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Inhibition of the Pin1 Prolyl Isomerase: A Novel Approach for DNA Checkpoint Abrogation in Breast Cancer Cell Lines

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We examined the hypothesis that the Pin1 prolyl isomerase plays a role in enforcing cell cycle checkpoints associated with DNA damage, and that inhibition of this enzyme would result in abrogation of the checkpoint. A set of synthetic peptides were prepared that would serve as competitive substrate inhibitors of Pin1 prolyl isomerase activity. To facilitate uptake into cells the peptides contained an amino terminal sequence of HIV-1 Tat. Negative and positive control peptides were also examined. We determined the effects of the synthetic peptide inhibitors on cell growth, abrogation of an S-phase checkpoint induced by hydroxyurea treatment, and abrogation of a G2 DNA damage checkpoint induced by bleomycin treatment. We found that none of the synthetic peptides had any effect on cell growth, nor were they effective in abrogating the S-phase checkpoint induced by hydroxyurea. However, a small but reproducible effect on DNA damaged cells was observed. The Pin1 peptide predicted to have the greatest activity induced a more rapid shift of G2 blocked cells into G1 phase after 48-72 hours following initial bleomycin exposure. Our results do not suggest that the peptide inhibitors examined here have therapeutic potential, but they do show that inhibition of Pin1 may play a role in checkpoint control. A second generation of more active Pin1 inhibitors may provide a new therapeutic approach.

Cell Cycle, DNA damage, Checkpoint, Synthetic Peptides

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19

20
<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover</td>
<td>1</td>
</tr>
<tr>
<td>SF 298</td>
<td>2</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>3</td>
</tr>
<tr>
<td>Introduction</td>
<td>4</td>
</tr>
<tr>
<td>Body</td>
<td>4-10</td>
</tr>
<tr>
<td>Key Research Accomplishments</td>
<td>10</td>
</tr>
<tr>
<td>Reportable Outcomes</td>
<td>10</td>
</tr>
<tr>
<td>Conclusions</td>
<td>10-11</td>
</tr>
<tr>
<td>References</td>
<td>11-12</td>
</tr>
<tr>
<td>Appendices</td>
<td>12-19</td>
</tr>
</tbody>
</table>
INTRODUCTION:

We examined the hypothesis that the Pin1 prolyl isomerase plays a role in enforcing cell cycle checkpoints associated with DNA damage, and that inhibition of this enzyme would result in abrogation of the checkpoint. Cell cycle checkpoints function to maintain genomic stability, therefore drugs that abrogate the checkpoint (in this case inhibit Pin1 function), should enhance the cell killing activity of chemotherapeutic agents that induce DNA damage. A set of synthetic peptides were prepared that would serve as competitive substrate inhibitors of Pin1 prolyl isomerase activity. To facilitate uptake into cells the peptides contained an amino terminal sequence of HIV-1 TAT. Negative control peptides for Pin1 inhibition, and a positive control peptide previously shown to inhibit the Chk1 kinase involved in maintaining the DNA damage checkpoint (1), were also examined. We determined the effects of the synthetic peptide inhibitors on cell growth, abrogation of an S-phase checkpoint induced by hydroxyurea treatment, and abrogation of a G2 DNA damage checkpoint induced by bleomycin treatment. We found that none of the synthetic peptides had any affect on cell growth, nor were they effective in abrogating the S-phase checkpoint induced by hydroxyurea. However, a small but reproducible effect on DNA damaged cells was observed. Bleomycin treatment induced a transient G2 block as a result of activation of the DNA damage checkpoint. The synthetic peptide predicted to have the greatest activity towards Pin1 induced a more rapid shift of the G2 blocked cells into G1 phase after 48-72 hours following initial bleomycin exposure. While these results do not suggest that the Pin1 peptide inhibitors examined here have therapeutic potential, the results do indicate that inhibition of Pin1 may play a role in DNA damage checkpoint control. Therefore, our results support the investigation of a second generation of more active and specific Pin1 inhibitors. Pin1 inhibitors of sufficient specificity may provide a new approach to override DNA damage checkpoints and enhance the activity of current chemotherapeutic agents.

BODY:

Synthetic Peptides

A series of 4 synthetic peptides were designed to serve as potential competitive inhibitors of the Pin1 prolyl isomerase and the appropriate negative controls. Each of the peptides contained an N-terminal sequence of 11 amino acids of YGRKKRRQRRR, which corresponded to the HIV-1 TAT protein transduction domain (2). This sequence was used to facilitate delivery of the peptides into target cells. The inhibitory sequence was based upon the Pin1 substrate peptide WFYSPR (3), which requires a phosphorylated serine residue N-terminal to the proline residue for activity. In addition, a fifth peptide was prepared to serve as a positive control peptide for inhibition of the DNA damage checkpoint. This peptide was previously shown by Sugumana et al. to inhibit the activity of Chk1 kinase (1), which is required to maintain the DNA damage checkpoint (4). The peptides used in this study are listed in Table 1.
### TABLE 1

Pin1 inhibitory peptides and predicted function.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Designation</th>
<th>Sequence</th>
<th>Predicted activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide 1</td>
<td>P1</td>
<td>YGRKKRRQRRRWFYAPR</td>
<td>Inactive control peptide with Ser to Ala substitution at position 15</td>
</tr>
<tr>
<td>Peptide 2</td>
<td>P2</td>
<td>YGRKKRRQRRRWFYSPR</td>
<td>Inactive peptide with non-phosphorylated Ser at position 15</td>
</tr>
<tr>
<td>Peptide 3</td>
<td>P3</td>
<td>YGRKKRRQRRRWFYEPR</td>
<td>Some activity possible, contains Ser to Glu substitution at position 15 that mimics charge of phosphorylated Ser</td>
</tr>
<tr>
<td>Peptide 4</td>
<td>P4</td>
<td>YGRKKRRQRRRWFYpSPR</td>
<td>Active inhibitory peptide, contains phosphorylated Ser at position 15</td>
</tr>
<tr>
<td>Peptide 5</td>
<td>P5</td>
<td>YGRKKRRQRRRLYRSPMPENL</td>
<td>Positive control, inhibitor of Chk1</td>
</tr>
</tbody>
</table>

### Analysis of the T47D spindle checkpoint

We chose three breast cancer cell lines, MCF-7, MDA-MB-231, and T47D, to examine the effects of Pin1 synthetic peptide inhibitors on growth properties and cell-cycle checkpoint abrogation. The MCF-7 cells are known to be p53 positive while the MDA-MB-231 and T47D cell lines are reported to be p53 negative, allowing for a comparison of Pin1 inhibition effects based upon p53 differences. In addition, the T47D cell line had been reported to have a defective spindle assembly checkpoint (5), which would allow us to examine the contribution of a spindle assembly checkpoint defect to the effects resulting from Pin1 inhibition. Since we have previously characterized a spindle assembly defect in the A427 lung adenocarcinoma cell line (6), we examined the T47D cell line using similar techniques.

Upon exposure to microtubule inhibitory drugs, cycling cells will enter a state of mitotic arrest lasting up to 24 hours or longer depending on the cell type if the cells have an active spindle assembly checkpoint. Thus accumulation of mitotic cells over time followed by an increase in multinucleated cell at later time points is a characteristic of most cultured cells following treatment with microtubule inhibitory drugs. Cells lacking
a spindle assembly checkpoint do not arrest in mitosis following treatment with microtubule inhibitors. Rather these defective cells proceed through the mitotic stage (usually within 1-2 hours), similar to spindle assembly checkpoint positive cells, but fail to assemble a normal spindle or segregate chromosomes properly. Often, and as observed in the A427 cells, spindle assembly checkpoint negative cells also fail cytokinesis. The resulting cells are easily detected as multinucleated cells.

Following an 8-24 hour treatment of T47D cells with nocodazole, a microtubule depolymerizing agent, or taxol, a microtubule stabilizing agent, an increase in the mitotic index of the population was not observed. These results suggested that the cells were not arrested in mitosis and had a defect in the spindle assembly checkpoint as reported (5). However, upon more careful morphological examination of the treated cell population we did not detect a significant increase in multinucleated cells. Some mitotic cells were also present in the population, but a typical distribution of mitotic stages, as present in untreated populations, was not observed. We carried out a series of experiments to examine the effects of nocodazole and taxol on the T47D cells, and have come to the conclusion that these cells in fact have a functional mitotic spindle checkpoint. This conclusion is based on the following evidence. First, cells entering mitosis at or shortly after application of the microtubule inhibitory drugs remain arrested in mitosis. Second, cells do not appear to move through the mitotic arrest, as there is no morphological evidence for cells undergoing cytokinesis due to a lack of telophase cells in the population. Third, the generation of multinucleated cells is not observed. As a control we examined the MDA-MB-231 cells and showed the accumulation of mitotic and micronucleated cells following treatment with microtubule inhibitors. Data is summarized in Table 2.

If the T47D cells have a functional mitotic checkpoint, why does the population not show an increase in mitotic index after treatment with microtubule inhibitors as expected? We believe that disruption of microtubule morphology in these cells results in a cell cycle block prior to mitosis. Therefore, previous studies that looked only for an increase in mitotic index as an indicator of a functional mitotic spindle checkpoint would have erroneously concluded that the T47D cells exhibited a spindle assembly checkpoint defect.

Our results indicate that the T47D cell line is not a suitable marker cell line to examine spindle assembly checkpoint defects. The cell line may still be a useful human breast tumor cell line to examine the effects of the Pim1 inhibitors, however.
TABLE 2
Effect of Microtubule Inhibitors on T47D and MDA-MB cells

<table>
<thead>
<tr>
<th>Time</th>
<th>Cells</th>
<th>Drug</th>
<th>Mitotic Index</th>
<th>Multinucleation Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>T47D</td>
<td>None</td>
<td>1.2%</td>
<td>Not detected</td>
</tr>
<tr>
<td>8 hr</td>
<td>T47D</td>
<td>Nocodazole</td>
<td>2.5%</td>
<td>Not detected</td>
</tr>
<tr>
<td>24 hr</td>
<td>T47D</td>
<td>Nocodazole</td>
<td>3.2%</td>
<td>&lt;1.0%</td>
</tr>
<tr>
<td>8 hr</td>
<td>T47D</td>
<td>Taxol</td>
<td>3.6%</td>
<td>Not detected</td>
</tr>
<tr>
<td>24 hr</td>
<td>T47D</td>
<td>Taxol</td>
<td>4.9%</td>
<td>2.5%</td>
</tr>
<tr>
<td>0</td>
<td>MDA</td>
<td>None</td>
<td>2.8%</td>
<td>Not detected</td>
</tr>
<tr>
<td>8 hr</td>
<td>MDA</td>
<td>Nocodazole</td>
<td>16.7%</td>
<td>&lt;1.0%</td>
</tr>
<tr>
<td>24 hr</td>
<td>MDA</td>
<td>Nocodazole</td>
<td>1.6%</td>
<td>79.0%</td>
</tr>
<tr>
<td>8 hr</td>
<td>MDA</td>
<td>Taxol</td>
<td>13.9%</td>
<td>&lt;1.0%</td>
</tr>
<tr>
<td>24 hr</td>
<td>MDA</td>
<td>Taxol</td>
<td>2.2%</td>
<td>75.6%</td>
</tr>
</tbody>
</table>

**Effects of synthetic peptides on the growth of human breast cancer cell lines**

The effects of the Pin1 inhibitory peptides on cell morphology were examined following incubation with 10 or 100 μM concentrations of each peptide for 24-72 hours. In addition to the three breast cancer cell lines (MCF-7, MDA-MB 231, and T47D), we also examined the effects on Chinese hamster ovary (CHO) and human HeLa cells. Microscopic examination showed normal cell morphology at all time points with all cell lines examined.

To examine any effects of the synthetic peptides on cell growth, we used a modification of the MTT growth assay as described (7). Briefly, cells were seeded into wells of a 96-well plate overnight and then incubated in the presence of each synthetic peptide (10 μM) for 48 hours. No change in cell growth was detected for any of the peptides.

These results indicate that the Pin1 inhibitory peptides at concentrations up to 100 μM had no deleterious effects on cell morphology or cell growth under normal culture conditions.

**Effects of the synthetic peptides on S phase checkpoint abrogation of human breast cancer cell lines**

The concentration of hydroxyurea required to block cell growth was determined for each cell line using the MTT growth assay (see above). Cells were seeded and grown overnight prior to the addition of hydroxyurea at concentrations ranging from 0.25 to 8.0
mM. Cell growth in comparison to a control population of cells was determined after 48 hours of additional incubation. T47D, MCF-7, and MDA-MB 231 cells were arrested in S phase following an overnight incubation in the presence of 2 mM hydroxyurea. Pin1 inhibitory peptides were then added at a concentration of 10 and 50 μM, and after further incubation of 4, 8, and 24 hours cells were examined for induction of premature mitosis by fluorescence microscopy after fixation and labeling of DNA with DAPI. No observable changes were detected in the appearance of the cells following incubation with any of the Pin1 inhibitory peptides.

These results indicated that the Pin1 inhibitory peptides were unable to abrogate an S phase arrest induced by hydroxyurea in the breast cancer cell lines. Since there was an absence of growth inhibition, morphological alteration, or abrogation of the S phase checkpoint by the Pin1 inhibitory peptides, we decided to compare the effects of the Pin1 inhibitory peptides with a known S phase abrogating agent. We have shown that the kinase inhibitor UCN-01 is capable of abrogating the S phase arrest induced by hydroxyurea in both CHO and HeLa cells. Checkpoint abrogation by UCN-01 has not been observed in p53 positive cells such as the MCF-7 cell line. Therefore, we decided to limit our experiments with the Pin1 inhibitory peptides to the CHO, HeLa, and T47D cell lines. If and when the Pin1 inhibitory peptides were shown to be effective in any of these cell lines, we would then examine their effects using the two other breast cancer cell lines.

**Effects of the synthetic peptides on S phase checkpoint abrogation: comparison with UCN-01**

We first examined the effects of the Pin1 inhibitory peptides on cell growth and morphology using HeLa and CHO cells. Neither cell line was affected by concentrations of the inhibitory peptides up to 100 μM. The Pin1 inhibitory peptides were also unable to abrogate an S phase arrest induced by 2 mM hydroxyurea in these two cell lines. No evidence for checkpoint abrogation including presence of premature mitosis, formation of multinucleated cells, or induction of apoptosis was detected following examination of the S phase arrested cells by fluorescence microscopy. In contrast, exposure of HeLa or CHO cells to 5 nM UCN-01 readily induced premature mitosis as indicated by induction of mitotic figures and multinucleated cells (Figure 1, appendix).

Interestingly, the T47D breast cancer cells were not sensitive to UCN-01 induced S phase checkpoint abrogation. These results are more typically associated with p53 positive cells like the MCF-7 cell line (8), and bring into question the p53 status of the T47D cell line.

The results obtained from this analysis indicated that the Pin1 inhibitory peptides had no effect on S phase checkpoint abrogation in any of the cell lines tested. Further experiments to analyze potential effects of the peptides on the S phase checkpoint were abandoned. Since the mechanisms regulating the S phase checkpoint are likely different than those involved in maintaining the G2 phase DNA damage checkpoint, further experiments were designed to examine the possible effects of the Pin1 inhibitory peptides on abrogation of the DNA damage checkpoint.

**Effects of UCN-01 on G2 DNA damage checkpoint abrogation**
As a control for the potential effects of the Pin1 synthetic peptides on G2 checkpoint abrogation, we examined the effects of UCN-01 on HeLa cells arrested at G2 following DNA damage induced by bleomycin. Cells were treated with bleomycin (40 nM) for 24 hours followed by the addition of UCN-01 (5 nM) for specified times. Indirect immunofluorescence microscopy of cells labeled for microtubules, kinetochores, and DNA showed that UCN-01 induced premature mitosis within 2 hours of exposure (Figure 2, appendix). The temporal induction of premature mitosis and subsequent multinucleation following UCN-01 treatment of G2 DNA damage checkpoint arrested was quantified (Figure 3). Further we examined the DNA profiles of bleomycin and UCN-01 treated cells by flow cytometry (Figure 4). The immunofluorescence data clearly shows that UCN-01 treatment rapidly abrogated the DNA damage checkpoint inducing premature mitosis within 1 hour of exposure. Abnormal cytokinesis and appearance of multinucleated cells became apparent within 3-4 hours. Abrogation of the checkpoint was confirmed by the flow cytometry data. Bleomycin treatment induced a G2 arrest as shown in panel B of Figure 4. By 8 hours a significant increase in the G1 DNA peak was apparent, which increased further by 12 hours.

The results obtained from the bleomycin and UCN-01 treatment sequence was used as the basis for a comparative analysis of cells treated with bleomycin followed by exposure to the Pin1 synthetic peptides.

**Effects of the synthetic peptides on DNA damage checkpoint abrogation**

HeLa cells examined by immunofluorescence microscopy after exposure to the Pin1 synthetic peptides following bleomycin induced DNA damage and arrest in G2 phase showed no signs of altered morphology within 24 hours (data not presented). This result was in stark contrast to the results obtained following UCN-01 treatment as described in the previous section. Mitotic cells and apoptotic cells were present in the samples following extended periods of treatment (48 and 72 hours), however, similar morphologies were also observed in the control population of cell exposed only to bleomycin. The G2 arrest induced by DNA damaging agents is known to be transient (9), and the increase in mitotic and apoptotic cells we observed at the later time points were the result of the ‘natural’ breakdown of the G2 phase DNA damage checkpoint.

Analysis of the flow cytometry data, however, indicated that the Pin1 peptides were having an effect on the bleomycin treated cells. A sequence of flow cytometry data from cells treated with bleomycin only shows the transient nature of the G2 arrest (Figure 5). The DNA profiles show a loss of the G2/M phase peak after extended time periods. When compared with the DNA profiles of cells exposed to bleomycin for 24 hours and then treated with Pin1 inhibitory peptide, a more rapid shift or loss of the G2/M peak was apparent (Figure 6). The greatest effect was observed with the P4 peptide (Figure 6), which was predicted to have the greatest potential for inhibition of Pin1. In contrast, peptide P1 treatment had no effect on the temporal sequence of DNA profiles following bleomycin treatment. The P1 peptide was predicted to have the least potential activity as a Pin1 inhibitor.

We conclude from this analysis that the Pin1 peptides have a weak, but reproducible, effect on the G2 phase DNA damage checkpoint. It appears that inhibition
of Pin1 may enhance the temporal decay of the DNA damage checkpoint. At the present time we are referring to this effect as being a modulatory effect on the DNA damage checkpoint. Our results indicate, therefore, that Pin1 plays some role in DNA damage checkpoint maintenance. However, to further explore the role of Pin1 in this process will require a more extensive analysis of the inhibitory activity of the synthetic peptides, and design of more effective inhibitors, both of which were beyond the scope of the present proposal.

KEY RESEARCH ACCOMPLISHMENTS:

- Demonstrated lack of spindle checkpoint defect in T47D cell line

- Demonstrated lack of toxicity associated with treatment of cells with the Pin1 inhibitory peptides

- Demonstrated failure of the Pin1 inhibitory peptides to abrogate S phase checkpoint

- Demonstrated ability of the Pin1 inhibitory peptides to modulate the G2 arrest following induction of the DNA damage checkpoint after bleomycin treatment.

REPORTABLE OUTCOMES:

At the present time none of the data obtained from these studies has been prepared for publication. Since almost all of the results obtained in this study were negative or of minimal effect, it is unlikely that a publication will be forthcoming unless additional experiments with more effective inhibitory peptides are carried out. It is likely that the results obtained from the DNA damage checkpoint studies will be used as preliminary data to support portions of a new research grant application to the NIH.

A single poster presentation entitled "Analysis of UCN-01 Abrogation of DNA Damage Checkpoint in HeLa Cells", which reported some of the data obtained in these studies was made internally at Ohio State University College of Medicine

CONCLUSIONS:

Our results indicate that inhibition of Pin1 prolyl isomerase using synthetic peptides designed as competitive substrate inhibitors has only limited effects on checkpoint regulation of the cell cycle in human cells. We found no evidence that the Pin1 inhibitory peptides had any effect on the S phase checkpoint induced following hydroxyurea treatment. In comparison, UCN-01 was a very effective S phase checkpoint-abrogating agent. Limited DNA damage checkpoint modulating activity was observed following exposure of bleomycin treated cells to the Pin1 inhibitory peptides. Bleomycin treatment causes DNA damage that activates the DNA damage checkpoint and induces a transient G2 phase arrest. We demonstrated that the Pin1 inhibitory peptides could shorten the length of the bleomycin induced G2 arrest. However, the effects of the Pin1 inhibitory peptides were relatively mild in the sense that there was
only a minor temporal effect on the transient nature of the bleomycin induced G2 arrest. In comparison, UCN-01 treatment caused a rapid abrogation of the DNA damage checkpoint G2 arrest induced by bleomycin. Despite the lack of significant effect, the results do show that the Pin1 inhibitory peptides examined in this study had some modulatory role. At the present time we have not determined the effectiveness of the inhibitory peptides at inhibiting Pin1 activity in vitro, nor have we determined the effective concentration of peptide in the treated cells. Therefore future studies may be warranted that examine the Pin1 inhibitory activity of the peptides used in this study. In addition, a second generation of inhibitory peptides with greater Pin1 inhibitory activity could be designed that may have greater activity when applied to cells in culture. The present studies do not support the utilization of the Pin1 inhibitory peptides tested as potential chemotherapeutic agents, but do support the possibility that Pin1 may play a role in DNA damage checkpoint maintenance. This would support the development of more active Pin1 inhibitory compounds for future testing.

REFERENCES:


APPENDICES:

Figure 1. Induction of premature mitosis by UCN-01 in hydroxyurea treated S phase arrested HeLa cells. Cells were stained for microtubules (Panels A, D, and G), kinetochores (Panels B,E, and F), and DNA (Panels C, F, and I). Premature mitotic metaphase cells (Panels A, B, and C) formed spindles with the kinetochores aligned at the metaphase plate (Panel B arrow). Some kinetochores failed to align properly (Panel B, arrowheads). Most of the DNA (Panel C, arrow) was not associated with the kinetochores (Panel C, arrowhead). Defective cytokinesis was common (Panels D, E, and F). Many kinetochores were caught in the midbody (Panel E, arrow), as well as some of the chromosomal DNA (Panel F, arrow). The completion of premature mitosis resulted in the generation of multinucleated cells (Panels G, H, and I). As can be observed most of the kinetochores are present in clusters not associated with the bulk of the DNA (Panels H and I, arrowheads). These results show that induction of premature mitosis from S phase causes the formation of detached kinetochores.

Figure 2. Induction of premature mitosis by UCN-01 in bleomycin treated G2 arrested HeLa cells. Cells were stained for microtubules (Panels A, D, and G), kinetochores (Panels B,E, and F), and DNA (Panels C, F, and I). Control cells (Panels A, B, and C) show normal bipolar metaphase spindles and alignment of kinetochores and DNS along the metaphase plate. Bleomycin only treated cells (Panels D, E, and F) show enlarged nuclei and duplicated kinetochores, but few mitotic figures indicative of the G2 phase arrest. Following treatment with UCN-01 for 2 hours (Panels G, H, and I) premature mitotic cells are present. Kinetochore and chromosomal alignment within the bipolar spindle is defective.

Figure 3. Effects of UCN-01 after bleomycin induced G2 checkpoint arrest. Morphological evaluation of HeLa cells treated with bleomycin or bleomycin plus UCN-01 for various periods of time was determined. As can be seen, premature mitotic cells were induced by UCN-01 within 1 hour of exposure to the G2 arrested cells. Peak induction of mitotic figures was observed after 2 hours. Subsequently there was an increase in telophase and multinuclear cells indicating completion of mitosis.

Figure 4. Flow cytometry analysis of bleomycin and bleomycin plus UCN-01 treated cells. Panel A shows the DNA profile of a control population of cells. Panel B shows cells treated only with bleomycin for 24 hours. Panels C, D, and E show bleomycin arrested cells treated with UCN-01 for 2, 8, and 12 hours respectively. As can be seen, bleomycin effectively arrests cells in G2 (Panel B). escape from the G2 arrest following UCN-01 treatment is apparent by the increased G1 DNA peak shown in panels D and E.
Figure 5. Flow cytometry analysis of bleomycin treated cells. Panel A shows the DNA profile of a control population of cells. Panels B, C, D, and E show cells treated only with bleomycin for 24, 48, 72, and 96 hours respectively. In this experiment, bleomycin affectively arrests cells in G2 (Panel B), although some G1 cells remained in the population at this time point. By 48 hours, the G2 arrest is complete (Panel C). Escape from the G2 arrest and generation of apoptotic cells is shown by the increase in the sub G1 peak of DNA in Panels D and E. Note that in this experiment a significant population of G2 arrested cells remained even after 96 hours of bleomycin treatment (Panel E).

Figure 6. Flow cytometry analysis of cells treated with bleomycin and Pin1 inhibitory synthetic peptide 4. The samples examined were collected from the same set of cells depicted in Figure 5. Bleomycin and the inhibitory Pin1 peptide were added together at time 0, and samples were collected at 24, 48, 72, and 96 hours (Panels A, B, C, and D respectively). After 24 hours a slight increase in S and G2 DNA profiles were present (Panel A). At subsequent time points (Panels B, C, and D) an increase in sub G1 DNA is observed when compared to the corresponding samples treated with bleomycin alone (see Figure 5, Panels C, D, and E). Note that few if any G2 arrested cells are present after 96 hours, whereas a significant G2 population is still present in the cells treated with bleomycin alone. These results indicate that inhibition of Pin1 as a result of inhibitory peptide treatment was capable of enhancing the breakdown of the DNA damage G2 arrest induced by bleomycin.
Figure 5