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Gene Delivery

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 NO in overcoming MDR and recent progress of combined NO and drug delivery systems.

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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

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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-κB, and drug efflux-associated proteins (Fig. 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].

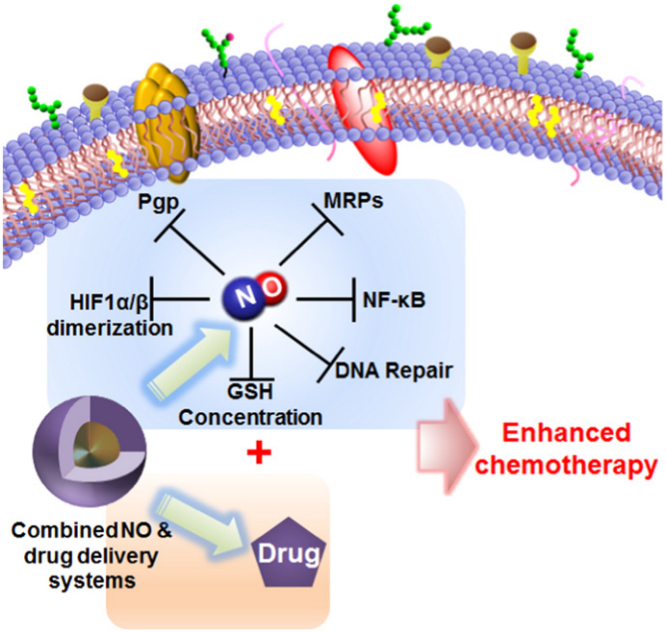

Fig. 1. Schematic summarizes the potential mechanisms of NO in overcoming MDR.

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 HIF1α/β induces the expression of MDR-associated proteins (MRPs), destabilization or inactivation of HIF-1α 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-1α in human hepatoma Hep3B cells, but also suppresses the induction of the *C*-terminal transactivation domain of HIF-1α, 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-1α [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 chemosensitizers certain cancer cells *via* NF-κB-associated pathways. NF-κB is known to not only modulate survival and metastatic pathways, but also to regulate drug resistances [55]. As the functions of NF-κB 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-κB 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-κB, 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].