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Pharmacology of a Mimetic of Glutathione Disulfide, NOV-002

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Abstract

NOV-002 is a novel therapeutic agent in development for oncology indications used in combination with chemotherapy. Clinical trials in Russia and the USA have demonstrated clinical activity and the present focus is in non-small cell lung cancer (NSCLC) patients. The active component of the drug is oxidized glutathione (GSSG) and this imparts multiple effects upon redox pathways both at the cell surface and inside the cell. The drug induces S-glutathionylation of some proteins and impacts kinase/phosphatase regulated signaling pathways. Induction of myeloproliferation is believed to contribute to the clinical advantages provided by NOV-002 that include improved tolerance of chemotherapy and increased survival.

Keywords

glutathione; redox; S-glutathionylation; thiols

Introduction

NOV-002 is a complex of glutathione disulfide (GSSG) with cis-platinum at an approximate 1000:1 ratio. The cisplatin may serve to stabilize the GSSG, but does not assert any therapeutic effect. Unusually, the availability of clinical data has preceded full preclinical evaluation of the drug. Significant clinical trial results have been generated from earlier Russian investigations and indicate a unusual clinical profile in oncology indications, combining improved tolerance of standard chemotherapeutic drugs with increased efficacy [1]. While the Russian results were positive, a number of U.S. clinical trials are ongoing in several oncology indications in order to gain FDA approval. Modulation of GSH/GSSG levels and of glutathione S-transferase (GST) has been attempted as a means to improve response to cancer drugs. Use of buthionine sulfoximine (BSO) and ethacrynic acid, while effective preclinically [2], were not successful enough in the clinic to merit continued development [3,4]. However, one consequence of these studies was the design of a peptidomimetic inhibitor of GSTπ, TLK199 (γ-glutamyl-S-(benzyl)cysteinyl-R(−)phenyl glycine diethyl ester). Where preclinical and mechanism of action studies revealed an unanticipated myeloproliferative activity in rodents [5,6]. The discovery of a protein:protein interaction between GSTπ and c-jun NH_2 terminal kinase (JNK; [7,8]) provided a model for how TLK199 could produce proliferation in marrow progenitor cells [6]. Since NOV-002 affects bone marrow in both preclinical and clinical

studies, there is reason to conclude that thiol manipulation may be a common factor in influencing myeloproliferation. Indeed, there are examples where glutathione is directly implicated in regulation of immune response. Manipulation of blood GSH/GSSG levels by administration of n-acetyl cysteine has been shown to influence survival and quality of life in HIV infected patients [9,10]. With NOV-002 it is possible to prescribe general, pleiotropic mechanisms linking GSH/GSSG with myeloid proliferation and immune regulation.

GSH homeostasis is maintained in cells by a complex series of balanced pathways. *De novo* synthesis can occur through the γ-glutamyl cycle [11], where the three constituent amino acids (glu-cys-gly) are combined with rate limiting catalysis through glutamate-cysteine ligase (GCS) and glutathione synthetase. Salvage of GSH can occur through the cleavage activity of the membrane associated γ-glutamyl transpeptidase (GGT) that can recycle constituents. While intracellular concentrations of GSH may vary considerably, 0.1 to 10mM are commonly found in mammalian cells (10 to 30 μM in plasma). Glutathione can occur in reduced (GSH), oxidized (GSSG) or in mixed disulfide forms and its ubiquitous abundance is testament to its biological importance. More recently, S-glutathionylation of proteins has been recognized as an important post-translational modification. S-glutathionylation can influence conformation of various structural proteins including actin [12] or other clusters of proteins that can be grouped into, energy metabolism/glycolysis, cell signaling, calcium homeostasis, protein folding and redox homeostasis (for review see [13]). Since GSSG can be a proximal donor in the S-glutathionylation reaction, the implications are that NOV-002 may also provide donor substrate. As a consequence of this and other effects on cellular redox balance, NOV-002 influences multiple cell processes and functions, including critical proliferation pathways.

Mechanism of action

NOV-002 is not cytotoxic alone even at high doses, and while NOV-002 causes protein S-glutathionylation, the platinum component does not [14]. Instead, the capacity for modulation of redox conditions at the cell surface and/or intracellularly may underlie the pharmacological properties of NOV-002. NOV-002 has pleiotropic effects on preclinical model cell systems. For example, NOV-002 treatment of HL60 cells alters a number of cellular redox parameters both at the cell surface and intracellularly [14]. It is generally accepted that GSSG does not passively cross the cell membrane [15]. Thus, the effects of NOV-002 on cells may be mediated via direct effects on cell surface targets. Alternatively, the interaction of NOV-002 with a membrane-associated enzyme such as γ-glutamyl transpeptidase (GGT; for which both GSSG and NOV-002 are substrates) could result in hydrolysis into constituent amino acids, whereupon extra availability of cysteine could stimulate GSH metabolism. In addition, there is evidence that GGT, through Fenton chemistry, can eventually increase levels of hydrogen peroxide (H_2O_2), which is cell permeant and capable of transmitting an oxidative signal into the cell [16]. The increased bioavailability of GSSG through NOV-002 administration could increase the flux of H_2O_2 as a consequence of stimulating the GGT activity. In addition, GSSG can directly impact GSH levels through increasing H_2O_2 and stimulating glutamate-cysteine ligase [17].

Cell surface protein thiols are believed to act as sensors of extracellular redox status, and their modification has been linked to regulation of cell signaling and other functions in a variety of cell types [18]. NOV-002 treatment of HL-60 cells reduced cell surface thiol content through oxidative modification of cell surface proteins [14]. A growing body of evidence suggests that redox-based modulation of surface proteins is capable of regulating a variety of cell functions [19]. One potential target of such modification is cell surface protein disulfide isomerase (PDI) that regulates, for example, viral entry into cells (e.g. HIV-1 as a consequence of redox modulation of CD4 on lymphocytes and the HIV-1 envelope glycoprotein gp120 [20]), cell-mediated adhesion by integrins [21] and tumor cell invasiveness [22]. Since NOV-002 inhibits

PDI activity, this enzyme may represent a cell surface target for this drug. NOV-002 also produces multiple intracellular changes indicative of alterations in redox balance against the backdrop of stimulating the rate of cell proliferation. Redox conditions can regulate a number of signaling pathways and directly control cell division and differentiation responses. A fine-tuning of the redox balance has led some investigators to suggest that as little as a 15% increase/decrease in cellular redox can activate pathways that direct cells towards either proliferation or differentiation [23,24]. Absolute levels of GSH and GSSG increased transiently following NOV-002 treatment. The ratio of GSH:GSSG was decreased by approximately 36% after NOV-002 treatment, indicating the generation of a mild oxidative signal within the cell interior. In addition, NOV-002 treated HL60 cells showed an ~ 2.5-fold increase in H_2O_2 production compared with untreated cells. These results were consistent with a time and concentration dependent increase in the phosphorylation of three kinases that in bone marrow, can play direct roles in cell proliferation (JNK, p38 and ERK) and in AKT, a kinase that acts in concert with JAK2 and STAT5 to regulate marrow proliferation. The JAK-STAT signaling pathway is intimately involved in governing the response of cells to cytokines and growth factors [25]. Activation of the pathway by NOV-002 indicates that the drug is impacting those pathways that lead to hematopoiesis/myeloproliferation. These stimulations were also coincident with the induction of S-glutathionylation of actin and could suggest a cause: effect relationship between signaling and cytoskeleton morphology. S-glutathionylation occurs to certain target proteins when a cell is exposed to oxidative or nitrosative stress [26]. Filomeni and colleagues have used a number of model systems to demonstrate that under certain conditions, GSSG can act as a pro-oxidant activator of the p38 MAPK death pathway in some tumor cell lines [27, 28]. While the fate of cells affected by these kinases is tissue dependent, there is some general concurrence amongst the specific kinases targeted. Their studies generally reflect the important role that GSSG has in mediating early response pathways through redox changes that transduce to a kinase signaling cascade and impact cell survival pathways. As such, the concomitant changes in intracellular GSSG, actin S-glutathionylation, kinase phosphorylation and cell proliferation in HL-60 cells treated with NOV-002 seem to be causally interrelated.

Pharmacogenetic correlations are becoming more important in the establishment of new cancer therapeutics. Surrogate biomarkers can be potentially useful in establishing pharmacokinetic and pharmacodynamic properties. For NOV-002, there is the possibility that the S-glutathionylation pattern of plasma proteins may provide such information. Early analysis of murine serum data revealed that four protein bands are prominently S-glutathionylated. For three of these proteins, the intensities of labeling increase following treatment of the animals with a comparatively low dose of 15mg/kg i.p. of NOV-002. These biomarkers may eventually prove to be valuable pharmacodynamic indicators in humans.

Clinical Results

NOV-002 has undergone a number of Phase II oncology studies in the Russian Federation and the US, primarily in NSCLC. In Russia, a multi-center, randomized, open-label, 12-month study was conducted with first-line chemotherapy (cisplatin + etoposide for the first two cycles, with additional agents employed in subsequent cycles) in patients with advanced NSCLC. NOV-002 was administered intravenously (60–80 mg) on days of chemotherapy and intramuscularly (10 to 20 mg) on days between chemotherapy cycles. One-year survival was significantly improved from 17% in the chemotherapy alone group to 63% in the NOV-002 plus chemotherapy group ($p<0.01$) and patients receiving NOV-002 were able to receive more cycles of chemotherapy. Improved tolerance of chemotherapy in NOV-002-treated patients was also evidenced by increased peripheral blood counts of leukocytes, monocytes, erythrocytes/hemoglobin and lymphocytes compared to chemotherapy alone. In addition, performance status (using Karnofsky Score) was significantly improved in the NOV-002 plus chemotherapy group compared to the chemotherapy alone group.

In a US Phase I/II trial [1], 44 chemotherapy-naïve, Stage IIIB/IV NSCLC patients were randomized into: Groups A and B: NOV-002 in combination with carboplatin and paclitaxel (C+P). For each nominal 21-day chemotherapy cycle, these groups received 60 mg of NOV-002 i.v. daily for the first 4 days, then 60 mg i.m. (A) or s.c. (B) weekdays for the next 17 days. Group C: C+P alone. Primary study endpoints included tumor response and safety. 11 out of 16 (69%) Group B patients demonstrated >50% tumor shrinkage versus 5 out of 15 (33%) in the control group (C). Six out of 13 (46%) patients in Group A demonstrated an objective response. In addition, 100% of NOV-002 treated patients in Group B and 85% in Group A completed four cycles of C+P compared to 50% of control patients (Group C). NOV-002 is the subject of a pivotal ongoing Phase III trial in advanced NSCLC. Full patient accrual is complete and results are expected in 2009.

Conclusion

NOV-002 is a well-tolerated therapeutic adjuvant to standard cancer drug therapies. It enhances myeloproliferation primarily as a consequence of redox-induced changes that occur at both the cell surface and inside the cell. Clinical testing in Russia produced positive survival data in NSCLC patients. Further trials are ongoing in the USA and a critical Phase III trial is in progress in parallel with Phase II trials in other oncology indications.

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