Synergistic antiproliferative activity of the RAD51 inhibitor IBR2 with inhibitors of receptor tyrosine kinases and microtubule protein

Peter J. Ferguson, Mark D. Vincent, and James Koropatnick

Journal of Pharmacology and Experimental Therapeutics

Supplemental Data
Supplementary Figure 1. Compilation of data from numerous experiments demonstrating the concentration-dependent ability of IBR2 to inhibit the proliferation of a variety of cancer cell lines. Cells were grown in 96-well plates, and treated with a range of concentrations of IBR2. The relative cell density was determined after 4 days by viability staining. These data were obtained from control treatments for cells receiving a combination of IBR2 and another agent. Panel A, lung cancer cell lines; Panel B, gastric and colon cancer cell lines; Panel C, leukemia and HNSCC cancer cell lines, plus HEK293; Panel D, breast and prostate cancer cell lines.
Supplementary Figure 2. Effect of IBR2 on cell cycle progression. Panel A: A549b cells were incubated in IBR2 for 3, 4 or 5 d, at which time cell density was determined using alamarBlue and plotted as a percent of untreated controls. Determination of cell cycle distribution was undertaken simultaneously (Panel B): A549b cells were incubated in IBR2 for 24 h, followed by harvesting with trypsin, washing with PBS, and fixing in 70% ethanol. Fixed cells were stained with propidium iodide and analyzed with a flow cytometer (10,000 events). The percent of cells in each of G1, S, and G2/M phases was determined using the curve-fitting program FlowJo. Data are means of 3 separate experiments; error bars, SD.
Supplementary Figure 3. Quantification of enhancement in drug toxicity caused by presence of a second agent. The relative cell density of combination-treated, A549b cells was determined as a percent of the respective control, such that the values for the cells treated with imatinib plus 10 μM IBR2 were normalized to those of the cells treated with 10 μM IBR2 alone. Therefore, each IBR2 treatment appears as 100%. If IBR2 has no effect on inhibition of proliferation caused by the second agent, the 2 dose-response curves overlap. In this example, IBR2 caused a leftward shift in the dose-response of imatinib, meaning that the cells were rendered more sensitive to imatinib by the presence of IBR2. The concentration of imatinib that reduced the final cell density by 50% (IC_{50} value) was determined by interpolation. The change in this value was calculated as a percent of the original IC_{50} value, and this number was used to generate subsequent figures. It is understood that the choices of “drug” and “enhancing agent” (or “second agent”) are arbitrary, and the numbers could be used to indicate that imatinib in fact enhances IBR2 cytotoxicity. IBR2, as well as verapamil and B02, are considered to be the enhancing agent for 2 reasons: (1) they were tested in combination with many different chemotherapy drugs, and so for purposes of clarity they were designated as the second agent in each of these assays, to facilitate comparisons between experiments; (2) it was observed that in many instances the second agent is able to enhance drug toxicity when the second agent is used at concentrations that are on their own non-toxic. This suggests enhancement of the drug toxicity. Note also that the second agent often has no effect on the drug, in which case the change in IC_{50} value is zero, and in some instances the second agent interferes with or antagonizes the toxicity of the drug, in which case the change in IC_{50} value is a positive value (for examples see Supplementary Figure 9).
Supplementary Figure 4. Representative experiments demonstrating the ability of IBR2 to enhance the antiproliferative activity of imatinib against a variety of cancer cell lines: Panel A, AGS gastric adenocarcinoma; Panel B, HT-29 colon carcinoma; Panel C, H69 NSCLC. Cells were grown in 96-well plates, and treated with simultaneous exposure to the drugs at the concentrations indicated. The relative cell density was determined after 4 days by viability staining. The relative density of cells treated with both agents was normalized to the density observed for the respectively treated IBR2 control. The concentration of drug that inhibited proliferation by 50% was interpolated from plotted data. The change in the IC$_{50}$ value caused by the presence of IBR2 was determined as a percent of the IC$_{50}$ of imatinib in the absence of IBR2, as described in Supplementary Figure 1, to generate the graphs presented in Figures 2 - 7. Points are means of triplicate values within one experiment.
Supplementary Figure 5. Representative experiments demonstrating the ability of IBR2 to enhance the antiproliferative activity of regorafenib against a variety of cancer cell lines: Panel A, MCF7 breast carcinoma; Panel B, Hs746T gastric carcinoma; Panel C, K562 CML; Panel D, H69 NSCLC. Cells were grown in 96-well plates, and treated with simultaneous exposure to the drugs at the concentrations indicated. The relative cell density was determined after 4 days by viability staining. The relative density of cells treated with both agents was normalized to the density observed for the respectively treated IBR2 control. Points are means of triplicate values within one experiment.
Supplementary Figure 6. Representative experiments demonstrating the ability of IBR2 to enhance the antiproliferative activity of gefitinib against a variety of NSCLC cancer cell lines: Panel A, A549b; Panel B, H1650; Panel C, H1975. Cells were grown in 96-well plates, and treated with simultaneous exposure to the drugs at the concentrations indicated. The relative cell density was determined after 4 days by viability staining. The relative density of cells treated with both agents was normalized to the density observed for the respectively treated IBR2 control. Points are means of triplicate values within one experiment.
Supplementary Figure 7. Enhancement of olaparib, but not IBR2, cytotoxicity by anti-BRCA2 siRNA. A549 NSCLC or DU145 prostate carcinoma cells were grown in 25-cm² flasks, and allowed to plate overnight. siRNA [scrambled control (sc-2) or anti-BRCA2 (BR-a)] was introduced into cells with the use of LFA2K. The siRNA concentration was 3 nM on A549b or 5 nM on DU145. After incubating (37°C) on the cells for 4 hours, a second volume of medium was added. Cells were then incubated for 20 h, after which the siRNA-containing medium was replaced with growth medium with or without (siRNA-only control) 1 μM olaparib (Panel A) or a range of concentrations of IBR2 (Panel B). Cell number was determined after 4 d by enumeration with a particle counter. Proliferation (fold-increase in cell number) was calculated as a percent of that of the respective drug-free, siRNA-treated control.
Supplementary Figure 8. Representative experiments demonstrating the interaction between IBR2 and olaparib against a variety of cancer cell lines: Panel A, A549b NSCLC; Panel B, DU145 prostate carcinoma; Panel C, HT-29 colon carcinoma; Panel D, MDA-MB-468 breast carcinoma. Cells were grown in 96-well plates, and treated with simultaneous exposure to the drugs at the concentrations indicated. The relative cell density was determined after 4 days by viability staining. The relative density of cells treated with both agents was normalized to the density observed for the respectively treated IBR2 control. The effect of IBR2 on olaparib cytotoxicity was negligible under most conditions. Points are means of triplicate values within one experiment.
Supplementary Figure 9. IBR2 causes an increase in IC\(_{50}\) value for some drugs, depending on the cell line, indicative of antagonism against the cytotoxic activity. Cultured cells were exposed to drugs as described in the legend of Suppl. Fig 4, and the change in IC\(_{50}\) value was quantitated as described in Suppl. Fig. 3. A positive value for the change in IC\(_{50}\) (i.e., increase in the IC\(_{50}\)) is indicative of antagonism. Experiments are of limited number: paclitaxel - A549b and HT-29, n=2 or 3 per point, HN-5a/V15e, n = 1 or 2; irinotecan and melphalan - n = 1 or 2; cisplatin - A549b, n=2, HT-29, 1. The IC\(_{50}\) values were: for paclitaxel, A549b – 3.2 +/- 0.3 nM (4), HT-29 – 3.8 +/- 0.2 nM (4), HN-5a/V15e, 27.2 +/- 10.5 nM (3); for irinotecan, A549b – 2.59 +/- 0.05 μM (2), HT-29 – 4.63 μM (1); for melphalan, A549b – 8.5 +/- 1.1 μM (2); for cisplatin, A549b – 2.9 +/- 0.6 μM (2), HT-29 – 2.1 μM (1).
Supplementary Figure 10. Effect of 17-AAG on proliferation of cancer cell lines or transformed human embryonic kidney cell line (HEK293). Cells were cultured in 96-well plates, and exposed for 4 days to 17-AAG. Live cell density was determined using the vital stain alamarBlue, and relative density calculated as a percent of that of untreated cells. Points are means of up to 13 determinations; bars, SD. IC$_{50}$ values (Panel B, n= 8 to 13) were determined by interpolation of plotted data, as demonstrated in Supplementary Figure 3.
Supplementary Figure 11. IC\textsubscript{50} values for chemotherapy drugs used in combination experiments with 17-AAG. Cells were cultured in 96-well plates, and exposed for 4 days to the drug indicate. Live cell density was determined using the vital stain alamarBlue, and relative density calculated as a percent of that of untreated cells. Points are means of up to 15 determinations; error bars, SD. IC\textsubscript{50} values were determined by interpolation of plotted data, as demonstrated in Supplementary Figure 3.
Supplementary Figure 12. Effect of 17-AAG on inhibition of proliferation by anticancer agents. Cells cultured in 96-well plates were incubated with a combination of 17-AAG and the indicated drug. IC$_{50}$ values were interpolated from plotted data, and the change in IC$_{50}$ caused by 17-AAG was determined as described in Supplementary Figure 3. IC$_{50}$ values for imatinib are indicated in Figure 1. 17-AAG did not significantly alter the IC$_{50}$ value for afatinib against HEK293 (10.8 +/- 1.4 μM) (n=2). IC$_{50}$ values for the drugs are indicated in Figure 1 and Supplementary Figure 11. For this set of experiments, the IC$_{50}$ value of vincristine against A549b was 4.7 +/- 0.3 nM (n=3).
Supplementary Figure 13. Representative experiment demonstrating the effect of combining vincristine with IBR2 or 17-AAG against HEK293. Vincristine was much less inhibitory of proliferation against HEK293 than against the cancer cell lines, such that it was not possible to determine an IC$_{50}$ up to 150 nM. Neither IBR2 at concentrations that inhibited proliferation by up to 40%, or 17-AAG, which inhibited proliferation by 10-15%, enhanced the activity of vincristine. At some combinations of concentrations, the inhibitory effect of vincristine was decreased. Each point is the mean of triplicate wells in a single experiment. Errors are smaller than the size of the symbol.