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Basic Properties of Anthocyanin for Pain Management

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Abstract

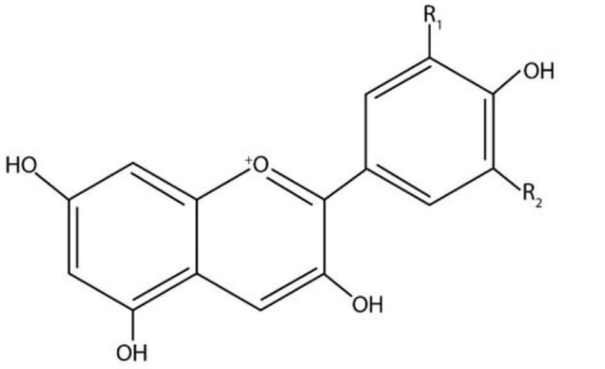
Inflammation and oxidative stress is both two important key players in the development, enhancement, and maintenance of both nociceptive and neuropathic pain. They are almost invariably involved in pain-related diseases, such as all-cause low back pain, diabetic neuropathy, neurodegenerative diseases, myocardial ischemia, cancer, and various autoimmune disorders, among others. They act synergistically and their presence can be beneficial, yet detrimental to neurons and nerves if they are in overdrive state. Meanwhile, anthocyanin, a group of flavonoid polyphenols, is very common in nature and can be easily derived from fruits and vegetables. Accumulating evidence has shown that anthocyanin possesses potent anti-inflammatory and anti-oxidant effects through numerous mechanisms and that its proof-of-concept in ameliorating various pathology of disease states have been extensively documented. Unfortunately, however, the empirical evidence of anthocyanin for alleviating pain has been very minimal to date, despite its potentials. Herein, we discuss the basic properties of anthocyanin and its relevant pain mechanisms which could become potential targets for pain management using this natural compound.

Introduction

Pain is a universal feeling innately presents in almost every human being. Naturally, pain is designed to protect our body from various harmful insults, yet pain can often be problematic during persistent pathological states, such that seen in the majority of diseases. Further complicating matters, abnormal processing of pain signals either in the periphery or in the central nervous system (CNS) in the presence of ongoing noxious insults can lead to the development of neuropathic pain. Neuropathic pain is difficult to manage due to multiple issues, including inadequate diagnosis, high complexity, and less understanding of the mechanisms involved, inappropriate treatment selections and outcome reporting, and inadequate comorbidity management [1]. In fact, the limited options for neuropathic pain have led to the surge of opioid addiction and overdose epidemic in the U.S [2]. Given the account of the extremely common prevalence of pain (i.e., more than one-third of Americans suffer from acute or chronic pain [3]) and the desperate needs for novel pain therapeutic strategies, it is worth to take a look on an abundantly available natural substance with strong anti-inflammatory and anti-oxidant properties such as anthocyanin (ANC). ANC has been studied extensively over the past decades and has been proven to either mitigate or alleviate a vast array of diseases, including infections (e.g., common colds, and urinary tract infections), cardiometabolic and degenerative diseases (e.g., hypertension, and myocardial infarction), and to autoimmune disorders (e.g., ulcerative colitis, and systemic lupus erythematosus) [4], [5], [6]. Extensive studies have proven that ANC exerts a clinically significant effect as an anti-inflammatory and anti-oxidant against those aforementioned diseases. Given its potential protective effects toward inflammation and oxidative stress, ANC, thus, is also potential for the treatment of pain, including nociceptive and neuropathic pain. In fact, ANC has been shown to reduce inflammation-induced pain behavior in animal study with similar efficacy as to NSAID, as well as demonstrating proof-of-concept prevention and treatment for diabetic neuropathy [7], [8]. We, therefore, would like to discuss the relevant aspects of ANC and its potential use to be incorporated in the management of pain.

ANC's baseline characteristics

ANC is a group of flavonoid polyphenols regarded as the most abundant water-soluble pigments of plants on earth. ANC is derived from two Greek words which mean red blue [9]. According to its name, ANC gives multiple pigments to many fruits, flowers, and other vegetation's, ranging from red, blue, and orange, to purple [10]. ANCs are naturally found as glycoside and bound to sugar groups, thus are also known as anthocyanidins [11]. There are six types of widely spread and readily available anthocyanidins, which are arguably of importance to human diet, comprises: Cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin (Figure 1) [9], [10]. In addition, glycosylation is essential to increase anthocyanidin's molecular stability and water-soluble capacity [12].



R_1	R_2	ANC type	Pigment
H	H	Pelargonidin	
OH	H	Cyanidin	
OCH_3	H	Peonidin	
OH	OH	Delphinidin	
OCH_3	OH	Petunidin	
OCH_3	OCH_3	Malvidin	

Figure 1: Different types of anthocyanin molecular structure with its associated pigment (adapted from Pojer et al. [9] and Bowen-Forbes et al. [16])

ANC's molecular structure and color are greatly influenced by pH changes. For instance, it is red to orange in color with eight conjugated double bond-carrying cations under very acidic pH (i.e., 1–3) [13], [14], it turns into quinodal blue on pH 4 [10], becomes colorless and form a chalcone at pH 5–6 [9], and ultimately turns to blue-purple with quinodal base at pH 7–8 [15] [16].

ANC has been extensively studied and further processed to become natural food additives [17], as well as food supplements for health-related purposes [18]. In fact, there is plenty of valid evidence supporting the wide-range beneficial effects of ANC in promoting health through various organ systems with numerous molecular mechanisms. For instance, it has been shown through laboratory and epidemiologic studies that ANC consumption can lower the risk of multiple degenerative diseases, ranging from ischemic heart disease to cancer, and helps alleviating generic pain due mainly to its anti-oxidant, anti-inflammatory, and immunomodulation properties.

ANC-rich dietary sources can be easily found in the environment, thanks to its common availability. Among those with highest content are (comprising but not limited to) bilberries (*Vaccinium myrtillus*) with 405 mg of glucoside-type ANC/100 g, elderberries (*Sambucus nigra*) with 794.13 mg of glucoside-type ANC and 462.96 sambubioside-type ANC/100 g, chokeberries (*Aronia melanocarpa*) with 557.67 mg of galactoside-type ANC/100 g, and billberries (*V. myrtillus*) with 405 mg of galactoside-type ANC/100 g, among others [9], [19], [20]. Given its widespread availability, abundant contents, relative affordability, and well-known health promoting effects, ANC merit further studies and perhaps, to some extent, to be incorporated into the treatment of various diseases of which it has been proven to have protective and/or alleviating effects. In this review, we focus to discuss the pharmacokinetics and pharmacodynamics of ANC, especially for its ability to reduce acute and chronic pain transmission.

ANC's pharmacokinetics (absorption, distribution, metabolism, and excretion)

ANC has been demonstrated to be rapidly absorbed, that is, it can be detected in the portal and systemic blood plasma within 6–20 min post-ingestion [21], [22]. Interestingly, it can be absorbed intact, regardless of its molecular sizes and structures or its attached acylated components, although recent evidence seems to confront these findings [23], [24], [25], [26], [27], [28], [29], [30]. The time to reach maximum plasma concentration (T_{max}) of ANC varies, depending on the types of active substance, as well as the dietary source of it. In general, based on animal studies, the T_{max} ranges from 15 min (as seen in bilberries, and elderberries with cyanidine 3-glucoside), 30 min (as seen in blackcurrants with cyanidine 3-glucoside and cyanidine 3-rutinoside, and 4–8-week-blueberries), an hour (as seen in dry marrion blackberries with 3-glucoside), and to 2 h (as seen in delphinidin 3-rutinoside) [9], [24], [23], [31]. It was proposed that the rate and extent of its absorption depend on the structure and composition of glycine, sugar moiety, and its acylation components [31], [32], [33], [34]. It was generally presumed the more complex of an ANC structure, the less rate for it being absorbed [35].

The systemic bioavailability of ANC was reported to be relatively low, with animal studies varied between 0.26% and 1.8% [34], [36], [37], [38], [39], [40], [41]. Some data suggested an estimated range of bioavailability between less than 1 and 2%, with only trace amount

of the substance detected in organs [42]. This finding was supported by the use of radioactive labeling of cyanidin 3-O-glucoside (Cy3G) fed to the mice which showed minimal accumulation of the traced substance outside the gastrointestinal tract tissue (i.e., only 0.76% of radioactivity was detected), suggesting that Cy3G was poorly absorbed by other organs, despite high accumulation in GI tract (44.5%) [43]. However, it was suggested that ANC's bioavailability could be much greater when taking into account of the pre-systemic metabolism, such as Phases I and II metabolism, conjugation, microbe-assisted metabolism, as well as enterohepatic recycling [42], [44].

ANC undergoes specific bodily activities which varied between organs it passes. For instance, the exposure of saliva to ANC in the mouth could degrade approximately 50% of its total amount as a combined result of oral microbes' enzymatic activities, high temperature, and salivary protein bindings [42], [45]. In addition, it also experienced several processes which results in modified ANC structure. For example, oral pH exposure of 6.78 leads to a significant transformation of native ANC to chalcone (which was reported to be up to 30% from total ANC content), or its deglycosylation by oral microbes transform it into aglycones [42], [46].

It is suspected that ANC was significantly absorbed in the stomach as opposed to the small intestine. The notion was based on the observation that *in situ* gastric administration of ANC glucoside and galactoside on rats can readily be observed in the blood plasma in the form of malvidin 3-glucosidase after only 6 min post-administration [21]. ANC absorption in humans has also been confirmed within minutes of its ingestion [47]. In fact, ANC can be found in portal and systemic circulation through translocation activities using bilitranslocase, an organic anion carrier that can be found both in the stomach and liver [21], [48], [49], [50], [51]. Due to the nature of highly acidic gastric content (pH between 1.5 and 4), ANC is stabilized as well as found in the forms of quinodal as well as the flavylium species which are eligible to be transported to the liver by means of bilitranslocase [9], [42].

ANC can also be absorbed from small intestine through various transport mechanisms. Some of them were sodium-dependent glucose co-transporter-1 or other glucose-associated transporters and intestinal bilitranslocase [42], [49], [52]. It had been reported that ANC absorption was greater in the jejunum (roughly 55% from total content) followed by duodenum, although to a smaller extent (i.e., 10%), with no observable absorption through ileum or colon [53]. In total, small intestine was estimated to be responsible for up to 7.5% absorption of ingested ANC, a 3–7-fold higher than the estimated bioavailability of ANC [9], [54]. The size of the molecule also matters for absorption. ANC, being a large water-soluble molecule, should be transported by means of active diffusion, as opposed to its aglycone version (anthocyanidin) which is known to be hydrophobic, thus can passively diffuse across the enterocytes [9], [55], [56].

In addition to being absorbed in the small intestine, ANC also undergoes metabolism, for example, being hydrolyzed to aglycone by the action of various intestinal enzymes, such as β-glucosidase, β-glucuronidase, and α-rhamnosidase [9], [57], [58]. The purpose of the transformation is suspected to ease the transport of these molecules. ANC also undergoes methylation by catechol-O-methyl transferase (COMT), an enzyme responsible for the degradation of catecholamines [26], [59], [60]. It occurs in the kidney tubular epithelial cells, as well as in the vascular endothelial cells, after the release of ANCs in the bloodstream [61].

ANC then reaches the liver wherein it undergoes various metabolic processes, including hydroxylation and glucuronidation through Phases I and II metabolisms [28], [33], [42]. However, the extent of ANC metabolism by cytochrome P450 was unknown, but it was assumed to be related to the hydroxylation of nonreactive carbons [42], [62]. Whereas, phase II metabolism consists of conjugation and glucuronidation, with the latter being assisted by Uridine 5'-diphospho - (UDP) glucoronosyl transferase and UDP dehydrogenase enzymes [42], [58], [63].

ANC also undergoes sulfation by phenol sulfotransferase (SULT1) enzyme which can be found in the small intestine, liver, and platelet [38], [64], [65]. The resulting sulfoconjugate formation of ANC (in this case: Cyanidine and pelargonidin) can readily be found in human urine after ingestion of ANC-rich dietary sources [38], [66].

Although ANC absorption was deemed next to none in the large intestine, the organ plays an important role in enterohepatic recycling of bile-containing ANCs [39]. Native ANC can be readily detected in rat's bile after 20 min of its ingestion [22]. It is also interesting to note that bile can undergo a recycling process for more than 20 times, suggesting a prolonged transit time of ANC in the body, along with its potential extended Phase II metabolism [42]. Given the evidence that ANC was found in bile, it is prudent to assume that its excretion was done through urine and feces. Indeed, a human study using blueberry juice demonstrated that only 4% of native ANC content was found in the urine, with the rest of it was found in the forms of its metabolites [67]. A study using radioisotope-labeled cyanidine 3-glucoside also found that approximately 44% of ANCs were elimited by means of urine, breath, and feces [68]. The study also found that ANC was degraded into various metabolites, such as phenolic, hippuric, and phenylacetic acids. This is in accordance with another finding in which more than 371 ANC metabolites can be detected in the urine [69].

As mentioned previously, organ uptake of ANC was relatively small. A study using rats fed by

blackberry (*Rubus fruticosus* L.) known for its rich ANC content (i.e., 14.8 mmol/kg diet) compared with those of control diet for 15 days was shown to have highest accumulated ANC in the jejunum (605 nmol/g), followed by kidney (3.27 nmol/g), liver (0.38 nmol/g), and brain (0.25 nmol/g) [22]. A greater amount of ANC accumulation in the kidney was reported by the administration of 500 mg/kg of cyanidine 3-glucoside, that is, 700 nmol/g, whereas in the prostate gland was found to be roughly 400 nmol/g [40]. Another study using mice fed with 0.5% bilberry extracts for 2 weeks demonstrated highest ANC accumulation in the liver by 173 pmol/g wet weight of tissue [70]. Moreover, this comprises for up to 51.5% of the total ANC distribution in the body.

Pathophysiology of pain and its relevant targets for ANC

Pain is defined as an unpleasant sensory and emotional experience that is commonly associated with actual or potential tissue damage [71]. Based on its neurophysiological mechanism, pain can be classified as nociceptive and non-nociceptive. Nociceptive pain is elicited by noxious stimuli (i.e., mechanical, temperature, and chemical) to the peripheral tissue in the body. Whenever the threshold for the stimuli is lowered and/or the magnitude of noxious stimuli increases, sensitization occurs [72], which, in turn, transmits the pain impulse to the dorsal horn of the spinal cord to be further modulated and processed into the thalamus and higher cortical function, respectively [73]. On the other hand, the most relevant non-nociceptive pain is neuropathic pain. Neuropathic pain is defined as ongoing pain after injury to the central or peripheral nervous system (PNS) (including but not limited to trauma, metabolic imbalance, ongoing viral infections, and exposure to chemotherapies) [72]. Neuropathic pain is thought to result from abnormal somatosensory processing in the central and PNS. There are several underlying mechanisms, comprising aberrant activation of transducers and membrane instability (due to biophysical changes of ion channels) induced by the previous nerve injury.

The pathophysiology of pain is very broad and complex; therefore, due to space constraints and relevancy, we focus our discussion on the potential prospects of ANC in pain intervention, especially linked to inflammation and oxidative stress.

Pro-inflammatory cytokines and chemokine

Inflammation has long been recognized to play key roles in the occurrence and maintenance of various pathological pains [74]. Inflammation may occur following tissue injury by obnoxious stimuli, that is, through mechanical, thermal, or chemical exposure. Inflammation is marked by the production of various cytokines and/or chemoattractant proteins by the peripheral nerves [74], spinal cord [75], the dorsal root ganglion (DRG) [76], cutaneous source [77], resident or recruited macrophages (including astrocytes and microglia) [78], mast cells [79], endothelial cells [80], and Schwann cells [81] (Figure 2). In addition, cytokines can be delivered to the DRG and dorsal horn of spinal cord by means of retrograde axonal or non-axonal mechanisms, thus further extending its coverage [74].

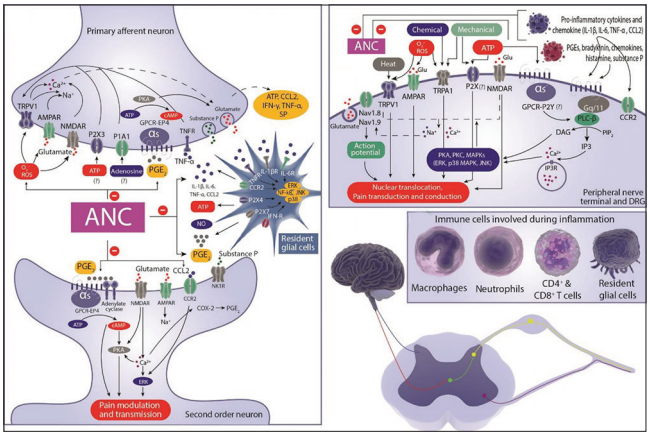


Figure 2: The pathophysiology of neuropathic pain (a) pain is transmitted by means of transduction, conduction, transmission, and modulation, before finally conceived as pain stimulus by brain (perception), (b) various immune cells take roles in these phases, including glial activation in the PNS and central nervous system, (c) various substances and receptors are involved in pain transmission and modulation, including ATP and ROS and its receptors (TRPA1, TRPV1, and purinergic receptors), which were augmented by pro-inflammatory cytokines during inflammation, all of which could be inhibited by ANC at the level of peripheral nerve and dorsal root ganglion, and (d) second-order neuron (some picture materials were taken and modified from the library of science and medical illustrations by sommersault 18:24, licensed under CC BY-NC-SA 4.0, materials are available under Public License)

Among those cytokines, the most critical group being pro-inflammatory cytokines. There is accumulating evidence demonstrating the involvement of various pro-inflammatory cytokines in the initiation, exacerbation, and maintenance of pathological pain, for example, interleukin 1β (IL-1β). It is commonly synthesized and secreted by the recruited and activated macrophages in the vicinity of inflamed area and even in the DRG neurons [82]. IL-1β has been proven to exert hyperalgesia on *in vivo* administration [83]. Furthermore, inhibition of IL-1 receptor by an antagonist was able to attenuate hyperalgesia and nerve injury-induced allodynia [84], [85]. In addition, IL-1β was also responsible to increase certain neuropeptide and lipid compound productions which are strongly associated with pathological pain and inflammation, such as substance P and prostaglandin E_2 (PGE_2) [86], [87].

Another example is IL-6. This cytokine has pleiotropic effects with regard to nerves and pain. IL-6 plays an important role in CNS axonal regeneration, but also can be destructive during inflammatory period [88], [89]. IL-6 also activated astrocytes and microglia, two resident glial cells which possess several critical interplays with neurons during either

physiological or pathological states [90]. Indeed, IL-6 immunoreactivity was observed in the dorsal and ventral horns of rats following nerve injury (e.g., sciatic cryoneurolysis/SCN), whereas its intrathecal administration could mimic and potentiate pain behavior after SCN [91]. In addition, spinal nerve lesion on IL-6 knockout mice demonstrated delayed response of mechanical allodynia [92], suggesting that IL-6 may contribute to the development of neuropathic pain. On the other hand, increased PGE_2 levels were shown to increase the secretion of IL-6 from injured nerve, while the administration of selective PGE_2 receptor 4 antagonist (EP4) and protein kinase C (PKC) inhibitor attenuated this effect, suggesting that PGE_2 may increase IL-6 secretion (or even production) by the recruited macrophages and resident glial cells in an inflamed area by means of PKC pathway and EP4 receptor [93].

IL-6 also plays an important role in cancer pain. An animal study with implanted tumor cell demonstrated an increased level of IL-6 mRNA expression and another study confirmed elevated levels of IL-6 following tumor cell implantation [94]. The mechanism of IL-6-induced cancer pain was thought due to trans-signaling pathway in the DRG, thus inducing its hyperexcitability which in turn upregulates transient receptor potential vanilloid channel type 1 (TRPV1) through Janus-activated kinase (JAK)/Phosphoinositide 3-kinase (PI3K) signaling pathway [95].

Tumor necrosis factor-alpha (TNF-α) is another critical cytokine in the pathophysiology of inflammatory pain. It was predominantly secreted by microglia under the influence of interferon gamma (IFN-γ) during neuroinflammatory events. Intraplantar injection of TNF-α had been shown to induce mechanical and thermal hyperalgesia. TNF-α bound to TNF receptor 1 (TNFR1) and TNFR2, both of which found in both neurons and glial cells [96], which in turn activated NF-κB following peripheral nerve injury [93]. Furthermore, the administration of TNF-α antagonists was shown to attenuate inflammation in human intervertebral disk cells and hyperalgesia from two independent studies [83], [97]. In addition, TNF-α was thought to play a role in the neuropathic pain by increasing Ca^{2+}-permeable AMPARs insertion in the spinal cord neurons which, in turn, induced mechanical allodynia [98]. It was also shown that TNF-α upregulated IL-6 expression in the DRG on binding with TNFR1 through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation [93]. It is also interesting to note that TNF-α induced Wallerian degeneration on nerve injection, as well as demonstrated the appearance of neuropathic pain behaviors [74].

Besides pro-inflammatory cytokines, chemoattractant cytokines (or also known as chemokines) also play a critical role in the modulation of pain. This is especially true for chemokine (C-C motif) ligand 2 (CCL2) (previously known as monocyte chemoattractant protein 1/MCP-1), a chemokine which acts to recruit and regulate migration and infiltration of monocytes, neutrophils, and glial cells [99]. CCL2 and its receptor (CCR2) are upregulated in peripheral nerve injury as well as other cases, including neuroinflammation, or CNS trauma [74]. CCR2s were identified in DRG neurons and an animal model lacking these receptors was shown to be protective from developing mechanical allodynia, whereas a persistent upregulation of CCR2s in DRG and peripheral nerve was seen following injury [74]. These findings suggest that CCL2 is likely involved in mediating nociceptive and chronic neuropathic pain, as well as present in various other neuroinflammatory conditions. It is also relevant to note that CCL2-CCR2 interaction was shown to induce hypersensitivity in a demyelinating neuropathic pain in a time-dependent manner, suggesting that chronic pain should be staged as different molecular interplays and mechanisms take place over variable timing periods [72].

ANC has been able to suppress the production of multiple aforementioned pro-inflammatory cytokines and chemokines, including IL-1β, IL-6, TNF-α, and CCL2, as well as inhibit its corresponding receptor (i.e., G-coupled protein receptor/GPCR and CCR2). In addition, ANC was also demonstrated to inhibit ATP production, ameliorate ROS in the event of oxidative stress, and suppress glutamate (Glu) release from inflamed neurons. These multifaceted mechanisms were relevant in the pathophysiology of nociceptive and neuropathic pain, thus explaining its mechanism of action for this pathological event (discussed further in section 2.3.4).

Glial activation in CNS and PNS

Two types of resident glial cells in the CNS, i.e., microglia and astrocytes are activated by various neuropeptides and neurotransmitters secreted from nearby neurons. Under normal physiological conditions, glial cells are important by acting as a physical support for neurons, improving synaptic transmission efficacy, preserving tissue integrity during nerve injury, facilitating neuronal ionic exchange, and continuously communicating with neurons to modulate neuronal transmission [90]. However, during inflamed states, the corresponding neurons may secrete EEA, PGEs, substance P (SP), ATP, and nitric oxide (NO), all of which induces glial cell activation. Those activated glial cells in turn are recruited to the injury site by the role of the previously described chemokines (esp. CCL2) and secrete various pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α [74].

In addition to pro-inflammatory cytokines, activated glial cells also increase PGEs production through increased cyclooxygenase (COX) enzymes activity [100]. The resulting PGEs, in turn, promote positive feedback to the dorsal root neurons to increase NO production. Interestingly, activated glial cells

also release NO under the influence of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and metabotropic glutamate (mGluRs), as well as responsible in the production of ATP [90].

NO has been well-demonstrated to play a role in dorsal horn neurons sensitization during inflammatory tissue damage and primary afferent fiber (PAF) injury by means of cyclic guanosine monophosphate (cGMP) formation and PKG-mediated phosphorylation of specific membrane associated proteins (MAPs) [90]. In addition, NO was reported to increase nociception by transported retrograde into the presynaptic PAF terminals and release glutamate, SP, and calcitonin gene-related peptide (CGRP) via cGMP.

PGEs are also critical in the pathological pain state. Prostaglandin G2 and H2 are synthesized from arachidonic acid in which its processes are catalyzed by COX enzymes [101]. In general, COX-2 enzyme is thought to be responsible in generating a large amount of prostaglandin H2, which, in turn, converted into PGE_2 by prostaglandin E synthase (PGES) and –synthase 2 (PGES2) [102]. In fact, COX-2 existence predominates in the spinal cord, especially in regions receiving nociceptive inputs, such as laminae I, II, and X [90]. PGE_2 binds to its receptors (EP2, EP3, and EP4) and are coupled to G-protein coupled receptor (GPCR) alpha-s which results in adenylate cyclase stimulation and increased cyclic adenosine monophosphate (cAMP) levels with subsequent protein kinase A (PKA) activation [103]. PGE_2 signaling is thought to increase peripheral nociceptors responsiveness through capsaicin receptor (TRPV1) and tetrodotoxin-resistant sodium channel SCN10A [104], in which its activations are responsible to increase excitability of peripheral nociceptors and facilitate the propagation of nociceptive impulses along the peripheral nerve.

Increasing evidence has demonstrated the role of p38 mitogen-activated protein kinase (MAPK) activation in the event of nociceptive and neuropathic pain [105], [106], [107]. MAPK plays a crucial role in the generation of pain due to its ability to be activated by several microglial receptors, as well as to regulate many inflammatory mediators important in pain facilitation. For example, p38 MAPK pathway was activated following spinal nerve ligation and the administration of its antagonist was proven to prevent allodynia [108]. In addition, minocycline, which is known for its microglial activity inhibitor, was shown to elicit its action through p38 MAPK pathway with the resulting pain attenuation in various pathological states [109]. In neuropathic pain model, activated microglia by ATP was shown to release brain-derived neurotrophic factor (BDNF), wherein it was involved in the shifting of neuronal anion gradient in the event of neuropathic pain [110].

Purinergic pathways and its relations to pain

Purinergic pathways have been well-documented to be involved in both nociceptive and neuropathic pain transduction, conduction, and transmission, along with its apparent physiological roles in hollow organs. Purinergic pathways involve purinergic receptors which exist in several different types and are found in peripheral nociceptive sensory neurons in the DRG, trigeminal, nodose, and petrosal ganglia [111], [112].

Purinergic receptor nomenclature is defined by the type of the activating cotransmitters, with P1 and P2 receptor family being activated by adenosine and ADP/ATP, respectively [113]. The P2 receptor family is further divided into two subtypes, P2X and P2Y, by means of pharmacological classification [114] and the mechanisms of signal transduction [115]. P2X subtypes are those with ligand-gated ion channel receptors, whereas P2Y subtypes are those with G-protein coupled receptors (GPCRs) [113], [115].

Under normal physiological conditions, purinergic pathway is involved in various organ signaling. For instance, distended urinary bladder caused epithelial cells to release ATP which readily bound to the purinergic receptor $P2X_3$ and assisted the normal voiding reflex. Another instance was the involvement of both P1 and P2 receptor families in the intestinal peristalsis regulation, as well as its serotonin secretion [116], [117]. Other purinergic signaling was found on uterine cervical dilation during late pregnancy as mediated by ATP release and subsequent interaction with $P2X_3$ receptors, as well as in the lung's vagal sensory fibers which play a role in airway's smooth muscle hyperactivity during asthma and chronic obstructive pulmonary disease [118], [119].

Regarding nociceptive stimuli, it was proposed that the pain transduction involves the sensitization of $P2X_3$, $P2X_{2/3}$, $P1A_2$, and $P2Y_1$ receptors in the sensory neurons found in the peripheral nociceptive terminals of cutaneous region and other organs by the released adenosine and/or ATP/ADP from sympathetic nerves, mechanoreceptors (i.e., Merkel cell), vascular endothelial cells, and even cancer cells. The binding, in turn, generates nociceptive impulse and conducted to the first order neuron in the dorsal column of spinal cord through DRG, then continue to the thalamus and somatosensory cortex after once again modulated by purinergic receptors ($P2X_2$, $P2X_4$, and $P2X_6$)-ATP interaction. It was also proposed that purinergic receptors upregulation was positively associated with TRPV1, suggesting their functional interaction and subsequent sensitization of nociceptive sensory neurons which underlies the development of mechanical hyperalgesia [113], [120], [121].

In fact, $P2X_3$ receptors were found to be expressed in the cutaneous sensory nerve fibers and the subsequent binding of ATP and α,β-methylene ATP can activate the Aδ and C fiber nociceptors [122], [123]. It was proposed that ATP also mediates the upregulation of $P2X_2$ and $P2X_3$ receptors in the DRG during cutaneous inflammation and subsequently contributes to a more effective nociceptive sensitization [113], [124]. In

addition, purinergic mechanism is also involved even in a disease with autoimmune etiology such as inflammatory bowel disease (IBD), wherein the noxious stimuli arise from the intrinsic immune system. It was found that $P2X_3$-immunoreactive neurons were significantly higher in myenteric plexus of inflamed colon as opposed to normal control [125]. Interestingly, other types of purinergic receptors ($P2Y_6$ and $P2X_7$) were also found to be strongly expressed and upregulated in the resident CD4+ and CD8+ T cells in the medullary thymus and spleen of IBD cases and subsequent administration of $P2X_7$ receptor antagonist decreased NF-κB activation in lamina propria immune cells, as well as downregulation of pro-inflammatory cytokine production in colon tissues and put murine colitis into halt [126], [127]. These findings suggest that purinergic signaling mechanisms are not only limited to the peripheral nociceptive sensory neurons but also activated in various immune cells in the event of inflammation, which almost invariably occurs during nociceptive sensitization. In the heart, $P2X_3$ as well as adenosine (A_1 and A_2) receptors were important in the nociceptive transmission through nodose ganglion afferent neurons during myocardial ischemia [128], [129]. Moreover, the $P2X_7$ and $P2Y_2$ receptors in the heart were found to be upregulated and downregulated, respectively, in the event of ischemia-reperfusion injury, suggesting the harmful role of $P2X_7$ and protective effect of $P2Y_2$ purinergic signaling pathway [130]. Purinergic and adenosine receptors ($P2X_3$, $P2X_{2/3}$, $P2Y_2$, and A_1 subtype) were also found in the articular joints of the temporomandibular [131], knee [132], [133], and ankle [134], anatomical locations that are often be problematic and associated with combined mechanical and inflammatory nociceptive sensitization.

Purinergic signaling also plays a major role in neuropathic pain. Several receptors including $P2X_4$, $P2X_7$, and $P2Y_{12}$ receptors expressed by the activated microglia have been linked to the development of neuropathic pain. ATP was suspected to play a role through binding with these receptors [135]. One mechanism for neuropathic pain signaling modulated by purinergic receptors is through the synthesis and release of BDNF and disinhibition of pain transmission activity by neurons located in the lamina I [136]. The involvement of BDNF was also confirmed by the attenuation of mechanical hyperalgesia-induced neuropathic pain in the absence of $P2X_4$ receptors [137]. Interestingly, activated $P2X_4$ receptors require calcium ion and p38 MAPK pathway for the synthesis and release of BDNF from microglia [138], and that this mechanism also applied to the long-term potentiation (LTP) induction of C-fiber by ATP in the dorsal horn of spinal cord [139].

It is also well noted that $P2X_7$ receptors which are commonly expressed in resident glial cells also contribute to neuropathic pain, particularly through inflammation [140]. ATP dependent-activated $P2X_7$ receptors were known to upregulate pro-inflammatory cytokines, mainly IL-1β [141], [142], TNF-α [143], and IL-6 [144]. IL-1β and TNF-α productions were increased through p38 MAPK pathway, whereas the underlying mechanism of IL-6 upregulation is still unclear [143], [144]. These findings reiterate the importance of inflammation-mediated nociceptive and neuropathic pain and that to successfully manage pathological pain; we shall always take inflammation into account.

The anti-inflammatory properties of anthocyanin

ANC derived from various dietary sources has been studied extensively, particularly with regard to its anti-inflammatory effects. ANC extracted from red raspberries has been demonstrated to suppress COX-2, IL-1β, and IL-6 expression, as well as reducing NO synthesis (through inhibited expression of iNOS) in RAW264.7 macrophages [145]. Its subsequent administration to a mouse model with colitis was shown to ameliorate the associated weight loss and cellular damage. ANC exerts its anti-inflammatory effects through inhibiting NF-κB, p38 MAPK, JNK, and Akt signaling pathways [146], [147], [148], [149]. In addition, ANC is also a strong COX enzyme inhibitor. The COX inhibitory capacity was even comparable to NSAIDs such as ibuprofen or naproxen at certain concentrations [150]. In fact, ANC derived from red sweet cherry water was shown to inhibit COX enzymes by 80–95% at 250 μg/mL [151]. Furthermore, ANC appears to have an increased tendency toward COX-2 rather than COX-1 inhibition. ANC derived from black soybean seed coats has been shown to inhibit UV-induced COX-2 expression [152]. Similarly, ANC-derived purple sweet potato also reduced the expression of COX-2 and iNOS expression in rats with liver injury [153]. This is perhaps due to the heavy involvement of COX-2 in various pathological states, such as ischemia-reperfusion injury [154], intestinal inflammation [155], and tumor [156], [157]. Fortunately, both nociceptive and neuropathic pain also have a strong connection with COX-2 as its mediators [158], [159], [160], thus the ability of ANC to inhibit COX-2 and relieve both types of pain would be greatly appreciated. Predictably, strong COX-2 inhibition results in reduced PGE-2 production and release [152], [161], [162], hence, abridging the inflammation and nociceptive sensitization.

The resulting inhibition of inflammatory pathways by ANC was supposedly demonstrated by reduced pro-inflammatory cytokines production and secretion. Indeed, ANC derived from soybean seed coat was shown to inhibit TNF-α expression through NF-κB-dependent pathway [163], whereas ANCs-rich extract from bilberry was shown to reduce mRNA levels of iNOS, TNF-α, IL-1β and IL-6, and reduce protein levels of iNOS, TNF-α, and NF-κB [164]. In addition, ANC metabolites also reduced IL-6 and VCAM-1 levels in oxidized LDL challenged vascular endothelial cells [165].

It is also worth noted that ANC-rich black elderberry administration to hyperlipidemic mice was shown to reduce CCL2 serum levels [166]. Purified ANC supplementation for 24 weeks could reduce plasma CCL2 levels by approximately 11% [167]. Similarly, delphinidin 3-sambubioside and delphinidin downregulated MEK1/2-ERK1/2 and NF-κB signaling pathway and lead to decreased IL-6, TNF-α, and MCP-1 (also known as CCL2) levels [168]. Reduction in chemokine levels, thus, can potentially reduce resident glial activation and prevent the exacerbation of inflammatory nociceptive sensitization. Indeed, the combined suppression of pro-inflammatory cytokines and chemokine gene expression, production, and release of strawberries-derived ANC extract has been shown to ameliorate reactive astrogliosis, delay disease onset, and extend survival in mice with amyotrophic lateral sclerosis (ALS) [169]. Moreover, ANC can also block glutamate-induced AMPK activation [170], suggesting that ANC may act indirectly in mitigating excessive neuronal excitations.

The various mechanisms of anti-inflammatory actions elicited by ANC should, therefore, be readily demonstrated on a clinical basis. Indeed, a double-blind RCT of tart cherry drink administration before long distance running was shown to reduce muscle pain significantly among runners when compared to those who did not consume it [171]. A double-blind randomized controlled trial (RCT) assessing the efficacy of cherry juice to osteoarthritis (OA) patients also demonstrated the same outcome, which is a significant reduction of both pain score and highly specific C-reactive protein (hsCRP) inflammatory marker among treatment group [172]. To be specific, ANC has been demonstrated to attenuate inflammation-induced thermal hyperalgesia, mechanical hyperalgesia, and edema in rat model, with similar efficacy to indomethacin at its highest dose (400 mg/kg BW) [173].

Finally, the direct involvement of ANC on purinergic receptors with regard to pain attenuation has yet to be investigated because purinergic receptors have gained accumulating evidence and important roles in both nociceptive and neuropathic pain, and that its inhibition by ANC seems plausible.

Oxidative stress

Oxidative stress is a condition wherein there is an imbalance between the amount of free radicals and antioxidants. The imbalance can be in the form of excessive free radicals in the presence of relatively inadequate antioxidants. Under normal physiological states, aerobic metabolism as a consequence of various mitochondrial enzymes produces free radicals in the form of reactive oxygen intermediates and nitrogen species (RNI). These are also produced through ultraviolet, ionizing radiation, and air pollution exposures [174]. In addition to these, the electron transport chain activity in mitochondria also generates reactive oxygen species (ROS) [175]. All of those molecules belong to free radicals. A free radical has a free electron on its external orbit and always attempts to find another electron to stabilize itself. Hence, a free-radical may forcefully take an extra electron from nearby biomolecules [174].

Another example of ROS generation is through the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, cell membrane enzyme complexes to produce ROS which involves in cellular signaling and tissue homeostasis (Figure 3). The enzymes were expressed in the neurons, astrocytes, and microglia. Under abnormal states (e.g., infections), NADPH oxidases can be highly activated (especially NADPH oxidase 2/NOX2 in the CNS), thus resulting in high levels of ROS which is associated with oxidative stress and neurodegeneration [176].

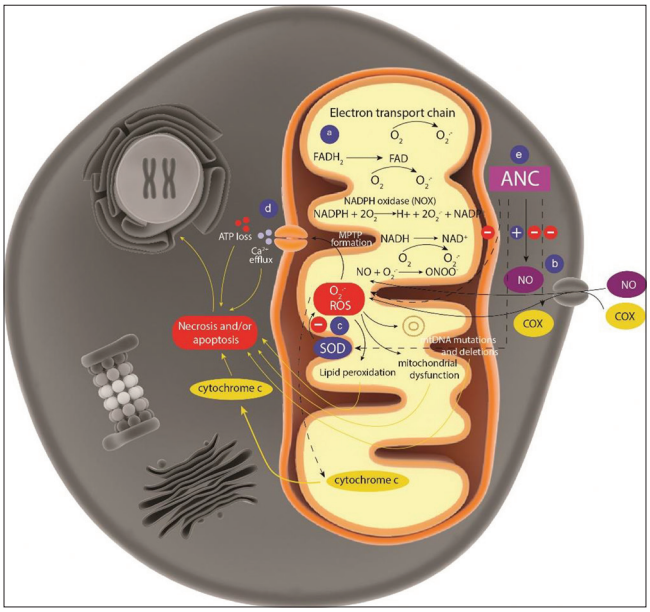


Figure 3: The generic roles of mitochondrial ROS in cell death (a) mitochondrial electron transport chain and a series of chemical reaction to generate ATP lead to ROS (O2−) accumulation. (b) In addition, external environment can become another source of free radicals, such as the involvement of COX and NO, with the latter being associated with the production of peroxynitrite. (c) Free radicals (mostly ROS) in the presence of imbalance endogenous antioxidant enzymes (primarily SOD), will induce oxidative stress, all of which injures cell and causes cellular death through various mechanisms, including mitochondrial DNA (mtDNA) mutations and deletions with subsequent mitochondrial dysfunction, lipid peroxidation, and release of cytochrome c to the cytoplasm. (d) ROS also induces mitochondrial permeability transition pore formation, an opening in the mitochondrial outer membrane facilitating the efflux of calcium ions and ATP into the cytoplasm, leading to cellular death. (e) ANC directly suppresses ROS production from various sources, as well as inhibits other molecules with pro-ROS formation such as NO and COX. It also boosts endogenous antioxidant levels (e.g., SOD and GSH) to neutralize ROS. All of them ultimately result in reduced oxidative stress and protects cells from necrosis and/or apoptosis. (Some picture materials were taken and modified from the library of science and medical illustrations by sommersault 18:24, licensed under CC BY-NC-SA 4.0, materials are available under Public License)

The existence of a free radical may be omitted on an individual basis, but it can be problematic when massive free radicals appear simultaneously over a prolonged period of time, which is usually found during oxidative stress. Oxidative stress can be detrimental to the cells and tissues because free radicals damage all of the cellular components (including organelles, lipids, proteins, and DNA), inducing apoptosis. This is especially relevant for neurons in the CNS in which they contain high levels of polyunsaturated fatty acids, thus is prone to cellular damage [177]. It is also important to note that mitochondrial DNA is more vulnerable to ROS than the nuclear DNA, thus mitochondrial dysfunction may result in more ROS production [178].

Even worse, oxidative stress can initiate an inflammatory cascade wherein the surrounding cells, including neurons and resident glial cells, to be activated and further produce various pro-inflammatory molecules, along with increased production of more free radicals. The activation of glial cells by means of toll-like receptors has been shown to induce the release of various pro-inflammatory cytokines and ROS at the same time [179]. On chronic activation of these glial cells, they are known to secrete more superoxide which can react to NO (which is also produced by glial cells) to generate peroxynitrite which has been shown to be detrimental to neurons [177]. Moreover, NF-κB, a protein complex responsible for the activation of glial cells, is also a strong inducer of NOX2 and inducible NO synthase/iNOS (an enzyme that catalyzes NO production), thus also responsible to the generation of ROS and subsequent cellular damage [180]. On the other hand, as previously described, NF-κB was also responsible for the upregulation of COX-2 enzyme, thus increases PGEs production, and subsequently elevates the superoxide levels as a byproduct of PGEs production, further damaging the cells [177]. This positive feedback can maintain a vicious cycle with linear increase in its magnitude with regard to inflammation and tissue damage, thus initiating, maintaining, and increasing the intensity of nociceptive stimuli.

The *folie à deux* actions between oxidative stress and inflammation also affect the pathophysiology of pain in various pathological states. For example, they have been linked as a culprit for diabetic neuropathy, wherein a chronic persistent hyperglycemic state induced peripheral nerve damage, increased accumulation of advanced glycation end products and activated various inflammatory pathways [181]. Another example is cancer that metastasize to bone was known to upregulate glutamate release responsible for cancer-induced bone pain and attenuation of its release could reduce the pain [182]. In addition, cancer chemotherapy has also been identified to produce oxidative stress and inflammation, further complicating cancer treatment through peripheral nerve damage and the resulting neuropathy [183]. Oxidative stress is also thought to contribute to the development of osteoarthritis by inducing telomere instability, replicative senescence, and chondrocyte impairment in the affected cartilage [184]. Furthermore, oxidative stress also triggers an inflammatory state and thus, initiating nociceptive stimulation in OA as predicted [185].

Furthermore, ROS has been shown to induce and maintain central sensitization in the spinal cord through the regulation of N-methyl-D-aspartate and AMPARs and its downstream effect of LTP [186], [187]. ROS also activated TRPA1 and TRPV1, ion channels known to involve in membrane depolarization and consequent nociceptive sensitization in the spinal cord, in chronic pain after spinal cord injury (SCI) [72], [188], [189], [190]. Similarly, hydrogen peroxide (H_2O_2), a product of cellular respiration, has been demonstrated to modulate synaptic plasticity and affecting the release of calcium ions in the interneurons of spinal cord dorsal horn neurons with the consequent nociceptive sensitization [188], [191], [192], [193]. Interestingly, nociceptive pain like capsaicin-induced hyperalgesia was shown to cause superoxide accumulation and concomitant reduction of antioxidant superoxide dismutase (SOD)-2 activity [194], suggesting a direct causative link between oxidative stress and antioxidant imbalance with the generation of nociceptive and neuropathic pain.

The anti-oxidant properties of anthocyanin

ANC exerts potent antioxidant activities. Its antioxidant potential depends on its chemical structure and the subsequent hydroxylation, methylation, acylation, and glycosylation patterns [195], [196], [197]. ANC has potent superoxide- and peroxynitrite-scavenging activities [198]. It, therefore, also inhibits lipid peroxidation [199]. It was reported that different ANC variants exert different magnitude of antioxidant capacities. Accordingly, ANC's antioxidant power can be ranked as follows (from strongest to weakest): Delphinidin, petunidin, malvidin (equals to cyanidin), peonidin, and pelargonidin [9], [198]. On raw foods (e.g., in vegetables, and fruits), ANCs were commonly found alongside with other compounds, such as Vitamin C and phenol, thus enabling them to act synergistically as antioxidants [200].

It was proposed that ANC acts through both direct and indirect mechanisms. It has a direct free-radical scavenging activity because it can donate the electron to the reactive free radicals, thus stabilizing it [9]. In fact, ANC can bind with superoxide, singlet oxygen, peroxide, hydrogen peroxide, and hydroxyl radicals [201], [202], [203], [204], [205]. Whereas indirectly, it increases *in vivo* endogenous antioxidant activities, such as increasing intrinsic SOD and glutathione peroxidase gene expressions and levels or reducing ROS formation through inhibiting NADPH oxidase [206], [207]. These comprehensive mechanisms ergo reduce as well as neutralize various free radicals.

Clinically, ingestion of ANC derived from blueberries was shown to increase serum antioxidant status in human subjects [208], [209]. Likewise, administration of cyanidin 3-glucoside to rats was shown to reduce thiobarbituric acid reactive substances (TBARS) and protection against lipid peroxidation amidst no elevation of endogenous antioxidants. This study, therefore, is in accordance with the direct free-radicals scavenging mechanisms of ANC [210]. Furthermore, ethanol and water extract of purple sweet potato (PSP) had been demonstrated to suppress malondialdehyde levels in cell culture medium [211]. PSP is known for its ANC-rich content [212], whereas malondialdehyde, similar to TBARS, is the most frequently used biomarker for oxidative stress in the presence of various diseases [213].

There has been no direct study which links ANC with its potential ability to reduce free radicals in the event of inflammatory pain. However, some evidence can be used to infer several notions. First, ANC has specific neuroprotective mechanisms against antioxidants. ANC was shown to be protective against apoptosis of mitochondrial oxidative stress-induced cerebellar granule neurons through increased glutathione (GSH) levels, inhibiting lipid peroxidation and cardiolipin oxidation, and prevention of mitochondrial fragmentation [214]. Second, ANC is permeable to viable neurons. It was shown that ANC metabolites can prevent ROS formation on both the cellular membrane and cytosol of human neuronal cell line, suggesting that it can be up taken by neurons in the CNS and PNS [215]. Indeed, ANC was shown to readily penetrate blood–brain barrier [216], [217] and could be identified in the cortex, cerebellum, hippocampus, and striatum of rats fed daily with 2% blueberry for 10 weeks [218]. Third, ANC can act centrally to attenuate CNS demyelination, reduce inflammation, and scavenge free radicals. ANC can be detected in the peripheral interstitial fluid and its administration to ethidium bromide-induced pontine demyelination rat models has been shown to restore the Na^+/K^+ ATPase pump activities of these cells (suggesting protection against demyelination), reduce pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, and IFN-γ) levels, and combat oxidative stress by increasing SOD levels [219]. Fourth, ANC can act in the PNS as it was demonstrated to promote myelination in the peripheral nerve through increase Sirt2 protein known to involve in myelination in mouse embryonic Schwann cell culture model [220].

If we take a look at these notions, it is prudent to assume that ANC could attenuate both nociceptive and neuropathic pain through its neurotrophic and neuroprotective mechanisms against direct noxious insults, free radicals, and inflammation. In fact, it is known that central as well as peripheral demyelination is associated with pain due to direct nerve injury or prolonged immobilization [221]. Thus, the fourth and fifth notion could actually serve a template model in which *in vivo* ANC might attenuate nociceptive and/or neuropathic pain. In fact, administration of pelargonidin, a subtype of ANC, has been proven to alleviate chemical and thermal hyperalgesia and reduce ROS formation in streptozotocin-induced-diabetic rat models [222]. The last study is technically a proof-of-concept of ANC's capability in alleviating pain.

Conclusion

Inflammation and oxidative stress precipitate and maintain of both nociceptive and neuropathic pain. The mechanisms are pleiotropic, including activation of inflammatory pathways leading to increased production and secretion of various pro-inflammatory cytokines and chemokines, upregulation of nociceptive receptors and its associated transcription factors, as well as specific interaction with certain receptors and pathways, such as purinergic pathway (particularly for neuropathic pain). Oxidative stress also plays a major role in the pathophysiology of both pain types. ANC's protective effects against inflammation and oxidative stress had been described meticulously in this review and shown to exert multiple simultaneous actions against these pathological events. In fact, the proof-of-concept study had been translated into meaningful clinical impacts toward the treatment of several pathological pains. Given its excellent safety profile and the nature of its massive anti-inflammatory and anti-oxidant properties, ANC should be further investigated in more clinical settings for treating various nociceptive and/or neuropathic pain originated from multiple diseases.

A potentially useful approach would be to incorporate ANC as an adjunctive treatment along with currently accepted medication regimens and to compare it with the conventional medication alone in the setting of RCT whenever possible. The currently available data favors the promising results of such studies, and if it is proven so, the benefits would not only limited to alleviating the pain but also to ameliorate, if not accelerating recovery, against the primary disease itself.

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