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REVIEW

Naltrexone at low doses (LDN) and its relevance to cancer therapy

Wai M. Liu and Angus G Dalgleish

Institute for Infection and Immunity, St George's University of London, London, UK

ABSTRACT

Introduction: Naltrexone was designed to inhibit opioid receptors without activating them and hence used to block the stimulatory effects of morphine and heroin. It was noted that in certain patients being treated with naltrexone for an opioid addiction many reported significant secondary benefit when being weaned off naltrexone. This group of patients had chronic inflammatory and autoimmune conditions and reported improvements whilst using the lower dosages of naltrexone. There have also been recent anecdotal reports of cancer resolution following the use of low doses of naltrexone (LDN). However, the mechanism of action is unclear.

Areas covered: We review three mechanisms through which LDN can influence cancer progression; namely, (a) antagonism of receptors to which LDN binds, which include toll-like receptors 7–9 that lead to IL-6 suppression b) modulation of immune function in patients; and c) direct inhibition of signaling pathways involved in cancer cell control, including the priming of pro-apoptotic pathways.

Expert opinion: Considering the increase in the number of anecdotal reports of activity, there will likely be a bigger drive toward using LDN in the oncological setting. These reports support clinical trials of LDN in cancer, especially when given in combination with certain chemotherapy.

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1. Introduction

Naltrexone was discovered as an orally available analogue of Naloxone, which was developed as an intravenous drug capable of reversing the negative side effects of morphine [1]. In addition to desired effects of pain control, included severe respiratory depression, constipation, addiction and death by overdose. Morphine is one of a number of opiate drugs originally derived from or based on the poppy (*Papaver somniferum*) that includes heroin, methadone and pethidine. These drugs, which are all very useful for numbing pain, lead to addiction and withdrawal side effects, in addition to tolerance requiring bigger doses to achieve the same level of pain control [2]. These drugs mimic the endogenous neuropeptides, which act throughout the peripheral and central nervous system by stimulating several types of opioid receptors. There are many types that constitute the super-family of receptors to which opioids can bind, which share similarities and includes the somatostatin receptor and the toll-like receptor (TLR).

Naltrexone is an opiate receptor antagonist preventing opiate stimulation; it has been used for decades as a treatment for addiction to opiates as it prevented the euphoria induced by recreational use of morphine and heroin [1,2]. Mechanistically, naltrexone interfered physically with the interaction between opiate and receptor, and by doing so neutralized their action [2]. In reality, however, opiate receptor expression in cells is both complex and malleable, and repeated and chronic stimulation/blockade by naltrexone could lead to changes in the expression and distribution of the receptors. Indeed, in some instances, blocking these receptors to negate opiate action could actually result in a compensatory increase in other receptors [3]. This introduces the interesting possibility that a key 'MOA' for naltrexone could actually be to increase the expression of related receptors. However, this could also pose a concern; not only would complicate the treatment of addiction for which naltrexone was initially used but these receptors could provide new targets for other ligands. The implication would be using naltrexone to counteract opiate addiction could unintentionally increase the action of endogenous ligands. It is thus conceivable that naltrexone could influence more than just disorders of addiction. Of particular note, and relevance to the current review, endogenous opioids were reported to be able to influence the immune system to enough of an extent to be considered as immune modulators and a role as immunotherapy was initially considered in the early 1980s [4].

The first clinical extrapolation of this effect was made by Dr Bihari who noted that sick HIV/AIDS patients had low measurable endorphin levels which could be enhanced by naltrexone in low doses. This increase was subsequently shown in a small randomized trial to prevent opportunistic infections [5]. A series of papers by Zagon and McLoughlin quickly followed that demonstrated the presence of opioid receptors in and on multiple types of immune cells as well as the existence in these cells of mRNA coding for these receptors [6]. In over 300 papers, Dr Zagon and colleagues confirmed that the endorphin-receptor system is involved in every biological system that regulates the immune response. Blocking opioid receptors briefly with naltrexone could cause

CONTACT Wai M. Liu wliu@sgul.ac.uk Institute for Infection and Immunity, St George's University of London, 2nd Floor, Jenner Wing, Cranmer Terrace, London SW17 0RE, UK

an upregulation in the production of endorphins, which ultimately acted to correct immune system dysfunction.

The aim of the current review is to discuss the importance of the opioid receptor in determining the ultimate anticancer action of naltrexone. The anticancer effects of LDN will also be discussed, which appear to work along two streams. We will focus on the direct effect of LDN and how it is able to arrest tumor growth and enhance apoptosis and will also detail the effect that LDN has on supporting the anticancer actions of the immune system. Attention will also be paid to the interactions with other receptors to which LDN can bind and elicit therapeutic function.

2. Opioid receptors

Opioids exert their effects by engaging a super-family of G-protein-coupled receptors (GPCRs), of which there are many. The three archetypal ones named μ, δ and κ are structurally similar and vary in their tissue distribution. This means their activities and actions can be distinct and tissue specific; indeed, they can interact with a variety of agonists and other drugs and all with differing affinities [7]. Binding to these receptors affects the action of the cell through well understood processes, which ultimately engage central signaling pathways, such as the PI3 kinase and RAS pathways, which together influence cell fate.

The activation of the central signaling pathways is not an effect unique to any one of the opioid receptors, but most likely a generic feature of GPCR activation. Similarly, binding through these receptors can be promiscuous, with multiple ligands capable of binding to many receptors with varying affinities. Furthermore, this plasticity in ligand binding is not limited to binding to the opioid receptor. For example, the chronic administration of the cannabinoid receptor antagonist SR141716A can modify the action of the opioid receptors [8], through a spill-over effect of the cannabinoid into the semi-homologous opioid receptor. Similarly, morphine can elicit a physiological response by cross-reacting with the somatostatin receptor [9]. Taken together, it is easy to see how exogenous sources of opioids and their related compounds could modify the natural functions of ligand-receptor systems in the body; a number of which may not be canonical.

This can often result in confusion when trying to understand the relationship between ligand and receptor binding, and how this can affect overall cell functioning. This has only added to the confusion in the evidence that shows the effects of opiates can be both anticancer in nature as well as cancer-supporting. Furthermore, the manner in which the receptors are activated and react can be different depending upon the ligand. The binding affinities of differing ligands, their spatial and temporal engagement profiles, these all affect the way key intracellular cascades are activated, leading to differences in the overall response.

Furthermore, naltrexone has multiple chiral centers that lead to a number of stereoisomers. Like a number of other compounds, this stereogeneity can determine ultimate function, as binding to or antagonizing of cognate receptors can vary according to the spatial orientation of the ligand. For example, it has been reported that the two main enantiomers of naltrexone antagonize different receptors; with levo-naltrexone working on opioid receptors and dextro-naltrexone preferring TLRs [10]. Thus, depending on which enantiomer is present, and which receptor is involved, it is clear that naltrexone can have such varying responses.

These responses vary depending upon the nature of the ligand:receptor engagement as well as the intracellular cascades involved. Thus, for example, when morphine binds to receptors, it does so in a way that elicits an intracellular response that ultimately lead to a modification of synaptic functioning. Conversely, when naltrexone binds to the same receptors, the way in which they interact will differ, with the consequential effect that fundamentally differs from morphine [11]. In addition to the physical antagonism of morphine, the effect of naltrexone can include responses such as desensitization of receptor, activation of ancillary signaling systems, and initiation of cellular proliferation elements.

Taken together, it is clear that two different drugs that bind to the same receptor can have different effects.

3. LDN and cancer

The scientific rationale for LDN in cancer patients is compelling either alone or in combination. Nevertheless, the high cost of a clinical trial to justify registration, together with the fact that LDN is not protected, means that there have been no significant randomized studies to date. However, the numerous anecdotal responses justify further clinical studies. Of particular note, a number of these anecdotal reports of response to LDN have been reported both administered as single agents or more usually in combination with another agent. Activities have been seen in lung adenocarcinoma [12]; adenoid cystic tongue carcinoma (in combination with vitamin D3) [13]; renal cell cancer (together with Alpha Lipoic Acid (ALA)) [14]; and pancreatic cancer (with ALA) [15,16]. The potential for combination is even more intriguing from a clinical perspective, Lissoni et al. report four partial responses and one stable disease in nine patients with renal cell cancer treated with IL-2 and LDN. Significantly, however, these patients had disease progression when using IL-2 alone [17].

This small selection of examples highlights activity in a range of cancer types, with no one type appearing to be more receptive to LDN treatment. This suggests a broad mechanism of action. Nevertheless, a small number of processes appear to be impacted more often, which suggests that anticancer activity is achievable via modulation of immunity, and activation of cell signaling cascades underpinning cell proliferation and death.

A number of papers have highlighted an ability of naltrexone to suppress tumor growth [18]. These studies from both *in vitro* and animal studies have not established an explicit mechanism of action, but in broad terms can involve two areas. LDN can directly interfere with intracellular signaling pathways that result in an arrest of cell proliferation and up-regulation of proteins associated with promoting apoptosis. LDN is also able to modify immune-function, which can ultimately enhance the cytotoxic activity of immunity. To complicate the narrative, there is also a plethora of papers that show

alterations to cell signaling and immune modulation in a similar fashion way LDN does can actually enhance cell growth [19,20]. So, taking the effects of LDN in isolation, it is difficult to establish the principal mechanism of action. What seems to be important though is the ultimate outcome of treatment with naltrexone is critically determined by the dose and schedule by which it is used. Indeed, we have compared the effect on the gene expression profile of cancer cells of low and high doses of naltrexone, and shown the profiles are completely different depending on the dose used. Specifically, gene ontology analysis showed low doses of naltrexone had a greater impact on genes associated with cell cycle control and the immune responses, and that these effects were unique to this lower dose [21].

In vivo studies performed in 1980s, highlighted the importance of dose in determining the overall effect as mice that were treated with clinically conventional doses of 10 mg/kg induced a continuous occupancy of the opioid receptors, which was associated with increased tumor growth [22]. However, if doses were reduced to 1 or 0.1 mg/kg, the receptor blockade was incomplete. Binding sites were thus available to exogenous opiates and endogenous endorphins, resulting in activation of their anti-tumor actions. In addition to dose, the schedule of naltrexone administration was also crucial, with intermittent administration of low-dose naltrexone achieving the greatest anti-tumor response. The reason for this still remains elusive, but it has been suggested that the extent to which opioid receptors are antagonized, can induce changes in the types and numbers of opioid receptors expressed. For example, a study in albino mice reported LDN was able to increase the expression of the opioid growth factor receptor (OGF-R), which was also associated with alterations to key signaling pathways, a number of which were directly linked to cell growth and death [23].

An increase in expression of another type of receptor in a compensatory manner to make up for the loss of another has been seen in other ligand:receptor relationships. As this receptor is of a different type, its action would be different to the one it replaced and so ultimately the ligand/receptor relationship at this level would change. Thus, it would be feasible that a particular ligand with a conventional set of actions elicited through a particular type of receptor could inadvertently activate other cellular processes via this compensatory change in receptor distribution. Indeed, our own studies reported in 2016 have shown that a different group of genes can become activated, which fundamentally differ depending upon the particular dose of naltrexone used and the level of receptor antagonism [21]. In a similar note, the effect of naltrexone can differ in individuals as the compensatory changes that influence intracellular signaling can differ.

Our studies have also highlighted anticancer action of LDN is associated in part with changes to pERK and PI3-K signaling. Additionally, as these cascades are inextricably linked to apoptosis and the mechanisms that regulate it, we and others have shown LDN is capable of altering the balance of pro and anti-apoptotic proteins that regulate cell killing. Specifically, our *in vitro* and *in vivo* models show how the pro-apoptotic proteins BAX and BAD can be enhanced by a short-term exposure to LDN, which in turn can sensitize cancer cells to the cytotoxic effects of common chemotherapy agents [21]. Crucially, others have shown similar apoptosis-enhancing effects via engagement of parallel systems [24].

4. Cancer inflammation

As discussed, there is good reason to suggest that LDN has a potential role in anticancer therapeutic regimens. Indeed, the effects it has on intracellular signaling pathways that support oncogenesis is a means by which LDN can be used to disrupt aberrant cell growth. However, the effects that LDN also has on the immune system can also contribute to its anticancer action. Inflammation, particularly chronic inflammation forms the basis of a number of diseases. Indeed, we and others have described how chronic inflammation, arising as a result of chronic exposure to a non-infective irritant, may support cancer development. Examples of this include the long-term irritation and exposure to asbestos fibers leading to mesothelioma, chronic bronchitis and emphysema as a predisposing factor to lung cancer, and the association between chronic inflammatory bowel disease and colon cancer [25]. Drugs that target particular elements of inflammation, such as the inhibitors of cyclo-oxygenase (COX), have shown activity and potential clinical benefit in a cancer setting [26]. Similarly, there is considerable epidemiological evidence supporting the effectiveness of the ubiquitous non-steroidal anti-inflammatory drug aspirin as a preventative for cancer development [27].

LDN has potent anti-inflammatory qualities, it appears to modulate and modify different elements of the immune system. *In vitro* investigations using models of individual components of immunity have described naltrexone altering the intracellular signaling in and subsequent cytokine output of certain immune cells. Although immunity as a whole is more complex and cannot be simply considered a collection of individual cells working in isolation, it is interesting to note that in patients administered LDN, the systemic levels of cytokines that drive both humoral and cell mediated inflammation, such as G-CSF, IL-4, IL-6, IL-10, IFN-alpha and TNF-beta, were significantly reduced after eight weeks [28]. Moreover, LDN has been reported as having a marked clinical effect on a number of clinical conditions whose shared pathology is chronic inflammation, which include Crohn's disease and psoriasis, as well as numerous inflammatory autoimmune diseases, such as arthritis, SLE and multiple sclerosis. Thus, it is this ability to dampen down cytokines driving key elements of immunity that lends support to the growing view that LDN is immune-modulatory.

Additionally, LDN is also thought to improve adaptive immune responses by enhancing the maturation of professional antigen presenting cells, as studies have shown increased expression of maturation markers on dendritic cells (DCs) following culture with LDN [29]. More significantly, DCs were able to elicit responses in autologous T-cells.

Although it is unclear how naltrexone, which is fundamentally an opioid antagonist, can modify the levels of cytokines that influence immune function, what is clear is that opioids such as morphine have been known for some time to be immunosuppressive [30]. Opioid receptors have been found

on immune cells [31], which have a role in regulating immunity [32]; specifically, studies have indicated that antagonism of opioid receptors can affect the activities if a range of immune cells. Indeed, the upregulation of OGF-R, which has been discussed by Zagon's group, is intimately associated with the ability to suppress the ability of colony formation, migration and invasion in cervical cancer cells. These effects were associated with reduced expression of P13-K, AKT and mTOR *in vivo* and *in vitro*, which are central signaling cascades regulating immune function [33]. Similarly, LDN also, by means of enhancing OGF-R, can suppress the epithelial mesenchymal transition of cervical cancer cells, which has an indirect effect on tumor associated macrophages associated with reduced IL-10 expression in nude mice [34].

The similarity between opioid receptors and other GPCRs suggests the possibility of other receptors being responsible for naltrexone action. Therefore, disruption of signaling via receptors of the same super-family or those that modify signaling through them is also thought to contribute to the mechanism by which naltrexone imparts its immune-modulatory effects. One such receptor that has been described to be part of this response is a distinct class of pattern-recognition receptors called the toll-like receptors. These have a central role in initiating immune responses by serving to recognize specific cellular and molecular patterns of cells damaged by pathogens. Activation of these TLRs, which exist in different classes, varies according to the stimulus, leads ultimately to changes to signaling cascades that orchestrate an immune response.

These responses, which are part-modulated by GPCRs, are an essential part of the innate immune system, providing a first line of defense against microbial invasion and present on all major immune system cell types. Activation of a TLR, of which there are ten, leads to the production of proinflammatory cytokines, often involving NF-KB production, which is a recognized target for autoimmune disease and cancers. NF-KB can enhance cancer oncogene activity, which is a major mechanism whereby it can enhance cancer progression. Importantly, we and others have shown that naltrexone can disrupt immune responses by inhibiting cytokine production by peripheral blood mononuclear cells by antagonizing TLRs [35]. More specifically, we screened a panel of available inflammation receptors and confirmed that naltrexone could completely block TLR-9 on immune cells, with some activity in TLR-7 and TLR-8. We could not detect activity on TLR-4 (which is on the cell membrane) and whose activity was previously reported on glial but not immune system cells. Parenthetically, TLR- 7, 8 and 9 are all intracellular receptors.

An important relevance of TLR-9 inhibition is that it is associated with chronic inflammatory states such as Crohn's and psoriasis. It is also accepted that chronic inflammation is a precursor for many tumor types, whether caused by chronic infections (e.g. HBV, HCC, HPV or EBV) or by chronic irritation (e.g. smoke or diet) [25]. Of further relevance is that TLR-9 stimulation leads to the production of IL-6, which is the cytokine most closely associated with cancer progression.

Taken together, cancers are often associated with inflammation that can lead to suppression of cell mediated immunity, as well as angiogenesis. Therefore, LDN can exert a positive effect on cancer control by (i) inhibiting chronic inflammation and NF-κB activated oncogenic pathways by TLR antagonism; (ii) upregulating immune responses by modulating opioid; and (iii) directly inhibiting cell signaling pathways in tumor cells that support the oncogenic process.

5. Expert opinion

Recent years have seen a rapid increase in the number of reports highlighting a role for LDN in immunological and oncological conditions. These reports present tantalizing glimpses into different ways the drug can be used. Although naltrexone was first employed as a means to support patients with addictive disorders, it was discovered, albeit serendipitously, if used at lower dosages, it could also help with other indications. However, this dose range was very narrow, typically one between 3–5 mg per day for patients. The dosage appeared not to be dependent upon body weight, but more with daily dose, as patients using doses outside of this range commonly reported a loss of activity, which was restored once the dose was re-adjusted to between 3–5 mg/day. More importantly, most of these conditions have a strong inflammatory component and where the effect can be observed directly such as patients with psoriasis, the benefit observed at the commonly used 4.5 mg dose disappears if it is raised to even 6 mg. Reassuringly, however, clinical benefit and activity are quickly restored when the dose is dropped back to 4.5 mg.

The existence of real-world cases describing therapeutic activity by using LDN has led to a number of lab-based studies that have confirmed an immune-modulatory element of LDN. The fact that so many cases have been recorded where adding the agents has improved and/or supported the actions of other treatments highlights the potential of utilizing a drug that is safe and cost-effective. Although there have been a number of randomized trials in some indications showing some benefit, none were large enough to lead to a formal approval. The need for these larger trials would require backing from industry, and understandably, the risks associated with promoting a drug that is generic can be off-putting. Hopefully, the increased understanding of mechanisms of action will present IP and licensing opportunities that will attract the support necessary to deliver a therapeutic product.

Gene analysis of LDN action has identified mechanisms of action, which suggest novel approaches to enhancing its activity. Cancer cells are often resistant to chemotherapy as they possess dysfunctional apoptosis pathways. Studies have shown LDN is capable of altering the balance of proteins that determine cell death in cancer cells, and by swinging apoptosis toward a pro-apoptotic setting, LDN possesses the ability to prime cancer cells to cytotoxic chemotherapy drugs. Studies have also shown that the sequence in which it is given can influence overall activity, and it is the sensitization element of LDN's activity that is an area of work that is currently being explored in more depth. This is something we and others have highlighted and discussed previously [36]. Hopefully, this will allow for new treatment regimens to be developed that can employ LDN more effectively. Ultimately, these combination approaches mean LDN may be able to partner with a wide

range of drugs, and potentially be employed as an 'universal adjuvant.'

There are a number of conditions for which remarkable activity is hard to explain based upon LDN's ability to modulate opioid receptors. This led to a search for additional receptors through which LDN could work, and it was discovered naltrexone could inhibit IL-6 production through TLR-7,8 and 9. This effect is also more likely to explain the reported benefits of LDN in Crohn's Disease and psoriasis, which both over-express TLR-9. The fact that IL-6 is a major promotor of cancer progression and metastatic spread is yet another reason to explore LDN in a range of oncological conditions.

Conflict of interest

WM Liu receives research funding from LDN Pharma Ltd. AG Dalgleish receives funding from the Institute for Cancer Vaccines and Immunotherapy. Both authors are named inventors on a number of patents related to the use of LDN as potential therapy. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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