

# Pregabalin in Neuropathic Pain: Evidences and Possible Mechanisms

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**Abstract:** Pregabalin is an antagonist of voltage gated  $Ca^{2+}$  channels and specifically binds to alpha-2-delta subunit to produce antiepileptic and analgesic actions. It successfully alleviates the symptoms of various types of neuropathic pain and presents itself as a first line therapeutic agent with remarkable safety and efficacy. Preclinical studies in various animal models of neuropathic pain have shown its effectiveness in treating the symptoms like allodynia and hyperalgesia. Clinical studies in different age groups and in different types of neuropathic pain (peripheral diabetic neuropathy, fibromyalgia, post-herpetic neuralgia, cancer chemotherapy-induced neuropathic pain) have projected it as the most effective agent either as monotherapy or in combined regimens in terms of cost effectiveness, tolerability and overall improvement in neuropathic pain states. Preclinical studies employing pregabalin in different neuropathic pain models have explored various molecular targets and the signaling systems including  $Ca^{2+}$  channel-mediated neurotransmitter release, activation of excitatory amino acid transporters (EAATs), potassium channels and inhibition of pathways involving inflammatory mediators. The present review summarizes the important aspects of pregabalin as analgesic in preclinical and clinical studies as well as focuses on the possible mechanisms.

**Keywords:** Pregabalin, neuropathic pain, diabetic neuropathy, post-herpetic neuralgia.

## 1. INTRODUCTION

Neuropathic pain as defined by The International Association for the Study of Pain is “pain initiated or caused by a primary lesion in the nervous system”. In other words, neuropathic pain may be defined as the pain originated from the pathology of the nervous system. It involves alterations in the function, chemistry and structure of neurons. Spontaneous pain, hyperalgesia and allodynia are the common symptoms observed in neuropathic pain. Spontaneous pain is characteristically burning or shooting in nature. Hyperalgesia is an increased pain response to supra-threshold noxious stimulus, while allodynia is a sensation pain elicited by a non noxious stimulus (e.g. the gentle touch of clothes, bending of cutaneous hairs by a puff of wind). Spontaneous pain may be simply conceptualized as “stimulus independent” whereas hyperalgesia and allodynia as “stimulus dependent”. Neuropathic pain is a result of various mechanisms operating at the peripheral, spinal cord and supra-spinal levels, which cause alterations in the pain conduction pathway. This may also develop secondary to some other pathological conditions such as diabetes mellitus, cancer, herpes infection, autoimmune diseases and HIV infection etc. [1].

Pregabalin, (S)-3-(aminomethyl)-5-methylhexanoic acid, is a pharmacologically active S-enantiomer of a racemic 3-isobutyl gamma amino butyric acid analogue. It is a well established anticonvulsant and analgesic agent. In fact pregabalin is the first drug to receive an approved labeling from Food and Drug Association (FDA) for the treatment

of diabetic neuropathy and post-herpetic neuralgia [2]. Preclinical and clinical studies have shown the effectiveness of pregabalin in managing the neuropathic pain. Animal based studies have helped to describe the mechanisms for its anti-hyperalgesic and anti-allodynic action. Clinical studies have also shown the efficacy and dose dependent effects of pregabalin either as monotherapy or in combination with analgesics in relieving pain and related symptoms [3, 4]. The major advantage of pregabalin is its relative reliability, easy use and high tolerance in patients with neuropathic pain [5]. As a successor of gabapentin, pregabalin has been shown to be effective in several models of neuropathic pain, incisional injury and inflammatory injury. The present review summarizes the preclinical and clinical outcomes of pregabalin with the possible underlying mechanisms.

## 2. EVIDENCES FOR ITS PAIN ATTENUATING EFFECTS FROM PRECLINICAL STUDIES

There have been a number of studies showing the prominent neuroprotective action of pregabalin in different models (Table 1) [6]. As neuronal injury/damage is an essential component of neuropathic pain therefore, the neuroprotection provided by pregabalin may be linked to its neuropathic pain attenuating effects. Accordingly, various preclinical studies have documented the analgesic actions of pregabalin in different pain models including neuropathic pain. Administration of pregabalin (3-30 mg/kg, oral) after 2.5 hrs of carrageenan injection (after full induction of inflammation) has been shown to alleviate the carrageenan-induced thermal hyperalgesia in a dose dependent manner with  $ED_{50}$  value of 6 mg/kg in comparison to gabapentin with  $ED_{50}$  of 19.2 mg/kg [7]. Administration of pregabalin (30 mg/kg or 300  $\mu$ mol/kg, s.c.) has been shown to significantly block the late-phase (phase-2) nocifensive

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**Table 1. Summarized Data from the Preclinical Reports Showing the Effectiveness of Pregabalin in Neuropathic as well as in Inflammatory Pain Models**

S.No.	Animal Model	Doses of Pregabalin and Outcomes	References
1.	Chronic constriction injury model (CCI)	30-100 mg/kg, s.c./oral/4-10 mg/kg i.v Attenuation of allodynia and spontaneous pain	[8, 11, 13]
2.	Spinal nerve injury (SNI)/ spinal nerve ligation (SNL)/ spinal cord contusion (SCC)/ weight drop spinal cord injury model	10-30 mg/kg, i.p./ 10-300 $\mu$ mol/kg, oral Single dose administration produced significant reduction in mechanical hypersensitivity threshold in a dose dependent manner	[9, 10, 12]
3.	Cancer Chemotherapy (oxaliplatin/ docetaxel) induced neuropathy model	10-30 mg/kg, oral/ 2 mg/kg i.v. Amelioration of heat hyperalgesia, cold allodynia, impairment of sciatic nerve,	[16, 17]
5.	Infraorbital nerve injury model	10% topical application Reduction in Orofacial pain intensity	[15]
6.	Carrageenan induced thermal hyperalgesia	3-30 mg/kg, oral 3 times more potent than gabapentin in hyperalgesia attenuation	[7]
7.	Formalin test	30 mg/kg or 300 $\mu$ mol/kg, s.c. Blockade of phase-2 nocifensive response	[8, 9]

response in formalin-induced nociception [8-9]. In neuropathic pain models, it has also been reported to significantly attenuate the pain symptoms. In chronic constriction injury (CCI) model, administration of pregabalin (30-100 mg/kg, s.c.) after 10 days of nerve injury (stable allodynia achieved) has been shown to produce a dose-dependent inhibition of punctuate allodynia (assessed with von Frey filaments application) in mice [8]. Even a single dose of pregabalin (30, 100 and 300  $\mu$ mol/kg, oral) has been shown to reverse cold allodynia in spared nerve injury (SNI) and spinal nerve ligation (SNL) models of neuropathic pain [9]. In weight-drop spinal cord injury model in mice (which mimics the symptoms of neuropathic pain due to spinal cord injury in human), a single dose administration of pregabalin (10 and 30 mg/kg, i.p.) on the 28<sup>th</sup> day of injury is reported to significantly attenuate mechanical hypersensitivity (allodynia) in a dose-dependent manner [10]. Acute administration of pregabalin (4 mg/kg and 10 mg/kg, i.v. infusion) for 2 h is reported to significantly attenuate the static allodynia (measured in term of paw withdrawal threshold) in a CCI model [11]. A single dose administration of pregabalin (30 mg/kg, i.p) has been shown to be effective in decreasing mechanical sensitivity in the spinal cord contusion (SCC) model of central neuropathic pain in rats [12].

A recent study has revealed the effectiveness of pregabalin (30 mg/kg, oral) in reducing the frequency of spontaneous pain behavior (by assessing the abnormal limb movements including lifting/guarding, flinching/shaking and licking in the operated limb using a magnet implanted in injured limb) in a CCI model [13]. Pregabalin has also been shown to attenuate mechanical allodynia in the tibial neuroma transposition (TNT) model [14]. The topical application of pregabalin (10 %) for 4 consecutive days after 7 days of surgical nerve injury is reported to reduce neuropathic orofacial pain intensity in an infraorbital nerve injury model in rats [15]. Administration of pregabalin (2

mg/kg, i.v.) has also been reported to attenuate chemotherapy (oxaliplatin, 6 mg/kg i.p)-induced neuropathic pain symptoms especially cold allodynia [16]. Pregabalin (10 mg/kg and 30 mg/kg, oral) administration for 8 consecutive days has been shown to significantly attenuate docetaxel-induced neuropathy (heat hyperalgesia, cold allodynia) along with reduction in substance P and calcitonin gene-related peptide (CGRP) release suggesting pregabalin as potential agent against chemotherapy-induced neuropathy [17].

Apart from the studies showing its effectiveness as monotherapy, there have also been reports suggesting its synergistic actions with other pain relieving drugs. Its synergism with tapentadol (a  $\mu$ -opioid receptor agonist and noradrenaline reuptake inhibitor) for analgesic actions (in terms of ED<sub>50</sub> value) has been demonstrated. The ED<sub>50</sub> value of the combination of pregabalin and tapentadol (in the ratio of 2.5:1) was reported to be 0.83 mg/kg in comparison to ED<sub>50</sub> value of 4.21 mg/kg (for pregabalin) and 1.65 mg/kg (for tapentadol) assessed using ipsilateral paw withdrawal threshold with electronic von Frey filaments [18]. The synergistic interaction may be possibly due to enhancement of pregabalin-mediated descending pain inhibitory signals (involving the spinal mechanisms) in the presence of increased noradrenaline in the brain (due to decreased noradrenaline re-uptake) by tapentadol. Administration of pregabalin and naproxen (NSAID) in 10:1 ratio is shown to act synergistically to reverse thermal hyperalgesia in the inflamed hind paw. The synergistic actions may be due to potentiation of pregabalin-mediated inhibition of glutamate release *via* Ca<sup>2+</sup> channel inhibition by naproxen to inhibit the release of glutamate through inhibition of PGE<sub>2</sub> synthesis in the dorsal horn neurons of the spinal cord. The reduced glutamate release-induced reduction in the post-synaptic excitation of dorsal horn neurons may ultimately result in decreased central sensitization to alleviate hyperalgesia [7]. The combination of pregabalin with synthetic cannabinoid

WIN 55212-2 mesylate at a fixed ratio of 1:1 has also been shown to exert synergistic interaction in a mouse model of acute thermal pain by unknown mechanisms [19].

Pregabalin has emerged as the most effective drug to manage various neuropathic pain states in comparison to its predecessor analgesic agents. According to preclinical data, pregabalin has turned out to be 3- to 10-fold more potent than gabapentin as an antiepileptic [20] and 2- to 4-fold more potent as an analgesic in treating neuropathic pain [21]. Hurley and co-workers also demonstrated that pregabalin reduces hyperalgesia to an equivalent extent at one third dose as compared to gabapentin [7]. The antinociceptive testing in oxaliplatin-induced neuropathic pain model for various drugs (assessed using the rat tail immersion test in cold water) ranked pregabalin (2 mg/kg) as the most efficacious drug as compared to lidocaine (3 mg/kg) and morphine (4 mg/kg) on the basis of the ratio of the pharmacological effect (versus time) to dose [16].

### 3. EVIDENCES FROM CLINICAL STUDIES

#### 3.1. Diabetic Neuropathic Pain

The various animal experimental studies suggesting the efficacy and safety of pregabalin as monotherapy or as combination have encouraged further research in the clinical settings (Table 2). There have been a number of clinical studies showing its effectiveness in diabetic neuropathic pain [22-25]. Pregabalin at fixed dosages of 300 and 600 mg/day (three times daily), is reported to be superior to placebo in relieving pain and improving pain-related sleep interference

in three randomized, double-blind, multicentre studies of 5-8 weeks duration in patients with painful diabetic peripheral neuropathy (pDPN) [26-27]. The efficacy results from a 6-week multicenter study have indicated that 600 mg/day pregabalin significantly decreases mean pain score and increases the proportion of patients with  $\geq 50\%$  decrease from baseline pain (39% vs. 15% for placebo) [28]. The retrospective analyses of daily mean pain scores from nine controlled trials of pregabalin at 150, 300, or 600 mg/day also reported a significant reduction in pain during the first 2 weeks of treatment of painful diabetic peripheral neuropathy [29]. The pooled analysis of seven studies has shown that pregabalin (150, 300, and 600 mg/day TID) significantly reduces pain and pain-related sleep interference associated with pDPN with the highest efficacy at the dose of 600 mg/kg BID [30]. The higher doses of pregabalin produce a faster reduction in pain scores as compared to lower doses, and the most rapid pain reduction is generally observed in patients receiving pregabalin 600 mg/day, divided into 2 or 3 doses [31]. The pooled data from 11 double-blind, randomized clinical studies of pregabalin (75-600 mg/day) in older patients (age  $\geq 65$  years) with pDPN or PHN (Post-herpetic neuralgia) shows significant reduction in neuropathic pain and improvement in pain compared to those observed in younger patients [32]. The results obtained from a 12-week randomized, double-blind, multicentre, placebo-controlled, parallel-group study further support the efficacy, tolerability, and safety of pregabalin in flexible- or fixed-dose regimen with its ease of application in clinical settings and project it as a better treatment option in elderly patient,

**Table 2. Clinical Reports of Pregabalin in Various Forms of Neuropathic Pain**

S.No.	Type of Pain	Dose, Duration and Salient Outcomes	Reference
1.	Diabetic painful neuropathy	150-600 mg/kg, 4-14 weeks Improvement in pain and related symptoms within 2 weeks of treatment with sustained and long lasting effects throughout study Mild to moderate side effects like dizziness, somnolence edema, weight gain, motor incoordination etc. Exacerbation of CVS dysfunctions particularly in previously cardiac compromised patients	[5, 22, 27, 30, 33, 37, 38, 39, 40]
2.	Cancer chemotherapy induced neuropathic pain	75-300 mg/day, 2-8 weeks Effective in oxaliplatin, docetaxel, fluorouracil/cisplatin refractory pain in cancer patients of all age groups as monotherapy as well as in combination to other analgesics.	[3, 4, 41, 42, 43, 44, 45, 46]
3.	Post-herpetic neuralgia	150-600 mg/day, 8-13 weeks Highly significant pain reduction with mild to moderate side effects 6 times more potent than gabapentin Mild to moderate dizziness	[47, 48, 49, 50, 51, 52]
4.	Fibromyalgia	150-600 mg/day, 4-12 weeks Significant and long lasting pain relief with improvement in other global parameters. Cost effective and good tolerability Common side effects like dizziness, somnolence, edema, dry mouth etc.	[54, 55, 56, 57, 58, 59, 60]
5.	Trigeminal neuralgia	150-600 mg/kg, 8 week Long lasting pain relief	[61]
6.	Post-operative pain	300-600 mg/day, pre-operatively/post-operatively, Attenuation of acute pain Reduced the consumption of opioids	[62, 63, 64, 65, 66]

who represent a large proportion of the populations with neuropathic pain syndromes [5].

Clinical trials for comparative efficacy and safety analyses of pregabalin and amitriptyline have projected pregabalin as a better treatment option in diabetic neuropathy due to lesser proportion of side effects (25%) than amitriptyline (65.4%) [33]. The comparative study of pregabalin (75-300 mg/day), duloxetine (20-120 mg/day) and gabapentin (300-1800 mg/day) in the treatment of diabetic neuropathy for 12 weeks shows that monotherapy with pregabalin is superior in terms of onset of pain reduction [34]. In a 6 week study, pregabalin is shown to be equally effective as gabapentin for reduction of pain intensity and improvement in sleep quality with painful diabetic neuropathy [35]. A study by Saldaña and co-workers reported that pregabalin produces relief in terms of pain perception, disability level, anxiety, depression, sleep quality and quality of life in gabapentin refractory neuropathy suggesting pregabalin as a valid treatment alternative (either as monotherapy or in combination with other analgesics) for the management of patients with gabapentin-refractory peripheral neuropathic pain [36]. Apart from better pain relieving actions of pregabalin, its cost-effectiveness as monotherapy or as add-on therapy in comparison to existing treatment (antidepressants, opioids, anticonvulsants different than pregabalin and/or analgesics) in the management of refractory diabetic neuropathy has also been demonstrated [37]. The cost effectiveness of pregabalin in treating various types of neuropathic pain has also been supported by various other studies [38-40].

### 3.2. Cancer and Chemotherapy-induced Neuropathic Pain

There have been studies suggesting the usefulness of pregabalin in cancer-induced pain as well as in anti-cancer agents (chemotherapy)-induced neuropathic pain. A retrospective investigation of Japanese patients revealed the effectiveness of pregabalin (> 300 mg/day) in attenuating cancer-induced neuropathic pain [41]. Pregabalin in combination with oxycodone successfully attenuated neuropathic pain after chemoradiation therapy in patients with non-small cell lung cancer in comparison to a combination of the transdermal fentanyl patch, oxycodone and gabapentin [3]. Shibahara and co-workers further demonstrated the efficacy of pregabalin as adjuvant (75-300 mg/day) with oxycodone in 5-FU/CDDP (5-fluorouracil + cisplatin)-induced neuropathic pain, not responding to lornoxicam (NSAID) and oxycodone (semisynthetic opioid) suggesting its efficacy as compared to other commonly used analgesics [4]. Administration of pregabalin (150 mg/tid) for 2-6 weeks has been shown to produce significant improvement in oxaliplatin-induced sensory neuropathy (from grade 2 and 3 to grade 1 and 2) in about 48 % patients [42]. Administration of pregabalin (75-300 mg/day) for 8 weeks has also been shown to produce a significant and long lasting pain relief in chemotherapy-induced neuropathic pain in majority of pediatric oncological patients (86%) with infrequent and transient side effects [43]. Administration of pregabalin (75-150 mg/day) has been shown to significantly reduce paclitaxel (of gemcitabine/paclitaxel regimen)-induced sensory neurotoxicity in patients with left renal pelvic cancer [44].

Amongst the different analgesics, the relative efficacy of pregabalin (in comparison to amitriptyline and gabapentin) for the management of cancer-induced neuropathic pain was demonstrated in 120 patients having severe cancer-associated neuropathic pain. Pregabalin treated patients were shown to exhibit maximum improvement in terms of pain evaluation parameters (pain score and secondary outcome measures such as intensity of lancinating, dysesthesia and burning) along with clinically significant morphine sparing effect [45]. The post hoc analysis of pregabalin vs. non-pregabalin-treated cancer pain patients (n=273) in a 2-month prospective, multicentre study also reported that pregabalin treatment produces more satisfactory improvement to usual treatment (92.6% vs. 78.9%) in terms of decreased pain intensity, interference with normal daily activities and sleep quality [46].

### 3.3. Post-herpetic Neuralgia (PHN)

An 8-week, multicenter, parallel-group, double-blind, placebo-controlled, randomized clinical trial in 173 PHN patients has shown significant pain reduction in pregabalin-treated (300 and 600 mg/day) patients (starting from the first day of treatment and sustained throughout the study) assessed using the McGill Pain Questionnaire to calculate total (sensory and affective) pain scores. The greater proportion of patients with  $\geq 30\%$  and  $\geq 50\%$  decrease in mean pain scores were observed in pregabalin treated as compared to the placebo group (63% vs 25% and 50% vs 20%,  $p = 0.001$ ) along with significant improvements in other factors associated with neuropathic pain such as sleep, quality of life, mood and overall global improvement [47]. Two multicentred, double blind, placebo-controlled trials in 370 and 238 in PHN patients for 13 weeks and 8 weeks respectively, reported the efficacy of different doses of pregabalin (150, 300, 600 mg/day) within 1 week of treatment with mild to moderate side effects including dizziness, somnolence, peripheral edema, headache, dry mouth and ataxia [48-49]. A meta-analysis using MEDLINE and AMBASE databases has also shown the efficacy of pregabalin in reducing pain intensity in patients with PHN associated neuropathic pain [50]. An 8 week open randomized comparative study of 50 PHN (thoracic, cervical, trigeminal and lumbosacral types) patients reported satisfactory improvements of pain perception ( $>75\%$ ) with 75 mg twice daily pregabalin with dizziness as the most common side effect which was found within tolerable levels as none of the patient stopped treatment due to adverse reaction [51]. In comparison to gabapentin, pregabalin was shown to produce similar analgesic effects at  $1/6^{\text{th}}$  dose of gabapentin with exacerbation of side effects with an increase in its dose [52].

### 3.4. Fibromyalgia

Fibromyalgia is characterized by chronic widespread musculoskeletal pain due to lowered pain threshold along with disordered sleep and fatigue [53]. Pregabalin has shown efficacy in treating fibromyalgia with rapid and clinically significant improvements in several outcome measures related to core symptoms of the syndrome in patients with long-standing fibromyalgia [54]. Eight-week, multicentred, double-blind, randomized clinical trial demonstrated the

efficacy of different doses of pregabalin (150, 300, and 450 mg/day) in pain alleviation in 529 patients with fibromyalgia. Pregabalin the doses 300 and 450 mg/day was shown to significantly reduce the average severity of pain (more patients had  $\geq 50\%$  improvement in pain at the end point, i.e., 29%, versus 13% in the placebo group) and improve the health related quality of life with tolerable mild or moderate side effects among which dizziness and somnolence were the most frequent [55]. The meta-analysis of classical trials reports from Pfizer (four randomized trials including 2754 patients) has shown significant benefit in terms of pain relief with pregabalin (300, 450 and 600 mg/day) for the treatment of fibromyalgia. The maximum response rate with pregabalin was observed after 4-6 weeks and remained constant thereafter [56]. A post hoc analysis of data from a multicenter, double-blind, placebo-controlled, randomized trial designed to evaluate the efficacy of pregabalin monotherapy in fibromyalgia pain (based on the pain and Patient Global Impression of Change criteria) demonstrated significant and long lasting clinical improvement in pain and other associated global parameters [57]. The pooled data from 3 open-label extension studies of pivotal randomized clinical trial including 1206 patients (mostly female, 92.4%) also showed that pregabalin (75-300 mg BID) is safe and well tolerated among fibromyalgia patients with dizziness, somnolence, headache, peripheral edema, and increased weight as common side effects of mild to moderate intensity [58]. A 4 week randomized, double-blind, placebo-controlled, 2-period crossover polysomnographic (PSG) study to assess the effect of pregabalin on sleep, tiredness, and pain in fibromyalgia patients revealed that pregabalin at 300-450 mg/day significantly improves these symptoms with good tolerability [59]. A decision-analytic model demonstrated pregabalin (225 mg BID) as cost saving and more effective as compared to duloxetine, tramadol, milnacipran, and gabapentin for the treatment of severe fibromyalgia [60].

### 3.5. Trigeminal Neuralgia

A one year follow-up, open label study to check the efficacy of pregabalin treatment in patients suffering from trigeminal neuralgia with or without concomitant facial pain revealed that pregabalin (150-600 mg/day) significantly reduces pain intensity and frequency ( $>50\%$ ) after 8 weeks. The pain relief was long lasting and sustained even after 1 year, thus suggesting pregabalin as the drug of choice for management of trigeminal neuralgia [61].

### 3.6. Pre-/post-operative Pain

A randomized controlled trial including 91 female patients scheduled for laparoscopic hysterectomy demonstrated that administration of maximally recommended dose of pregabalin (600 mg/day) pre-operatively was more effective than diazepam (10 mg), and reduced the consumption of opioids (oxycodone) postoperatively for reduction of pain [62]. Another randomized, double-blinded, placebo-controlled trial (including 99 patients) aimed to investigate the efficacy and safety of pregabalin in reducing both acute post-operative pain (up to 48 hr after surgery) and chronic pain (measured as hypoesthesia in the anterior chest after 3 months of surgery) in patients after robot-assisted endoscopic

thyroidectomy showed that pre-operative (1 hr before surgery) administration of pregabalin (initial dose 150 mg, followed by same dose twice a day) significantly improves the pain scores and reduces the need for additional analgesics during 48 hr after the surgery. However, there was no significant difference between two groups related to chronic pain and chest hypoesthesia at 3 months after surgery showing that pregabalin was mainly effective in reducing early postoperative pain, but not chronic pain [63]. Patient outcome with different treatments after lumbar discectomy for radicular low back pain is variable and the benefit is inconsistent and many patients continue to experience pain even after 3 months of surgery. On the contrary, administration of pregabalin (300 mg at 90 minutes preoperatively and 150 mg at 12 and 24 hours postoperatively) in lumbar discectomy for radicular low back has demonstrated a greater decrease in pain intensity after 3 months in pregabalin receiving patients with lesser disability score and greater pain tolerance [64]. Another randomized, placebo-controlled prospective trial in 32 patients (18-65 years) with radicular low back pain undergoing elective lumbar discectomy revealed that a single pre-operative pregabalin administration at 300 mg dose 1 hour before surgery did not reduce the intensity of pain, but decreases the morphine consumption [65]. A randomized controlled trial including 108 patients (who underwent lumbar spinal surgery) conferred superior analgesic benefits with combined administration of pregabalin (150 mg twice daily) and dexamethasone (16 mg) for 4 days starting from 1 hour before the induction of anesthesia on postoperative pain [66].

### 3.7. Other Study Reports

In a prospective randomized trial including 36 patients with 4-week treatment suggested that the combination of celecoxib (cyclooxygenase-2 inhibitor) and pregabalin is more effective than monotherapy for the treatment of chronic low-back pain assessed by using a visual analogue scale (VAS, 0-100 mm) and the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) pain scale [67]. A prospective analysis of 683 patients (aged  $\geq 18$  years) with a 6-month history of chronic refractory low back pain showed higher reduction in severity of pain with pregabalin as compared to usual care (61.6% vs. 37.3%) with significant reduction in overall cost [68]. A randomized, double-blind, placebo-controlled trial including 64 patients with chronic pancreatitis-associated intense abdominal pain showed positive effects on secondary outcome measures (change in individual dermatomal thresholds) with pregabalin (75-300 mg BID) after three weeks treatment as compared to primary outcome measure (change in the sum of thresholds). It suggests that pregabalin is more effective in reducing skin sensitization as reflected by electric thresholds as compared to deep tissue sensitization (reflected by pressure thresholds) [69]. However, a randomized, double-blind, placebo-controlled, parallel-group trial has shown that pregabalin (150-600 mg/day BID) is well tolerated in HIV patients, but exhibits no superiority to placebo in the treatment of painful HIV neuropathy measured in terms of mean Numeric Pain Rating Scale (NPRS) score, Patient Global Impression of Change (PGIC) and sleep measurements [70].

#### 4. SIDE EFFECTS

The majority of clinical studies involving administration of pregabalin for the treatment of various types of neuropathic pain states have shown dizziness, somnolence, headache, dry mouth, peripheral edema, weight gain, blurred vision, motor in coordination and ataxia as the most common side effects (occurred in 1-10% patients) at lower doses. The intensity of these side effects is generally increased with an increase in dose and at highest prescribed dose (600 mg/day), the incidences of side effects increase with higher frequency of dizziness (70%), blurred vision (63%) and headache (31%). Therefore, administration of pregabalin needs careful monitoring to take care of these side effects at higher doses [62]. There is clinical report documenting that patient withdrawal from the clinical trial is very insignificant despite the presence of some side effects, which may be possibly due to high efficacy of pregabalin [71]. The occurrence of some of these adverse effects may be attributed directly to its primary mechanism of action. It inhibits the various types of calcium channels viz., P, Q, and N- type located in the different brain areas to decrease depolarization-dependent neurotransmitter release [72]. The highest level of expression of these channels has been found in the cerebellum and in the hippocampus, and their dysfunction/decreased activity may affect the vestibulocerebellar/brainstem structures and higher cortical functions leading to dizziness, blurred vision, ataxia [73, 74] and cognitive impairment [75]. Blum and co-workers reported the development of hyponatremia, confusion and disorientation in a 74 year old patient (suffering from cardiovascular problems) treated for diabetic neuropathic pain with pregabalin and these symptoms were resolved within 2 days after discontinuation of drug [76].

Apart from these common side effects, a few number of patients may also develop cardiovascular abnormalities (<1%) with pregabalin usage, particularly in patients with previous history of cardiovascular dysfunction. Pregabalin treatment has been shown to cause chronic heart failure possibly due to calcium channel blockade mediated deleterious effect on myopathic ventricles, especially in patients with left ventricular systolic dysfunction [77]. A 92 year old patient with a history of paroxysmic fibrillation was shown to develop sinus tachycardia followed by atrial fibrillation and congestive heart failure due to pregabalin [78]. Erdoğan and co-workers reported a case report profile of a 54 year old patient who developed peripheral edema after 2 weeks of pregabalin treatment (150 mg/day for 1<sup>st</sup> week and 300mg/day for 2<sup>nd</sup> week). After discontinuation of pregabalin, peripheral edema reduced suggesting a direct casual relationship. This fluid retention may be possibly attributed to inhibition of calcium channel which causes peripheral vasodilation and fluid leakage into the interstitial area. It may be further linked to the unexplained weight gain and may contribute to exacerbate congestive heart failure [79]. Aksakal and coworkers have recently reported that pregabalin (300 mg/day) administration for 8 months in a patient with diabetic neuropathic pain results in complete atrioventricular blockade, which is resolved within 4 days after pregabalin discontinuation. The cardiovascular side

effects of pregabalin have been attributed to its inhibitory effects on L-type calcium channels, predominantly located on the myocardium [80]. These cardiovascular events may be linked to the mechanisms through which other calcium channel blockers such as nifedipine produce increased mortality in susceptible cases, i.e., through coronary steal phenomenon associated with vasodilation and reflex increase in sympathetic activation [81]. In the heart,  $Ca^{2+}$  is essential for electrical activity and is a direct activator of the myofilaments in contraction. Therefore, long term pregabalin administration for the management of neuropathic pain may lead to various cardiac disorders such as arrhythmias or contractile dysfunction by blocking L-type  $Ca^{2+}$  channels, especially in patients with a history of CVS dysfunctions [82].

Pregabalin has an abuse liability because it produces the disturbances in central nervous system [83] and therefore, it needs careful administration in patients with alcohol and benzodiazepine dependence [84]. Administration of pregabalin with alcohol or benzodiazepines may synergistically depress neuronal conduction in the CNS possibly due to the enhancement of GABA mediated actions and inhibition of  $Ca^{2+}$  mediated glutamate release at synapses [85]. In preclinical studies, pregabalin has been shown to induce bone marrow changes, hepatic hypoxia, endothelial cell proliferation and hemangiosarcoma in mice [86-88]. The combination of hypoxia and sustained increase in endothelial cell proliferation, angiogenic growth factors, dysregulated erythropoiesis, and macrophage activation has been hypothesized as the key event for the hemangiosarcoma formation in mice [89]. However, there has not been any such correlation in humans for such incidents.

#### 5. MECHANISM OF ACTION

##### 5.1. Voltage Gated Calcium Channels

There have been a number of studies showing an up-regulation of voltage gated calcium channel (VGCC) in dorsal root ganglion and dorsal horn in neuropathic pain. These VGCC are composed of different subunits including  $\alpha_1$  (pore forming trans-membrane subunit),  $\alpha_2$ - $\delta$ ,  $\beta$  and  $\gamma$  (auxiliary subunits) [90]. The  $\alpha_2$ - $\delta$ -subunit is composed of extracellular ( $\alpha_2$ ) and membrane-spanning ( $\delta$ ) components that remain covalently associated with a disulfide bond and are required for proper cell-surface expression of VGCC [91]. The  $\beta$  subunit binds to the  $\alpha_1$  subunit and controls the VDI (voltage-dependent inactivation), CDI (calcium-dependent inactivation) and CDF (calcium-dependent facilitation) of VGCC [92]. The  $\gamma$ -subunit is mainly associated with skeletal-muscle calcium channels, but its general importance of other channel types is not clear [93].  $Ca_v$   $\alpha_2$ - $\delta$ -1 subunits are very critical for the effective functioning of VGCC. These subunits associate directly with pore forming  $Ca_v$   $\alpha_1$  subunit in 1:1 ratio [94]. The expression of  $Ca_v$   $\alpha_2$ - $\delta$  along with  $Ca_v$   $\alpha_1$  enhances the localization of VGCC to the plasma membrane [95]. Studies have shown that expression of  $Ca_v$   $\alpha_2$ - $\delta$ , rather than  $Ca_v$   $\alpha_1$ , is the rate limiting factor in modulating synaptic function. An important role of highly conserved MIDAS motif of  $Ca_v$   $\alpha_2$ - $\delta$  has been described in controlling the synaptic VGCC function. The forward

trafficking of VGCC from cytosol to the plasma membrane of presynaptic terminal is controlled by  $\alpha_2\text{-}\delta$ , but in MIDAS independent manner. The second local step for the proper VGCC function and coupling to exocytosis is MIDAS dependent. The expression of  $\alpha_2\text{-}\delta\text{-}1$  with an intact MIDAS motif leads to an 80% increase in release probability of neurotransmitter and its mutation leads to a corresponding decrease in exocytosis [96]. The experimental nerve injury models have shown an increased level of  $\alpha_2\text{-}\delta\text{-}1$  mRNA in the damaged sensory neurons (DRGs) as shown by *in situ* hybridization [97], microarray analysis [98] and quantitative PCR [99]. There is a corresponding increase in  $\alpha_2\text{-}\delta\text{-}1$  protein in DRGs and spinal cord, as determined by Western blot [100] and immunohistochemistry [99]. Furthermore,  $\alpha_2\text{-}\delta\text{-}1$  over-expressing mice showed a neuropathic phenotype of hyperalgesia and tactile allodynia even in the absence of nerve injury [101], indicating that  $\alpha_2\text{-}\delta\text{-}1$  causes excitability of DRG neurons and the expression of neuropathy.

Pregabalin is a calcium channel antagonist which shows specific binding affinity for the  $\alpha_2\text{-}\delta$  ( $\alpha_2\text{-}\delta\text{-}1$  and  $\alpha_2\text{-}\delta\text{-}2$ ) auxiliary subunits of voltage-dependent calcium channels particularly P/Q, N and L-type [82,101-103]. The primary evidence regarding  $\alpha_2\text{-}\delta$  as the primary target of pregabalin may be deduced from studies employing transgenic mice with mutant  $\text{Ca}_V$   $\alpha_2\text{-}\delta$  gene (R217A mutant mice). The autoradiographic study also revealed that the binding affinity of pregabalin was significantly decreased in the cortex (84 %), hippocampus (80 %), caudate putamen (66 %), lumbar dorsal horn (70 %) and cerebellum (37 %) of R217A mice. The analgesic effect of pregabalin was also found to be abolished in these mutant mice (in both CCI and formalin test) without any effect on the analgesic activity of morphine and amitriptyline, showing that pregabalin binding to  $\alpha_2\text{-}\delta\text{-}1$  subunit of VDCC is important for its analgesic activity [101]. It has been shown in *in vitro* studies (in synaptosomes) that pregabalin reduces artificially-stimulated calcium influx and reduces the neurotransmitter release within 10-30 min of application [104].

Bauer *et al* demonstrated the importance of increased trafficking of the  $\text{Ca}_V$   $\alpha_2\text{-}\delta\text{-}1$  subunit from the dorsal root ganglia to the dorsal horn in the development of neuropathic pain. Furthermore, the elevated  $\alpha_2\text{-}\delta\text{-}1$  protein in the dorsal horn, but not in the DRGs was significantly reduced by chronic treatment with pregabalin for 8 days following spinal nerve ligation, indicating that pregabalin interferes with transport of  $\alpha_2\text{-}\delta\text{-}1$  to its terminal zones [99]. Administration of pregabalin is also shown to partially reverse the up-regulated  $\text{Ca}_V$   $\alpha_2\text{-}\delta\text{-}1$  at the pre-synaptic nerve terminals in the dorsal horn of the spinal cord [104]. Hence, impaired anterograde trafficking and synaptic expression of VDCC mediated through auxiliary  $\alpha_2\text{-}\delta$  subunits present an important mechanism for analgesic action of pregabalin by inhibition of synaptic transmission, decrease in neurotransmitter release and reduction of spinal sensitization [99]. This may be concluded that pregabalin provides its analgesic action by binding to the  $\alpha_2\text{-}\delta$  subunit of VDCC and inhibits its functional expression (membrane and anterograde trafficking) with concomitant inhibition of  $\text{Ca}^{2+}$  mediated excitatory neurotransmitter glutamate release at neuronal synapse (Fig. 1).

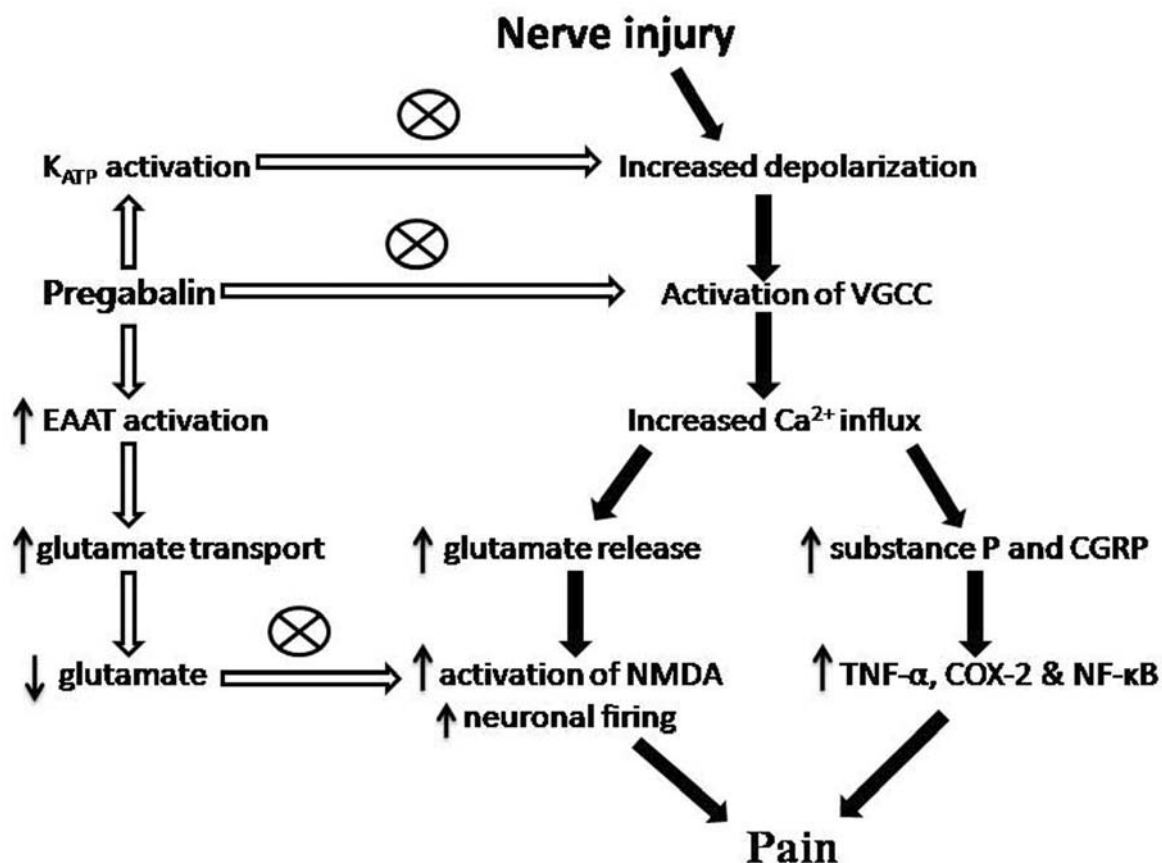
## 5.2. Glutamate Transporter

L-Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system and is stored in the synaptic vesicles. Excitatory amino acid transporters (EAATs), located on the plasma membrane of the neurons and glial cells, rapidly terminate the action of glutamate and maintain its extracellular concentration below excitotoxic levels [105, 106]. Amongst the five  $\text{Na}^+$ -dependent glutamate transporters (EAATs 1-5) [107], EAAT3 has been documented as a target of pregabalin. It is present mainly in the cytosol and only 20% of its expression is detected at the plasma membrane in normal state [108, 109]. A recent study has demonstrated that pregabalin increases the trafficking of EAAT3 from cytosol to the plasma membrane (as shown by an increase in  $V_{\text{max}}$ ) in a concentration dependent manner. Pregabalin mediated enhanced expression of EAAT3 at the plasma membrane of the neurons and the glial cells may significantly decrease the functional response of excitatory neurotransmitter (glutamate). Furthermore, pregabalin mediated enhancement in EAAT3 activity was abolished by PKC and P13K inhibitors suggesting that its action on EAAT3 may involve these signaling molecules [110]. Earlier studies had shown that EAAT3 activity and expression are highly regulated by these intracellular signaling pathways including PKC and PI3K [111, 112] (Fig. 1).

## 5.3. Potassium Channels

Pregabalin is shown to modulate different potassium channels including  $\text{K}_{\text{ATP}}$  channels which may be another mechanism responsible for its analgesic effect. It has been reported that  $\text{K}_{\text{ATP}}$  channel opening produces anti-nociceptive effects by reducing the neuronal excitability and inhibiting the release of different neurotransmitters including substance P in the spinal cord [113]. Kweon *et al* demonstrated the anti-nociceptive effect of pregabalin in the formalin test in rats. Intrathecal administration of pregabalin, through catheterization of spinal subarachnoid space, was shown to decrease formalin-induced frequency of flinching in a dose dependent manner. However, administration of glibenclamide reversed the analgesic effect of pregabalin suggesting that the anti-nociceptive effect of pregabalin in the rat formalin test may be associated with activation of  $\text{K}_{\text{ATP}}$  channels [114]. Earlier studies had shown that application of pregabalin to the intracellular surface of the excised patches activates  $\text{K}_{\text{ATP}}$  channels in the differentiated hippocampal neuron-derived H19-7 cells, suggesting that its binding site could be primarily on the intracellular site [115].

However, application of pregabalin either inside or outside DRG neurons has been shown to produce enhancement of  $\text{K}^+$  currents suggesting that the actions of pregabalin may involve both extracellular and intracellular target sites [116]. Pregabalin mediated enhanced  $\text{K}^+$  current may decrease neuronal excitability by decreasing norepinephrine and glutamate release in the rat neocortical tissue [117]. Studies have shown that administration of cAMP analogue prevents pregabalin-induced increase in  $\text{K}^+$  current suggesting that the intracellular response of pregabalin may be associated with activation of protein kinase A [116]. Other studies have also shown that inhibition of  $\text{Ca}^{2+}$  currents



**Fig. (1).** The mechanism of action for pain alleviation by pregabalin: Pregabalin blocks the VGCC and hence decrease glutamate and sensory neuropeptides (substance P and CGRP) release at synapse by decreasing  $\text{Ca}^{2+}$  influx. EAATs (excitatory amino acid transporters) activity is increased by pregabalin which caused more decrease in synaptic availability of glutamate. Decreased glutamate levels further inhibited the activation of NMDA and decreased the neuronal firing. Additionally pregabalin also activates the  $\text{K}_{\text{ATP}}$  channels, which also contributes to inhibition of neuronal excitation. Pregabalin through all these pathways ultimately provides significant pain relief in various neuropathic pain states.

by gabapentinoid drugs is sensitive to cAMP analogues capable of activating/inhibiting PKA (Fig. 1) [118].

#### 5.4. GABA

Although pregabalin is structurally related to GABA, it was not found to be metabolized to GABA and it also does not exhibit direct binding affinity for GABA receptors [119]. Pregabalin does not appear to mimic GABA or pharmacologically enhance its actions suggesting that direct or indirect effects on GABA receptors do not contribute significantly to its pharmacological actions. Furthermore, radioligand binding studies have shown that pregabalin has no affinity for GABA-A or GABA-B receptors [120]. Pretreatment with bicuculline (GABA-A antagonist) did not affect the antiallodynic effect of pregabalin suggesting that its therapeutic action is not mediated through GABA-A receptors [121]. Furthermore, pregabalin and gabapentin did not inhibit GABA transport in *in-vitro* settings [122]. The above findings suggest that there is no significant correlation between GABA and pregabalin action in alleviation of neuropathic pain. On the contrary, some reports suggest that the higher doses of pregabalin might increase the GABA content in the neuronal tissues through enhanced glutamic

acid decarboxylase activity, which results in decreased neuronal excitability and hence, may be an alternative mechanism by which pregabalin alleviates neuropathic pain [123, 124].

#### 5.5. Inflammation

Pregabalin modulates the release of sensory neuropeptides like substance P and CGRP in inflammation induced spinal cord sensitization [125]. Pregabalin inhibits lipid peroxidation, microglial cells and attenuates cellular apoptosis especially of oligodendrocytes suggesting that its neuroprotective action is mediated through anti-inflammatory effect [126]. Pregabalin suppresses substance P and other inflammatory neuropeptides induced NF- $\kappa$ B activation in both neuronal and glial cell lines non-specifically by inhibiting p65 nuclear localization. Furthermore, pregabalin also suppresses NF- $\kappa$ B-regulated gene products and COX-2 expression and thus, inhibits substance P-induced cytokine synthesis in the different neurological disorders [127]. Studies from our laboratory have shown that pregabalin mediated beneficial effects in vincristine and CCI-induced neuropathic pain is associated with decreased TNF- $\alpha$  expression and myeloperoxidase activity in the



sciatic nerve of rats. It suggests that pregabalin mediated anti-inflammatory actions including decreased release of pro-inflammatory cytokines play a key role in mediating its anti-nociceptive actions in different models of peripheral neuropathic pain (Fig. 1) [128, 129].

### 5.6. Central Versus Peripheral Action

Both systemic (intraperitoneal; i.p.) and local (intracerebroventricular or intrathecal; i.c.v. or i.t.) administration of pregabalin reduces thermal and mechanical hypersensitivity in partial ligation of the sciatic nerve (the Seltzer model) in mice, suggesting that pregabalin acts at both supraspinal and spinal loci. The depletion of spinal noradrenaline (NA) or pharmacological blockade of spinal  $\alpha_2$ -adrenoceptors with yohimbine (i.p. or i.t.) has been shown to reduce the analgesic effects of pregabalin (i.p. or i.c.v.) in thermal and mechanical hypersensitivity based tests. It has been proposed that pregabalin acts supraspinally to activate the descending noradrenergic pain inhibitory system coupled with spinal  $\alpha_2$ -adrenoceptors to ameliorate neuropathic pain [130]. An increase in  $\text{Ca}_v \alpha_2\delta$ -1 expression at the supraspinal location in response to adrenergic system stimulation is abolished in the presence (i.c.v administration) suggesting the possibility of its supra-spinal actions [10].

Electrophysiological studies in intact and spinalized rats have shown that pregabalin significantly inhibits C-fiber-mediated spinal nociceptive specific (NS) neurons with no significant inhibitory effects on A- $\delta$  fiber-mediated early responses. However, the pain inhibitory effects of pregabalin are abolished in the spinalized animals as compared to intact rats suggesting that pregabalin-induced selective anti-nociceptive effect involves the supraspinal centers [131]. Martinez and co-workers compared the central and peripheral contributions of pregabalin in treating neuropathic pain in two models including streptozotocin-induced diabetic peripheral neuropathy and traumatic injury (spinal nerve ligation). The sufficient doses of pregabalin provided through intranasal or intrathecal methods were shown to ameliorate tactile allodynia and thermal hypersensitivity in both spinal nerve ligation and diabetic peripheral neuropathy models. In contrast, administration of pregabalin at the level of the peripheral nerve did not attenuate pain behavior in either of the models again suggesting that the site of action of pregabalin is central, not peripheral [132].

### 6. CONCLUSION

Preclinical as well as clinical studies project pregabalin as a very effective agent for treating neuropathic pain with a very good safety profile. The inhibition of voltage gated  $\text{Ca}^{2+}$  channel is the most likely target for pregabalin action, which contributes to the reduction of excitatory neurotransmitters release and inhibition of synaptic transmission. The modulation of  $\text{K}^+$  channel conductance, excitatory amino acid transporter and inflammation are other possible mechanisms responsible for its analgesic actions. However, studies are still needed to elucidate its mechanism in a more detailed manner.

### CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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