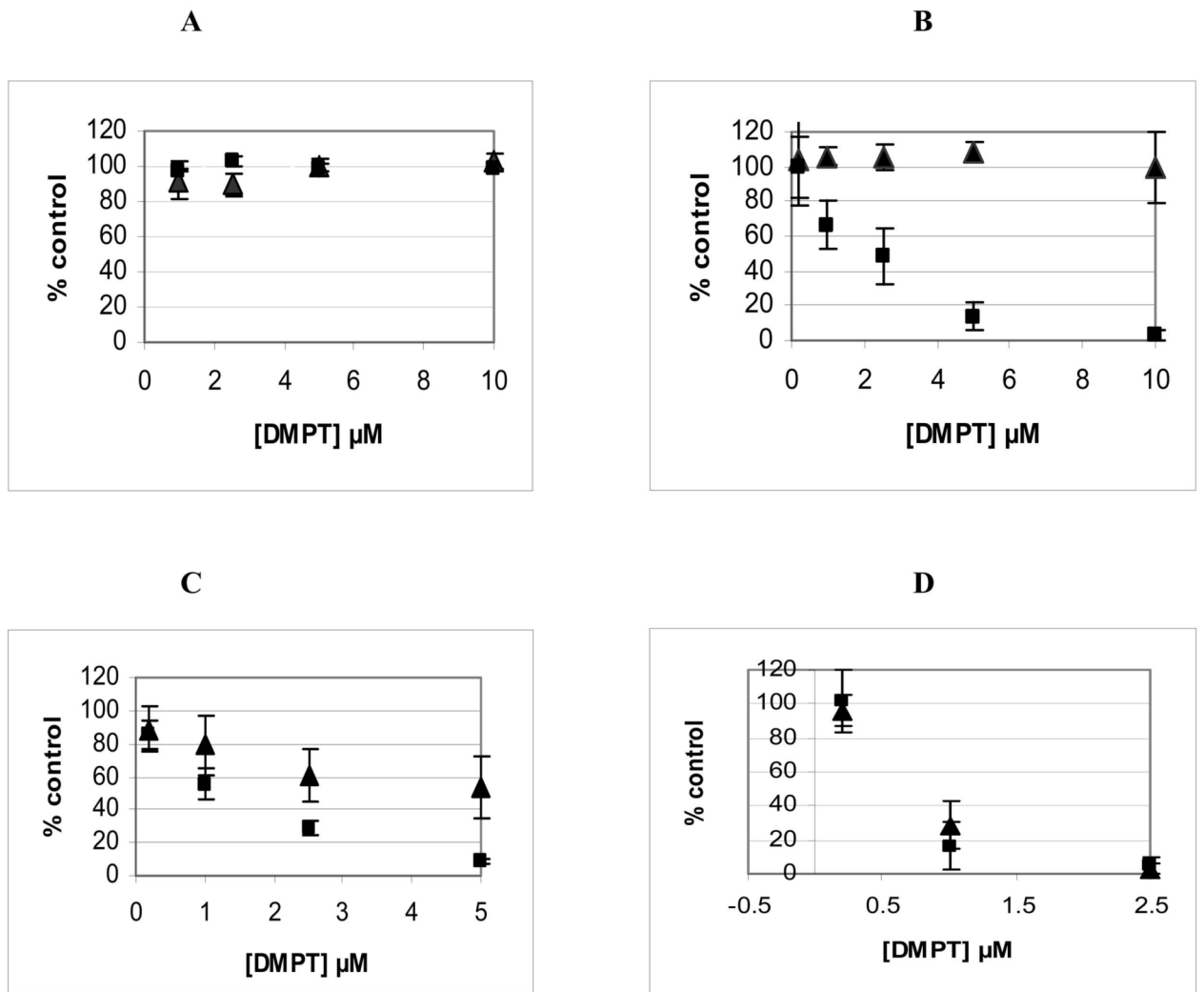
A

B

C

D

Figure 7.
Effects of exposures to DMPT on ATP levels (▲) and lytic function* (■) plotted on the same graph. A). 1 h exposure to DMPT, R^2=0.35; B). 24 h exposure to DMPT, R^2=0.18; C). 48 h exposure to DMPT, R^2=0.96; D). 6 d exposure to DMPT, R^2=0.96. *Lytic function data from Gomez et al. 2006.
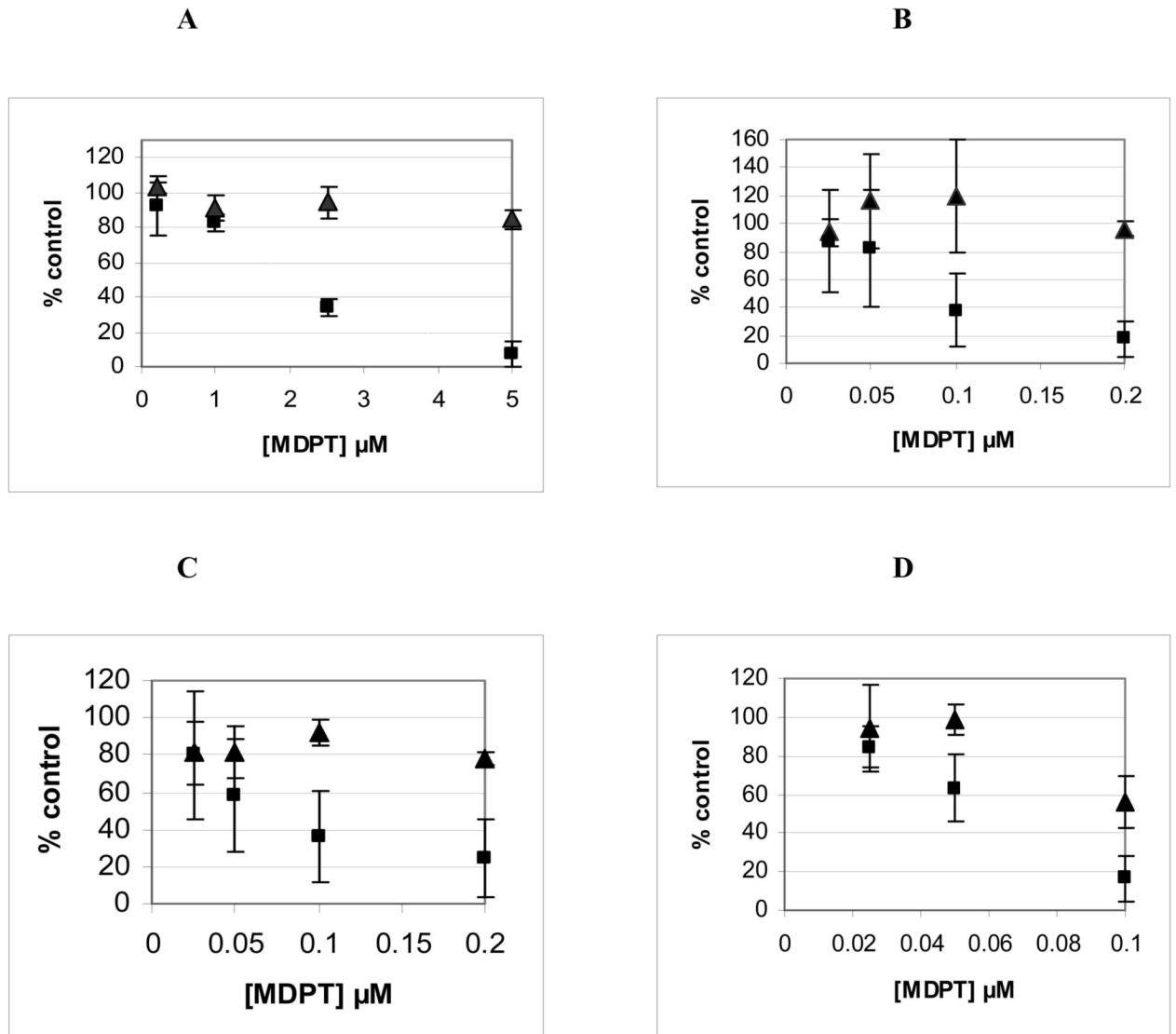
Figure 8.
Effects of exposures to MDPT on ATP levels (▲) and lytic function* (■) plotted on the same graph. A). 1 h exposure to MDPT, R^2=0.57; B). 24 h exposure to MDPT, R^2<0.001; C). 48 h exposure to MDPT, R^2=0.04; D). 6 d exposure to MDPT, R^2=0.84. *Lytic function data from Gomez et al. 2006.
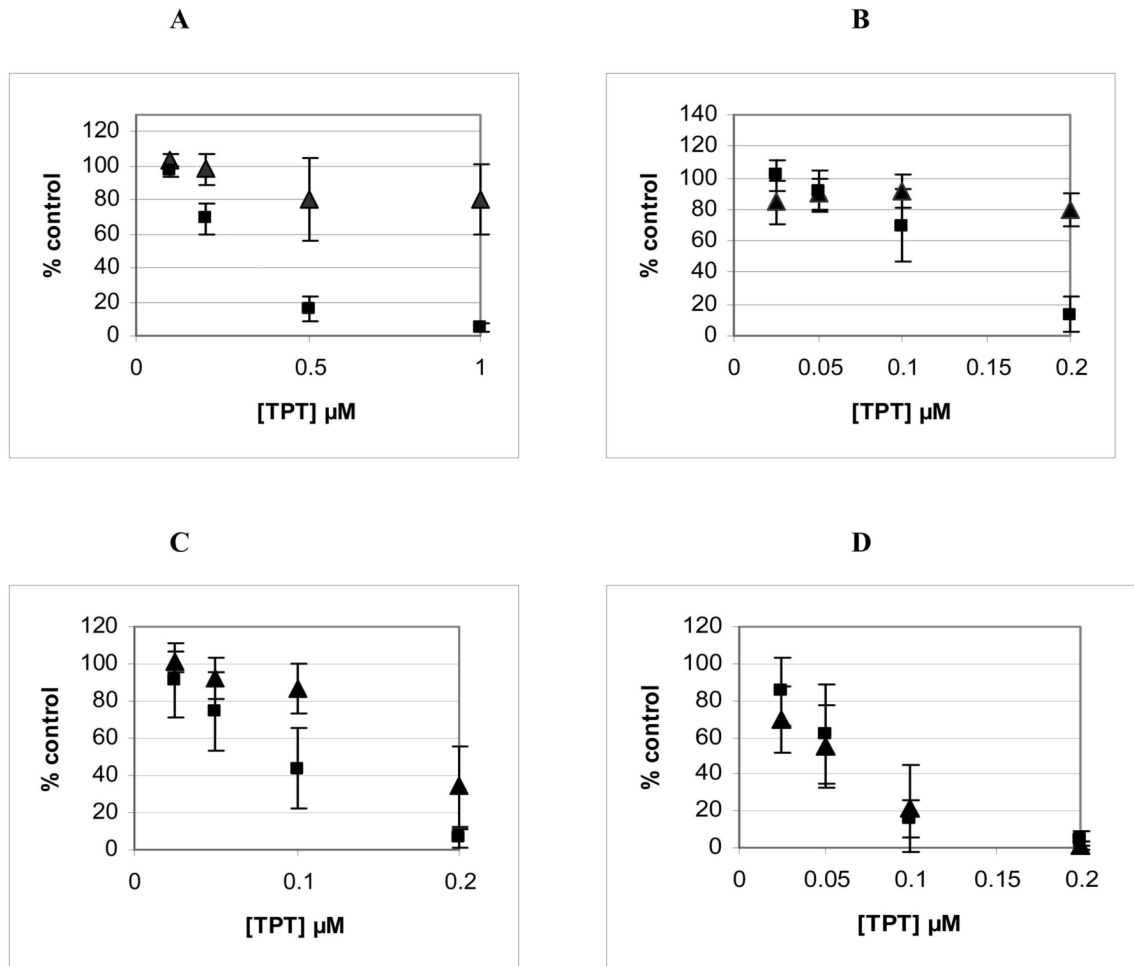
A

B

C

D

Figure 9.
Effects of exposures to TPT on ATP levels (▲) and lytic function* (■) plotted on the same graph. A). 1 h exposure to TPT, R^2=0.98; B). 24 h exposure to TPT, R^2=0.38; C). 48 h exposure to TPT, R^2=0.92; D). 6 d exposure to TPT, R^2=0.997. *Lytic function data from Gomez et al. 2006.

Table 1

Viability of lymphocytes following exposure to TMT, DMPT, MDPT and TPT

Compound	concentration	% viable cells as compared with control at each length of exposure			
		1 h	24 hrs	48 hrs	6 days
TMT	20 μM	98±26	98±4	96±6	
	10 μM	96±4	96±2	96±5	93±1 [*]
	5 μM	88±12	99±1	100±1	93±7
	2.5 μM		100±6	98±2	101±6
	1 μM				100±4
	0.5 μM				
DMPT	10 μM	95±1	102±5	90±3	89±11
	5 μM	98±1	100±4	93±4	94±4
	2.5 μM	102±13	99±5	91±6	98±2
	1 μM	100±6	101±2	95±6	98±3
	0.2 μM		102±4		
MDPT	5 μM	92±13			
	2.5 μM	95±12			
	1 μM	95±11			
	0.2 μM		92±12	100±4	90±10
	0.1 μM		94±3	98±6	104±2
	0.05 μM		96±3	102±3	99±4
	0.025 μM		96±4	100±4	
TPT	1 μM	92±5			
	0.5 μM	93±3	96±3	71±2 [*]	90±14
	0.2 μM	91±2	95±4	93±6	98±5
	0.1 μM	96±5	97±5	98±0	99±9
	0.0 5 μM		96±6	99±4	96±9
	0.025 μM				

[*] indicates a significant decrease in viability as compared to control. There was 100% recovery of cells following exposures.