The redox modulator NOV-002 inhibits proliferation of ovarian tumor cells but increases proliferation of myeloid cells

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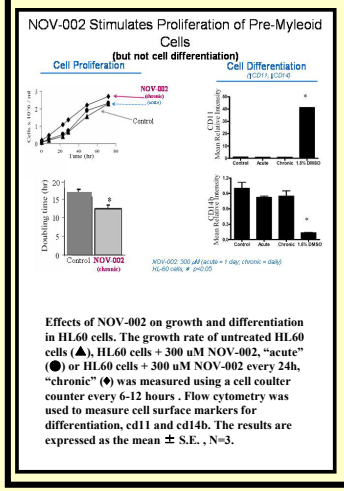
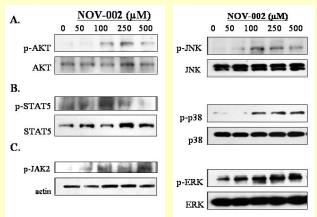
Abstract

NOV-002 is a glutathione disulfide mimetic that is in Phase II clinical trials for breast cancer. Non-clinical studies have interrogated the myeloproliferative and antitumor activity of the drug. NOV-002 alters redox homeostasis, at the cell surface and intracellularly, through kinase signaling culminating in differential effects on cell proliferation/survival in myeloid verses tumor cells. Thus, we previously showed that NOV-002 has positive growth effects on human myeloid lineage cells (HL60) and yet the drug leads to cell cycle arrest and apoptosis in human ovarian (SKOV3) cancer cells. In the present study, we sought to understand differences in redox-mediated signaling events that govern the opposing pharmacological properties of NOV-002. While the redox-signaling events are quite similar, the cellular consequences are distinct. Chronic treatment of cells with NOV-002 leads to enhanced proliferation in HL60 cells and growth arrest in SKOV3 cells. The alteration in growth rate occurs in both myeloid and tumor cell types in parallel with stress-induced S-glutathionylation and activation of MAP kinase pathways. In myeloid lineage cells, activation of MAP- and JAK/STAT-kinases leads to proliferation whereas activation of this pathway in SKOV3 cells leads to apoptosis. NOV-002 treatment also results in changes in plasma and mitochondrial membrane potentials in both cell models. These changes were concurrent with time- and dose-dependent increases in the accumulation of intracellular Ca^{2+}. Interestingly, dose- and time- dependent increases in nitric oxide (NO) generation were observed in HL60 cells and shown to be mediated through eNOS. SKOV 3 cells do not express eNOS and, consequently, NO generation was not detected. Finally, redox-modulation of cell surface thiols (via S-glutathionylation) occurs in both cell types but to different extents. NOV-002 treatment leads to a ~75% decrease in free sulfhydryls in myeloid cells whereas ovarian cancer cell surface free sulfhydryls were reduced by only ~5%. It remains to be determined whether these observed differences in cell surface protein glutathionylation and/or in mechanism of Ca^{2+} flux can be linked to the opposite effects of NOV-002 on proliferation/survival in myeloid and tumor cells. Regardless of the basis of this difference, it can be speculated that the differential effects of NOV-002 on myeloid and tumor cells *in vitro* may relate to the unique clinical profile that NOV-002 has demonstrated to date--increased survival and anti-tumor efficacy combined with mitigation of chemotherapy-induced hematological toxicity.



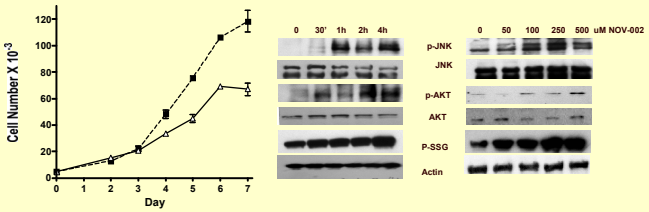
Effects of NOV-002 on growth and differentiation in HL60 cells. The growth rate of untreated HL60 cells (▲), HL60 cells + 300 uM NOV-002, "acute" (●) or HL60 cells + 300 uM NOV-002 every 24h, "chronic" (♦) was measured using a cell coulter counter every 6-12 hours . Flow cytometry was used to measure cell surface markers for differentiation, cd11 and cd14b. The results are expressed as the mean ± S.E. , N=3.

Cellular Kinase Signaling



HL60 cells were treated for 1h with 0-500 μ M NOV-002 in complete media. Actin S-glutathionylation was also seen under these conditions (data not shown) and represent another manifestation of oxidative signaling in HL60 cells after treatment with NOV-002.

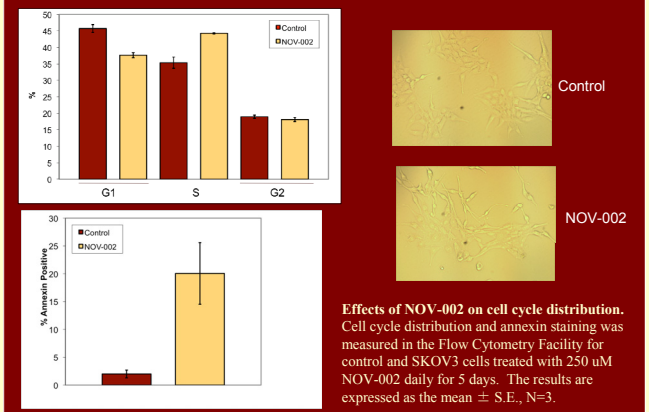
NOV-002 inhibits ovarian tumor cell growth



Effects of NOV-002 on in human ovarian cancer cells. The growth rate of untreated SKOV3 cells (■) or SKOV3 cells + 250 uM NOV-002 every 24h, "chronic" (◆) was measured using a cell coulter counter every 6-12 hours. The results are expressed as the mean ± S.E. , N=3.

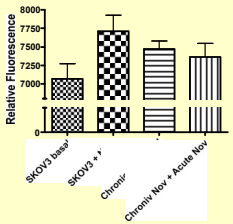
Time and concentration dependent effects of NOV-002 on stress kinases and their phosphorylated products. SKOV3 cells were treated for 1h with 0-500 μ M NOV-002 in complete media or 250 uM NOV-002 for 0-4h. NOV-002 induces protein S-glutathionylation and activates stress kinase pathways but the net result on proliferation is opposite to that observed in myeloid cells.

NOV-002 treatment leads apoptosis in SKOV3 cells



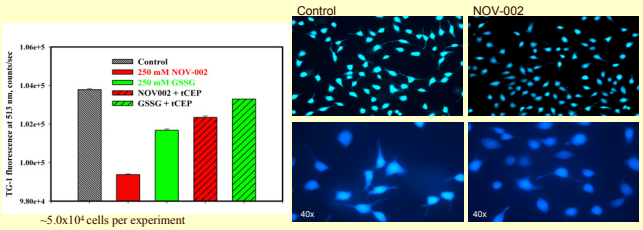
Effects of NOV-002 on cell cycle distribution. Cell cycle distribution and annexin staining was measured in the Flow Cytometry Facility for control and SKOV3 cells treated with 250 uM NOV-002 daily for 5 days. The results are expressed as the mean ± S.E., N=3.

NOV-002 treatment increases ROS levels



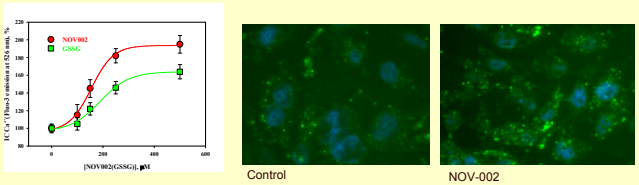
ROS determination by DCF-DA in SKOV3 cells treated acutely and chronically with NOV-002. 5-(and-6)-chloromethyl-2′,7′ -dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA) was used to determine the increase in reactive oxygen species in the five minutes following a 250 mM dose of NOV -002 in control and SKOV3 that were pretreated with 250 mM NOV-002 daily for three days.

Effects of NOV-002 on free sulfhydryls



Effects of NOV-002 on free sulfhydryls in SKOV3 cells. Free protein sulfhydryl content of intracellular proteins was measured at 0 and 30 min following 250 uM NOV-002 or GSS alone or following tCEP (reducing agent) treatment. Supernatants from cell lysates (standard lysis buffer) were passed through Biospin-6 (BioRad) size-exclusion columns and diluted 1:100 with 20 mM PB(pH=7.4) in quartz cuvettes. After the addition of TG-1 (final concentration 5 μ M, fluorescence at 513 nm (excitation at 379 nm) was recorded in real-time kinetics until saturation. The protein concentration of cell lysates was detected using the Bradford procedure (BioRad). The averaged emission saturation values were normalized for protein concentrations. The ThioGlo-1 emission (at 513nm) for each treatment group was averaged and plotted as mean ± SD (n = 6); (*=p≤0.001). Cell surface free sulfhydryls is represented on the right .

Effects of NOV-002 calcium mobilization



Effects of NOV-002 on calcium mobilisation in SKOV3 cells. The dynamics of intracellular Ca^{2+} changes were measured using a Modulus™ Microplate Multimode Reader (Turner BioSystems, Sunnyvale, CA) with a "Blue" optical kit (Ex = 490 nm, Em = 510-570 nm) and standard kinetics mode. The starting point for the kinetic experiments began ~ 2 min after the addition of NOV-002 or GSSG (e.g. the time necessary for additions of drug to all wells). All fluorescent measurements were corrected using the following controls: fluorescence of unlabeled cells, fluorescence changes in labeled cells without any addition of what, the effect of addition of solvents on the cellular fluorescence without probes. Each treatment group was averaged and plotted as mean ± SD (n = 6).

Conclusions

1)NOV-002 induces oxidative signaling in both tumor and myeloid lineage cells as evidenced by intracellular and cell surface protein S-glutathionylation and stress kinase activation.

1)However, the net effects on cell proliferation are opposite – inhibition in the ovarian tumor cells and stimulation in the HL60 myeloid lineage cells.

1)Such cell type-dependent consequences of oxidative signaling by NOV-002 may ultimately be of consequence to its clinical profile in cancer patients.