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Combination of nitric oxide and drug delivery systems: tools for overcoming drug resistance in chemotherapy

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Abstract

Chemotherapeutic drugs have made significant contributions to anticancer therapy, along with other therapeutic methods including surgery and radiotherapy over the past century. However, multidrug resistance (MDR) of cancer cells has remained as a significant obstacle in the achievement of efficient chemotherapy. Recently, there has been increasing evidence for the potential function of nitric oxide (NO) to overcome MDR. NO is an endogenous and biocompatible molecule, contrasting with other potentially toxic chemosensitizing agents that reverse MDR effects, which has raised expectations in the development of efficient therapeutics with low side effects. In particular, nanoparticle-based drug delivery systems not only facilitate the delivery of multiple therapeutic agents, but also help bypass MDR pathways, which are conducive for the efficient delivery of NO and anticancer drugs, simultaneously. Therefore, this review will discuss the mechanism of nitric oxide (NO) in overcoming MDR and recent progress of combined NO and drug delivery systems.

Graphical abstract

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Keywords

Multidrug resistance (MDR); nitric oxide (NO) donor; cancer; nanoparticle; chemosensitization

1. Introduction

Multidrug resistance (MDR) encompasses a broad spectrum of defense mechanisms by cancer cells, which makes them resistant to one or more chemotherapeutic drugs by decreasing uptake, increasing efflux, inactivating drugs, activating DNA repair mechanisms, up-regulating metabolism, and/or stimulating detoxification pathways [1–4]. This unexpected hurdle remains a major obstacle for efficient chemotherapy.

Ongoing research is investigating the molecular pathways mediating MDR in an effort to develop rational strategies for intervention. One of the most widely employed strategies is associated with the P-glycoprotein (Pgp) that mediates the efflux of cytotoxic agents [1–4]. Nanoparticles have been widely utilized for enhanced stability, controlled release, and targeted delivery of therapeutic agents where the primary mechanism of uptake is *via* phagocytosis, which bypasses the efflux action of Pgp [4–6]. Active targeting ligand on the surface of nanoparticles also facilitates the evasion of Pgp pathway *via* receptor-mediated endocytosis [4,7–10]. Pgp inhibitors including cyclosporin A [11], vitamin E [12] and verapamil hydrochloride [13] are used along with anticancer drugs to increase intracellular accumulation of drug and improve overall therapeutic response. Furthermore, curcumin [14] and small interfering RNA (siRNA) [15–18] have been exploited to suppress the expression of Pgp or MDR-related genes.

Nitric oxide (NO) is a vital endogenous gaseous mediator which participates in a variety of physiological and biological pathways associated with cardiovascular homeostasis [19–21], immune response [22–24], neurotransmission [25,26], cell apoptosis, and proliferation [27,28]. NO is considered to be a promising ideal drug that not only exerts therapeutic

effects, but also minimizes side effects because of its endogenous presence *in vivo* and rapid transformation into innocuous ions within six seconds or less after its action, as contrasted with other chemotherapeutics and nucleotide drugs [27–34]. Accordingly, numerous NO-donors defined as NO-releasing functional moieties or small molecule drugs have been developed and applied to biomaterials for evaluation as therapeutic modalities [29–38]. As the complicated functions of NO are highly dependent on its concentration and duration [28], NO delivery systems that can deliver NO donors to the target sites and control the NO release are prerequisite to realize the full potential of NO [34]. Current progress of NO delivery systems is summarized in details in references 31–34.

There are currently two approaches for the NO-mediated anticancer therapy, direct killing and chemosensitization. Direct killing of cancer cells can be achieved with high concentration of NO (μM – mM) [35–38], however, its practical applications are limited due to the poor bioavailability of NO-donors with low NO capacity and instability during storage and systemic circulation. On the other hand, broad ranges of NO concentrations have shown chemosensitizing effect to reverse MDR, which has recently inspired researchers to develop combined NO and drug delivery systems.

The primary purpose of this article is to introduce and discuss the recent efforts of the combined NO and drug delivery strategy to combat MDR. Prior to this discussion, we would like to provide a brief summary of the underlying mechanism behind NO mediated reversal of MDR for chemotherapy as a background for the subsequent discussion.

2. Mechanisms of NO in overcoming MDR

Although many controversial results have been published in the literature, there is an increasing body of evidence that NO plays an important role in reversing MDR effects. In this section, we would like to discuss the role of NO in DNA repair, depletion of thiols, glutathionylation of histone proteins, hypoxia-induced factors, NF- κ B, and drug efflux-associated proteins (Figure 1). It is advised that the following discussion cannot provide a generalized overview of NO effect on MDR because specified mechanisms are specific to a particular drug and NO-donor combinations.

NO is one of the representative reactive nitrogen species, which induces the nitrosation and denaturation of several proteins involved in DNA repair [39–43]. Several anticancer drugs target DNA or enzymes associated with transcript and replication [44,45] Accordingly, it is obvious that NO reduces DNA repair capacity and subsequently increases the cytotoxicity of anticancer drugs inducing DNA damage. Indeed, 1,1-diethyl-2-hydroxy-2-nitrosohydrazine (DEA/NO), a NO-donor, increased the cytotoxicity of 1,3-bis(2-chloro-ethyl)-1-nitrosourea (BCNU), a potent anticancer drug, by inhibiting DNA repair protein, *O*⁶-methylguanine-DNA-methyltransferase [43].

NO induces the depletion of glutathione (GSH) that generally inactivates platinum(Pt)-based drugs. As Pt has high affinity for sulfur groups, thiol-containing molecules cause the deactivation of platinum-based drugs [46–48]. In particular, several drug-resistant cancer cell lines are known to have high levels of GSH compared to drug-sensitive cancer cell lines,

where the depletion of GSH can contribute to enhancement of the anticancer effects of Pt-based drugs by sequestering Pt-GSH adducts [49]. Ignarro *et al.* reported that NCX-4016, which is a nitro derivative of aspirin, released NO by glutathione *S*-transferase (GST)-mediated reaction with GSH and reduced 50% of the cellular glutathione in cisplatin-resistant human ovarian cancer cells overexpressing GSH, thereby potentiating the cytotoxicity of cisplatin [50].

Lo Bello *et al.* proposed that NO enhances chemotherapeutic efficacy by improving the chance of doxorubicin binding to nucleic acids [51]. As contrasted with drug-sensitive breast cancer cells (MCF7), the glutathionylation of histone protein was significantly increased after the treatment of *S*-nitrosoglutathione (GSNO) in MDR breast cancer cells (MCF7/Dx) with high levels of glutathione and glutathione *S*-transferase P1-1 (GSTP1-1) expression. In the confocal laser scanning microscopy (CLSM) analysis, the exposure of GSNO to MCF7/Dx facilitated the accumulation of DOX in the nuclei, whereas most of DOX was localized in the cytoplasm without GSNO. On the other hand, in MCF7, DOX was localized in the nuclei regardless of GSNO treatment. In this study, a complex comprised of GSTP1-1, histone H3, and glutathione was observed after treatment of GSNO, however, its involvement in the nuclear transport mechanism is unclear and warrants further investigation into the mechanism of how glutathionylation of histone protein and its complex with GSTP1-1 induces nuclear localization of anticancer drugs. Nevertheless, the glutathionylation of histone proteins by NO is proposed as a critical route to reverse the MDR mechanism because histone proteins are important nuclear proteins regulating gene transcription.

NO inhibits the activation of hypoxia-induced factors (HIF) that regulate gene transcription and induce MDR under anaerobic conditions [52,53]. As the dimerization of HIF1 α / β induces the expression of MDR-associated proteins (MRPs), destabilization or inactivation of HIF-1 α triggers a reversal of MDR effects. Bunn *et al.* reported that sodium nitroprusside (SNP), a NO-donor, not only prevents the endogenous accumulation of HIF-1 α in human hepatoma Hep3B cells, but also suppresses the induction of the C-terminal transactivation domain of HIF-1 α , which inhibits the binding of HIF-1 to DNA for further transcription [52]. In addition, Brune *et al.* reported that NO contributes to the destabilization of HIF-1 α [53]. Although the chemosensitizing effects of NO were not investigated in this study, NO has been suggested to overcome hypoxia-induced MDR effects that are commonly found in solid tumors [54].

NO chemosensitizes certain cancer cells *via* NF- κ B-associated pathways. NF- κ B is known to not only modulate survival and metastatic pathways, but also to regulate drug resistances [55]. As the functions of NF- κ B can be inhibited by NO-mediated *S*-nitrosylation [56], Bonavida *et al.* hypothesized that the treatment of diethylenetriamine NONOate (DETA-NONOate), a NO-donor, facilitates the inhibition of NF- κ B as well as its downstream activities and reverses the MDR effect [57]. In order to demonstrate their hypothesis, they utilized a human prostate carcinoma cell line (PC-3) overexpressing Yin Yang 1 (YY1) and Bcl-2/Bcl_{XL} that regulate resistance to apoptosis. Indeed, treatment of DETA-NONOate induced the inhibition of NF- κ B, YY1, and Bcl_{XL}, which led to sensitization towards cisplatin.

Chigo *et al.* suggested that NO reduces the number of active Pgp and MRPs [58,59]. They reported that the MDR cells (HT29-dx) not only showed significantly lower NOS activity and NO production than normal cells (HT29), but also had no response in NOS activity and NO synthesis following the treatment of doxorubicin (DOX) that exerts its cytotoxicity *via* NO-dependent mechanism [58,60]. In analysis of the kinetics of drug efflux, the treatment of S-nitrosopenicillamine (SNAP), a NO-donor, led to the decrease in $V_{\max}$ without changing K_m in both cell types and induced the nitrosylation of MRP3 that is overexpressed in HT29-dx, indicating that NO induces the conformational change of MRP3 following inactivation of the transporters. As a result, enhanced DOX uptake was observed in the treatment of NO-donors or agents inducing iNOS. As contrasted with HT29-dx cells exhibiting high expression of MRP3 and low expression of Pgp, human malignant mesothelioma (HMM) cells showed reverse trends with high expression of Pgp and low expression of MRP3 [59]. In these cell lines, NO-inducing agents elicited a nitration of Pgp with insignificant detectable nitration of MRP3, which increased the uptake of doxorubicin [59]. These results imply that exogenously delivered NO facilitates sensitization of cancer cells by inactivating the Pgp or MRPs.

Taken together, NO enables to overcome MDR by reducing DNA repair and detoxification capacity, enhancing nuclear transport of drug, inhibiting HIF and NF- κ B, and/or inactivating drug efflux proteins. In addition, it is well known that drug delivery systems facilitate the bypass of MDR via passive and/or active targeting strategy as discussed in introduction section. Accordingly, it is natural that the combined NO and drug delivery systems have been developed as a way to surmount MDR in anticancer therapy.

3. Progress of combined NO and drug delivery systems

Although there have been numerous reports purporting molecular pathways for NO's mechanism of chemosensitization, few studies have applied the potential of NO for the purpose of improving therapeutic efficacy of anticancer drugs. In particular, the delivery systems capable of simultaneously delivering NO and chemotherapeutic drugs are a prerequisite to realize the full clinical potential of NO in anticancer chemotherapy not only because they allow prolonged circulation that facilitates the enhanced accumulation of drugs and NO and subsequent improved therapy, but also because spatio-temporal separation of NO and drug delivery prohibits synergistic effect. Therefore, in this section, we highlight recent progress in combined NO and drug delivery systems.

3.1. NO delivery systems for overcoming MDR

Davis *et al.* first introduced the potential of NO delivery systems in overcoming MDR effects to the field of biomaterials in 2013 [61]. They synthesized a block copolymer containing thiol groups in its hydrophobic segments by polymerizing oligoethylene glycol (OEG) and 2-vinyl-4,4-dimethyl-5-oxazolone (VDM) monomers *via* reversible addition-fragmentation chain transfer (RAFT) polymerization and then modified the thiol groups of the block copolymer with S-nitrosothiols, a NO-donor comprised of thiol and nitric oxide (-SNO). Micelles sequestering the S-nitrosothiol groups within the hydrophobic core were prepared by spontaneous self-assembly of the polymer in aqueous solution. Subsequently,

their redox-responsive NO releasing activity was demonstrated. Although the amount of NO released from the micelles was not enough to exert significant cytotoxicity, pretreatment of the micelles reduced the IC₅₀ of cisplatin by 5-fold compared to that of free cisplatin in neuroblastoma BE(2)-C cells. On the other hand, pretreatment of NO-releasing micelles led to negligible enhancement of IC₅₀ in non-cancerous fibroblast cells (MRC-5 cells). While the strategy established in this study utilized a NO delivery system and a drug separately with therapeutic evaluation at the *in vitro* level, but this preliminary study is valuable because it has been in the forefront of promoting the development of combined NO and drug delivery systems for efficient anticancer therapy.

Inspired by the Davis group, the Lee group employed a similar strategy in exploiting mineralized nanoparticles containing NO-donors to improve the therapeutic efficiency of doxorubicin (DOX) (Figure 2) [62]. According to Kim *et al.*, mineralization of NO-donors attenuates the NO release by protecting the NO-donors from premature triggers [63]. It has also been proven that acid-sensitive degradability of biominerals allows for pH-responsive NO delivery [63]. Considering these advantages, the Lee group developed *S*-nitrosoglutathione (GSNO)-loaded calcium carbonate (CaCO₃) nanoparticles (GSNO-MNPs). The nanoparticles exhibited pH- and redox-responsive NO release behavior owing to the pH-sensitivity of CaCO₃ and redox-responsiveness of *S*-nitrosothiol, respectively. Despite negligible cytotoxicity of GSNO-MNPs, the pretreatment of nanoparticles improved the anticancer effects of DOX by approximately 30%. Although the detailed mechanism was not investigated in this study, the authors postulated that NO released from the nanoparticles accelerates the formation of peroxynitrite (ONOO-) by reacting with superoxide produced by DOX in mitochondria, which enhances the anticancer efficiency of DOX [64,65].

The potential of NO delivery systems in overcoming MDR was first demonstrated *in vivo* by the Si and Zhang groups (Figure 1) [66]. They developed NO-releasing micelles (TNO₃) by modifying the hydrophilic polyethylene glycol (PEG) end group of FDA-approved D- α -tocopheryl polyethylene glycol succinate (TPGS) with NO-releasing nitrate (-ONO₂). Despite the external exposure of nitrate groups to the surrounding aqueous environments, the self-assembled micelles exhibited low amount of total NO release in PBS (10%) and in DMEM medium (16.6%) during 144 h and 96 h, respectively. However, the micelle showed burst NO release in the presence of glutathione (GSH) (~90%) and in the HepG2 cells (90.5%) due to the redox-responsiveness of the nitrate. The released NO not only induced cytotoxicity at higher concentrations (1 μ M NO), but also enhanced cellular uptake of DOX *in vitro*. In particular, the coefficient of drug interaction (CDI) values of the combined TNO₃ and DOX treatments was calculated to be lower than 0.7 (0.004, 0.026, and 0.046 for 24, 48, and 72 h, respectively), indicating that the released NO not only induced cytotoxicity, but also sensitized tumor cells to DOX to exert synergistic effects. *In vivo* study demonstrated that co-treatment of TNO₃ and DOX exhibited 1.4-fold higher DOX accumulations in tumors for the first 4 h, but similar levels after 8 h compared to the free DOX. Interestingly, the authors claimed that NO-induced dilation of blood vessel around solid tumors reduced interstitial pressure and increased tumor perfusion, which facilitated the accumulation of DOX at tumors in a short period. The co-treatment of TNO₃ and DOX showed a significant inhibition of tumor growth compared to the single administration of TNO₃ and DOX. These

results first demonstrated that co-treatment of NO and anticancer drug leads to synergistic therapeutic effects.

3.2. Combined NO and drug delivery systems

Hu *et al.* developed a combined NO and drug delivery system and evaluated the therapeutic potential *in vitro*. They devised a simple one-step nanoprecipitation/drug entrapment procedure to prepare nanoparticles containing *S*-nitrosothiol and DOX [67]. SNO-polysilsesquioxane was synthesized by acid-catalyzed polycondensation of 3-mercaptopropyltrimethoxysilane (MPTMS) in the presence of sodium nitrite in organic solution. The resultant mixture was added to an aqueous solution of DOX to afford nanoparticles (SNODOX) with *S*-nitrosothiol and DOX *via* nanoprecipitation. The cytotoxicity of SNODOX in MDA-MB-231 cells was confirmed to be higher than that of single treatment of DOX or SNO nanoparticles, owing to the additive cytotoxic effects of NO and DOX. It is noteworthy that the additive cytotoxic effects of NO did not significantly affect normal cells (H9c2 rat cardiomyoblast), implying that the potential applications of combined NO and drug delivery are conducive for tumor cell selective therapy. Balkus Jr. *et al.* employed a similar strategy in the synthesis of a silica based nanoparticle formulation [68]. They prepared amine-modified mesoporous silica nanoparticles (MSN), followed by loading of cisplatin and modification with diazeniumdiolate. The therapeutic index of MSN with both diazeniumdiolate and cisplatin was higher than that of MSN with only diazeniumdiolate or cisplatin, implying that the combined NO and drug delivery method can show synergistic therapeutic effect, although further investigation on its mechanism is necessary.

Recently, Chia and Sung groups reported an interesting microparticle as a combined NO and drug delivery system (Figure 2) [69]. They utilized a microfluidic device and water-in-oil-in-water (W/O/W) double emulsions method to prepare poly(D,L-lactic-co-glycolic acid) hollow microparticles (PLGA HMs) containing the anticancer drug irinotecan (CPT-11) and NO-donor diethylenetriamine diazeniumdiolate (DETA-NONOate) in its hydrophilic core. Diazeniumdiolates are one of the most widely used NO-donors, comprised of a diazen group ($-N=N-$) and two oxygen atoms [30,34]. As these functional groups are sensitive to acidic conditions, the pH of the inner aqueous phase was adjusted 8.0 to prevent the premature decomposition of DETA-NONOate. According to the design, protons in tumor acidic environments infiltrate the shell of PLGA HMs to allow DETA-NONOate to release NO bubbles. The resulting NO bubbles not only reverse Pgp-mediated MDR of tumor cells, but also create defects in the shell of the PLGA HMs to permit the release of CPT-11. Ultrasound images revealed that a pH of 6.6 induced the release of NO bubbles from hollow microparticles (HMs) and TEM images showed that pores and defects formed on the shell of HMs. As a result, a rapid CPT-11 release was observed under acidic conditions. Compared to the HMs at pH 6.6, NO bubbles and defects were not detected at pH 8.0 and relatively weaker ultrasound signals and smaller defects were found at pH 7.4. Accordingly, the release of CPT-11 was slower at pH 8.0 and pH 7.4 compared to the release at pH 6.6. Remarkably, the HMs exhibited an inverse relationship between pH and uptake of CPT-11 as well as expression of Pgp transporters. The acidic conditions (pH 6.6) led to the significantly reduced expression of Pgp (45%) compared to the non-treated MDR cells (MCF-7/ADR

cells), whereas the expression of Pgp (10%) was slightly reduced at physiological pH (pH 7.4). Compared to free CPT-11, HMs showed 100% enhanced uptake of CPT-11 at pH 6.6, whereas their uptake of CPT-11 increased by 13% at pH 7.4. The antitumor potential of HMs was evaluated in MCF-7/ADR xenograft mice by intratumoral injection. Compared to free CPT-11 or free CTP-11/NONOate, the significantly enhanced tumor regression with reduced Pgp expression was only observed in the mice treated with HMs. Although the practical applications of HMs are limited due to their poor bioavailability, this report is prominent because it first demonstrated that the NO released from combined NO and drug delivery systems actually enhances the uptake of drug by suppressing the Pgp transporters. It is also important to highlight here that the rationally designed combined NO and drug delivery system with pH-responsiveness allows for the tumor-selective suppression of Pgp transporters and subsequent enhanced chemotherapy.

The strategies discussed above exploited NO-donors responsive to internal stimuli, such as pH-responsive diazeniumdiolates or redox-responsive *S*-nitrosothiols. However, these types of NO-donors are commonly unstable under physiological conditions, which makes it difficult to store NO stably prior to their use and to prevent unintentional NO release during systemic circulation [30–34]. Accordingly, we would like to discuss the studies utilizing external stimuli-responsive NO-donors for combined NO and drug delivery systems.

Our group recently developed UV/vis light-responsive micelles for combined NO and DOX delivery (Figure 3) [70]. mPEG-PLGA-BNN6-DOX nanoparticles were prepared by co-encapsulating DOX and UV-responsive NO-donor, *N,N'*-di-*sec*-Butyl-*N,N'*-dinitroso-1,4-phenylenediamine (BNN6), into hydrophobic core of the PEG-PLGA micelles. 365 nm UV irradiation triggered immediate NO release from the micelles containing BNN6, whereas NO release was not detected under dark conditions in PBS. A mere 2 min UV irradiation was sufficient to induce defects in BNN6-loaded micelles, facilitating the burst release of DOX (60% at 48 h). Under dark conditions, NO release was not observed and the DOX release was only 28% at 96 h. The cellular uptake of DOX was marked by an initially increased rate of uptake, followed by a decreased rate in UV-treated mPEG-PLGA-BNN6-DOX in MDR ovarian cancer cell line OVCAR-8/ADR. Free DOX and mPEG-PLGA-BNN6-DOX under dark conditions showed a continuous decrease in cellular uptake over time. In particular, mPEG-PLGA-DOX-BNN6 exhibited significant anticancer effect towards the MDR cancer cells (~70% cytotoxicity), as contrasted with free DOX, mPEG-PLGA-DOX, and mPEG-PLGA-BNN6 which could not exert effective cytotoxic effect (20–30% cytotoxicity). mPEG-PLGA-BNN6-DOX system presents a conceptual strategy to prevent the premature release of NO and encourage the targeted delivery of NO and drug. However, further efforts exploiting other NO donors or development of advanced remote activation strategies for deep tissue penetration such as two-photon excitation laser [71] or upconversion nanoparticles [72] are needed and warrant exploration.

4. Conclusion and perspective

To date, there have been increasing reports demonstrating that NO contributes to a reversal of MDR by inhibiting DNA repair, depleting thiols, post-modifying histone proteins, hindering hypoxia-induced factors, inactivating NF- κ B, and reducing the number of active

drug efflux-associated proteins, which has inspired researchers to develop combined NO and drug delivery systems for the enhanced chemotherapy. Despite their nascent development, the combined NO and drug delivery systems have shown high potential in overcoming MDR with improved chemotherapy response compared to the single administration of corresponding drugs. However, there are several outstanding limitations to resolve for practical applications. First, a clear consequence of the requirements for combined NO and drug delivery is the need for technology capable of storing and delivering NO efficiently. Diazeniumdiolates and S-nitrosothiols are the most widely employed NO-donors to investigate the therapeutic potential of NO, however, they are too unstable to store prior to use and unstable in blood [34]. Although nitrobenzene and metal-NO complexes have been proposed as alternative NO-donors due to their stability in aqueous solutions, their practical applications also have been limited because the NO release from these NO-donors requires UV light that is not biomedically benign and has poor tissue penetration [34]. Accordingly, there have been continuous efforts to overcome the intrinsic drawbacks of NO-donors by developing formulations or functional groups capable of protecting unstable NO-donors from triggers and further restricting NO release to specific stimuli [34,62,63,73–78]. Second, it is paramount to investigate the mechanism of how the combined NO and drug delivery systems enable reversal of MDR effects and subsequently improve chemotherapy. Although several mechanistic studies with anticancer drugs and NO-donors have been demonstrated as discussed above, there is no guarantee that the combined NO and drug delivery systems utilizing nanoparticles also adhere to the same mechanisms. Indeed, only one research group demonstrated that the combined NO and drug delivery system inhibits the expression of Pgp [69]. However, these results have not been reported in the literature utilizing NO and drug without delivery systems. Third, it is largely unknown whether the combined NO and drug delivery systems can exert synergistic effect in anticancer therapy. Although all strategies discussed above showed higher cytotoxicity than NO or drug treatment alone, only one study demonstrated that co-treatment had a CDI of less than 0.7 [66]. Fourth, proper range of NO concentrations for overcoming MDR should be demonstrated. There is no explicit report of which concentration of NO is relevant to reversal of MDR although several studies reported whether and how NO affects MDR. The estimation of proper range of NO concentrations is very important in designing combined NO and drug delivery systems and evaluating their clinical potential. Accordingly, further investigations on the effects of NO concentration on overcoming MDR should be carried out. Fifth, successful clinical implementation of the combination of NO and drug is highly dependent on the technological progress of drug delivery systems. In particular, targeted accumulation of drug has been identified as a critical issue to be addressed for the practical application of the combined NO and drug delivery systems. In addition, the ability of the combined NO and drug delivery systems in overcoming MDR can be exhibited after their efficient accumulation at the target sites. The combined NO and drug delivery systems are expected to provide a solution to resolve this problem because NO has potential in increasing tumor perfusion, which can improve the enhanced permeation and retention (EPR) effect that allows macromolecules to penetrate leaky tumor blood vessels surrounding solid tumor [68,79]. In conclusion, the continuing endeavor to surmount these challenges will pave the way for achieving clinical applications of the combined NO and drug delivery system to enhance chemotherapy.

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Figure 1. Schematic summarizes the potential mechanisms of NO in overcoming MDR.

Figure 2. Schematic illustration of the development of NO-releasing mineralized nanoparticles. The pretreatment of this NO delivery system enhances the chemotherapy efficacy. Reprinted with permission from [62]. Copyright 2016, Elsevier.

Figure 3.

Combined NO and drug delivery system that was first evaluated *in vivo*. DOX is loaded into the micelles comprised of D- α -tocopheryl polyethylene glycol succinate (TPGS) with the NO-releasing nitrate (-ONO₂). The nanoparticles showed enhanced anticancer effects *in vivo* compared to the control groups. Reprinted with permission from [66]. Copyright 2014, American Chemical Society.

Figure 4. Schematic illustration of the synthesis of combined NO and drug delivery system utilizing cisplatin and diazeniumdiolate-modified MSN. Reprinted with permission from [68]. Copyright 2015, Elsevier.

Figure 5. The composition of microparticles for combined NO and drug delivery and its therapeutic mechanism. The PLGA shell of the microparticle contains CPT-11 and DETA-NONOate. The tumor's acidic pH induces the release of NO that forms defects on the shell of the microparticle to allow for drug release. In addition, the NO inhibits the expression of Pgp, which reverses the MDR effects. Reprinted with permission from [69]. Copyright 2015, WILEY-VCH Verlag GmbH & Co. KGaA.

Figure 6.

Schematic diagram of mPEG-PLGA-BNN6-DOX and its mechanism of drug release. The UV irradiation not only elicits NO release, but also induces defects in the nanoparticle. The NO inhibits the ABC transporter that effluxes anticancer drugs, which potentiates the effects of chemotherapy. Reprinted with permission from [70]. Copyright 2016, American Chemical Society.