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Full paper

Synergistic cytotoxicity from combination of imatinib and platinum-based anticancer drugs specifically in Bcr-Abl positive leukemia cells

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ABSTRACT

Imatinib, a multitargeted tyrosine kinase inhibitor, exhibits potent anticancer activity against leukemia harboring the Bcr-Abl oncogene and some solid tumors overexpressing c-kit and PDGFR. However, its clinical efficacy is severely compromised by the emergence of resistance primarily due to acquired mutations in the Bcr-Abl kinase domain. In this study, we showed that combination of imatinib with platinum (Pt)-based anticancer agents, including cisplatin and oxaliplatin, exhibited synergistic cytotoxic effect specifically in Bcr-Abl^{+} human chronic myeloid leukemia cell line K562 but not in Bcr-Abl^{-} RPMI8226 cells. Importantly, the synergistic effect was also found to circumvent imatinib resistance in an imatinib-selected resistant subline K562 ima1.0. The combination treatment increased apoptosis and DNA damage. Mechanistic study revealed that increased inhibition of Bcr-Abl and downstream ERK phosphorylation by the drug combination may contribute to the synergistic effect.
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1. Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder resulting from the clonal expansion of a transformed multipotent hematopoietic stem cell. CML is characterized by the Philadelphia chromosome (Ph) resulting from a balanced translocation between chromosome 9 and 22, which leads to the formation of the Bcr-Abl fusion oncogene. The dysregulated Bcr-Abl oncoprotein interacts with other cytoplasmic molecules and leads to activation of downstream signaling pathways, Ras–ERK (extracellular signal-regulated kinase), phosphatidylinositol 3-kinase (PI3K)–Akt and signal transducer and activator of transcription 5 (STAT5), thereby driving cancer cell survival and proliferation (1). Imatinib, approved by FDA in 2001, is an effective molecular targeted drug for treating CML. Working as a competitive ATP inhibitor, imatinib specifically binds to the inactive conformation of Abl (2), blocks all Bcr–Abl dependent phosphorylation and signaling pathways, leading to cell apoptosis (3).

Resistance to imatinib was evident not long after the introduction of the drug into clinical practice and it results in treatment failure. A number of resistance mechanisms have been reported (4). Combination of imatinib and multiple anticancer agents with differing mechanisms of action has been investigated to circumvent chemoresistance. While synergistic effect was observed in the combination between imatinib and other standard CML anticancer drugs (e.g. interferon-α and cytarabine), other drug combinations were only additive or even antagonistic (5). The combinations of imatinib with other molecularly targeted agents, including histone deacetylase inhibitors, proteasome inhibitors, aminopeptidase inhibitors, TNF-α related apoptosis inducing ligands (5), and COX-2 inhibitor (celecoxib), have also been studied (6).

Platinum (Pt)-based anticancer drugs, exemplified by cisplatin and oxaliplatin, are the mainstay of treatment for solid tumors. Pt-based drugs bind to N7 on purine nucleotides, and cause DNA damage, subsequently resulting in cancer cell apoptosis (7). Combinations of imatinib with Pt-based drugs have been studied in solid tumor cell lines. Synergistic cytotoxic effect has been reported for combination of cisplatin and imatinib in head and neck cancer (7) and metastatic nasophayngeal carcinoma (8). Combination of carboplatin/paclitaxel and imatinib was also found to produce synergistic effects in ovarian cancer cell lines (8). Imatinib can potentiate the anticancer activity of cisplatin in lung cancer *in vitro* (9), and the combination can synergistically inhibited the growth of

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the non-small cell lung cancer cell line A549 (10). Moreover, imatinib was reported to alleviate cisplatin-induced nephrotoxicity in rats, suggesting that imatinib may reduce the renal accumulation of cisplatin (11). As for hematological cancers, it was reported that imatinib could sensitize Bcr-Abl$^+$ cells to cisplatin by simultaneous inhibition of p53 transactivation and reduction of Bcl-xL (12). In Bcr-Abl$^-$ human leukemia cell line HL-60, imatinib and PRI-2191 (a calcitriol analog) were found to enhance the cytotoxic effect of cisplatin (13). The precise mechanism(s) contributing to the enhanced anticancer effect in some of these studies is not well defined.

In this study, the combination effect of imatinib and Pt-based anticancer drugs (cisplatin and oxaliplatin) was investigated in Bcr-Abl positive (+) (K562), negative (−) (RPMI8226) cells and in an imatinib-selected resistant Bcr-Abl$^+$ subline (K562 ima1.0). Combination of imatinib with cisplatin/oxaliplatin was found to exhibit synergistic cytotoxic effect in the Bcr-Abl$^+$ but not in the Bcr-Abl$^-$ cells. Importantly, the synergistic effect was found to circumvent imatinib resistance in the imatinib-selected resistant subline (K562 ima1.0). The combination treatment was found to increase apoptosis and DNA damage. Mechanistic investigation revealed that increased inhibition of Bcr-Abl and downstream ERK phosphorylation may contribute to the synergistic effect.

2. Materials and methods

2.1. Chemicals

Cisplatin and oxaliplatin were purchased from Acros Organics (Thermo Fisher Scientific, New Jersey, USA). Imatinib and MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) was purchased from FARCO chemical supplies. All other chemicals and solvents were of the best grade available.

2.2. Cell culture

Human leukemia cell lines K562 (Bcr-Abl$^+$ CML) and RPMI 8226 (Bcr-Abl$^-$ multiple myeloma plasma cell leukemia) were kind gifts provided by Dr Susan Bates (National Cancer Institute, NIH, USA). K562 was maintained in DMEM medium whereas RPMI8226 was maintained in RPMI1640 medium supplemented with 10% fetal bovine serum (Life Technologies, Grand Island, NY, USA), 100 units/mL streptomycin sulfate (Life Technologies) and 100 units/mL penicillin G sulfate (Life Technologies), and incubated at 37 °C in 5% CO_2. An imatinib-resistant subline of K562 (K562 ima1.0) was developed by exposing to increasing concentrations of imatinib, starting with an initial concentration of 0.1 μM and gradually increasing to 1 μM. At the time of experiments, K562 ima1.0 was around 15-fold resistant to imatinib, relative to the parental K562 cells.

2.3. Cytotoxicity of individual drugs and their combinations

The cytotoxicity of the drugs or their combinations were evaluated by the standard MTT assay (14). Cells were seeded in 96-well microtitre plates at 7500 cells/well, and all drug treatments were added immediately after cell seeding. The drugs were added simultaneously for the combination study. The ratio of the two drugs in combination was initially determined according to the relative ratio of the IC_{50} values of initial drug alone. Cells were treated with either individual drugs or their combinations for 72 h.

Combination index (CI) was used to evaluate the combination effect based on IC_{50} values obtained from individual drugs alone and combination treatment (15). CI was calculated according to the following equation,

$$CI = (D)com_1/(D)_1 + (D)com_2/(D)_2$$

in which $(D)com_1$ (or $(D)com_2$) is the IC_{50} value for drug1 (or 2) in the combination, $(D)_1$ (or $(D)_2$) is the IC_{50} value from individual drug treatment. The resulting combination index (CI) was then used to determine the outcome of the drug combination effect as (i) additive effect (CI = 1), (ii) synergism (CI < 1), or (iii) antagonism (CI > 1).

Dose-reduction index (DRI) was also calculated to indicate the extent of dose reduction of individual drugs that can be facilitated in a synergistic combination. DRI = 1 indicates no dose reduction, whereas DRI>1 and <1 indicate favorable and unfavorable dose-reduction, respectively. The equation used here is $DRI = (D)_1/(D)com_1$, in which $(D)com_1$ is the IC_{50} value for drug1 in the combination, $(D)_1$ is the IC_{50} value from individual drug treatment (16).

Potential nephrotoxicity of drug combination was also determined by the MTT assay on LLC-PK1 porcine kidney epithelial cell line. Confluent LLC-PK1 cells in 96 wells plates (10,000 cells/well seeded for one day) were treated by various drug combinations for 24 h. Percentage of surviving cells was then estimated by comparing with the no treatment control.

2.4. Western blot analysis

Whole cell lysates prepared from K562 cells were separated by SDS-PAGE and subjected to immunoblot analysis with the respective antibodies (phosphor-Bcr-Abl (Tyr-177) (Cell Signaling Technology, Danvers, MA, USA), PARP, phosphor-ERK1/2 (Thr177/Thr160), ERK1/2, phosphor-histone H_2Ax (Ser139) and GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA)). The blot was developed with WesternBright ECL Western blotting detection kit (Advansta, Menlo Park, CA, USA) and analyzed with the FluorChem Q Imaging System (Alpha Innotech, San Jose, CA, USA).

2.5. Apoptosis assay

K562 cells were treated for 48 h with 0.2 μM imatinib in the presence or absence of cisplatin (10 μM). At the end of the treatment, the cells were collected and washed twice with ice-cold PBS. The extent of apoptosis was determined by using the APC Annexin V apoptosis kit (BD Bioscience, San Jose, CA, USA) according to the manufacturer's instructions. Cells positive for both Annexin V and 7-AAD were considered apoptotic. One-way analysis of variance (one-way ANOVA) with Bonferroni's multiple comparison test was used to compare the differences among various treatment groups. A confidence level of $p < 0.05$ was considered significant.

2.6. Cellular Pt accumulation and DNA platination

Briefly, K562 cells were incubated with 100 μM of the tested Pt drugs at 37 °C for 4 h. In pilot study, both drug accumulation and DNA platination were found to be increased in a linear fashion with increasing drug concentration up to at least 400 μM. Afterward, the cells were washed twice with ice-cold PBS and then harvested in an appropriate lysis buffer. For the determination of drug accumulation, the cell lysates were resuspended in NETN buffer (100 mM NaCl, 20 mM Tris HCl (pH 8.0), 0.5 mM EDTA, 0.5% NP-40), sonicated, and subjected to concentrated HNO_3 digestion before analysis by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Perkin Elmer Optima 4300DV, UK). The absorbance of Pt at 265.95 nm was used for calculation. Protein concentration of the lysate was measured separately by the Bradford method for normalization purpose. For measurement of DNA platination, the cell pellets were resuspended in Tris-Triton buffer (10 mM Tris, 100 mM NaCl, 1% Triton X-100) and subjected to proteinase K/RNase inhibition before isolation of DNA by the standard phenol/chloroform/isoamyl alcohol method. The DNA isolated was then

reconstituted in 100 μL Tris–EDTA buffer before measurement of DNA-bound Pt concentration by ICP-OES as described above. DNA concentration of the samples was measured separately by UV spectroscopy for normalization.

3. Results

3.1. Combination of imatinib with Pt-based anticancer agents shows synergistic cytotoxic effect in K562 cells expressing the Bcr-Abl oncoprotein

3.1.1. Cell line models

Three leukemia cell line Bcr-Abl^+ K562, its imatinib-selected resistant subline (K562 ima1.0) and Bcr-Abl^- RPMI 8226 were used in this study. K562 ima1.0 selected cell line was obtained by incubating the parental K562 cells with increasing concentration of imatinib (from 0.1 μM up to 1 μM) over a period of six months. It has been reported that resistance to imatinib can be induced by different mechanisms, including acquisition of gene mutations, Bcr-Abl gene amplification, overexpression of antiapoptotic proteins, and increased drug efflux transporter P-glycoprotein (P-gp) (17). We have briefly characterized our imatinib-resistant K562 ima1.0 model. There was up-regulation of the phospho-Bcr-Abl protein in our selected resistant K562 ima1.0 cell line (Fig. 1A), suggesting Bcr-Abl overexpression. While the mRNA of MDR1/P-gp was upregulated 20 times in K562 ima1.0 than the parental K562 cells (Supplementary Fig. 1), this efflux transporter upregulation may not play a major role in the observed imatinib resistance because the use of a specific P-gp inhibitor (tariquidar) did not potentiate the cytotoxic effect of imatinib in K562 ima1.0 (Supplementary Table 1). On the other hand, sequencing of the Abl kinase domain revealed no mutation.

3.1.2. Determination of individual IC_{50} values

IC_{50} values for K562 (Bcr-Abl^+), K562 ima1.0 (imatinib-resistant) and RPMI 8226 (Bcr-Abl^-) cell lines after individual drug treatment (imatinib, cisplatin or oxaliplatin) were determined (Table 1). Combinations of imatinib with cisplatin or oxaliplatin at fixed ratios of their IC_{50} were then evaluated.

3.1.3. Synergistic cytotoxic effect of imatinib-Pt drug combinations was only achieved in K562 (Bcr-Abl^+) cells

Combinations of imatinib with cisplatin or oxaliplatin were evaluated in K562 (Bcr-Abl^+), K562 ima1.0 (imatinib-resistant) and RPMI8226 (Bcr-Abl^-) cells. First, equipotent dose ratio of imatinib and cisplatin (or oxaliplatin) was used in the combination treatment. The concentrations were chosen so that each drug in the combination contributed equally to the overall cytotoxic effect. Then, other fixed ratios of the two drugs were also evaluated to find out if the combination effect is universal. Combination effect was evaluated by calculating the combination index (CI) and dose-reduction index (DRI).

In K562 (Bcr-Abl^+), all combinations of imatinib with cisplatin (or oxaliplatin) gave rise to CI values significantly below 1 at fa (fractional growth inhibition) = 0.5, indicating synergistic effect. In contrast, in RPMI 8226 (Bcr-Abl^-), combinations of imatinib with Pt drugs only produced CI values of approximately 1, suggesting additive effect (Table 2). K562 ima1.0, which is an imatinib-selected resistant subline, showed resistance towards imatinib treatment. Compared with parental K562 cell line, IC_{50} value increased more than 10 times in the K562 ima1.0 cells (Table 1). Importantly, synergistic effect from cisplatin/oxaliplatin and imatinib combination was also evident in the imatinib-resistant K562ima1.0 cells (Table 2).

The DRI was also calculated to reveal if dose reduction is attainable in the drug combination. Consistent with the result in CI, drug combinations in Bcr-Abl^+ K562 cells made the DRI value remarkably greater than 1 (Supplementary Table 2).

3.1.4. Combination of Pt-based agents and imatinib did not increase nephrotoxicity

Nephrotoxicity represent a notorious toxicity of the classical Pt drug cisplatin. MTT assay was used to evaluate whether the drug combination between cisplatin and imatinib could affect the potential nephrotoxic effect. LLC-PK1 cells are widely used *in vitro* model for the investigation of nephrotoxic potential of drugs. When grown to confluent, they represent the physiologic features of the kidney epithelial cells in the body. As shown in Fig. 2, cisplatin was found to exhibit a concentration-dependent toxic effect in LLC-PK1 cells whereas imatinib did not significantly affect the cell viability. Importantly, combination of imatinib with cisplatin did not further increase the toxicity compared with cisplatin treatment alone.

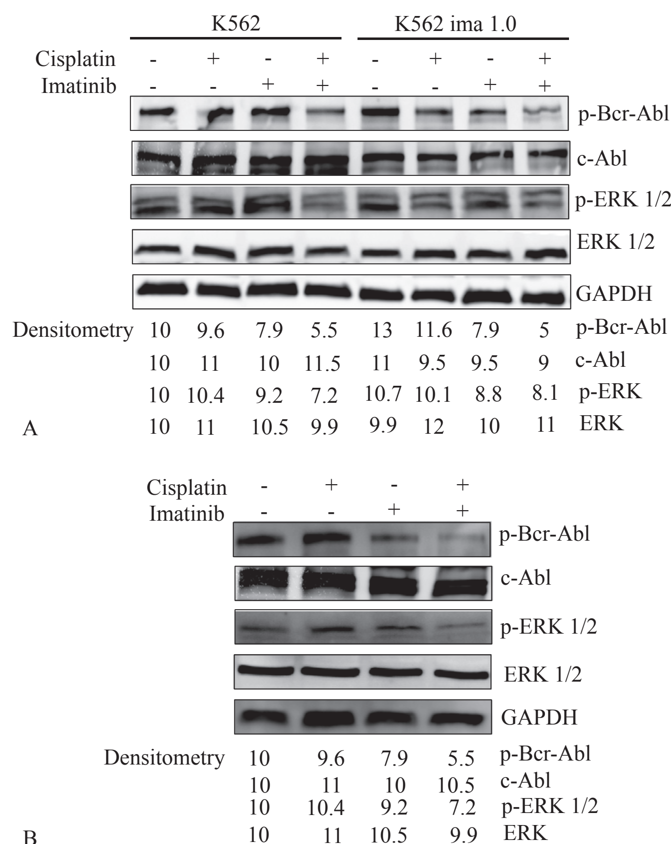

Fig. 1. Immunoblot analysis of inhibition of Bcr-Abl and ERK phosphorylation by combination of imatinib and cisplatin in (A) K562 ima1.0 imatinib-resistant cells; and (B) K562. The values under the image represent the mean densitometry-quantified band intensity from three reproducible experiments. GAPDH serves as the loading control for normalization.

Table 1
IC50 value of compounds on each cell line.

IC50 (μM)	K562 (Bcr-Abl)	K562 ima1.0 (Resistent)	RR	RPMI 8226 (non Bcr-Abl)
Imatinib	0.15 ± 0.02	2.08 ± 0.48	14	11.83 ± 0.91
Cisplatin	19.76 ± 1.42	17.47 ± 1.06	0.88	2.97 ± 0.82
Oxaliplatin	16.55 ± 2.08	16.43 ± 1.14	0.99	0.43 ± 0.04

RR (resistance ratio) was obtained by IC50 value of resistant cell line K562 ima1.0 divided by IC50 of parental K562.

Table 2
Combination index (CI) of each combination at fa = 0.5.

Cell line	Combination index	Pt_I 20:1	Pt_I 80:1	Pt_I 200:1
K562	CI (cisplatin-imatinib)	0.57 ± 0.09	0.33 ± 0.09	0.48 ± 0.10
(Bcr-Abl+)	CI (oxalipaltin-imatinib)	0.62 ± 0.05	0.35 ± 0.03	0.38 ± 0.07
Cell line	Combination index	Pt_I 1:10	Pt_I 10:1	Pt_I 100:1
K562 ima1.0	CI (cisplatin-imatinib)	0.46 ± 0.08	0.48 ± 0.11	0.51 ± 0.10
(Resistant Bcr-Abl+)	CI (oxalipaltin-imatinib)	0.55 ± 0.07	0.38 ± 0.10	0.52 ± 0.06
Cell line	Combination index	Pt_I 1:10	Pt_I 1:50	Pt_I 5:1
RPMI 8226	CI (cisplatin-imatinib)	1.30 ± 0.24	0.98 ± 0.31	1.14 ± 0.09
(Bcr-Abl-)	CI (oxalipaltin-imatinib)	1.41 ± 0.41	0.84 ± 0.12	0.90 ± 0.08

Pt–I: means "Pt-based compound: imatinib". Following ratios are the combination ratio of two components.

3.2. Drug combination increased apoptosis in K562 (Bcr-Abl positive) cells

Data from immunoblot analysis demonstrated that imatinib induced apoptosis in a concentration-dependent manner in Bcr-Abl positive K562 cells (Fig. 3A). 0.2 μM imatinib and 20 μM cisplatin (individually only induced a low degree of apoptosis) were chosen for the combination study. The drug combination was found to increase higher level of cleaved PARP (20%), compared with individual cisplatin (4%) and imatinib (9%) (Fig. 3B), indicating increased apoptosis. This is consistent with the data obtained from the Annexin V-7AAD apoptosis assay where combination of cisplatin and imatinib produced more apoptotic cells (Fig. 4).

3.3. Combinations of cisplatin and imatinib increased inhibition of Bcr-Abl and ERK phosphorylation

K562 cells are endowed with constitutively active Abl kinase activity because of the Bcr-Abl fusion oncoprotein. This activated Bcr–abl tyrosine kinase could lead to activation of its downstream signaling pathways, including the Ras–mitogen-activated protein kinase (MAPK), the Janus-activated kinase (JAK)–STAT pathway, and the phosphoinositide 3-kinase (PI3K)/AKT pathway (1), thereby driving cancer growth.

While imatinib works by specifically blocking Bcr–abl autophosphorylation and its downstream signaling pathways, cisplatin is devoid of this effect. Interestingly, the combination of imatinib with cisplatin was found to exhibit greater inhibition of Bcr-Abl and downstream ERK phosphorylation both on Bcr-Abl^{+} K562 and the selected resistance subline K562 ima1.0 (Fig. 1A,B), compared with imatinib treatment alone.

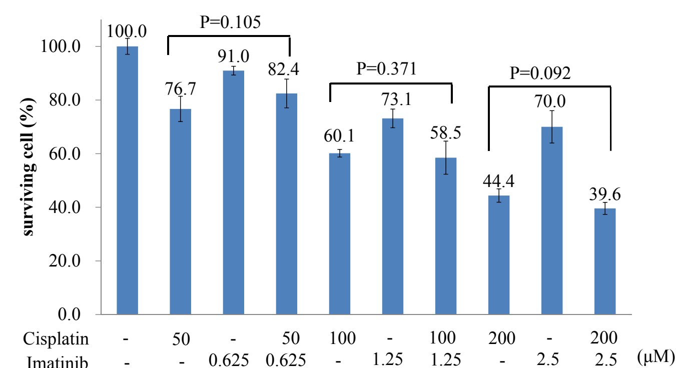

Fig. 2. Toxicity of combinations of imatinib and cisplatin in normal porcine kidney cell line LLC-PK1. Cisplatin exhibited a concentration dependent toxic effect while imatinib did not significantly affect the cell viability. Combination of imatinib with cisplatin at different ratios (cisplatin:imatinib = 100:1, 80:1) did not further increase the toxicity ($p > 0.05$).

3.4. Combinations of cisplatin and imatinib increased DNA damage in Bcr-Abl^{+} K562

3.4.1. Immunoblot analysis of DNA damage

Classical Pt-based compounds are known to induce DNA damage. The combination of cisplatin and imatinib was found to specifically increase the expression of the DNA damage marker phospho-γH_2Ax in the Bcr-Abl^{+} K562 but not in the Bcr-Abl^{-} RPMI 8226 cells (Fig. 5A). Immunofluorescence imaging of phospho-γH2AX labeled cells also confirmed that the drug combination gave rise to more punctate γ-H2Ax foci in the nucleus (Fig. 5B), consistent with more DNA damage relative to the individual drugs alone.

3.4.2. Platinum accumulation

The increased DNA damage in the drug combination may be mediated by a change in the drug accumulation of the Pt drug. Total Pt accumulation was therefore measured in K562 cells after incubation with cisplatin/oxaliplatin alone (100 μM) or their combination with 1.25 μM/5 μM imatinib. The equipotent concentration ratio (Pt drug: Imatinib = 80:1) or more of imatinib (20:1) in the drug combination was chosen for our experiments. No statistical difference was observed in the Pt accumulation between the Pt drug alone and the combination treatment (Supplementary Table 3).

Pt drugs are known to be actively pumped out by efflux transporters including ATP7A and MRP2 (18). The efflux of cisplatin in K562 cells was also monitored for 0, 2, or 4 h after a 4-h drug incubation with cisplatin alone (100 μM) or its combination with 1.25 μM imatinib. As expected, there was less Pt drug accumulation in the cells over time in drug-free medium because they are being actively pumped out. However, there was no statistical significant difference in drug efflux in the cisplatin alone and drug combination treatment (Table 3).

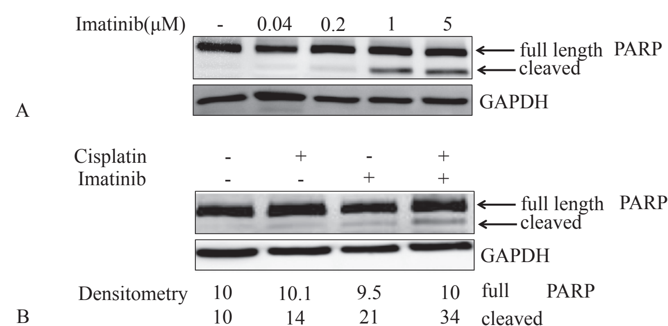

Fig. 3. Immunoblot analysis showing the apoptotic effect of imatinib and its combination with cisplatin. (A) Concentration dependent effect of imatinib; (B) combination of imatinib (0.2 μM) with cisplatin (20 μM).

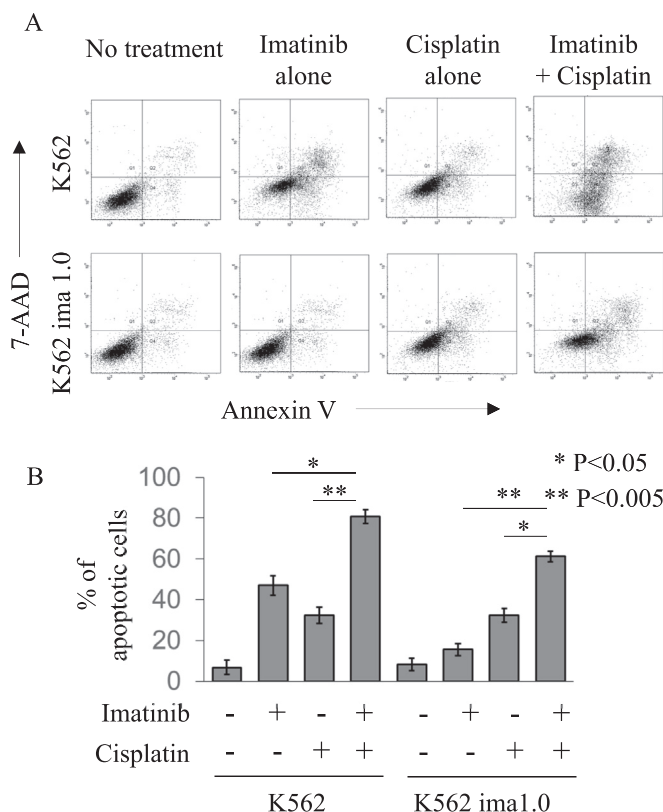

Fig. 4. Annexin V-7AAD assay for assessing the apoptotic effect of combinations of imatinib and cisplatin. (A) A representative set of data from three independent experiments is shown. (B) Summary of the apoptotic assay data from three independent experiments. Data are presented in histogram as means ± SD. $^{*}p < 0.05$; $^{**}p < 0.005$.

3.4.3. DNA platination

The extent of DNA platination was also evaluated after incubation of K562 cells in cisplatin/oxaliplatin alone (100 μM) or their combination with 1.25/5 μM imatinib for 4 h. The data showed that there is no statistical significant difference in DNA platination between the Pt drug alone and the combination treatment (Supplementary Table 4).

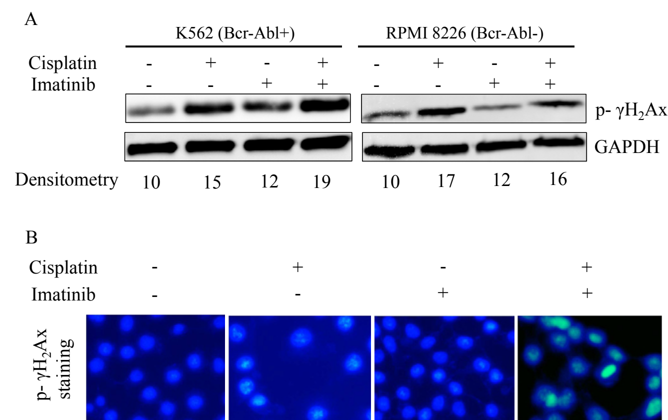

Fig. 5. (A) Immunoblot analysis showing the enhanced DNA damage by imatinib–cisplatin combination in Bcr-Abl^{+} K562 cells, but not Bcr-Abl- RMPI8226 cells. The values under the image represent the mean densitometry-quantified band intensity from three reproducible experiments. GAPDH serves as the loading control for normalization. (B) Immunofluorescence imaging of phospho-γH2Ax labeled K562 cells (Bcr-Abl+) after 24-h treatment with the indicated drugs or drug combination. More punctate γH2Ax foci were observed in the drug combination versus the two individual drugs alone.

Table 3
Platinum accumulation and efflux (ng Pt/mg protein).

Time/sample	Pt accumulation	Time/sample	Pt accumulation
0 h Cisplatin	27.85 ± 1.11	0 h Cisplatin:imatinib 80:1	23.96 ± 2.41
2 h Cispaltin	15.8 ± 2.11	2 h Cisplatin:imatinib 80:1	17.53 ± 1.97
4 h Cisplatin	14.0 ± 1.74	4 h Cisplatin:imatinib 80:1	16.00 ± 1.64

4. Discussion

Combination of imatinib with other anticancer agents have been investigated to circumvent drug resistance and to improve therapeutic response in CML treatment. In this study, drug cytotoxicity data demonstrated a synergistic cytotoxic effect between the Bcr-Abl-targeting imatinib and classical cytotoxic Pt-based anticancer drugs (cisplatin and oxaliplatin) specifically in K562 (Bcr-Abl^{+}) human leukemia cell line and its imatinib-selected resistant subline K562 ima1.0 (Table 2). Besides improving the therapeutic response in leukemia cells, the imatinib and Pt-drug combination may help circumvent imatinib resistance. The drug combination is also likely to be safe because toxicity in the combination groups produce the same extent of cytotoxic effect as cisplatin alone in a normal porcine kidney epithelial cell line LLC-PK1 (Fig. 2). In this study, we only applied the combination treatments on Bcr-Abl^{-} RPMI8226, Bcr-Abl^{+} K562 and its imatinib-selected resistant subline K562 ima1.0. For further investigation, other CML cell lines, especially those expressing resistance-causing mutant Bcr-Abl are needed. Moreover, examination of the combination effect of imatinib with other approved chemotherapeutic agents for CML on the above study models would provide more comprehensive references to evaluate the synergistic effect from combination of imatinib and Pt-based drugs.

Since the synergism effect was specifically observed only in Bcr-Abl^{+} K562 cells, Bcr-Abl and its downstream signaling molecules may play a key role in the observation. Western blot analysis showed more inhibition of Bcr-Abl (about 45%) and ERK (28%) phosphorylation by the drug combination treatment, than the individual imatinib alone (Bcr-Abl (about 21%) and ERK (8%)) in K562 sensitive cells (Fig. 1B).

Since more prominent DNA damage (indicated by the DNA damage marker phospho-γH_2Ax) was induced by the drug combination only in Bcr-Abl^{+} cell lines, cellular platinum accumulation and DNA platination were then studied. Our data indicated that imatinib had no effect on Pt-drugs accumulation and efflux. Consistently, the extent of DNA platination was also similar in the Pt-drug alone and the drug combination group. These results indicated combination with imatinib, Pt-drugs did not form additional Pt-DNA adducts. We speculate that the increased DNA damage may be contributed by the enhanced Bcr-Abl inhibition in the drug combination (Fig. 5). It has been reported that imatinib sensitized Bcr-Abl^{+} cells to cisplatin by simultaneous inhibition of p53 transactivation, induction of p53 accumulation predominantly in the cytoplasm, and reduction of Bcl-xL (12). These findings were obtained in BaF3 cells harboring wild type p53 and Bcr-Abl. However, it cannot completely explain the observation in K562 because K562 cells do not express functional p53 (19). In our study, compared with the individual Pt drug or imatinib, the drug combination did not further induce the p53-dependent proapoptotic genes BAX and PUMA (Supplementary Fig. 2). Thus, the observed synergistic effect may not be mediated by a p53-mediated mechanism. Interestingly, the anti-apoptotic gene Bcl2 was

downregulated by the drug combination versus imatinib treatment alone (Supplementary Fig. 2), which may contribute to the observed synergistic effect. Further mechanistic study, by which imatinib potentiated antitumor effect of cisplatin and vice versa, is needed to fully elucidate the specificity of our observed Bcr-Abl-specific synergistic combination cytotoxicity of imatinib with classical Pt-based anticancer drugs.

Our study demonstrated a Bcr-Abl-specific synergistic cytotoxic effect from combinations of imatinib and Pt-based anticancer drugs. The synergistic effect may be exploited to help circumvent imatinib resistance. More extensive inhibition of Bcr-Abl and its downstream ERK pathway may contribute to the synergistic effect. The results warrant further study to develop the drug combination to improve therapeutic efficacy in the treatment of CML.

Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jphs.2015.10.008.

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