Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting information

Enhanced proteotoxic stress: one of the contributors for hyperthermic potentiation of the proteasome inhibitor bortezomib using magnetic nanoparticles

1. Dose-response curve for BZ

Cells ($1x10^3$) were seeded in 96-well plates (Fisher Scientific, Suwanee, GA) for 48h in DMEM solution. Adhered cells were exposed to BZ concentrations ranging between 2-1000 nM for 48h. This exposure time was chosen because cells analyzed by clonogenic assay were exposed to BZ for 48 hr. Subsequently, cells were washed twice with HBSS buffer, stained with CellTiter Blue™ (Promega, Madison, WI) and analyzed using a spectrofluorometer (Spectra MAX Gemini EM, molecular devices, Sunnyvale, California).[4]

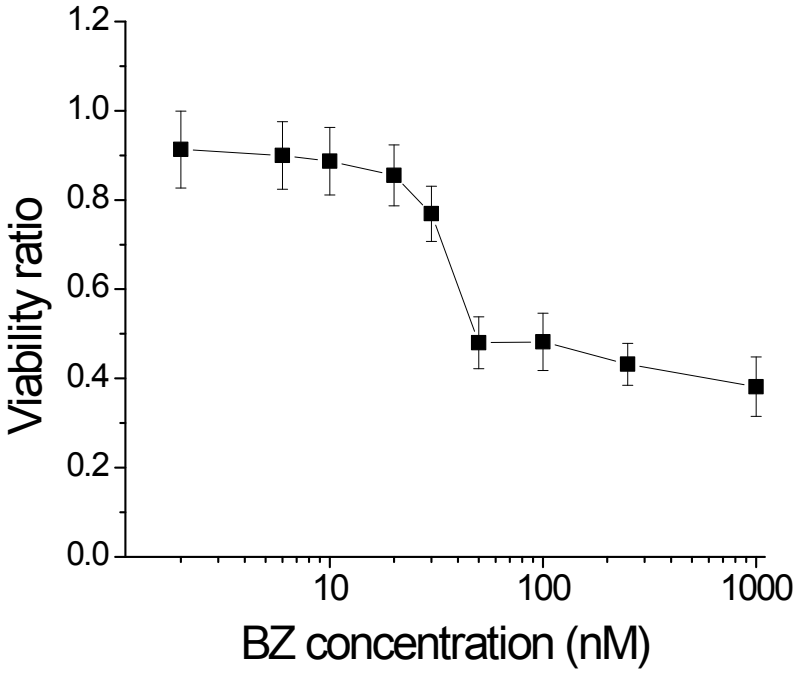

Figure 1. Dose- response curve for MCF-7 cells after exposure for 48 hours to various BZ concentrations (2-1000nM). Error bars represent the standard error of eight independent experiments.

MCF-7 cells had a low response to BZ in the concentration range between 2-30 nM. However, above 30 nM cell viability showed a significant decrease, with a minimum cell viability of approximately 40% at 1000 nM. MCF-7 cells showed an IC_{50} value of 100 nM, which coincides with previous studies.[13]

2. Proteasome activity

To obtain a BZ concentration that significantly inhibits the activity of the proteasome before hyperthermic treatment (cells were pretreated with BZ for 3hr), proteasome activity curve at 3hr was determined using a fluorogenic substrate.[4] Briefly, cells ($1x10^3$) were seeded in 96 well-

plates for 48h in DMEM solution. Then, cells were exposed to concentrations of bortezomib between 2nM and 1000nM for 3h. After this incubation period, 100 µL Proteasome-Glo™ Chymotrypsin-Like Assay solution (Promega, Madison, WI) was added and samples were incubated for 15 minutes in the dark at room temperature. Afterwards, luminescence was measured in a spectrofluorometer (Spectra MAX Gemini EM, molecular devices, Sunnyvale, California).

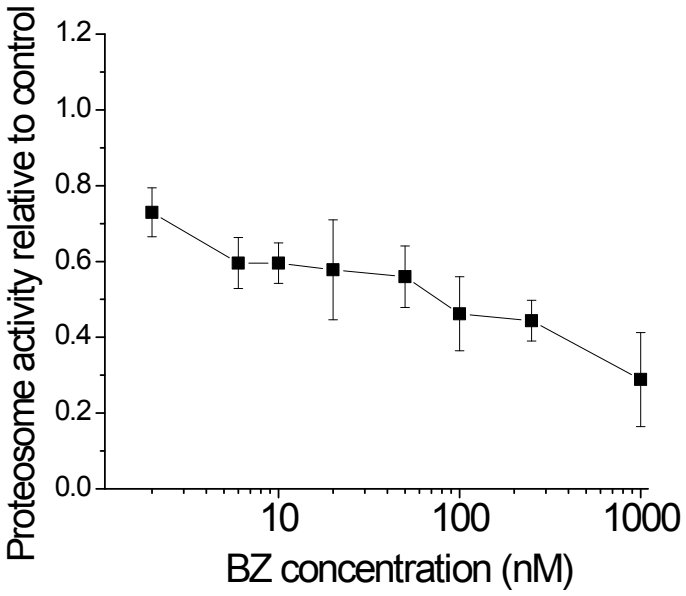

Figure 2. Proteasome activity curve as a function of BZ concentration for an exposure time of 3 hours. Error bars represent the standard error of the mean of three independent experiments.

Proteasome activity is concentration dependent, as illustrated in Figure 6. Higher BZ concentrations produced higher inhibition of the proteasome. For example, for this particular case, proteasome inhibition at a concentration of 2 nM was approximately 25%, whereas at 1000 nM it was found to be 70%. This behavior in proteasome activity as a function of BZ

concentration is in agreement with past studies, however at higher BZ concentration the viability is significantly affected.[4, 24] For subsequent experiments, the IC_{50} concentration (100 nM) was employed.

3. Cell cytotoxicity after 48 hr of recovery time in presence of BZ

After hyperthermic treatment, 100,000 cells were seeded in six-well tissue culture plates in presence of BZ and left in the incubator for 48 hours. Afterwards, cells were trypsinized and cell viability was measured by cell counting using trypan blue (Sigma, St. Louis, MO).

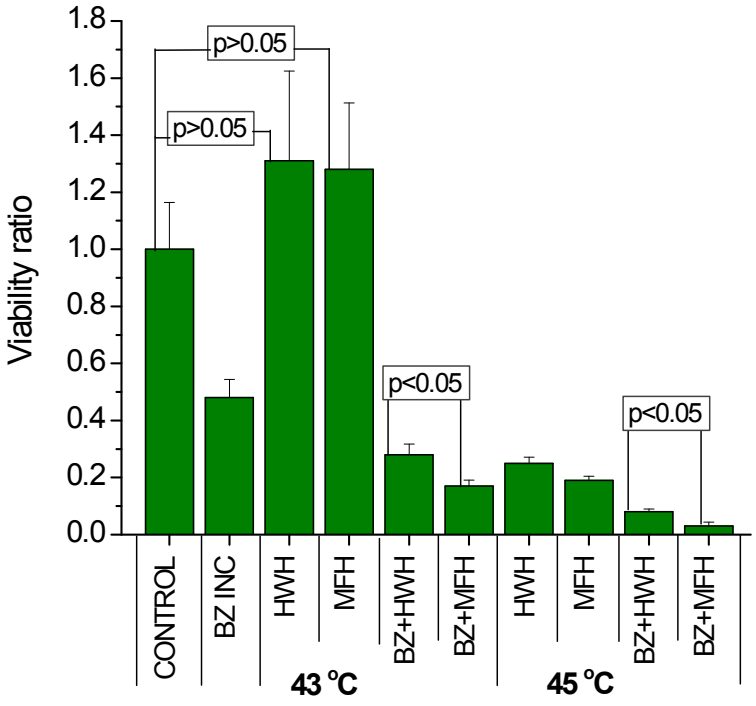

Figure 3. Viability ratio for MCF-7 cells pretreated or untreated with 100 nM of BZ for 3 hours, exposed to hyperthermia with or without BZ at 43 ºC or 45 ºC for 30 minutes and left to recover for 48 hr n presence of BZ. Error bars represent the standard error of three independent experiments.

Cell death was much greater for those samples treated by MFH or HWH in combination with BZ (Figure 7). At 43 ºC the viability ratio was 28 ± 10% for HWH+BZ and 17 ± 3% for MFH+BZ (statistically different with $p<0.05$) and at 45 ºC the viability ratio was 8 ± 1.7% for HWH+BZ and 3 ± 2.4% for MFH+BZ (statistically different with $p<0.05$).

4. Nuclear fragmentation analysis

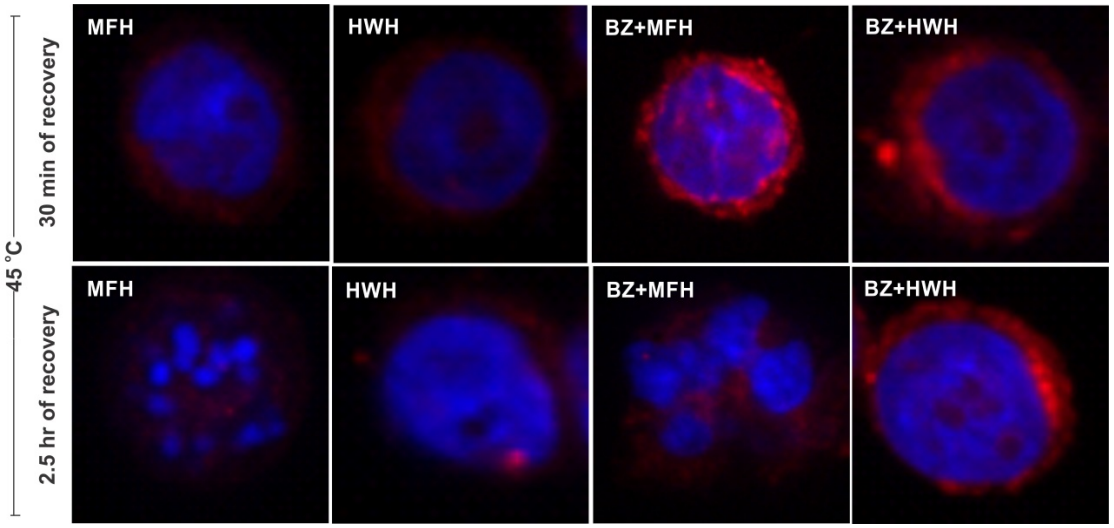

Figure 4. Illustration of nuclear fragmentation at 45 ^{o}C: Images were obtained by zooming in on individual cells at the different conditions.

At 30 minutes of recovery time cells treated by hyperthermic treatments with or without BZ did not show nucleus fragmentation. At 2.5 hr of recovery time cells treated by HWH or BZ+HWH apparent to have intact nucleus, however, nuclear fragmentation was evident in cells treated by MFH with or without BZ at this condition.

Table 1. Microtubles damage and aggresome formation at 45ºC

Treatment	Aggresome formation	Microtubules damage
HWH or BZ+HWH (45 ºC, 30 minutes of recovery time)	HWH alone did not induce aggresome formation (APF<25), however BZ+HWH had the capacity to form aggresome (APF>25)	HWH induces microtubule damage
MFH or BZ+MFH (45 ºC, 30 minutes of recovery time)	MFH alone did not induce aggresome formation (APF<25), however BZ+MFH had the capacity to form aggresome (APF>25)	MFH induces microtubules damage
HWH or BZ+HWH (45 ºC, 2.5 hr of recovery time)	No statistically significant difference was observed when compared to 30 minutes recovery time.	HWH induces microtubule damage
MFH or BZ+MFH (45 ºC, 2.5 hr of recovery time)	No statistically significant difference was observed when compared to 30 minutes recovery time.	MFH induces microtubules damage

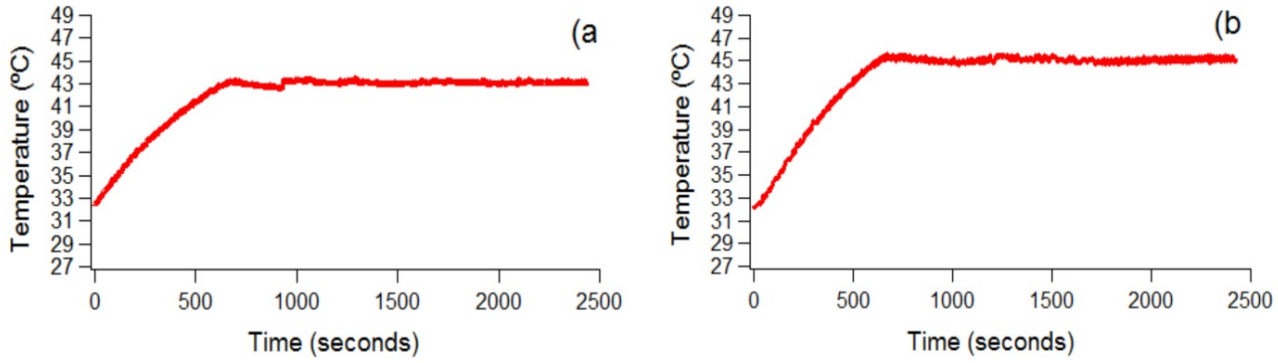

Figure 5. Temperature profile for cells treated by MFH at 43 ^{o}C (a) and 45 ^{o}C (b).