

Low-dose naltrexone: tricking the body to heal itself

Researchers at The Pennsylvania State University College of Medicine, Hershey, Pennsylvania have discovered the mechanism by which a low dose of the opioid antagonist naltrexone (LDN), an agent used clinically (off-label) to treat cancer and autoimmune diseases, exerts a profound inhibitory effect on cell proliferation. It has been postulated that opioid receptor blockade by LDN provokes a compensatory elevation in endogenous opioids and opioid receptors that can function after LDN is no longer available. Using a novel tissue culture model of LDN action, the mechanism of LDN has been found to target the opioid growth factor (OGF, [Met⁵]-enkephalin) and OGF receptor (OGFr) axis. This discovery, reported on page 1036 of this issue of *Experimental Biology and Medicine*, provides new insights into the molecular pathway utilized by an increasingly important clinically prescribed agent that serves as a basic biological regulator of cell proliferative events related to pathobiological states such as cancer and autoimmune diseases.

Although the antitumor effects of opioid antagonists were first noted by Drs Zagon and McLaughlin in 1981 (*Life Sci* 1981;28:1095–102), the first full reports about opioid antagonists modulating growth processes occurred in 1983 (*Science* 221:671–3; *Science* 221:1179–80). This led to the hypothesis that endogenous opioid systems play a role in cancer, development and cellular renewal (*Life Sci* 1984;35:409–16, *Life Sci* 1984;35:2057–64). These papers revealed that a short-term opioid receptor blockade with naltrexone (NTX), a general opioid receptor antagonist devoid of intrinsic activity, results in an elevation in endogenous opioids and opioid receptors in response to the opioid receptor blockade. Interference of opioid peptide–opioid receptor interactions for a short time each day (4–6 h) with LDN provided a subsequent window of time (18–20 h) for the increased levels of endogenous opioids and opioid receptors to interface and elicit a robust functional response: inhibition of cell proliferation. The question that now can be addressed is which endogenous opioid(s) and opioid receptor(s) are responsible for LDN's effects on cell proliferative processes.

The present study was structured to focus on the relationship of endogenous opioid pathways and the repercussions of intermittent opioid receptor blockade with regard to cell proliferation. A unique tissue culture model of LDN using a short-term exposure to NTX was developed, thereby avoiding the confounding variables introduced by systemic influences and allowing a dissection of the biological events involved. Screening of a wide variety of opioids (some selective for specific opioid receptors) revealed that only exogenous OGF had a profound effect on depressing cell proliferation. Removal of endogenous OGF by antibody neutralization in cultures given a short-term opioid receptor

blockade by NTX eliminated the repressive effects of this peptide on cell proliferation, indicating that the repercussions of short-term NTX exposure *in vitro* was dependent on OGF. Short-term NTX blockade continued to exert a negative effect on cell proliferation even when the classical opioid receptors, μ , δ and κ , were knocked down by siRNA technology. However, short-term NTX treatment did not repress cell proliferation when cells were subjected to siRNA to the non-classical opioid receptor, OGFr. These results indicate that the effects of short-term NTX *in vitro* are dependent on the OGF–OGFr axis. Previous studies have shown that the OGF–OGFr axis regulates cell proliferation by altering the G1/S phase of the cell cycle through the cyclin-dependent inhibitory kinases p16 and p21. Knockdown experiments with siRNA show that a short interval of exposure to NTX in tissue culture required p16 and/or p21 in order to have a functional outcome on cellular processes.

The research team was comprised of Dr Ian S Zagon, Distinguished University Professor, and Dr Patricia J McLaughlin, Professor, along with Dr Renee N Donahue, in the Department of Neural & Behavioral Sciences. Drs Zagon and McLaughlin not only discovered the phenomenon of LDN, and subsequently the OGF–OGFr axis, but have been at the forefront of translating their findings of LDN – and OGF – from the bench to the bedside. LDN has proven successful in Phase I and II clinical trials in the treatment of Crohn's disease, and OGF has been reported to be safe and efficacious for the treatment of advanced pancreatic cancer. Co-author Dr McLaughlin states: 'Now that we know LDN uses the OGF–OGFr axis as the pathway to control the cell cycle, this expands our arsenal of biological-based treatment modalities to bring about a change in disease states reliant on cell proliferation that not only includes LDN, but exogenous OGF and the imidazoquinoline, imiquimod. This information also provides the basis for a rational approach to the design of diagnostic tools and measures of therapeutic efficacy.' Dr Donahue, who has devoted a concentrated effort on improving the health of women through research explains: 'This study joins a series of other investigations (*Amer J Physiol* 2009;296:R1716–1725; *ibid*, 2009;297:R1154–R1161; *Gynecol Oncol* 2011;122:382–388; *J Cancer Ther* 2011;2:110–124; *Exp Biol Med* 2011;236:883–95) demonstrating that both LDN and OGF offer powerful treatments to combat a devastating cancer that strikes over 20,000 women in the US each year and stands as the fifth leading cause of cancer-related deaths in females. Thus, one should not lose sight that the OGF–OGFr axis is a new frontier in understanding the pathogenesis and treatment of a cancer that has been, up to now, recalcitrant to conventional therapies.' Dr Zagon adds that 'The exciting results that the mechanism of LDN

uses the OGF-OGFr axis, brings together two very important opioid-based treatment modalities under one umbrella. This common denominator in a physiological pathway does much in now directing our attention to how the OGF-OGFr system works, and explains why an opioid agonist (i.e., OGF) and antagonist (NTX in the form of LDN) have the same effects. Moreover, LDN is an oral medication, generic, inexpensive, and non-toxic, and has been documented to alter the course of both neoplasias and autoimmune diseases such as Crohn's and multiple sclerosis, making this drug especially attractive as a therapeutic agent. The fact that OGF has been found to be a potent anti-inflammatory agent (*Immunobiology* 2011;216:173–183; *ibid*, 2011;216:579–590) also opens the door to the potential

treatment of diseases of the immune system (HIV/AIDS) infections, hypersensitivity, and neurodegeneration, which involve cell proliferation, thereby widening the benefit from therapeutic manipulation of the OGF-OGFr axis by LDN.'

Dr Steven R Goodman, Editor-in-Chief of *Experimental Biology and Medicine* said 'These researchers from the Milton S Hershey Medical Center have made the important discovery of the mechanism by which a low dose of the opioid antagonist naltrexone (LDN) can suppress cell proliferative-related disorders such as cancer and autoimmune diseases. This is an exciting new direction for future therapy.'

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