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Effect of nitric oxide on cytotoxicity of Taxol: enhanced Taxol transcellular permeability

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Abstract

The present studies were aimed at testing the hypothesis that nitric oxide (NO) may enhance Taxol-induced cytotoxicity in carcinoma cells by increasing influx of Taxol into intracellular compartments. Prostate carcinoma cells (PC-3, LNCaP) and neuroblastoma cells (SKNDZ, CHP212) were used to investigate both transmembrane permeability and cytotoxicity of Taxol in the presence and absence of *S*-nitrosocaptopril (CapNO), a nitric oxide donating compound. The order of permeability rate of Taxol across the four cell lines was SKNDZ > LNCaP > PC-3 > CHP212. Pretreatment of the cell lines with CapNO (100 μM) enhanced permeability of Taxol across prostate PC-3 and LNCaP cells, but not neuroblastoma SKNDZ and CHP212 cells. Taxol inhibited cell growth at nanomolar levels with IC_{50}s of 0.21, 17.4, 96.4 and 842.9 nM corresponding to SKNDZ, PC-3, LNCaP and CHP212 cells, respectively. However, CapNO inhibited proliferation of the four cell lines at millimolar levels with IC_{50}s ranging from 0.3 to 1.1 mM. Enhancing effect of CapNO (100 μM) on Taxol cytotoxicity were found in PC-3 and LNCaP cells, but not in SKNDZ and CHP212. The findings suggest that the cytotoxic potency of Taxol is mainly dependent upon the cell membrane permeabilization to Taxol, and the enhancing effect of CapNO on Taxol-induced cytotoxicity is primarily mediated via the increased influx of Taxol by NO into intracellular compartments, while NO-induced cytotoxicity cannot be excluded.

Keywords: Taxol; Nitric oxide; *S*-Nitrosocaptopril; Permeability; Cytotoxicity

1. Introduction

Paclitaxel (Taxol) stabilizes microtubules and induces a well-defined G2-M arrest [1]. In addition, Taxol blocks mitosis at the metaphase–anaphase transition by interfering spindle formation [2]. The drug is used for treatments of a wide variety of cancers [3]. The clinical development of Taxol, however, has been obstructed because of its poor biopharmaceutical properties, including metabolic lability and poor cellular membrane permeation characteristics [4,5]. The problem of metabolic lability such as low oral availability and liver first-pass effect has, for all practical purposes, been resolved by intravenous administration of Taxol to avoid its intestinal permeation hurdles and liver first-pass metabolism. However, little progress has been made in developing strategies to improve the permeability properties of Taxol through the intestinal mucosa and tumor cell membrane in order to optimize the therapeutic index.

A new direction for cancer drug development has emerged from the discovery of NO as a major effector molecule in a diverse array of physiological and pathological processes. NO has drawn our great interest to exploit its activity in regulating cellular permeability and toxicity of other drugs. NO donating compounds are valuable tools for studying the physiology, pathophysiology, and pharmacology of NO, irrespective of NO synthases involved. We have recently demonstrated that CapNO, a well-documented NO donor [6–8], could significantly increase transmembrane permeability of Taxol across representa-

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Abbreviations: NO, nitric oxide; CapNO, *S*-nitrosocaptopril; RPMI, Roswell Park Memerial Institute; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrasodium bromide.

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tive colonic carcinoma Caco-2 monolayers [9], which have been used to predict the intestinal absorption of compounds. Both Caco-2 cells and tumor cells overexpress the apically polarized 170 kDa transmembrane protein, known as P-glycoprotein. Encoded by the human *MDR1* or the murine *mdr1a* and *mdr1b* genes, P-glycoprotein is a drug efflux pump belonging to the ABC protein superfamily [10,11]. P-glycoprotein functions as an active efflux transporter for cancer agents using the energy released from ATP hydrolysis, and it causes multidrug resistance in tumor cells. Taxol has shown to be a substrate of P-glycoprotein [4,12,13]. These findings provide us with a rationale that NO may enhance Taxol-induced cytotoxicity in tumor cells by increasing influx transport of Taxol into intracellular compartments of tumor cells, and thus overcome the acquired resistance of tumors against Taxol in combination chemotherapy. In addition, NO itself is a regulatory substance that plays a vital role in apoptotic signaling pathways. All of these form the basis of the present studies to test the aforementioned hypothesis by using human prostate LNCaP and PC-3 as well as neuroblastoma SKNDZ and CHP212 cell lines. Current chemotherapeutics for both cancers is unsatisfactory partially because prostate and neuroblastoma cells possess P-glycoprotein with varying degrees of activity in their cell membrane [14], which executes the detoxification mechanisms to lower intracellular accumulation of anticancer drugs.

2. Materials and methods

2.1. Chemicals

[^{3}H]-Taxol and [^{3}H]-mannitol were purchased from Moravek Biochemicals. Specific activity of Taxol was 10.1 Ci/mmol with radiochemical purity >99%. The majority of the tritium is in the *m*- and *p*-positions of the aromatic rings, with minor amounts in the 10-position of the taxane ring, and in either the 3′-position of the side chain, or less likely the 2-position of the taxane ring. The preparation of CapNO crystals was similar to that described previously [7,8]. The crystals are now available from Calbiochem-Novabiochem. RPMI-1640 medium and fetal bovine serum, L-glutamine, penicillin and streptomycin were obtained from Gibco-BRL, Life Technologies.

2.2. Cell culture

PC-3, LNCaP, SKNDZ and CHP212 cell lines were obtained from our in-house frozen stock cells cryopreserved in a large repository of ampules. Each cell line used for the assay was kept in a minimal number of passages. The PC-3, LNCaP, SKNDZ and CHP212 cells were maintained as monolayers in RPMI-1640 medium with heat-inactivated fetal bovine serum (10%), L-glutamine (2 mM), penicillin (100 mM), and streptomycin (0.1 mg/mL) at 37° in humidified 5% CO_2.

2.3. Permeability assay

Permeability assay was similar to that described previously [15–17]. Briefly, confluent cell lines growing in T75 tissue culture flasks were detached with a mixture solution containing 0.05% trypsin and 0.5 mM of EDTA-4Na in Hank's balance salt solution. Thereafter, trypsin was inactivated by addition of 5 mL of 5% serum-containing RPMI-1640 medium. Cells were seeded at a density of 10^5 cells/cm^2 onto the following Transwell after viability test determined by trypan blue exclusion. The Transwell inserts (No. 3460 Transwell; Corning Costar Co.) are featured as polyester membrane filters with a pore size of 0.4 μm and a diameter of 12 mm. Both membrane (10 μm thickness) and inside wall were pretreated with tissue culture for uniform cell attachment. Monolayers were incubated for 2 days to allow full maturation of the cells. The transepithelial electrical resistance of these cell lines ranged 20–35 Ω cm^2 when medium resistance was used as a basic value.

On the day of experiment, the old medium was aspirated. With the inserts suspended in the wells, the prewarmed fresh medium containing CapNO (100 μM) was added to the apical chamber (0.5 mL) while the basolateral chamber (1.5 mL) was filled with the same medium without CapNO. After pretreatment of the cell lines with CapNO for 30 min, [^{3}H]-Taxol (100 nM) was added to the apical chamber, and the medium samples (60 μL) were then taken from the opposite chamber at various time intervals over a 6-hr period. The samples were added to biodegradable Ultima Gold scintillation cocktail (Packard Inc.) to determine the [^{3}H]-Taxol radioactivity using a Beckman LS 1801 liquid scintillation counter (Beckman Instruments). Transwell without cells seeded was run concurrently as a control to measure the maximal amount of [^{3}H]-Taxol transported through the membrane when the permeation reached a plateau level. The apparent permeability coefficient (P_{app}) of [^{3}H]-Taxol was determined according to the following equation [16]:

$$P_{app}\,(\mathrm{cm/min}) = \frac{\Delta Q/\Delta t}{A \times C_0}$$

where $\Delta Q/\Delta t$ is the permeability rate (μmol/min) of [^{3}H]-Taxol across the microporous membrane. The rate was calculated from the initial straight slopes. A is the surface area of the membrane (i.e. 1 cm^2), and C_0 is the initial [^{3}H]-Taxol concentration in the apical chamber at $t = 0$ (μM). The results of experiments performed in six replicates were presented as means ± SD.

2.4. Cytotoxicity assay

Cells on microculture 96-well plates (1×10^5 cells per well) were preincubated for 24 hr at 37° and 5% CO_2 to

allow stabilization prior to addition of drugs. The individual cell lines were then treated with either CapNO (12 hr) or Taxol (48 hr) to determine dose-cytotoxic curves of the cell lines. For combined drug administration, the cell lines were pretreated with CapNO (100 μM) for 30 min. Six different concentrations of Taxol ranging from 0.1 nM to 5 μM were then added to the pretreated cell lines. The cell lines were incubated at 37° for 48 hr. Solution of MTT tetrazolium salt (1 mg/mL) was added to the cells. The cells were incubated again for 3 hr to allow for MTT metabolism to formazan by the succinate-tetrazolim reductase system active only in viable cells. A solution of 0.04 N HCl in isopropyl alcohol was added to stop the MTT assay. The supernatant was then aspirated and 150 μL of trituration was added to dissolve the water-insoluble blue formazan. The optical densities were read on a spectrophotometric plate reader at a single wavelength of 570 nm. The data were then analyzed using the KC-4 program to discern the IC_{50} value through a comparison of samples without cells (100% inhibition) and with cells not treated with drugs (control, 0% inhibition). IC_{50} values were determined for CapNO and Taxol, respectively, and then for the two drugs used in combination.

2.5. Statistical analysis

The results were expressed as mean values ± SD. The ANOVA was also applied to examine significance of differences, and $P < 0.05$ was considered to be significant.

3. Results

3.1. Comparison of Taxol cellular permeability

The permeability rate of Taxol across carcinoma monolayers of the tested cell lines was determined at its initial concentration of 100 nM for 6 hr. The integrity of cell monolayers is a prerequisite for meaningful permeability measurements under the current conditions. Therefore, it is critical to examine cell viability after incubation of the cell lines with Taxol for 6 hr to ensure that the incubation period and concentration of Taxol would not compromise the monolayers integrity and result in false increases in permeability. This was achieved in this study by using three approaches [15–17]. First, the integrity of each batch of cells was tested by measuring the leakage of [^{14}C]D-mannitol in representative cell monolayers. The permeability of this paracellular marker did not exceed values of 0.5%/hr. Second, MTT assay conducted on the cell lines exposed to Taxol (100 nM) for 6 hr indicated that the MTT conversion rate was similar to that of the control cells, suggesting that incubation of the cells with 100 nM of Taxol for 6 hr does not result in significant cytotoxicity. Third, transwell inserts without cells seeded were run concurrently as a control in the same transwell plate to measure the maximal permeability of Taxol. These measures assure that Taxol is freely permeable through the microporous membrane, and any delay in Taxol permeability is caused only by the diffusion barrier of cell monolayers. Under these well-controlled conditions, the P_{app} (cm/s) values of Taxol across SKNDZ, LNCaP, PC-3 and CHP212 were 10.56×10^{-6}, 4.13×10^{-6}, 2.03×10^{-6} and 0.29×10^{-6}, respectively. As can be seen in Fig. 1, the permeability rate of Taxol across neuroblastoma SKNDZ cells was higher than that across prostate PC-3 and LNCaP cells. The CHP212 cells showed the highest resistance to Taxol permeability. The differences in Taxol permeability may reflect the distinct membrane architecture of the individual cell lines, including protein array and P-glycoprotein activity.

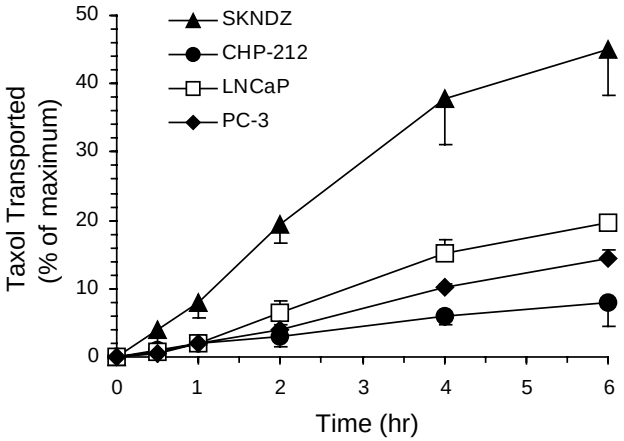

Fig. 1. Transcellular permeability of [^{3}H]-Taxol (100 nM) across neuroblastoma SKNDZ and CHP212 cell monolayers as well as prostate LNCaP and PC-3 cell monolayers. [^{3}H]-Taxol was added to the apical chamber, and the medium samples were collected from the opposite chamber for analysis. The data represent means ± SD (N = 6).

3.2. Effects of CapNO on Taxol permeability

The cell-free control revealed the maximum permeation of Taxol at a plateau of about 30 nM, which in theory was equal to the finally evenly-distributed concentration of Taxol in the Transwell. As can be seen in Fig. 2, pretreatment of PC-3 and LNCaP cells with CapNO (100 μM) significantly increased Taxol permeability across the two prostate cell lines, whereas pretreatment of SKNDZ and CHP212 cell lines with CapNO did not significantly enhance permeability of Taxol across the neuroblastoma cells.

3.3. Cytotoxic effects of Taxol

No significant growth inhibition of the tested cell lines occurred within 6 hr exposure of Taxol (100 nM), even when Taxol concentrations increased to 1 μM. Although cytotoxic effect of Taxol is dependent on both Taxol concentrations and duration of cell exposure, we found

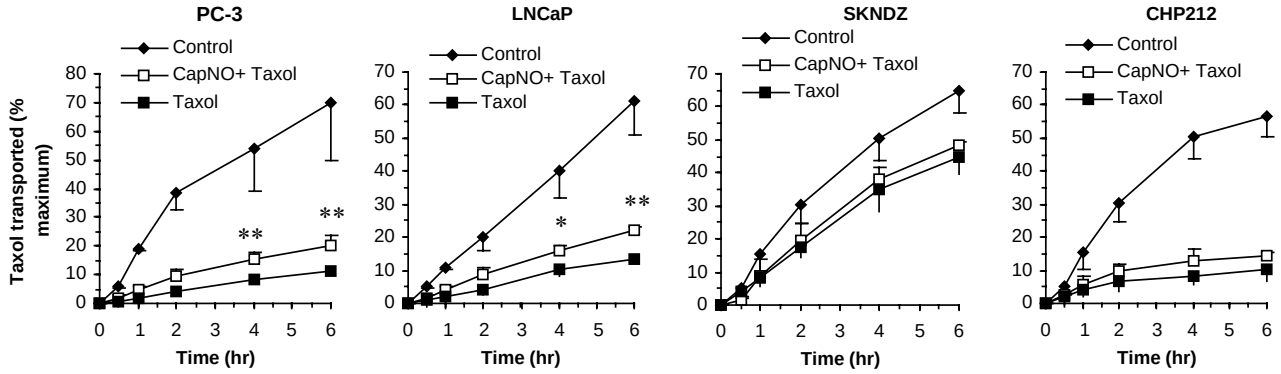

Fig. 2. Effects of CapNO (100 μM) on cellular permeability of [^{3}H]-Taxol (100 nM) across prostate PC-3 and LNCaP cell monolayers as well as neuroblastoma SKNDZ and CHP212 cell monolayers. The control experiments (no cell) were conducted concurrently in the same Transwell to determine the maximum amount (100%) of [^{3}H]-Taxol permeated when a plateau of the permeability was reached (~14 hr). Results are expressed as percentage of the maximum [^{3}H]-Taxol permeated (means ± SD, N = 6). The statistically significant differences are evaluated by ANOVA. $^*P < 0.05$ and $^{**}P < 0.01$, CapNO + Taxol vs. Taxol.

that Taxol cytotoxicity appeared to be more dependent on exposure duration than on dose, and only above a certain exposure time did cytotoxicity become dose-dependent. With the same exposure time of 48 hr, nanomolar concentrations of Taxol inhibited the proliferation of the four cell lines as shown in Fig. 3. An up to 4000-fold difference in sensitivity to Taxol was noted among the four cell lines. Very strong cytotoxic effects of Taxol were observed against neuroblastoma SKNDZ. By comparison, the two prostate cell lines showed a similar sensitivity to Taxol, and neuroblastoma CHP212 exhibited the least sensitivity in this respect. The IC_{50} values of Taxol against SKNDZ, PC-3, LNCaP and CHP212 cell lines were 0.21, 17.4, 96.4 and 842.9 nM, respectively. The cytotoxic potency of Taxol seems to be more dependent upon the cell membrane permeabilization to Taxol: the higher the cell membrane permeability to Taxol, the lower the IC_{50} value of Taxol is against the related cell line (e.g. SKNDZ).

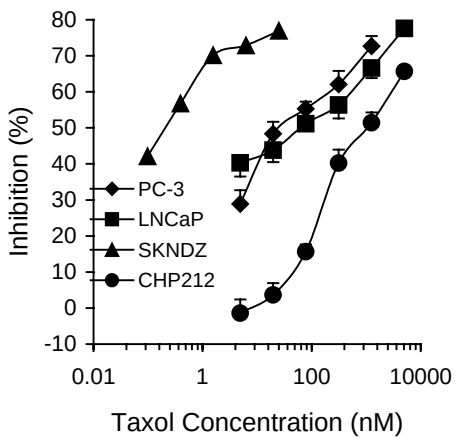

Fig. 3. Dose-inhibition curves of neuroblastoma SKNDZ and CHP212 cells as well as prostate PC-3 and LNCaP cells exposed to different concentrations of Taxol for 48 hr. The cytotoxicity was determined by MTT assay. The results are expressed as the percentage of inhibition compared with vehicle treatment (means ± SD, N = 6).

3.4. Cytotoxic effects of CapNO

Figure 4 shows the dose-inhibition curves of the four cancer cell lines obtained following 24 hr exposures to CapNO at concentrations ranging from 50 μM to 1 mM. Although SKNDZ appeared slightly more sensitive to CapNO than other cell lines, the differences in sensitivity to CapNO among the four cell lines were slight during the same exposure period. The IC_{50} values of CapNO in PC-3, LNCaP, SKNDZ, and CHP212 cell lines were 543, 1180, 332 and 940 μM, respectively.

3.5. Combination effects of Taxol and CapNO

To determine whether the effect of Taxol on cell growth could be increased by CapNO, we investigated the cytotoxic effects of Taxol in the presence and absence of CapNO (100 μM) for their potential enhancement at inhibiting cancer cell growth. The MTT assay performed on LNCaP and PC-3 cell lines showed that Taxol-induced cytotoxicity resulted in a dose-dependent inhibition of cell growth, which was even more pronounced when the cell lines were pretreated with CapNO (100 μM) for 30 min. The combination produced a shift of dose–response curves of the cell lines to the left (Fig. 5). The combination of Taxol with CapNO decreased the doses by 6–8 folds of Taxol required to induce 50% inhibition of LNCaP and PC-3 cell proliferation as determined by the MTT assay. In contrast, CapNO did not significantly enhance Taxol cytotoxicity on SKNDZ cells. And pretreatment of CapNO did not reinforce Taxol cytotoxicity on CHP212 at all. Taken together with the enhancing effects of CapNO on cell membrane permeability of Taxol across prostate LNCaP and PC-3, but not neuroblastoma SKNDZ and CHP212 cell lines, the present result suggests that the enhancing effect of CapNO on cytotoxicity of Taxol might be attributed to the increased influx of Taxol by CapNO into the prostate cell lines. Conversely, the lack of combination activity of Taxol with CapNO in the neuroblastoma cells might reflect

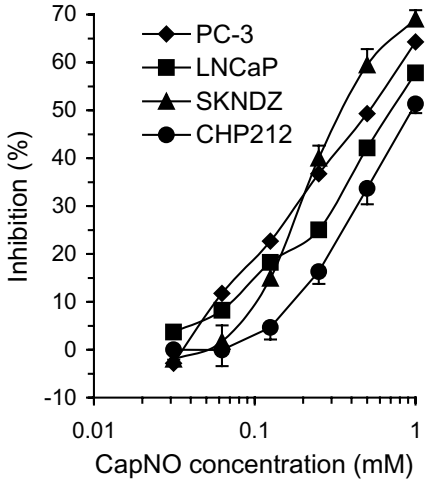

Fig. 4. Dose-inhibition curves of neuroblastoma SKNDZ and CHP212 cells as well as prostate PC-3 and LNCaP cells exposed to different concentrations of CapNO for 12 hr. The cytotoxicity was determined by MTT assay. The results are expressed as the percentage of inhibition compared with vehicle (means ± SD, N = 6).

a failure of CapNO to enhance permeability of Taxol into these cells.

4. Discussion

Permeation of drugs across cell membrane occurs via primarily the paracellular pathway or the transcellular pathway [16,18,19]. In general, hydrophilic drugs are restricted to the paracellular pathway, which consists of aqueous pores (average size approximately 7–9 μm) created by the cellular tight junctions. These aqueous pores restrict drug permeability based on the size and charge of the molecule. In contrast, hydrophobic drugs that lack charge and exhibit a low hydrogen-bonding potential can traverse the membrane by passive diffusion via the transcellular pathway. Studies have shown that Taxol, a substrate of P-glycoprotein [12,13,20], transports across cell membrane principally via the passive diffusion, which is counteracted by the efflux action of P-glycoprotein [4,5]. One of the critical determinants for the extent of cytotoxicity of Taxol is the net amount of Taxol transported into intracellular compartments and bound to microtubules to cause tubulin polymerisation. In the present studies, we found that permeability rate of Taxol across neuroblastoma SKNDZ is higher than that across prostate PC-3 and LNCaP cells, and neuroblastoma CHP212 cells (Fig. 1). Overexpression of the apically-polarized membrane P-glycoprotein is considered to be a primary defect of the architectural change, and is one of the major elements related to membrane resistance and failure of therapy for a majority of cancer drugs [11,21]. It is uncertain, however, to what extent the differences in Taxol permeability among these cell lines can be directly related to their acquired P-glycoprotein activity. Although the presence of P-glycoprotein has been demonstrated in these cell lines [14,22,23], no direct quantitative comparison about the activity of P-glycoprotein has been made among these cell lines.

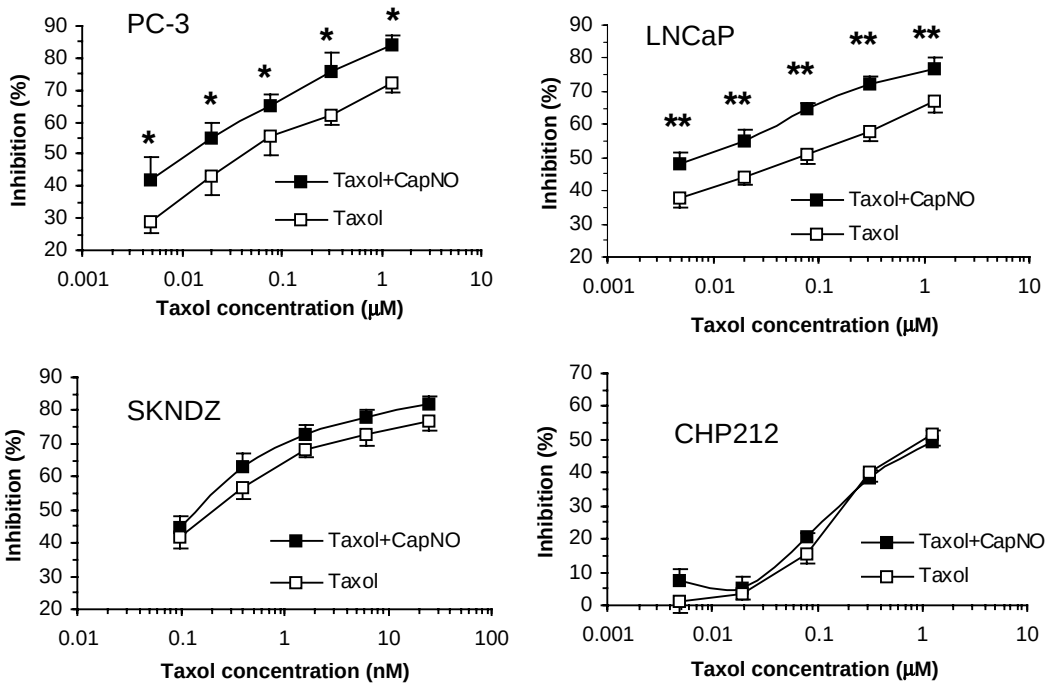

Fig. 5. Cytotoxic effect of Taxol on prostate PC-3 and LNCaP cells and neuroblastoma SKNDZ and CHP212 cells in the presence and absence of CapNO. The cells were pretreated for 30 min with CapNO of 100 μM before treatment with Taxol for 48 hr. The cytotoxic synergy between Taxol and CapNO occurred in the PC-3 and LNCaP cell lines. The results are expressed as the percentage of inhibition compared with vehicle treatment (means ± SD, N = 6). The statistically significant differences are evaluated by ANOVA. $^{*}P < 0.05$ and $^{**}P < 0.01$, CapNO + Taxol vs. Taxol.

Pretreatment of these cell lines with CapNO enhanced permeability of Taxol across the prostate PC-3 and LNCaP cells, but not the neuroblastoma SKNDZ and CHP212 cells (Fig. 2). It has become apparent that in neuroblastoma both P-glycoprotein and sphingolipid generation and metabolism play vital roles in drug resistance [24,25]. Unfortunately, there are no convincing evidences indicating that NO has a pronounced effect on metabolism of sphingolipid in neuroblastomas. However, prostate cancer cells do not exhibit significant sphingolipid characteristics [22]. P-glycoprotein-mediated drug efflux and high levels of glutathione (a cellular detoxification mechanism) are decisive factors of drug resistance phenotypes in prostate cancer cells [26]. NO has shown an inhibitory effect on multidrug resistance mechanisms: NO enhances cellular permeability via decreasing cellular ATP content [27], inducing cGMP-dependent protein kinase augmentation of ADP-ribosylation of monomeric actin [28,29], and tyrosine phosphorylation. These events may modulate P-glycoprotein by interaction with binding sites on P-glycoprotein, inhibition of ATP binding and hydrolysis, interaction with the lipid membrane of the cells. Cytoskeleton determines the ability of cells to change their shape in response to environmental and intrinsic stimuli. It has been shown that NO can lead to a more dynamic cytoskeleton and its rearrangements by augmenting ADP-ribosylation of monomeric G-actin, a major component of the cytoskeleton [28,29], and subsequently enhances cellular permeability to drugs. The formation of *S*-nitrosoglutathione resulted from *S*-nitrosation of glutathione by NO [30], or from the *trans*-nitrosation reaction between glutathione and CapNO [31] could effectively extend the life time of activity of NO under physiological conditions, and induce cytotoxicity over a longer period [27,32]. In addition, the conversion of glutathione to *S*-nitrosoglutathione could impair the glutathione-related cellular detoxification mechanism. These differences in cell membrane permeabilization, membrane components, and P-glycoprotein activity may explain the differential effects of CapNO on Taxol permeability between the two disease sub-panels.

In comparing Taxol and CapNO as cytotoxic agents used in present assay, we found a substantial difference in potency between the two differently-categorized compounds (Figs. 3 and 4). The finding suggests that the two compounds act at different cellular targets by distinct mechanisms. Taxol exhibits a peculiar mechanism of action by inhibiting microtubule disassembly with broad IC_{50}s covering from nanomolar to micromolar range. The cytotoxic mechanisms of Taxol in prostate cancer cells also include inhibition of protein isoprenylation and induction of apoptosis [33]. Whereas, CapNO inhibits the four cell lines at a narrow IC_{50} range around 1 mM, which is consistent with observed cytotoxic potency of NO and many NO donating compounds [32,34,35]. NO, released from CapNO after hemolytic cleavage of the nitrogen–sulfur bond, is generally recognized to have various effects on cellular functions including generation of toxic peroxynitrite [36], inhibition of Fe–S enzymes [37], deregulation of poly adenosine diphosphate (ADP)-ribosyl transferase, energy depletion, initiation of p53 expression [38,39] and oxidation of cellular sulfhydryl groups to trigger cellular suicide program [40]. The weak potency of NO and its donating compound CapNO at producing cytotoxicity may be attributed to the non-specific inhibition of NO on the above-mentioned cell targets. Consistent with observations that NO is a small molecule permeable to many cells [41,42], CapNO is a membrane-permeable NO donor that could readily permeate Caco-2 and endothelial cells by the passive diffusion [15]. In contrast, Taxol has limited transmembrane permeability [4,13,43] with permeability rate 10 times lower than CapNO [15]. The distinct transmembrane resistances could, therefore, significantly influence the potency of Taxol (but not CapNO) on different cell lines, resulting in a broad range of its IC_{50}s (Fig. 3). Differential sensitivity against Taxol is a common finding in neuroblastoma cell lines and reflects the natural phenotypic heterogeneity of the cell population [44,45]. Indeed, a significant difference in sensitivity to Taxol was found between neuroblastoma SKNDZ and CHP212 cells. It is conceivable that the weak sensitivity of CHP212 cells to Taxol is attributable to the high level of the cell's transmembrane resistance produced mainly by sphingolipid component, and thus less tubulin polymerization occurs due to relatively low levels of intracellular Taxol (Fig. 1).

Given that CapNO itself is capable of producing cytotoxicity and enhancing transmembrane permeability of Taxol, it is conceivable that CapNO might strengthen the cytotoxicity of Taxol against prostate PC-3 and LNCaP cells, but not neuroblastoma cells. This notion has been demonstrated (Fig. 5). The lack of properties for CapNO to reinforce cellular transport of Taxol across neuroblastoma cells may account for its failure to enhance cytotoxic effect of Taxol.

The studies described here may provide strategies to increase the bioavailability of Taxol, and overcome or alleviate cell resistance against Taxol in cancer chemotherapy. These findings provide insight into the mechanisms underlying the enhancing effect of NO on Taxol-induced cytotoxicity. It now seems probable that NO must be added to the list of factors capable of modulating the functions of P-glycoprotein and cell transport components.

Acknowledgments

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References

[1] Schiff PB, Horowitz SB. Taxol stabilizes microtubules in mouse fibroblast cells. Proc Natl Acad Sci USA 1980;77:1561–5.

[2] Horwitz SB, Lothstein L, Manfredi J, Mellado W, Parness J, Roy SN, Schiff B, Sorbara L, Zeheb R. Taxol: mechanisms of action and resistance. Ann NY Acad Sci 1986;466:733–44.
[3] Rowinsky EK, Donehower RC. Paclitaxel (Taxol). N Engl J Med 1995;332:1004–14.
[4] Sparreboom A, Asperen JV, Meyer U, Schinkel AH, Smith JW, Meijer DKF, Borst P, Nooijen WJ, Beijnen JH, Tellingen OV. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc Natl Acad Sci USA 1997;94:2031–5.
[5] Walle UK, Walle T. Taxol transport by human intestinal epithelial Caco-2 cells. Drug Metab Dispos 1997;26:343–6.
[6] Jia L, Blantz RC. The effects of *S*-nitrosocaptopril on renal filtration and blood pressure in rats. Eur J Pharmacol 1998;354:33–41.
[7] Jia L, Yang X, Guo W. Physicochemistry, pharmacokinetics and pharmacodynamics of *S*-nitrosocaptropril crystals, a new nitric oxide donor. J Pharm Sci 1999;88:981–6.
[8] Jia L, Wu C, Guo W, Young X. Antiangiogenic effects of *S*-nitrosocaptopril crystals as a nitric oxide donor. Eur J Pharmacol 2000;391: 137–44.
[9] Jia L, Garza M, Wong H, Wang Y, Weitman SD. Paclitaxel tumor cellular permeability: influence of plasma binding, P-glycoprotein and nitric oxide. Proc Am Assoc Cancer Res 2001;42:951.
[10] Higgins CF. ABC transporters: from microorganisms to man. Annu Rev Cell Biol 1992;8:67–113.
[11] Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 1993; 62:385–427.
[12] Budman DR, Calabro A, Wang LG, Liu XM, Steil L, Adams LM, Kreis W. Synergism of cytotoxic effects of vinorelbine and paclitaxel *in vitro*. Cancer Invest 2000;18:701–965.
[13] Crowe A. The influence of P-glycoprotein on morphine transport in Caco-2 cells. Comparison with paclitaxel. Eur J Pharmacol 2002; 440:7–16.
[14] Norris MD, Bordow SB, Marshall GM, Haber PS, Cohn SL, Haber M. Expression of the gene for multidrug-resistance-associated protein and outcome in patients with neuroblastoma. N Engl J Med 1996;334: 231–8.
[15] Jia L, Wong H. *In vitro* and *in vivo* assessment of cellular permeability and pharmacodynamics of *S*-nitrosylated Captopril, a nitric oxide donor. Br J Pharmacol 2001;134:1697–704.
[16] Jia L, Wong H, Cerna C, Weitman SD. Effect of nanonization on absorption of 301029: *ex vivo* and *in vivo* pharmacokinetic correlations determined by liquid chromatography/mass spectrometry. Pharm Res 2002;19:1090–5.
[17] Jia L, Wong H, Wang Y, Garza M, Weitman SD. Carbendazim: disposition, cellular permeability, metabolite identification and pharmacokinetic comparison with its nanoparticle. J Pharm Sci 2003;92: 161–72.
[18] Mullin JM, Fluk L, Kleinzeller A. Basal-lateral transport and transcellular flux of methyl α-D-glucoside across LLC-PK1 renal epithelial cells. Biochim Biophys Acta 1986;885:233–9.
[19] Pauletti GM, Okumu FW, Borchardt RT. Effect of size and charge on the passive diffusion of peptides across Caco-2 cell monolayers via the paracellular pathway. Pharm Res 1997;14:164–8.
[20] Casazza AM, Fairchild CR. Paclitaxel (Taxol) mechanisms of resistance. In: Hait WN, editor. Drug resistance. Boston: Kluwer Academic Publishers; 1996. p. 149–71.
[21] Gudmundsson O. Additional transporter characterization may lead to new pharmaceutical opportunities. Pharm Res 2001;18:1481–2.
[22] Theyer G, Schirmbock M, Thalhammer T, Sherwood ER, Baumgartner G, Hamilton G. Role of the *MDR-1*-encoded multiple drug resistance phenotype in prostate cancer cell lines. J Urol 1993;150: 1544–7.
[23] Bader P, Schilling F, Schlaud M, Girgert R, Handgretinger R, Klingebie T, Treuner J, Liu C, Niethammer D, Beck JF. Expression analysis of multidrug resistance associated genes in neuroblastomas. Oncol Rep 1999;6:1143–6.
[24] Ferte J. Analysis of the tangled relationships between P-glycoprotein-mediated multidrug resistance and the lipid phase of the cell membrane. Eur J Biochem 2000;267:277–94.
[25] Sietsma H, Dijkhuis AJ, Kamps W, Kok JW. Sphingolipids in neuroblastoma: their role in drug resistance mechanisms. Neurochem Res 2002;27:665–74.
[26] Suzuki Y, Kondo Y, Himeno S, Nemoto K, Akimoto M, Imura N. Role of antioxidant systems in human androgen-independent prostate cancer cells. Prostate 2000;43:144–9.
[27] Liang M, Knox FG. Nitric oxide enhances paracellular permeability of opossum kidney cells. Kidney Int 1999;55:2215–23.
[28] Clancy R, Leszczynska J, Amin A, Levartovsky D, Abramson SB. Nitric oxide stimulates ADP ribosylation of actin in association with the inhibition of actin polymerization in human neutrophils. J Leukoc Biol 1995;58:196–202.
[29] Gorodeski GI. NO increases permeability of cultured human cervical epithelium by cGMP-mediated increase in G-actin. Am J Physiol Cell Physiol 2000;278:C942–52.
[30] Jia L, Bonaventura C, Bonaventura J, StamLer JS. *S*-Nitrosohemoglobin: a dynamic activity of blood involved in vascular control. Nature 1996;380:221–6.
[31] Singh RJ, Hogg N, Joseph J, Kalyanaraman B. Mechanism of nitric oxide release from *S*-nitrosothiols. J Biol Chem 1996;271:18596–603.
[32] Khan S, Kayahara M, Joashi U, Mazarakis N, Sarraf C, Edwards AD, Hughes M, Mehmet H. Differential induction of apoptosis in Swiss 3T3 cells by nitric oxide and the nitrosonium cation. J Cell Sci 1997;110:2315–22.
[33] Danesi R, Figg WD, Reed E, Myers CE. Paclitaxel (Taxol) inhibits protein isoprenylation and induces apoptosis in PC-3 human prostate cancer cells. Mol Pharmacol 1995;47:1106–11.
[34] Sandau K, Brune B. The dual role of *S*-nitrosoglutathione (GSNO) during thymocyte apoptosis. Cell Signal 1996;8:173–7.
[35] Wink DA, Cook JA, Christodoulou D, Krishna M, Pacelli R, Kim S, DeGraff W, Gamson J, Vodovotz Y, Russo A, Mitchell JB. Nitric oxide and some nitric oxide donor compounds enhance the cytotoxicity of cisplatin. Nitric Oxide: Biol Chem 1997;1:88–94.
[36] Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch Biochem Biophys 1991;288:481–7.
[37] Cifone MG, Cironi L, Meccia MA, Roncaioli P, Festuccia C, Denuntiis G, D'Alo S, Santoni A. Role of nitric oxide in cell-mediated tumor cytotoxicity. Adv Neuroimmunol 1995;5:443–61.
[38] Messmer UK, Ankarcrona M, Nicotera P, BrUne B. p53 expression in nitric oxide-induced apoptosis. FEBS Lett 1994;355:23–6.
[39] Sandau K, Pfeilschifter J, Brune B. Nitric oxide and superoxide induced p53 and Bax accumulation during mesangial cell apoptosis. Kidney Int 1997;52:378–86.
[40] Melino G, Bernassola F, Knight RA, Corasaniti MT, Nistico G, Finazzi-Agro A. *S*-Nitrosylation regulates apoptosis. Nature 1997;388:432–3.
[41] Salzman AL, Menconi MJ, Unno N, Ezzell RM, Casey DM, Gonzalez PK, Fink MP. Nitric oxide dilates tight junctions and depletes ATP in cultured Caco-2BBe intestinal epithelial monolayers. Am J Physiol 1995;268:G361–73.
[42] Li B, Yao J, Morioka T, Oite T. Nitric oxide increases albumin permeability of isolated rat glomeruli via a phosphorylation-dependent mechanism. J Am Soc Nephrol 2001;12:2616–24.
[43] Walgren R, Walle T. The influence of plasma binding on absorption/exsorption in the Caco-2 model of human intestinal absorption. J Pharm Pharmacol 1999;51:1037–40.
[44] Spengler BA, Ross RA, Biedler JL. Differential drug sensitivity of human neuroblastoma cells. Cancer Treat Rep 1986;70:959–65.
[45] Riccardi A, Servidei T, Tornesello A, Puggioni P, Mastrangelo S, Rumi C, Riccardi R. Cytotoxicity of paclitaxel and docetaxel in human neuroblastoma cell lines. Eur J Cancer 1995;31A:494–9.