

Selectivity analysis of protein kinase CK2 inhibitors DMAT, TBB and resorufin in cisplatin-induced stress responses

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Received May 13, 2009; Accepted June 23, 2009

DOI: 10.3892/ijo_00000431

Abstract. Targeting protein kinases as a therapeutic approach to treat various diseases, especially cancer is currently a fast growing business. Although many inhibitors are available, exhibiting remarkable potency, the major challenge is their selectivity. Here we show that the protein kinase CK2 inhibitors DMAT, TBB and resorufin differ in their selectivity against PI3K family members, since PI3K and DNA-PK are subject to inhibition by DMAT and TBB, however, not by resorufin. TBB and DMAT treatment together with cisplatin lead to an inhibition of cisplatin-induced stress signaling (as detected by phosphorylation of JNK and H2AX). In the case of resorufin no interference with the stress-signaling pathway is observed, supporting the notion that TBB and DMAT interfere with upstream molecules involved in genotoxic stress signaling. We have also tested the protein kinase CK2 inhibitors with respect to cell viability and inhibition of endogenous CK2 activity in the absence and presence of the anti-cancer drug cisplatin. The strongest effect on viability was observed with resorufin. In contrast to resorufin, TBB protected cells from cisplatin-induced cell killing. Furthermore, the inhibition of endogenous CK2 activity was cell type-dependent as endogenous CK2 was inhibited to different degrees in the cell lines.

Introduction

Protein kinase CK2 is a ubiquitous, pleiotropic serine/threonine protein kinase distantly related to the CMGC subfamily of protein kinases. The heterotetrameric holoenzyme consists of two catalytic α -subunits and a non-catalytical dimer (β_2). The structure of the CK2 subunits and of the tetrameric holoenzyme have been solved (1-5) supporting previous biochemi-

cal data that this enzyme is, unlike most other protein kinases, not regulated by phosphorylation, second messengers or activating molecules such as cyclins as in the case of CDKs, and is believed to be constitutively active (6). Another peculiar characteristic of CK2 is its capability to use ATP and GTP as phosphoryl donors (7). It is now common belief that CK2 exhibits anti-apoptotic properties and that down-regulation of the enzyme activity favors programmed cell death (8).

Beside the CK2 α -subunit, which has been described in most cells and tissues, an isozyme, i.e., CK2 α' has been found to be predominantly expressed in brain and testes (9). Knockout experiments of protein kinase CK2 α and β -subunits were lethal in early embryonic stage of mouse development (10,11), whereas the knockout of CK2 α' led to a phenotype resembling globozoospermia in man (12), supporting the notion that the major CK2 subunits, i.e. α and β are essential for life.

Although it is the heterotetrameric holoenzyme that has been predominantly found and described, there are also reports suggesting the presence of free CK2 subunits or in association with various other proteins (reviewed in refs. 13-15). Moreover, protein kinase CK2 has been shown to be associated with many different diseases, especially cancer (16). Hence, it is not surprising that CK2 has become a 'druggable' kinase (6,17,18) and intensive efforts have been made in search for potent and specific small molecule inhibitors. Six major classes of ATP-site directed CK2 inhibitors (type I inhibitors) have been described (18-22): i) flavonoids (apigenin and quercetin), ii) hydroxyanthraquinones/xanthenones/hydroxycoumarines [emodin, 1,8-dihydroxy-4-nitroxanthene-9-one (MNX), 3,8-dibromo-7-hydroxy-4-methylchromen-2-one (DBC), 8-hydroxy-4-methyl-9-nitrobenzo(g)chromen-2-one (NBC)], iii) halogenated (aza)imidazoles [5,6-dichloro-1(β -D-ribofuranosyl)-benzimidazole (DRB), 4,5,6,7-tetrabromobenzotriazole (TBB), 2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole (DMAT), tetrabromocinnamic acid (TBCA)], iv) indoloquinazolines [(5-oxo-5,6-dihydroindolo-(1,2-a)quinazolin-7-yl)acetic acid (IQA)], v) pyrazolo(1,5-a)triazines and vi) carboxamides/carboxylic acids.

Among the most popular CK2 inhibitors are currently TBB and DMAT. Although both compounds exhibit a remarkable potency with respect to CK2 inhibition (23-25) their selectivity for CK2 is not optimal (26). Recently, a new and highly selective CK2 inhibitor resorufin was described (27). In

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Key words: DNA-PK, protein kinase inhibitors, CK2, stress kinases, cisplatin

contrast to the so far described CK2 inhibitors, resorufin is characterized by an exceptionally high degree of selectivity. The K_i values were determined for the holoenzyme and the free catalytic subunit to be 0.8 and 1.3 μM , respectively, which compares well with the best described CK2 inhibitors. Out of 52 kinases tested, only CK2 was inhibited by resorufin. In contrast, the most popular CK2 inhibitors, i.e., TBB and DMAT, also inhibited protein kinases DYRK1a, HIPK2, PIM2, PIM3 and PKD1 (26,27).

There is a controversy concerning the strategy of inhibition with respect to therapeutically rationale approaches, i.e., to focus on one kinase only, e.g., EGFR (Gefinitib), or rather to focus on multi-targets such as RAF kinase and VEGF (Sorafenib, Sutent) (28). However, irrespective which strategy is used, the compounds used should exhibit a high degree of selectivity towards the targets chosen.

The present investigations, where TBB, DMAT and resorufin were used in two different tumor cell lines in the absence and presence of the anti-cancer drug cisplatin, show that the lack of selectivity (as observed with TBB and DMAT) may interfere with major signaling pathways including PI3K (phosphoinositide-3 kinase) and DNA-PK (DNA-dependent protein kinase) regulated mechanisms.

Materials and methods

Materials. The anti-cancer drug cisplatin was obtained from Sigma-Aldrich (Taufkirchen, Germany). Phosphospecific antibodies p-JNK (p-T183/p-Y185) and p-p38 (p-T180/p-Y182) were from New England Biolabs GmbH (Frankfurt, Germany). The antibody detecting phosphorylated histone H2AX (p-S139) (γ -H2AX) was from Upstate (Hamburg, Germany) and ERK2 specific antibody was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA).

CK2 inhibitors TBB and DMAT were obtained from EMD (Nottingham, UK). Resorufin and the CK2 synthetic peptide (RRRADDSDDDDD) were from KinaseDetect ApS (Odense, Denmark). The synthetic peptide for DNA-PK (PESQEAFAADLWKK) was from EZ Biolabs (Westfield, IN, USA) and [γ - ^{32}P]-ATP (3,000 Ci/mmol) was obtained from Hartmann Analytic (Braunschweig, Germany).

Luminometric determination of PI3K activity. Determination of PI3K activity was performed by a luminometric approach as previously described (29) using 10 μM of inhibitor. Activity was calculated as the reduction in luminescence relative to samples without PI3K.

Cell culture and viability assay. Human breast adenocarcinoma cells MDA-MB-231 and human colorectal adenocarcinoma HT29 cells were grown in RPMI medium containing 10% fetal calf serum (Invitrogen, Carlsbad, CA, USA) at 37°C in an atmosphere containing 7% CO_2 . Cell viability was determined by use of the colorimetric WST assay (Roche Diagnostics, Mannheim, Germany). Treatment with CK2 inhibitors was performed 24 h after seeding of the cells into 96-well tissue culture plates (2.5×10^3 cells/plate). After further incubation for 72 h, cell viability was analyzed by assaying the cleavage of the tetrazolium salt WST to formazan according to the manufacturers protocol. Relative viability in untreated controls was set to 100%. For Western

blot analysis and protein kinase tests, cells were incubated for 16 h with 50 μM TBB, resorufin or 25 μM DMAT before addition of 50 μM cisplatin for an additional 8 h.

Western blotting. Activation of stress kinases by phosphorylation was measured by Western blot analysis using phospho-specific antibodies, which detect the dual phosphorylated (i.e. activated) form of JNK (T183/Y185) and p38 kinase (T180/Y182). Phosphorylated histone H2AX (γ -H2AX) was also assayed by Western blot using an S139 phosphospecific antibody. Cell extracts were prepared by lysing an identical number of cells in boiling SDS sample buffer. Proteins (20–30 μg) were separated by SDS gel electrophoresis and transferred onto nitrocellulose membrane. Subsequently, phosphorylated kinases and γ -H2AX were visualized by chemiluminescence using ECLTM detection reagent (GE Healthcare, Munich, Germany).

Radioactive kinase activity test. For measurements of protein kinase CK2 activity, cells were washed extensively in PBS, collected by centrifugation and resuspended in cold lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1 mM DTT, 30 mM Na_4PP_i , 10 mM NaF) containing a protease inhibitor cocktail (Roche). Lysates were cleared by centrifugation at 4°C for 20 min at 13,000 \times g. Protein kinase CK2 activity test was performed for 10 min at 30°C in a total volume of 50 μl containing 25 mM Tris-HCl pH 8.5, 150 mM NaCl, 5 mM MgCl_2 , 1 mM DTT, 125 μM ATP and 0.6 μCi [γ - ^{32}P]-ATP (3000 Ci/mmol), 200 μM CK2 peptide and 5 μg protein extract. Samples were spotted onto P81 phosphocellulose paper and washed extensively in 0.85 mM phosphoric acid. Incorporation of radiolabeled phosphate was measured by counting samples in a liquid scintillation counter (Canberra-Packard, Downers Grove, IL, USA).

DNA-PK activity was measured in a high-salt extract prepared as previously described (30). Extract (7 μg) was incubated for 5 min at 30°C in a total volume of 20 μl containing 25 mM HEPES pH 7.5, 25 mM KCl, 50 mM NaCl, 10 mM MgCl_2 , 0.2 mM EDTA, 1 mM DTT, 0.01 mg/ml sonicated salmon sperm DNA, 125 μM ATP and 1 μCi [γ - ^{32}P]-ATP (3000 Ci/mmol) and 250 μM DNA-PK peptide. Reactions were stopped by the addition of 20 μl stop solution (30% acetic acid, 5 mM ATP). Samples were spotted onto P81 paper and washed extensively in 15% acetic acid before liquid scintillation counting. For measurements of DNA-PK activity in lysates the reactions contained increasing amounts of DMAT, TBB or resorufin.

Results

TBB and DMAT inhibit PI3K and DNA-PK activity. Since both the protein kinase CK2 inhibitors TBB and DMAT, in contrast to resorufin, also inhibit other protein kinases (26,27) we initially tested the inhibition of the recombinant PI3K α -subunit (Fig. 1A). Indeed we showed that TBB and DMAT inhibited PI3K activity by 70 and 40%, respectively. Resorufin did not show any inhibitory influence on PI3K activity (Fig. 1A) supporting the notion that TBB and DMAT are also affecting the PI3K-related kinase family. It is not

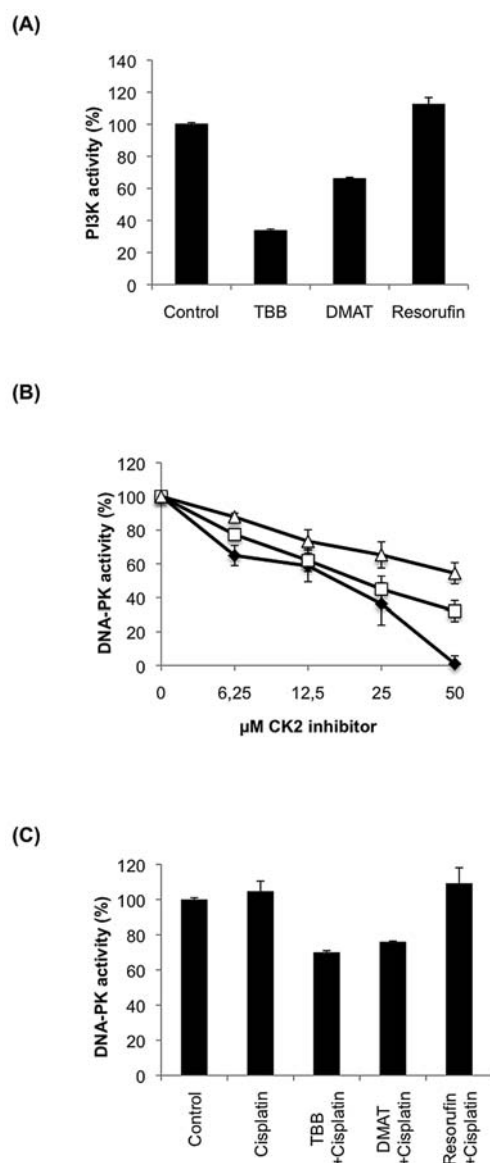


Figure 1. The CK2 inhibitors TBB and DMAT also inhibit PI3K and the PI3K-related kinase DNA-PK. (A) Luminometric kinase test with PI3K and the CK2 inhibitors TBB, DMAT and resorufin. The activity in the absence of inhibitors was set to 100%. (B) The activity of DNA-PK was measured in lysates from MDA-MB-231 cells in the presence of increasing concentrations of TBB (●), DMAT (□) or resorufin (Δ). The activity of DNA-PK in the absence of inhibitors was set to 100%. (C) MDA-MB-231 cells were incubated with 50 μ M TBB or resorufin and 25 μ M DMAT for 16 h before the addition of cisplatin (50 μ M) for an additional 8 h. The activity of DNA-PK was tested against the synthetic peptide PESQEAFADLWKK.

unlikely that these compounds might interfere with the catalytic subunit of DNA-dependent protein kinase (DNA-PK), which like ATM (ataxia telangiectasia-mutated protein) and ATR (ATM and Rad3-related), belong to the PI3K family of protein kinases. Hence, we tested DNA-PK activity in cellular MDA-MB-231 lysates in the presence of increasing concentrations of TBB, DMAT and resorufin (Fig. 1B). The results show that TBB inhibits DNA-PK activity significantly, whereas resorufin showed only a modest inhibition, especially at higher inhibitor concentrations; again the inhibition by DMAT is intermediate.

The results are further strengthened by experiments, where intact MDA-MB-231 cells were challenged with the three

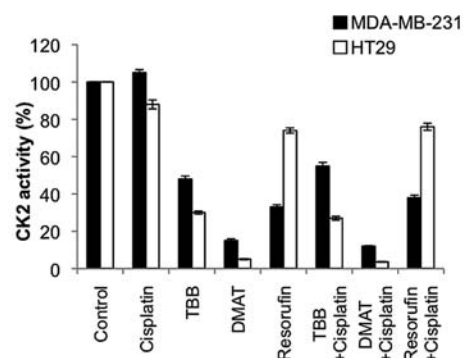


Figure 2. Inhibition of endogenous CK2 by TBB, DMAT and resorufin. Human breast carcinoma MDA-MB-231 cells and human colorectal carcinoma HT29 cells were pre-incubated with 50 μ M TBB or resorufin or 25 μ M DMAT. After 16 h cisplatin was added to a final concentration of 50 μ M for an additional 8 h. CK2 kinase activity in cell lysates was determined against the synthetic peptide RRRADSDDDDD.

CK2 inhibitors as shown in Fig. 1C. DNA-PK is activated by DNA breaks (30), hence, MDA-MB-231 cells were incubated with the DNA-damaging drug cisplatin alone or in combination with the three inhibitors. Whereas cisplatin treatment did not show any effect on DNA-PK activity (probably because the measured activity of DNA-PK represents the amount of DNA-PK that can be activated by exogenous DNA, but not the amount of DNA-PK that is actually active in the cells), TBB and DMAT caused a reduction by 40 and 30%, respectively. Resorufin did not affect DNA-PK activity at all (Fig. 1C).

Inhibition of endogenous CK2. We also measured endogenous CK2 activity in MDA-MB-231 and HT29 cell lines in the absence and presence of cisplatin (Fig. 2). Treatment with cisplatin alone did not have any effect on endogenous CK2 activity. However, treatment together with TBB led to a reduction of CK2 activity in the case of the MDA-MB-231 and HT29 cell lines by 50 and 70%, respectively; yet treatment with DMAT led to a reduction of 90 and 95%, respectively. Resorufin treatment reduced CK2 activity in the case of the HT29 cells by 25% and in the case of the MDA-MB-231 cells by ca. 70%. Co-treatment with cisplatin did not lead to a further decrease of CK2 activity (Fig. 2). Hence, the most potent inhibition of endogenous CK2 activity was obtained by DMAT. These results again show that the efficiency of the inhibitors is strongly cell type-specific, as has been described previously (27). CK2 activity measured in HT29 cells was approximately twice as high as in MDA-MB-231 cells.

Monitoring the activation of stress kinases upon cisplatin treatment. It is believed that one of the major components in deciding cell fate in response to cisplatin are the stress-activated protein kinases (reviewed in ref. 31) and it has previously been shown that DNA-dependent protein kinase (DNA-PK) is required for the late-activation of JNK (Jun-N-terminal kinase) upon genotoxic stress using the alkylating agent methyl methanesulfonate (MMS) (32). Since TBB and to a lesser degree DMAT, in contrast to resorufin, inhibited besides CK2 also DNA-PK, we next analysed cisplatin-induced stress signaling by monitoring the phosphorylation of JNK and p38.

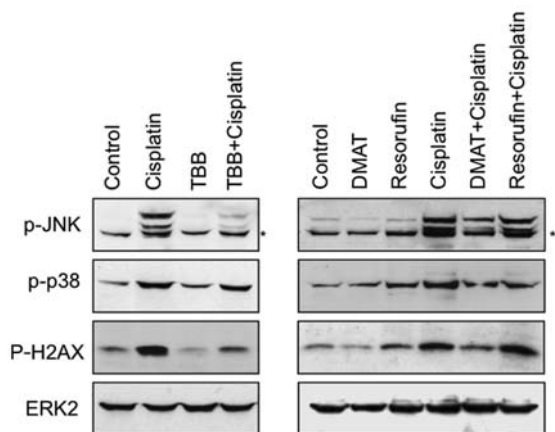


Figure 3. Effect of CK2 inhibitors on the cisplatin-induced phosphorylation of the stress kinases JNK1/2, p38 and histone H2AX. Western blot analysis of breast carcinoma MDA-MB-231 cells pre-incubated with 50 μ M TBB or resorufin or 25 μ M DMAT. After 16 h cisplatin was added to a final concentration of 50 μ M for an additional 8 h. As internal protein loading control, ERK2 protein expression was used.

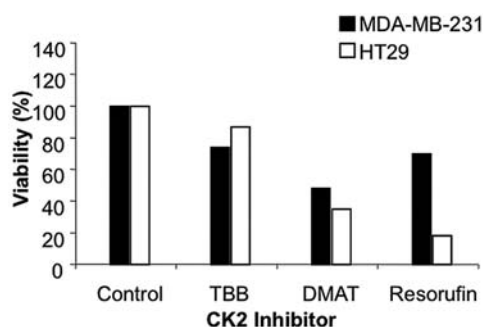


Figure 4. Effect of the CK2 inhibitors (TBB, DMAT and resorufin) on cell viability in human breast adenocarcinoma MDA-MB-231 cells and human colorectal adenocarcinoma HT29 cells. Cells were pre-incubated with 50 μ M TBB or resorufin or 25 μ M DMAT for 5 and then 72 h using 50% of the pre-treatment dose. At the end of incubation viability was determined using the WST-1 assay.

MDA-MB-231 cells were incubated for 24 h with TBB, DMAT or resorufin; for the last 8 h cisplatin was added to a final concentration of 50 μ M. Activation of the stress kinases (JNK and p38) was monitored by Western blot analysis using phosphospecific antibodies. Treatment of cells with cisplatin led to phosphorylation of JNK and p38, in agreement with published data (31). H2AX was also included to monitor DNA-damage-related histone phosphorylation at S139 (33,34), since it is phosphorylated in response to cisplatin and has been found to be a substrate for DNA-PK upon DNA-damage (35,36).

Treatment with TBB or DMAT alone has no effect on the phosphorylation status of P-JNK and P-p38, but slightly decreases the phosphorylation of H2AX. Treatment with TBB together with cisplatin (Fig. 3, lane 4), however, shows an attenuation of the phosphorylation of JNK and H2AX. The inhibitory effect of TBB is specific for JNK and H2AX, as it was not observed for p38. DMAT also partially attenuated cisplatin-induced phosphorylation of JNK and H2AX, whereas resorufin did not. The slight increase in phosphorylation of

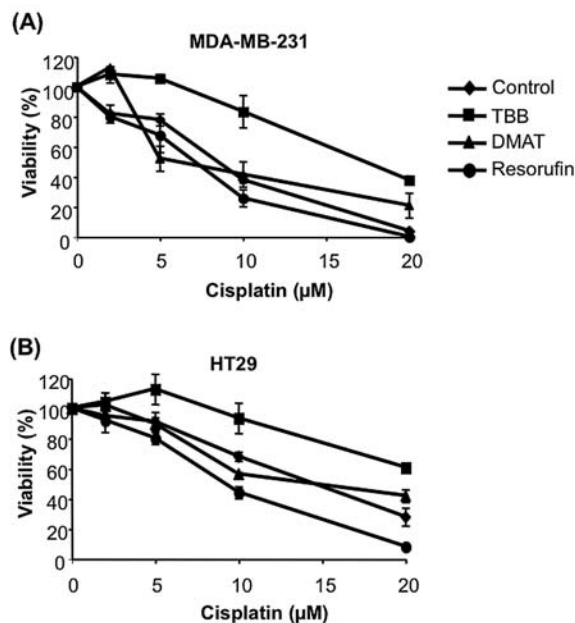


Figure 5. Effect of the combination of CK2 inhibitors and cisplatin on cell viability. Cells were pretreated with 50 μ M TBB or resorufin or 25 μ M DMAT [or no inhibitor (control)], followed by incubation with different concentrations of cisplatin and 50% of the pre-treatment dose of CK2 inhibitors. After 72 h, cell viability was measured using the WST-1 assay. Viability was set to 100% for samples only treated with the inhibitor.

H2AX by resorufin alone could be explained by the observation that slightly more protein was loaded in this lane, since also intensity of the unspecific band detected by the P-JNK antibody is increased.

There is no correlation between inhibition of CK2 in MDA-MB-231 cells using the different inhibitors (Fig. 2) and the observed decreased phosphorylation of H2AX and JNK. However, there is a good correlation between inhibition of DNA-PK (Fig. 1) and the attenuated phosphorylation of the stress kinase JNK and histone H2AX (Fig. 3). TBB was the most effective and resorufin the least effective, DMAT showed an intermediate inhibitory status both with respect to attenuation of phosphorylation and also to inhibition of DNA-PK.

CK2 inhibitors affect cell viability. To examine the response of the different CK2 inhibitors (TBB, DMAT and resorufin) on cell viability of the human tumor cell lines, MDA-MB-231 and HT29. The cells were pretreated with 50 μ M TBB or resorufin or 25 μ M DMAT for 5 h followed by incubation with 50% of the pretreatment dosage. After 72 h, cell viability was measured using the WST-1 assay [although resorufin in its oxidized form is used in colorimetric bioassays, there was no interference with the WST-assay (results not shown)]. Fig. 4 shows that TBB treatment affected the viability of the cell lines maximally at 25%. DMAT treatment impaired cell viability of MDA-MB-231 and HT29 cells by ~50 and 60%, respectively.

Resorufin treatment affected the cell line MDA-MB-231 at ~30%. However, in the case of the HT29 the effect on cell viability was 80%, supporting the notion that the effects of TBB, DMAT and resorufin on cell viability are dependent on the cell lines used.

Cisplatin-induced cytotoxicity in the presence of CK2 inhibitors. Next we investigated the effect of the CK2 inhibitors on the viability of the two cell lines in the presence of increasing concentrations of the anti-cancer drug cisplatin (Fig. 5). The cells were again pretreated with 50 μ M TBB or resorufin or 25 μ M DMAT, followed by increasing concentrations of cisplatin and 50% of the pretreatment dosage of the CK2 inhibitors. After 72 h, cell viability was measured using the WST-1 assay. The CK2 inhibitors affected viability to different degrees (Fig. 4). The starting point (100%) is viability only after treatment with the inhibitor.

Cisplatin treatment in the absence of CK2 inhibitors (control) affected cell viability of both cell lines investigated (Figs. 5A and B). In the case of the MDA-MB-231 and HT29 cells 10 μ M cisplatin treatment alone led to a reduction of cell viability by 60 and 30%, respectively. Co-treatment with TBB seemed to have a 'rescuing' effect in both cell lines. Resorufin in the presence of 10 μ M cisplatin, caused attenuation of cell viability in the case of MDA-MB-231 and HT29 cells by 70 and 50%, respectively (Fig. 5). DMAT-treated cells, when challenged with 10 μ M cisplatin differed only slightly in cell viability from resorufin-treated cells. The results demonstrate that there is a strong dependency of the inhibitor effect depending on the cell lines used and that the cell lines exhibit different cellular viability in response to the different inhibitors when used in combination with cisplatin.

Discussion

In search for the 'magic bullet', for better and more effective disease therapies, industry and academia are currently screening for compounds interfering with impaired signaling pathways, thereby, especially, focusing on protein kinases. Protein kinases belong to the second largest family of enzymes and have in common a rather conserved ATP-binding domain. Hence, it does not come as a surprise that most of the compounds found to interfere with kinase activities are targeting this site (class I inhibitors). Despite the remarkable potency of some inhibitors, one of the pitfalls of these inhibitors is that they target not only one specific kinase but also a whole spectrum of others. Monitoring these compounds with scrutiny *in vitro* and *in vivo* should therefore be mandatory. Although the panel of recombinant protein kinases for specificity screening of potential kinase inhibitors is steadily increasing, protein kinases such as ATM/ATR, DNA-PK and others are still unavailable as recombinant proteins and hence, escape routine testing with respect to being potentially targets of many kinase inhibitors applied.

Here, we have investigated the effect of three established protein kinase CK2 inhibitors, i.e. TBB, DMAT and resorufin by testing their influence on PI3K, DNA-PK and CK2 activity and activation of stress kinases, e.g. JNK, p38 after induction of DNA damage by the anti-cancer drug cisplatin. In addition, we have tested the effects on cell viability in combination with cisplatin.

Cell viability investigations revealed that these compounds challenged the two investigated cell lines to different degrees (Figs. 4 and 5). TBB had the least effect on cell viability in both cell lines and resorufin exerted the strongest effect. These results support the notion that the choice of cell lines

for particular *in vivo* investigations with respect to the effect of potential kinase inhibitors should not be underestimated. Hence, the NIH 60 cell line test is a very reasonable approach to monitor, in a first attempt, the efficiency of a potential compound candidate, since there are large differences with respect to drug and the cell line used (37).

Endogenous protein kinase CK2 activity is affected by the three compounds. DMAT proves to be the most effective compound interfering with CK2 activity, irrespective of which cell line was used. In the case of TBB and resorufin, inhibition of endogenous CK2 activity varies considerably between HT29 and MDA-MB-231 cells. Yet, for all three compounds no direct correlation between endogenous CK2 inhibition and cell viability can be established, supporting the notion that beside CK2, other molecules involved in cell survival, are targeted by TBB, DMAT and resorufin.

Additionally, we investigated the effect of the CK2 inhibitors on the activity of the kinases PI3K and DNA-PK. Although a limited primary sequence homology between PI3K and DNA-PK exists, the two are coined pharmacological homologs since PI3K inhibitors usually also target DNA-PK (38), which is also supported by our results. TBB inhibited both PI3K and DNA-PK, whereas resorufin only marginally affected DNA-PK and PI3K. DMAT showed an intermediate behavior.

We have also inspected the effect of the three CK2 inhibitors in the absence and presence of the anti-cancer drug cisplatin. DNA-damaging agents induce DNA damage response (DDR) involving activation of DNA-repair mechanisms and cell-cycle 'checkpoint' signaling (39). As a consequence of the formation of DNA lesions, in particular DNA double-strand breaks (DSB), ATM, ATR and DNA-PK, which are PI3K-related kinases (PIKKs), are activated and phosphorylate H2AX as well as many other DNA-repair and checkpoint proteins (36,40,41). Late-activation of stress kinases (JNK, p38) by the genotoxin MMS requires the DNA-repair protein DNA-PK and CSB (32). Furthermore, inhibition of PI3-kinases by low dose of wortmannin is known to attenuate activation of JNK by genotoxic stress (42). Hence, in order to exclude interference between the CK2 inhibitors TBB, DMAT and resorufin with class I PI3K and the PI3K-related kinase family member DNA-PK, which so far has not been tested *in vitro* with these inhibitors, we have monitored the phosphorylation of H2AX, p38 and JNK.

These analyses revealed that TBB and DMAT, but not resorufin, were able to attenuate cisplatin-induced signaling to JNK and H2AX. Interestingly, inhibition of cisplatin-triggered stress response by TBB and DMAT coincides with the inhibition of PI3K (α -subunits) and DNA-PK. These results are especially interesting in the light of recent reports of protein kinase CK2 phosphorylation of MDC1, a protein that has been shown to interact with several DDR proteins, including the MRE11-RAD50-NBS1 (MRN) complex (40,41,43,44). In these studies protein kinase CK2 inhibitors (e.g. DMAT and TBB) and CK2 down-regulation experiments by siRNA were used to demonstrate a role for CK2 in the phosphorylation of MDC1. Protein kinase CK2 has been shown to facilitate repair of chromosomal DNA single-strand breaks (45). However, great care should be taken when using 'so-called highly specific CK2 inhibitors' such as TBB, and

assigning a role for CK2 in an orchestra of molecules involving pleiotropic PI3K-related kinases and their downstream substrates.

In conclusion, apart from CK2, TBB and DMAT inhibit additional protein kinases, including the DNA damage sensing and repair protein DNA-PK, thereby having impact on stress signaling and cell death induced by the anti-cancer drug cisplatin.

Acknowledgements

We appreciate the competent and excellent work of Tine Rasmussen and Mia S. Andersen. This work was supported by a grant from the Danish Cancer Society to BBO (DP06083), by grants to OGI from the Danish Cancer Society (DP07109) and from NOVO NORDISK FONDEN and by a grant from the Deutsche Forschungsgemeinschaft to GF (DFG; FR 1241/5-3).

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