Tripolitana Medical Journal; 2025, Vol. 14, No. II, pp. 83 - 94

Research Article

ISSN 2077-5628

Analgesic and Anti-Inflammatory Effects of Celecoxib in the Presence of Lithium Carbonate Using the Formalin Test in Albino Mice

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Received 17 August 2025/ Accepted 5th October 2025; Published 10 October2025

ABSTRACT

Drug-drug interactions occur when a drug's effect on the body changes in the presence of another drug. Drug-drug interactions can be either pharmacokinetic or pharmacodynamic. The clinical consequences of drug-drug interactions could lead to reduced efficacy or increased toxicity. Lithium carbonate is a monovalent cation with a narrow therapeutic index. Lithium is a first-line treatment for Bipolar Affective Disorder. Celecoxib is a selective COX-2 non-steroidal anti-inflammatory drug, which is responsible for prostaglandin synthesis, an integral part of the pain and inflammation pathway.

It is indicated in conditions like osteoarthritis, rheumatoid arthritis, acute pain, and familial adenomatous polyposis. This work aims to investigate the effects of lithium carbonate on the analgesic and anti-inflammatory action of celecoxib by applying a formalin test using albino mice.

Mice were divided into 6 groups. Group 1 (control) administered 1% tween 80 at a dose of 5ml/kg; group 2 received celecoxib (50mg/kg); group 3 received lithium carbonate (10 mg/kg); while group 4 received lithium carbonate (50mg/kg); group 5 received combined treatment of lithium carbonate (10mg/kg) with celecoxib; while group 6 received combined treatment of lithium carbonate (50mg/kg) with celecoxib. Drugs used in mice as a standard, Tramadol (5 mg/kg) for phase I (analgesic), and Acetylsalicylic acid (200 mg/kg) for phase II (anti-inflammatory), for the formalin test. All drugs were injected subacutely (three doses); mice were intraperitoneally administered at 24, 5, and 1 hours before scoring. All drugs were administered as a suspension in 1% Tween 80. It was injected in a volume of 5ml/kg. All drugs were prepared freshly before use. Celecoxib exerts analgesic and anti-inflammatory effects as it decreases the duration of licking in both early and late phases after s.c. administration of formalin in the left hind paw. Lithium carbonate exerts analgesic and anti-inflammatory effects, and it decreased the duration of licking in both the early and late phases after s.c. administration of formalin in the left hind paw. The combined treatment of lithium and celecoxib has analgesic and anti-inflammatory action, but less than the additive effect; both partially antagonize each other.

Conclusion: Celecoxib and lithium carbonate each produce analgesic and anti-inflammatory effects. Lithium in the presence of celecoxib, and as a result of celecoxib›s effect on the renal system, lithium may partially act as a proinflammatory and nociceptive agent. Understanding and managing drug interactions is critical for optimizing patient outcomes and minimizing risks associated with pharmacotherapy.

Key words- *Celecoxib; Lithium carbonate; Formalin test; Analgesic; Anti-inflammatory.*

INTRODUCTION

Drug-drug interactions (DDIs) occur when a drug's effect on the body changes in the presence of another drug; this can be either pharmacodynamic or pharmacokinetic.[1,2] Types of Drug-Drug Interactions:

Pharmacokinetic Interactions: These involve changes in the absorption, distribution, metabolism, or excretion of a drug, leading to altering its concentration in the circulation;[3] for example, absorption: One drug may affect the gastrointestinal absorption of another, e.g., antacids reducing the absorption of tetracyclines.[4]

Distribution: Competition for plasma protein binding sites, e.g., warfarin and NSAIDs.[5] Metabolism: Enzyme induction or inhibition of cytochrome P450 enzymes; for example, rifampin induces CYP3A4, reducing the efficacy of oral contraceptives.[6,7] Excretion: Drugs may change renal excretion, e.g., probenecid, delaying penicillin excretion.[8,9]

Pharmacodynamic Interactions: Occur when drugs interact at the same or related target sites, leading to additive, synergistic, or antagonistic effects.[10]

Additive/Synergistic Effects: Increased therapeutic or toxic effects, e.g., combining sedatives like benzodiazepines and alcohol.[11]

Antagonistic Effects: One drug reduces the effect of another drug, e.g., beta-blockers and beta-agonists.[12]

Pharmaceutical Interactions: Occur outside the body,

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such as when drugs are mixed in the same IV solution, leading to physical or chemical incompatibilities, e.g., precipitation or degradation.[13]

The clinical consequences of Drug-drug Interactions are as follows: *Reduced efficacy*. One drug may diminish the therapeutic effect of another, e.g., antiepileptics, reducing the effectiveness of oral contraceptives.[14-16]

Increased Toxicity: Enhanced adverse effects, e.g., combining warfarin and aspirin increases bleeding risk.[17,18]

Unexpected Side Effects: New adverse reactions due to drug interactions may be observed.[19]

Lithium Carbonate is a monovalent cation that was first reported beneficial in psychiatric patients in 1949 by Cade. Its FDA-approved indications include the acute treatment of mania and maintenance treatment of bipolar disorder;[20] it has a narrow therapeutic index and numerous toxic effects in various organ systems.[21] Now, Lithium is a first-line treatment for Bipolar Affective Disorder.[22,23]

The exact mechanism of action in mood disorders is not unknown. Among the old mechanisms, lithium may affect the purinergic system, electrolyte metabolism, membrane transport, and second messenger systems (cyclic nucleotide and phosphatidylinositol), glycogen synthase kinase-3beta (GSK-3β), brain-derived neurotrophic factor, and neurotransmitters; while the new mechanisms showed the effects of lithium on the immune system, biological rhythms, telomere functions, and mitochondria.[24,25]

Celecoxib is a selective (COX-2) non-steroidal anti-inflammatory drug (NSAID), which is responsible for prostaglandin synthesis, an integral part of the pain and inflammation pathway. It is indicated in conditions like osteoarthritis, rheumatoid arthritis, acute pain in adult women, primary dysmenorrhea, and familial adenomatous polyposis.[26,27]

This research study aimed to investigate the effect of celecoxib as an analgesic and anti-inflammatory drug in the presence of lithium carbonate.

Aim of the work

To investigate the effects of lithium carbonate on the analgesic and anti-inflammatory action of celecoxib by applying formalin test using albino mice.

MATERIALS AND METHODS

Materials

Tween 80 was obtained from Merck-Schuchardt Company, Hohenbrunn, Germany; formalin was purchased from Shijiazhuang Xinlongwei Chemical Co., China; Lithium carbonate was obtained from Riedel-de Haen Company, Ph. France.

Animals

The experiments were carried out using male albino mice (25-40 g) bred in the animal house of the Faculty of Pharmacy- University of Tripoli. Standard food pellet diet and water were freely available for mice. The animals were kept at room temperature in a humidity-controlled room with 12:12 hrs in polypropylene cages. Animals were acclimated to laboratory conditions one week before the initiation of experiments.

Design of the work

Mice were divided into 6 groups. Group 1 (control) administered 1% tween 80[28] at a dose of 5ml/kg;[29] group 2 received celecoxib (50mg/kg);[30] group 3 received lithium carbonate (10 mg/kg); while group 4 received lithium carbonate (50mg/kg);[31] group 5 received combined treatment of lithium carbonate (10mg/kg) with celecoxib; while group 6 received combined treatment of lithium carbonate (50mg/kg) with celecoxib.

Drugs used in mice as a standard, Tramadol (5 mg/kg)[32,33] for phase I (analgesic), and Acetylsalicylic acid (200 mg/kg)[34] for phase II (anti-inflammatory) for the formalin test. All drugs were injected subacutely (three doses); mice were intraperitoneally administered at 24, 5, and 1 hours before scoring. All drugs were administered as a suspension in 1% Tween 80.[28] It was injected in a volume of 5ml/kg.[29] All drugs were prepared freshly before use.

Formalin test

Mice were placed carefully inside the restraint module with the selected (i.e., left) hind paw exposed outside. Subcutaneous injection of 0.1ml of (3%) formalin solution, using an insulin syringe in the mid-plantar surface of the hind paw. The needle is placed between the toes and ankle and inserted beneath the surface of the skin; thus, any tissue damage is avoided. The injection should be done firmly and time-optimized. The duration of licking during 0 to 5 minutes (phase I), and the duration of licking during the last 15 minutes of half an hour (phase II) are scored.[35] Scoring phase I (neuropathic pain) by the duration of licking in the first 5 minutes, while scoring phase II (pain due to inflammation) by the duration of licking during the last 15 minutes of the 30-minute test.

RESULTS

Phase I (Neuropathic pain): Table 1 showed that celecoxib (50 mg/kg), tramadol (5 mg/kg), and Lithium carbonate (10 and 50 mg/kg) decreased the duration of licking compared to the control-treated group ($P = 0.000$, 0.000, 0.000, 0.000, respectively).

Lithium carbonate produces a dose-dependent decrease in the duration of licking with the increase of dose compared to the control-treated group; lithium with a dose of 10 mg/kg showed a significance with $P = 0.037$, while lithium carbonate with a dose of 50 mg/kg showed a significance with $P = 0.000$.

Lithium carbonate in a dose of 50 mg/kg showed an insignificant decrease in the duration of licking compared to the mice treated with 10 mg/kg lithium carbonate ($P = 0.873$).

The combined treatment with lithium carbonate in a dose of 10 mg/kg and celecoxib produced a significant decrease in the duration of licking ($P = 0.000$) compared to the control-treated group. These combined treatments (lithium carbonate with 10 mg/kg and celecoxib) showed an insignificant decrease in the duration of licking compared to mice treated with lithium carbonate alone ($P = 0.227$); and also showed an insignificant increase in the duration of licking compared to mice treated with

celecoxib with a dose of 50 mg/kg ($P = 0.682$).

The combined treatment with lithium carbonate in a dose of 50 mg/kg and celecoxib showed a significant decrease in the duration of licking ($P = 0.000$) compared to the control-treated group. These combined treatments (lithium carbonate with 50 mg/kg and celecoxib) showed an insignificant decrease in the duration of licking compared to mice treated with lithium carbonate alone with a dose of 50 mg/kg ($P = 0.493$); and also showed an insignificant decrease in the duration of licking compared to mice treated with celecoxib alone with a dose of 50 mg/kg ($P = 0.675$).

Phase II (Inflammatory pain): Table 2 shows that celecoxib produces a decrease in the duration of licking compared to the control-treated group at $P = 0.000$. Acetyl salicylic acid, as a standard, also produced a decrease in the duration of licking compared to the control-treated mice ($P = 0.00$).

Lithium carbonate-treated mice produced a dose-dependent decrease in the duration of licking (lithium at a dose of 10mg/kg, $P = 0.006$, while at a dose of 50mg/kg, $P = 0.000$) compared to the control-treated mice.

The combined treatment of lithium carbonate (10mg/kg) and celecoxib showed a significant decrease in the

Table 1: Effect of lithium on the celecoxib analgesic effect in albino mice (phase I), applying the formalin test

Treatments	Duration of licking (Seconds)	P compared to control	P compared to Coxib	P compared to lithium 10mg/kg	P compared to lithium 50mg/kg
Control 1% T80	168.1 ± 14.11	0.00			
Tramadol 5 mg/kg	64.3 ± 5.07	0.00			
Celecoxib 50 mg/kg	18.6 ± 5.32	0.00			
Lithium 10 mg/kg	44.6 ± 33.59	0.037			
Lithium 50 mg/kg	24.0 ± 7.89	0.00		0.873	
Lithium (10mg/kg) + Coxib	26.8 ± 5.69	0.00	0.682	0.227	
Lithium (50mg/kg) + Coxib	10.3 ± 4.04	0.00	0.675		0.493

Table 2: Anti-inflammatory effect of celecoxib in the presence of lithium carbonate in albino mice using the formalin test (phase II)

Treatments	Duration of licking (Seconds)	P compared to control	P compared to Coxib	P compared to lithium 10mg/kg	P compared to lithium 50mg/kg
Control 1% T80	236.16 ± 37.276				
Acetyl salicylic acid 200 mg/kg	109.83 ± 31.592	0.00			
Celecoxib 50 mg/kg	33.00± 16.637	0.00			
Lithium 10 mg/kg	31.16 ± 18.314	0.006			
Lithium 50 mg/kg	22.50 ± 8.636	0.00		0.629	
Lithium (10mg/kg) + Coxib	27.16 ± 11.808	0.00	0.861	0.871	
Lithium (50mg/kg) + Coxib	40.00 ± 30.030	0.01	0.833		0.589

duration of licking compared to the control-treated mice (P = 0.000); also this combined treatment of lithium carbonate (10mg/kg) and celecoxib showed insignificant decrease in the duration of licking compared to lithium carbonate (10mg/kg), and compared to celecoxib-treated mice (P = 0.871, and 0.861, respectively).

Mice administered a combined treatment of lithium carbonate (50mg/kg) and celecoxib produced an insignificant increase in the duration of licking compared to lithium carbonate (50mg/kg) treated mice alone, also compared to celecoxib-treated mice only (P = 589, 0.833, respectively).

DISCUSSION

In this study, celecoxib exerts analgesic and anti-inflammatory effects as it decreases the duration of licking in both early and late phases after s.c. administration of formalin in the left hind paw.

Celecoxib is a nonsteroidal anti-inflammatory drug (NSAID);[27] it blocks the enzyme cyclooxygenase-2 (COX-2) responsible for prostaglandin (chemicals that play a role in pain and inflammation) synthesis.[36] Celecoxib weakly inhibits the COX-1 enzyme, which is involved in the production of prostaglandins[27] that protect the lining of the stomach against stomach ulcers.[37] Prostaglandin is the end product of fatty acid metabolism, produced by tissue-specific COX enzymatic activity. These products are important physiological and pathological mediators that are involved in a wide range of biological processes, including inflammation, pain, cancer, glaucoma, osteoporosis, cardiovascular diseases, and asthma.[38-40] In this study, Celecoxib may produce analgesic and anti-inflammatory effects through the inhibition of cyclooxygenase-2.

The mechanical hyperalgesia induced in rat paws by the carrageenan model showed that, in this model of inflammatory hyperalgesia, the anti-nociceptive effect of selective COX-2 inhibitors involved the participation of endogenous opioids.[41] At the spinal level, it was found that a selective COX-2 inhibitor effectively relieved inflammatory pain in rats. The delta and kappa opioid receptors are involved in the activity of the COX-2 inhibitor.[42] Celecoxib may mediate endogenous opioids, presumably indirectly, involving release from opioidergic nerves because celecoxib is not a direct agonist of opioid receptors.[43] It was found that the analgesic effects of celecoxib were prevented by selective μ and δ, but not κ opioid antagonists. This is evidence for the involvement of endogenous opioids in the mechanism(s) underlying celecoxib-induced analgesia.[42,44] A study demonstrated that the analgesia produced by NSAIDs may be caused by the release of the endogenous opioid, methionine-enkephalin.[45] These data indicate that there is a link between the opioid system and COX-2 inhibitor antinociception. However, the sites and mechanisms of any such connection are not yet clear.

Celecoxib analgesic effect was prevented by cannabinoid CB_1 and CB_2 receptor antagonists, respectively. This is evidence for the involvement of the cannabinoid component of the mechanism(s) underlying celecoxib-induced analgesia.[44] Formalin test using rats showed that the antinociceptive effect of celecoxib may be mediated partly through the cannabinoid system. These effects are possibly due to inhibition of endocannabinoid degradation and consequently, enhancement of endocannabinoid concentration at the spinal cord level.[46] Therefore, celecoxib may produce analgesic effects by enhancing the endocannabinoid activity and indirectly activating opioid receptors.

Celecoxib's anti-inflammatory effect may be produced through a blockade of $K_V1.3$ channels, which is associated with suppression of inflammatory immune reactions.[47]

Lithium carbonate exerts analgesic and anti-inflammatory effects, and it decreased the duration of licking in both the early and late phases after s.c. administration of formalin in the left hind paw.

Previous studies on analgesia indicated that lithium alone, when given in a single dose, produced analgesia or at least lowered the animal's response to pain stimuli.[48,49] Lithium produces analgesia by increasing the release of met-enkephalin, dynorphin, and β-endorphin from rat hypothalamic slices by interacting with a pertussis toxin-sensitive G-protein.[50] Lithium may produce an analgesic effect through the opioid system.

Lithium inhibits Glycogen Synthase Kinase 3 (GSK3β) and produces antinociceptive effects.[51,52] Glycogen Synthase Kinase 3-specific inhibitor decreases neuropathic pain in mice. Inhibition of GSK3β activities can prevent the development and reverse the existence of neuropathic pain.[53,54] It is known that activation of GSK3 will stimulate the production of pro-inflammatory cytokines (e.g., IL-6, IL-1β, and TNFα) while decreasing anti-inflammatory cytokines like IL-10.[55] Lithium's antioxidative and anti-inflammatory effects in an animal model demonstrate its ability to reduce inflammatory cytokine levels.[56] Lithium's inhibition of GSK3 is a mechanism contributing to its therapeutic effects in mood disorders and neuroinflammation.[55] GSK-3β inhibition by lithium decreased (lipopolysaccharide, LPS)-induced production of TNF-α while increasing IL-10 level in monocytes. The effect of lithium on IL-10 was found to increase IL-10 production, attesting to a strong anti-inflammatory action of the drug.[57]

Lithium inhibits GSK-3β,[58] which reduces NF-κB activation, leading to a reduction in TNF-α, IL-6, and COX-2;[55,59] as a result, lithium attenuates inflammatory pain (e.g., rheumatoid arthritis).[60]

Lithium shifts microglia from pro-inflammatory (M1) to anti-inflammatory (M2) phenotype via GSK-3β inhibition.[61-63] A pro-inflammatory (M1) macrophage releases IL-1β and TNF-α, which sensitize nociceptors

and amplify pain signals.[64] In neuropathic pain, M1 polarization in spinal cord/ dorsal root ganglia (DRG) maintains central sensitization,[65,66] while COX-2 inhibitors reduce M1-derived PGE_2.[67,68] Lithium reduces neuroinflammation in chronic pain (e.g., fibromyalgia).[69] There is clinical evidence that lithium shows efficacy in neuropathic pain.[70-72] Lithium suppressed spinal astrocyte activation via GSK-3β inhibition, reducing IL-1β and TNF-α.[70,71] Lithium may produce analgesic, anti-inflammatory, and antioxidant effects through the inhibition of GSK3β.

Lithium increases 17-hydroxy-docosahexanoic acid (17-hydroxy-DHA) levels, leading to anti-inflammatory properties, and this contributes to its neuroprotective effects.[73] 17-hydroxy-docosahexanoic acid (17-hydroxy-DHA) is a bioactive lipid derived from DHA; it has a role in inflammation resolution and neuroprotection.[74] Lithium may produce its anti-inflammatory effect by increasing the level of 17-hydroxy-DHA.

Evidence that chronic administration of lithium to rats, produce an anti-inflammatory effect by decreasing arachidonic acid turnover by reducing the expression of cytosolic phospholipase A2 (cPLA2), which releases arachidonic acid from membran phospholipids[75,76] and PGE2 concentration in rat brain; also, lithium reduced the activity of COX-2.[76,77] Therefore, Lithium may have its anti-inflammatory effect by decreasing arachidonic acid turnover and decreasing PGE2 concentration in the rat brain; also, it may produce its anti-inflammatory effect through the reduction of the activity of COX-2.

Lithium's effects on ion dynamics (Na^+, Ca^{2+}) and cellular excitability influence pain pathways and inflammation, though indirectly. Lithium's small ionic radius and high hydration energy allow it to partially block voltage-gated Na^+ channels by competition with sodium.[78] Lithium's impact on pain, by Na^+ channel blockade, reduces neuronal hyperexcitability in neuropathic pain.[79]

Lithium slowed Ca^{2+} re-equilibration; it inhibits inositol monophosphatase (IMPase), reducing IP_3-mediated Ca^{2+} release from endoplasmic reticulum.[80] Lithium impacts on pain by reducing Ca^{2+} spikes in dorsal horn neurons, leading to reduced central sensitization.[81] Also, lithium reduces glutamate release (Ca^{2+}-dependent) from primary afferents.[82] Lithium may produce analgesic effects through Na channel block or/and reduce Ca^{2+} spikes.

In phase I (neuropathic pain), Tramadol, as a standard, produces an analgesic effect.

Tramadol is a centrally acting analgesic; it produces analgesic activity via different mechanisms. It acts as an agonist of the mu opioid receptor.[83] Tramadol has been described to stimulate P-endorphin production in rats; it is speculated that this mechanism is involved in the anti-inflammatory action of tramadol.[84] It produced a significant reduction of PGE concentration in the inflammatory exudate.[85]

Tramadol modifies the transmission of pain impulses by inhibition of monoamine reuptake;[86] it inhibits serotonin reuptake and inhibits norepinephrine reuptake, enhancing inhibitory effects on pain transmission in the spinal cord.[83]

Tramadol is primarily known as a centrally acting analgesic; it also has a potential anti-inflammatory effect through its modulatory effects on inflammatory pathways. Although tramadol is not classified as an anti-inflammatory drug, it has been shown to reduce the levels of prostaglandins, interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α), which are key players in inflammation; also, it may influence prostaglandin synthesis indirectly, and may reduce nitric oxide (NO) production, which plays a role in inflammatory responses.[87-89]

Tramadol's interaction with NMDA receptors plays a role in its pain modulation effects, particularly in neuropathic and chronic pain conditions. Tramadol enhances serotonin (5-HT) and norepinephrine (NE) levels, which can indirectly reduce NMDA receptor-mediated excitatory transmission. Tramadol may modulate NMDA receptor activity, reducing hyperexcitability of nociceptive neurons.[90] Tramadol's NMDA modulation may contribute to its effectiveness in neuropathic pain, where glutamatergic hyperactivity is a major factor.[91] Tramadol may produce analgesic effects through the opioid system, and as an anti-inflammatory, inhibition of monoamine reuptake, and reducing hyperexcitability of nociceptive neurons through NMDA.

In phase II (antiinflammatory pain), acetyl salicylic acid, as a standard, produces an analgesic effect.

In phase II, acetyl salicylic acid has an anti-inflammatory effect, which is through the inhibition of prostaglandin synthesis through the inhibition of cyclooxygenase (COX) enzymes.[92] Acetyl salicylic acid (400mg/kg) has an antinociceptive effect toward neuropathic pain, in phase I, because acetyl salicylic acid significantly increases brain serotonin content and decreases the number of 5-HT2 receptors in the cortical brain membrane. These changes in serotonin and its receptors are thought to contribute to the analgesic effect.[93,94] Acetyl salicylic acid produces an anti-inflammatory effect through the inhibition of prostaglandin synthesis and increases brain serotonin content.

The combined treatment of lithium and celecoxib has analgesic and anti-inflammatory action, but less than the additive effect; both partially antagonize each other.

Celecoxib is a selective anti-inflammatory; it blocks the enzyme cyclooxygenase-2 (COX-2) that is responsible for prostaglandin (chemicals that play a role in pain and inflammation) synthesis. PGE2 (the main product of COX-2) can stimulate the production of IL-6 and has a close association with inflammation.[95,96]

In patients with bipolar disorder, an elevation of the pro-inflammatory markers, such as IL-4, TNFα, IL-1β, and CCL2 cytokine were observed; these markers have

an established role in inflammation in neuronal damage and degeneration.[97-100] In a meta-analysis of patients with bipolar disorder, CRP levels were also identified.[101,102]

Lithium, a well-established mood stabilizer, reduces IL-2, IL-6, and IL-10 levels after long-term use; this may be its mechanism of action through anti-inflammatory processes.[103] There is some evidence that lithium treatment of bipolar patients may be associated with changes in concentrations of pro-inflammatory cytokines interleukin-1-beta (IL-1β) and IL-6. Lithium treatment increased IL-1β and decreased IL-6 production.[104] Interleukin-1 beta (IL-1β) is a pro-inflammatory cytokine that plays a crucial role in the body's immune response and contributes to inflammatory pain hypersensitivity by inducing cyclooxygenase-2 (COX-2) expression in the central nervous system.[105] There is evidence that indicates that under certain experimental conditions, lithium exhibits pro-inflammatory properties (e.g., induction of IL-4, IL-6, and other pro-inflammatory cytokines).[59] Lithium inhibits GSK-3β, which enhances nuclear factor (NFκB) activity and increases the expression of COX-2.[106] In the case of the combined treatment, lithium may have proinflammatory properties; this leads to producing less analgesic and anti-inflammatory effect compared to the expected additive effect of lithium carbonate and celecoxib together.

Serum lithium concentration increases of up to 99% and 448% with concomitant celecoxib use. Celecoxib increases blood lithium levels and may lead to lithium toxicity.[107] Celecoxib-mediated inhibition of renal prostaglandins followed by a decrease in the blood flow into the renal system, and this may decrease renal lithium excretion.[108,109] Lithium is eliminated through glomerular filtration, but some is then reabsorbed together with sodium through the proximal tubule. Its level is, therefore, sensitive to water and electrolyte balance.[110,111] Renal system condition induced by celecoxib, which leads to an increase in lithium levels by decreasing its excretion; this condition will lead to the release of proinflammatory cytokines. Therefore, the combined treatment of celecoxib and lithium carbonate will lead to analgesic and anti-inflammatory effects less than the additive effect of each alone.

Lithium, a primary treatment for bipolar disorder, modulates both noradrenergic and serotonergic systems, contributing to its mood-stabilizing and antidepressant effects. Lithium increases noradrenaline release and synthesis by enhancing the tyrosine hydroxylase (TH) activity.[112] Chronic lithium treatment increases noradrenaline turnover in the prefrontal cortex and hippocampus.[113,114] Lithium increases tryptophan availability, enhancing 5-HT synthesis.[115] Serotonin plays a dual role in pain modulation, acting as both a pro-nociceptive (pain-enhancing) and anti-nociceptive (pain-suppressing) agent, depending on the receptor subtype, anatomical location, and pain state (e.g., acute vs. chronic). Serotonin produces anti-nociceptive (Pain-Suppressing) effects by descending pain inhibition from the brain to the spinal cord.[116]

The rostroventromedial medulla and dorsal raphe nucleus release 5-HT into the spinal dorsal horn, inhibiting pain signals via 5-HT$_1$A/1B receptors, reducing glutamate release from primary afferents,[116] and through 5-HT$_2$A/2C receptors, activate inhibitory interneurons (enhance GABA/glycine release).[117,118] Synergy with noradrenaline, 5-HT, and NE co-release in the spinal cord amplifies pain suppression.[82] Peripheral anti-inflammatory effects of serotonin, through a decrease in pro-inflammatory cytokines (TNF-α, IL-6) via 5-HT$_7$ receptors, reducing neuropathic pain.[119]

The pro-nociceptive (Pain-Enhancing) effects of 5-HT are mediated by peripheral sensitization. In injured tissues, 5-HT (released from platelets) activates 5-HT$_3$ receptors on nociceptors, leading to an increase in the excitability.[120-122] Activation of 5-HT$_2$A receptors leads to an increase in the inflammatory mediators (e.g., prostaglandins).[123-125] In chronic pain states (e.g., fibromyalgia), 5-HT$_3$ receptors in the spinal cord enhance NMDA signaling.[122,126] In the case of the combined treatment with celecoxib and lithium carbonate, lithium may stimulate $5_{\text{-HT3,}}$ leading to a decrease in the analgesic and anti-inflammatory effect. Therefore, the result of the combined treatment is less than the additive effect of each.

The interesting mechanism is that lithium upregulates NO signaling. The nitric oxide (NO) signaling pathway is thought to play a role in neural plasticity and the antidepressant effects of lithium. The NO system is crucial for neural plasticity, and its involvement in the antidepressant effect of lithium has been observed in studies like the Porsolt forced swimming test in mice;[127] also, lithium may produce an antidepressant effect through the blockage of NMDA receptors, in the mouse forced swimming test.[128]

The effects of lithium on NO are complex and context-dependent, involving both upregulation and downregulation of NO production depending on the experimental conditions and brain region. Nitric oxide (NO) plays a dual role in pain modulation, acting as both a pro-nociceptive (pain-promoting) and anti-nociceptive (pain-relieving) agent depending on the context, concentration, and location in the nervous system.[127]

Lithium reduces NO overproduction in neuroinflammatory conditions by downregulating inducible nitric oxide synthase (iNOS), particularly in microglia and astrocytes. This effect is linked to lithium's anti-inflammatory and neuroprotective properties. Lithium suppresses nuclear factor (NF-κB) signaling, a key regulator of iNOS expression.[69] NO enhances endocannabinoid signaling, contributing to pain relief.[129] Blocking nNOS/iNOS reduces inflammatory and neuropathic pain.[130]

A study by Bagetta *et al*.[131] found that, in the hippocampus of rats treated with lithium chloride, mRNA expression of

constitutive brain nitric oxide synthase can be augmented, with a consequent increase in nitric oxide synthesis. NO, as a pro-nociceptive agent (Pain Enhancer), NO is released by inflammatory cells (macrophages, neutrophils) and damaged tissues, contributing to hyperalgesia (increased pain sensitivity). NO enhances the sensitivity of TRPV1 and TRPA1 channels in nociceptors.[132] In this research, in the case of the combined treatment, the effect of lithium on NO may lead to a pain enhancer instead of pain relief. Therefore, the analgesic and anti-inflammatory effect of the combined treatment with lithium carbonate and celecoxib will produce an effect less than the additive action of both, each alone.

It was suggested that long-term administration of lithium treatment downregulates the cortical, but not cerebellar, α 1 -ARs, α 2 -ARs, β 1 -ARs, and β 2 -ARs. It is concluded that lithium induces region-specific and differential functional downregulation of α-AR and β-AR subtypes in the rat brain.[133]

It was found that β_2-agonists reduce NF-κB activation and cytokine production in immune cells.[134] β_2-AR is involved in peripheral pain modulation.[135] Activation of β_2-AR showed strong anti-inflammatory effects via cytokine suppression.[136] It was reported that α_2-AR activation suppresses macrophage-driven inflammation;[137,138] α_2-agonists can reduce inflammation by modulating sympathetic activity and mediated analgesia via spinal inhibition.[116,138] In this research, lithium in the presence of celecoxib may down regulate the adrenergic receptors, decreasing the analgesic and anti-inflammatory effect; therefore, the analgesic and anti-inflammatory effect of the combined treatment of lithium carbonate and celecoxib is less than the additive effect of both, each alone.

CONCLUSION

Celecoxib and lithium carbonate each produce analgesic and anti-inflammatory effects. Lithium in the presence of celecoxib, and as a result of celecoxib's effect on the renal system, lithium may partially act as a proinflammatory and nociceptive agent. Understanding and managing drug interactions is critical for optimizing patient outcomes and minimizing risks associated with pharmacotherapy.

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