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Thiol Modification By Pharmacologically Active Agents of the Diazeniumdiolate Class

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Abstract

Promising drug candidates of the diazeniumdiolate (NONOate) chemical family include several types of thiol modification among their mechanisms of action: (1) drugs designed to release nitric oxide (NO) on reaction with the thiol group of glutathione (GSH) arylate the GSH, a step that removes reducing equivalents from the cell; (2) a similar reaction of the drug with the thiol group of a protein changes its structure, leading to potentially impaired function and cell death; (3) the NO generated as a byproduct in the above reactions can undergo oxidation, leading to S-nitrosylation and S-glutathionylation; and (4) diazeniumdiolates can also generate nitroxyl, which reacts with thiol groups to form disulfides or sulfinamides.

Keywords

thiol; nitric oxide; diazeniumdiolate

I. Introduction

JS-K (*O*²-(2,4-dinitrophenyl)1-[(4-ethoxycarbonyl) piperazin-1-yl]diazen-1-ium-1,2-diolate) and related diazeniumdiolates represent a class of promising anticancer drugs, showing potent, broad-spectrum, tumoricidal activity against rodent models of prostate cancer,¹ leukemia,¹ liver cancer,² multiple myeloma,³ ovarian cancer,⁴ glioma,⁵ and lung cancer.⁶ Studies to date have revealed multicomponent mechanisms of action for these compounds, the sum of which couples antiangiogenic⁷ and reactive oxygen species-mediated effects⁶ with antimetastatic action⁸ and remarkable selectivity^{3,9} for inducing cytostasis and death in tumor cells while sparing their normal counterparts. Many of these mechanistic attributes depend on modification of cellular thiol groups. Here we review the types of thiol modification that can arise as a result of treating cells with compounds of the diazeniumdiolate family, including all those in Table 1.

II. S-ARYLATION

The O²-arylated diazeniumdiolates were designed to be activated metabolically by reaction with glutathione (GSH) in the cell to generate arylated GSH plus an ionic diazeniumdiolate species that spontaneously hydrolyzes to produce 2 molecules of nitric oxide (NO) for each molecule of drug thus the best-studied member of this family, and its *in vivo* metabolism, according to the mechanism shown in Fig. 1, is well established.

Transfer of the 2,4-dinitrophenyl (DNP) group from JS-K to GSH to form *S*-(2,4-dinitrophenyl) glutathione (DNP-SG) (Fig. 1) is an example of thiol S-arylation. It essentially is irreversible under physiological conditions, meaning that each molecule of JS-K effectively consumes a molecule of GSH during this step. If the thiol that activates JS-K for NO release belongs to a protein cysteinyl residue, the structure of the protein will be altered, often with significant disruption of normal cellular function. In principle, this could happen to any protein or peptide in the organism that bears a free thiol substituent. We view protein S-arylation as a likely major player in the cytotoxic action of JS-K and related O²-arylated diazeniumdiolates.

III. S-NITROSYLATION

The by-product of DNP-SG formation initiated by the S-arylation step of Fig. 1, the 1-[(4-ethoxycarbonyl) piperazin-1-yl]diazen-1-ium-1,2-diolate ion (CEP/NO), spontaneously hydrolyzes at physiological pH to generate 2 molecules of NO. This in turn undergoes aerobic oxidation to nitrous anhydride (N₂O₃), a well-known nitrosating agent that can react rapidly with a variety of cellular nucleophiles. Figure 2 shows this sequence of events as it applies to protein S-nitrosylation. Evidence for a protein S-nitrosylation pathway in the pharmacologic action of JS-K recently has been published.¹⁰

IV. S-GLUTATHIONYLATION

A posttranslational modification of growing interest in the arena of protein signaling is S-glutathionylation.¹¹ This involves formation of a disulfide bond linking the protein thiol group with a GSH sulfur atom, the product being represented as a protein-SSG grouping. One mechanism by which S-glutathionylation can occur is by nucleophilic attack by GSH on an S-nitrosylated protein, as in Fig. 3. Unlike S-arylation, the modification of protein-SSG can be reversed easily by 2-electron reduction.

V. SULFINAMIDE FORMATION

Nitroxyl (HNO, the by-product of the S-glutathionylation mechanism illustrated in Fig. 3)¹² dimerizes so fast that it cannot be isolated, meaning that its involvement in a given reaction must generally be inferred indirectly. A standard means of doing so is to rely on the high reactivity of HNO with thiols; thus, quenching experiments in which a given effect is disrupted by the addition of GSH can be taken as evidence for the intermediacy of HNO.

Figure 4 illustrates the formation of sulfinamides in such a quenching reaction. If RSH represents a protein with a free thiol group, a posttranslational modification of unknown toxicological consequences¹³ would result. Ionic primary amine diazeniumdiolates have been shown to produce HNO on hydrolysis at physiological pH, an effect that is blocked when GSH also is present in the reaction mixture.^{12,14,15} Although this transformation has not been demonstrated to be induced by JS-K, it should be considered as a possible player in the pharmacological action of O²-substituted primary amine diazeniumdiolates.

VI. CONSUMPTION OF GLUTATHIONE AND PERTURBATION OF CELLULAR REDOX BALANCE

An important conclusion arising from consideration of the above pathways concerns the extent to which they work together to consume GSH and increase intracellular oxidative stress. A decrease in the level of reduced GSH and an increase in its oxidized form were observed in non-small-cell lung cancer cells treated with JS-K.⁶ Because cellular redox status is, in part, a function of the GSH-to-glutathione disulfide ratio, this may constitute a major change in intracellular redox potential. In addition to direct depletion of GSH through the initial arylation reaction and cascading release of reactive nitrogen species, JS-K has indirect, mitochondria-mediated effects on cellular redox status. Depletion of GSH leads to oxidative stress that activates the intrinsic apoptotic pathway initiator Bax by its oxidation-dependent dimerization and consequent translocation to mitochondria. We have observed increases in total Bax and Bax dimers in the mitochondrial fraction of H1703 cells treated with JS-K.⁶ This functional translocation leads to the loss of mitochondrial membrane potential and cytochrome *c* release from mitochondria, initiating the intrinsic apoptotic pathway.

VII. CONCLUSIONS

The ability of JS-K to induce S-arylation, S-nitrosylation, S-glutathionylation, and multiplicative GSH consumption in tumor cells leads to a variety of deleterious reversible and irreversible changes in protein structure and function, dysregulated cellular signaling pathways, cytostasis, and death. Future studies in our laboratories and others should be aimed at addressing remaining questions about mechanisms, such as the origins of the remarkable selectivity of JS-K toward tumor cells relative to corresponding normal cells. This could be partially explained by the fact that cancer cells often exhibit stress-related phenotypes that are associated with oncogenic transformation. Levels of reactive oxygen species, DNA damage, or metabolic stress in cancer cells often are elevated compared with their nonmalignant counterparts. JS-K is clearly an agent that further enhances these effects, leading to overload of the cells' capacity to defend themselves, and thus cell death. We are hopeful that these features, together with the successful completion of the preclinical development program currently in progress, will lead soon to the introduction of JS-K as a first-in-class anticancer drug.

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ABBREVIATIONS

CEP/NO	sodium 1-[(4-ethoxycarbonyl)piperazin-1-yl]diazene-1,2-diolate
DNP	2,4-dinitrophenyl
DNP-SG	S-(2,4-dinitrophenyl)glutathione
GSH	glutathione
HNO	nitroxyl
JS-K	<i>O</i> ² -(2,4-dinitrophenyl) 1-[(4-ethoxycarbonyl)piperazin-1-yl]diazene-1,2-diolate

NO nitric oxide

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FIGURE 1.
Glutathione (GSH)-induced activation of JS-K as a nitric oxide (NO) donor

FIGURE 2.

Protein S-nitrosylation initiated by aerobic oxidation of the nitric oxide (NO) produced upon activation of diazeniumdiolate NO donors at a physiological pH

FIGURE 3.

A possible mechanism of protein S-glutathionylation. GSH, glutathione; HNO, nitroxyl

FIGURE 4.

Reaction of a thiol-containing compound (RSH) with nitroxyl (HNO) to produce the corresponding sulfinamide

TABLE 1

Mechanisms of Thiol Modification That Can Be Involved in the Action of Diazeniumdiolates That Release Nitric Oxide

S-Arylation
S-Nitrosylation
S-Glutathionylation
Formation of sulfinamide
Consumption of glutathione and perturbation of cellular redox balance