Temozolomide-perillyl alcohol (NEO212) is effective against MGMT positive and negative nasopharyngeal carcinomas

Thomas C. Chen [1], Weijun Wang [1], Hee-Yeon Cho [1], Florence M. Hofman [1], Axel Schonthal [2]

1. Dept. of Neurological Surgery, 2, Molecular Biology and Immunology, Keck School of Medicine at USC

Keck School of Medicine of USC

Abstract

Despite multimodality therapy involving surgery, radiation, and chemotherapy, the treatment of nasopharyngeal carcinoma (NPC) is still problematic. Recently, we have conjugated the monoterpene perillyl alcohol (POH; delivered intranasally for malignant glioma patients; Phase II) with the alkylating agent temozolomide (TMZ; chemotherapy for gliomas) via an unique carbamide bond. This new compound TMZ-POH (NEO212; NeOnc Technologies, LA, CA) was developed with the goal of intranasal administration in NPC patients.

NEO212 was used to assess activity against two NPC cell lines (NPC TW1-MGMT negative; NPC TW4-MGMT positive; Professor Lin, Taiwan University) both in-vitro and in-vivo. Acute cytotoxicity assay was performed using Cell Death Elisa assay with apoptosis detected within 24 hours (IC50 50 uM). Chronic cytotoxicity assays (7-10 days) using Colony Formation Assays (CFA) demonstrated IC50 of 50 uM for both cell lines. Mechanism of cytotoxicity was DNA damage (irregardless of MGMT status), with cleavage of caspase 7 and CHOP expression. In-vivo activity was demonstrated by implanting luciferase transfected NPC TW4 cells into the subcutaneous flank of nude mice and treating with subcutaneous injections of NEO212 once a palpable tumor was detected. NEO212 was administered at concentrations of 5 mg/kg/day or 30 mg/kg/day x 20 days. Micro PET imaging demonstrated good control or shrinkage of the tumors over those 20 days. Future experiments will focus on an orthotopic nasopharyngeal model and nasal delivery of NEO212. If successful, these experiments will provide proof that a non-invasive intranasal route for NEO212 may be a novel potential therapy for NPC patients.

Introduction

NEO212 is a novel chemical entity that was generated by covalently linking two different molecules, perillyl alcohol (POH) and temozolomide (TMZ). *Fig. 1* shows the chemical structure of NEO212 (also described as (S)-Perillyl Alcohol Temozolomide Carbamate)

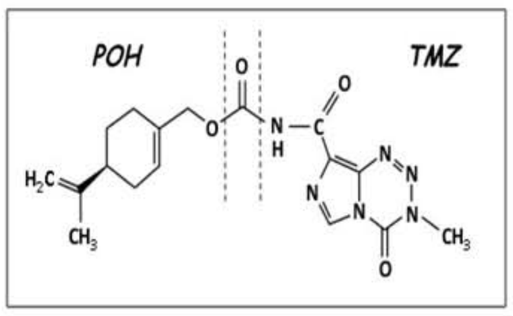

Fig. 1: Chemical structure of NEO212. Perillyl alcohol (POH) was covalently conjugated to temozolomide (TMZ) via a carbamate bridge

Results

Figure 2

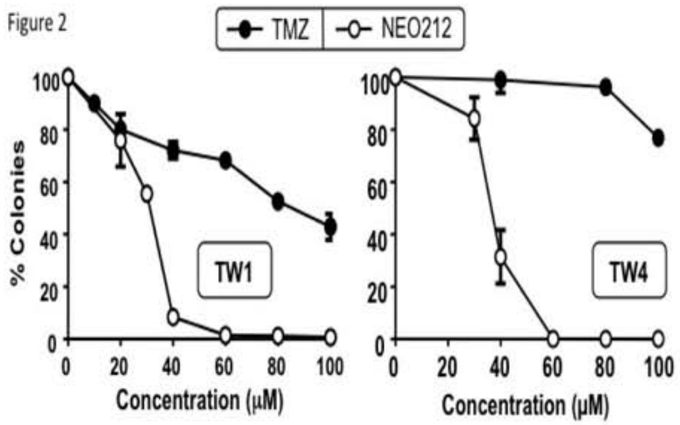

Fig. 2: Survival and colony-formation of the NPC cell lines. TW1 and TW4 cells were treated with increasing concentrations of NEO212 or TMZ. After 48 hours, the drugs were removed and the cells received fresh medium without any drugs added. The cells were left undisturbed for 12 days, after which time the number of newly formed colonies was determined. The number of colonies from those cultures that had not received any drugs, or had received vehicle only, was set at 100%. As can be seen, NEO212 at a concentration of 60 μM completely prevented any and all colony formation (0% survival) in both cell lines, whereas the cytotoxic effects of TMZ were substantially weaker.

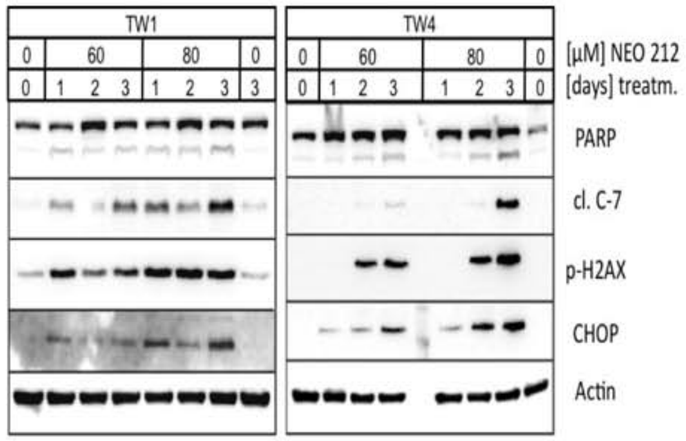

Fig. 3: Induction of molecular indicators of cell stress, DNA damage, and cell death/apoptosis. TW1 and TW4 cells were treated with 60 or 80 μM NEO212 for 1, 2, or 3 days. Cellular proteins were harvested and analyzed for PARP cleavage (i.e., appearance of a faster migrating band) and cleaved caspase 7 (cl. C-7), both of which are indicators of an ongoing cell death process. Stronger signal for p-H2AX in NEO212-treated cells demonstrates DNA damage induced by drug treatment. Increased signal for CHOP in NEO212-treated cells demonstrates severe cellular stress, in particular endoplasmic reticulum stress.

Figure 5

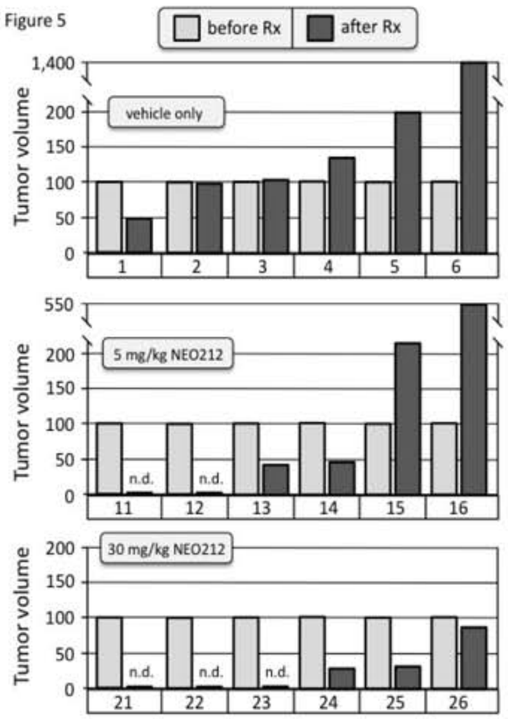

Fig. 4: Tumor growth during 28-day treatment period. Shown is tumor size before and after treatment for each animal. Animals #1–6 received vehicle only (no NEO212); animals #11–16 received 5 mg/kg NEO212; animals #21–26 received 30 mg/kg NEO212. All animals received once-daily injections for 28 days. Tumor volume was measured by bioluminescent imaging of all animals (see also Fig. 6) before the onset of treatment (light gray bars, before Rx) and again one day after the termination of the 28-day treatment regimen (dark gray bars, after Rx). The extent of bioluminescence (total light flux) for each animal was converted to 100% before drug treatment, for easier comparison purposes. n.d.: not detectable.

Figure 6

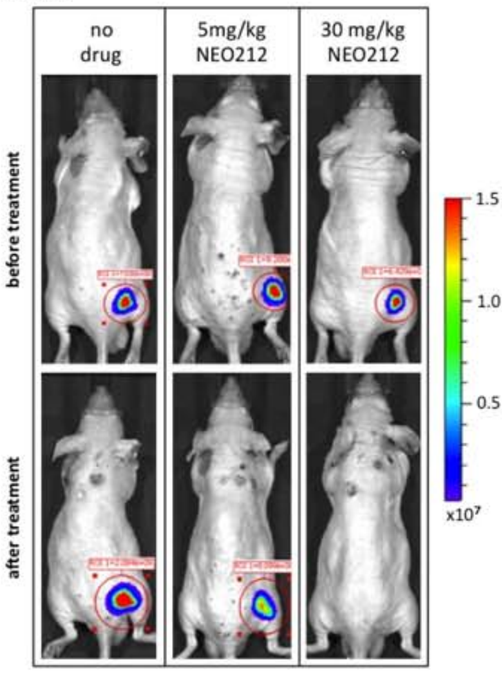

Fig 5. Whole-body bioluminescent imaging. Shown are three representative animals from each of the three treatment groups, where top and bottom photo is from the same animal. Top photos were taken before the onset of treatment. Bottom photos were taken one day after the 28-day treatment regimen. The heat (color) bar to the right shows the scale of light flux, where red represents stronger flux (more tumor growth) than blue (less tumor growth).

Discussion

We have generated a novel chemical entity, NEO212, by covalently linking perillyl alcohol (POH) to temozolomide (TMZ). This new compound has revealed striking anticancer activity in preclinical nasopharyngeal carcinoma (NPC) models. In vitro, NEO212 exhibited substantially greater tumor cell killing potency than either of its individual components, POH or TMZ alone. In an in vivo mouse model, treatment with NEO212 resulted in clear therapeutic outcomes, in the absence of conspicuous side effects. Thus altogether, NEO212 harbors potential for the treatment of patients with NPC, and clinical trials should follow.