

Neurotoxic assessment of chronic abuse of pregabalin in experimental model

Abstract:

Pregabalin (Lyrica) is an analogue of the gamma-amino butyric acid neurotransmitter, approved for the treatment of epilepsy, generalized anxiety disorder, neuropathic pain and fibromyalgia. The possibility for abuse and/or dependence of pregabalin was risen recently. Pregabalin is controlled in many countries including Saudi Arabia. However, unofficial use of this substance is also on the increase. The purpose of this study was to assess the potential neurotoxic effects associated with overdose prolonged pregabalin supplementation. Forty male wistar rats were divided into Group (1) normal control received distilled water, Group (2) received pregabalin (150mg/kg), Group (3) received pregabalin (300 mg/kg), and Group (4) received pregabalin (600 mg/kg). pregabalin supplementation in different doses resulted in significant dysregulation in neurotransmitters release, upsurge oxidative stress markers via enhancing lipid peroxidation and depleting antioxidant markers. Also, pregabalin doses evoked brain tissue inflammation through elevating TNF- α , IL-1 β and MCP-1. Moreover promoted brain tissue apoptosis by activating caspase -3 and suppresses Bcl2.

Pregabalin effects on the aforementioned parameters were dose dependent. These findings could highlight the potential neurotoxic effect of prolonged abuse of pregabalin supplementation through dysregulating brain neurochemical, inflammatory, oxidant/antioxidant and apoptotic mediators

Keyword:

Pregabalin - Abuse – Neurotransmitters – Oxidative stress – Euphoria – Apoptosis.

1. Introduction:

Pregabalin[(S)-3-(aminomethyl)-5-methylhexanoic acid] is an alkylated analogue of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) designed to diffuse across blood brain barrier and act as a central neuromodulation agent[1]. It is one of the newest antiepileptic drugs used to treat partial epilepsy and also manage generalized anxiety disorder; neuropathic pain, fibromyalgia and post-herpetic neuralgia[2]. A substantial off-label use has been materialized, such as hypnotic-dependent insomnia[3], withdrawal of benzodiazepines[4] and alcohol dependence[5].

Pregabalin (Trade name Lyrica®) exerts its mechanism of action via selective binding to the alpha2-delta subunit of presynaptic voltage-gated calcium channels in the central nervous system.[6] This potent binding of pregabalin at the calcium channel of neurons causes an inhibition of calcium dependent release excitatory neurotransmitters leading to attenuation of post-synaptic excitability[7] related to pain pathway, including glutamate, noradrenaline and substance P[8] and increases neuronal GABA levels

without direct effect on GABAA or GABAB or GABA uptake or degradation[9].

The recommended daily dose of pregabalin is 150-600 mg divided into two or three smaller doses, and the defined daily dose by the World Health Organization is 300 mg [1]. Upon oral administration, Pregabalin is rapidly absorbed and its bioavailability reaches approximately 90%. Although pregabalin is not very lipophilic but able to cross the blood brain barrier(BBB)and a steady state is attained within 24-48 hours with repeated administration and less than 2% of pregabalin is metabolized and it is excreted virtually unchanged in the urine and half-life of pregabalin is 6.3 hours[10].

pregabalin is considered well tolerated. Consequently, Pregabalin was classified as schedule V of the Controlled Substances Act[11]. But, similar to other compounds structurally related to neurotransmitter GABA, there were arising concerns regarding pregabalin addictive liability. There were several case reports addressing its recreational misuse[12]. Pregabalin misusers reported entactogenic, euphoric, and dissociative feelings when administered in doses exceeding therapeutic dosages[13].The routes of abuse of pregabalin include oral, intravenous, nasal insufflation, rectal (“plugging”), smoking and “parachuting” (emptying the content of the capsule into a pouch)[14].The WHO report describes diaphoresis, tachycardia, hypertension, tremors, diarrhea, anxiety, auditory hallucinations as symptoms of pregabalin withdrawal[1].

Pregabalin abuse for recreational intention has also been associated with several adverse effects which involve the central nervous system including dizziness, confusion, psychosomnolence , ataxia , cognitive disorders, CNS depression and coma[15].Moreover , those neurotoxic effects

were reported to be dose dependent[13]. However, the magnitude of the abuse potential and the mechanism behind it are not fully known.

The aim of the current study is to investigate the potential neurotoxic effect of chronic abuse of high doses of pregabalin and to explore the underlying biochemical aspects that are related to oxidative stress, inflammation, and apoptosis in brain tissue.

2. Materials and Methods

2.1. Animals:

Forty male albino rats (200 g \pm 50 g) were recruited in this study. Rats were obtained from the Animal House Colony of King Fahd Medical Research Center, Jeddah. Rats were kept on a cycle 12:12 light/dark, in a controlled temperature room (25 \pm 2°C). Rats had access to food and water ad libitum at King Fahd Medical Research Center Animal Facility Breeding Colony. All the methods used in the present study were approved by the Ethical Committee of King Fahd Medical Research Center, Jeddah, KSA and followed the recommendations of the National Institutes of Health Guide for Care and Use Committee (IACUC) of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Chemicals:

Pregabalin 150 mg /capsule was purchased from Pfizer Pharmaceutical Industries (Rabigh, Saudi Arabia).

Three pregabalin doses were used in this study: 150, 300 and 600 mg/kg/day. The doses were given orally by intra-gastric gavage for 90 consecutive days to evaluate chronic abuse of pregabalin.

These doses are approximately equivalent to 1500 mg ,3000 mg and 6000 mg in humans which are known to produce the euphoric and dissociative effects desired by addicts, and as reported by abusers in case reports for recreational uses [16,17]according to the conversion equation[18]:

Human equivalent dose (mg/kg) = animal dose (mg/kg) × (animal Km / human Km).

Km is the average body weight (kg) of species divided by its body surface area (m²).

2.3. Study design:

Rats were randomly divided into four groups with (n=10).group 1 received 1ml distilled water , group 2 received pregabalin in distilled water (150 mg/kg/day), group 3 received pregabalin in distilled water (300 mg/kg/day), group 4 received pregabalin in distilled water (600 mg/kg/day) for 90 consecutive days.

At the end of the experimental period, all animals were euthanized and sacrificed by decapitation. Brains were excised, washed with ice – cold saline (0.9%) and homogenized with 0.1 M phosphate buffer saline at pH 7.4, to give a final concentration of 10% w/v for the biochemical assays.

2.4. Biochemical analysis:

2.4.1. Brain Monoamines neurotransmitters

Brain serotonin,dopamine, and norepinephrine weredetermined using high-performance liquid chromatography (HPLC) system, Agilent technologies 1100 series equipped with a quaternary pump (Quat pump, G131A model). Separation was achieved on ODS-reversed phase column (C18, 25 x 0.46 cm i.d. 5 μm). The mobile phase consisted of potassium phosphate buffer/methanol 97/3 (v/v) and was delivered at a flow rate 1

ml/min. UV detection was performed at 270 nm, and the injection volume was 20 μ l. The concentration of the neurotransmitters was determined by external standard method through using peak areas. A linear standard curve was constructed where samples concentration was obtained directly from the curve [19].

2.4.2. Oxidative stress markers:

Lipid peroxidation malondialdehyde (MDA) was determined in brain tissue according to Ohkawa[20]. Brain reduced glutathione (GSH) was evaluated as per Ellman [21] and superoxide dismutase (SOD) activity was estimated in brain tissue as previously described by Nishikimi[22]. The determination of catalase (CAT) was carried out following Clairborne[23].

2.4.3. Inflammatory mediators and apoptotic markers

Brain inflammatory cytokines : tumor necrosis factor alpha and interleukin one beta (TNF- α , IL-1 β), inflammatory chemokine monocyte chemoattractant protein (MCP-1) and apoptotic markers caspase 3 (Cas3) and B- cell lymphoma (Bcl₂) were determined by enzyme-linked immuno-sorbent assay using ELISA kits (Invitrogen Corporation Camarillo, CA, USA) and microtiter plate reader (Fisher Biotech, Germany) according to the manufacturer's instructions.

2.4.4. Statistical analysis:

The obtained data were presented as Mean \pm SE. Homogeneity of variance for each variable was analyzed using the Levine test. One-way analysis of variance (ANOVA), followed by Duncan's multiple rank test were performed using the mSTAT-c computer program to determine the statistical

significance between the different groups. The difference was considered significant at $P < 0.05$.

3. Results

3.1 Brain monoamine neurotransmitters:

Rats treated with pregabalin at different doses (150, 300 and 600 mg) showed a significant decline in both dopamine (-49% , -81% and -91% respectively) and adrenaline (-56%, -80% and -97% respectively) as compared to the control group. whereas, a significant upregulation in serotonin levels was observed following pregabalin doses administration by 1.75, 2.98 and 4.17 folds versus control group. (fig.1)

3.2 oxidative stress markers:

Administration of pregabalin at doses (150, 300 and 600 mg/kg) induced a significant increment in brain tissue MDA level by (4.5, 8.8 and 12.4 folds, respectively) as compared to the control group. While significantly attenuated brain tissue antioxidants level of GSH (-37.7 % , -68.2% and -84.7% , respectively) ,SOD activity (-37.9% , -58.8% ,and -79.7% ,respectively) and CAT level (-38.4%, 61.5% , and -84.7% , respectively) in comparison with the control group. (fig.2)

3.3Inflammatory mediators:

The present data revealed a significant upsurge in brain tissue inflammatory mediators in a dose dependent manner as evidenced by a significant elevation in brain tissue TNF- α , IL-1 β and MCP-1 levels (109.7%, 125.3%, and 59.1% respectively) after pregabalin (150mg/kg) treatment as compared to the control group. This elevation reached (228.1%271.5% and104.1%

respectively) by increasing pregabalin dose to 300mg/kg and (378.3%,479.7% and 164.5% respectively)following administration of pregabalin (600 mg/kg) as compared to control group. (fig.3)

3.4 Apoptotic markers

The data showed a significant elevation in brain tissue Caspase 3 levels (2.4 folds, 4.5 folds and 6.4 folds) after administration of pregabalin three doses (150mg/kg, 300mg/kg and 600mg/kg respectively) as compared to the control group. While there was a significant gradual decrease in Bcl2 levels in brain tissues (0.6 folds, 0.3 folds and 0.08 folds) after administration pregabalin (150mg/kg, 300mg/kg and 600mg/kg respectively) versus the control group. (fig.4)

The changes in all the aforementioned parameters were dose dependent and became more observable by increasing the dose of the drug.

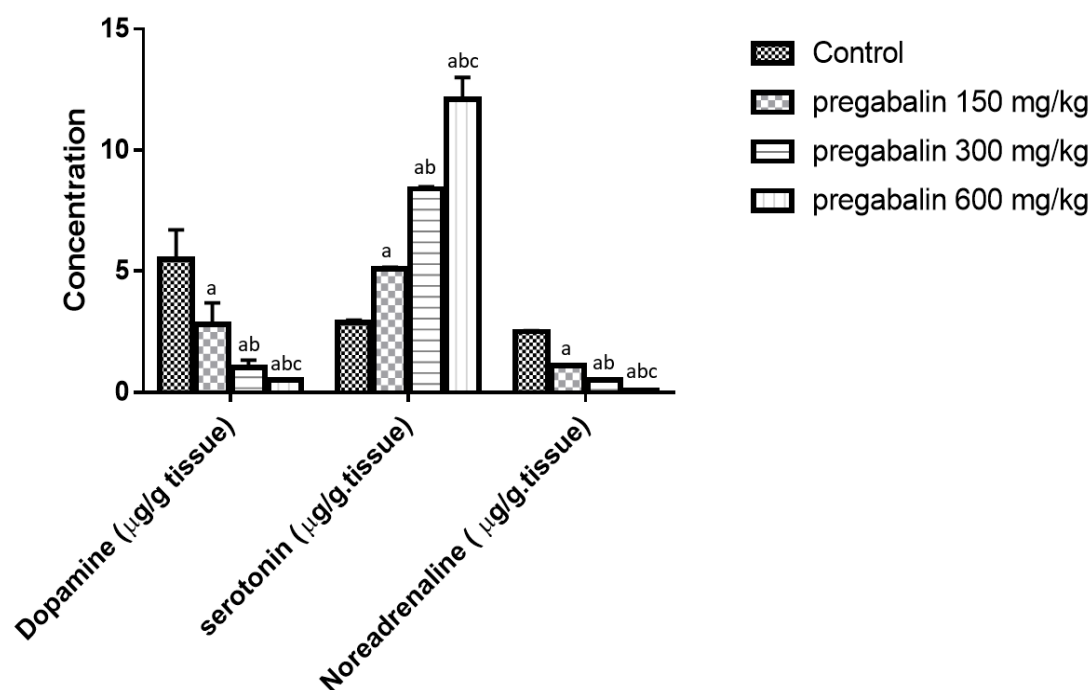


Fig.1.Effect of different doses of pregabalin on brain tissue Dopamine, Serotonin andNoradrenaline (n=10, Mean \pm SEM) ^a significant from control group, ^b significant from pregabalin (150 mg/kg), ^c significant from pregabalin (300 mg/kg).

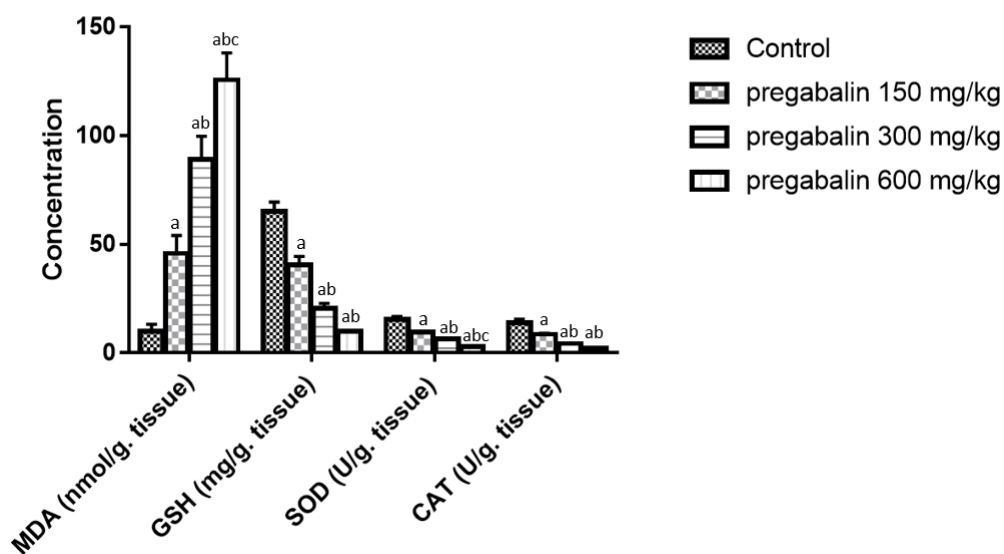


Fig.2. Effect of different doses of pregabalin on brain tissue Malondialdehyde level (MDA), Reduced glutathione (GSH) , superoxide dismutase (SOD) and catalase (CAT) ,(n=10, Mean \pm SEM) ^a significant from control group , ^b significant from pregabalin (150 mg/kg) , ^c significant from pregabalin (300 mg/kg).

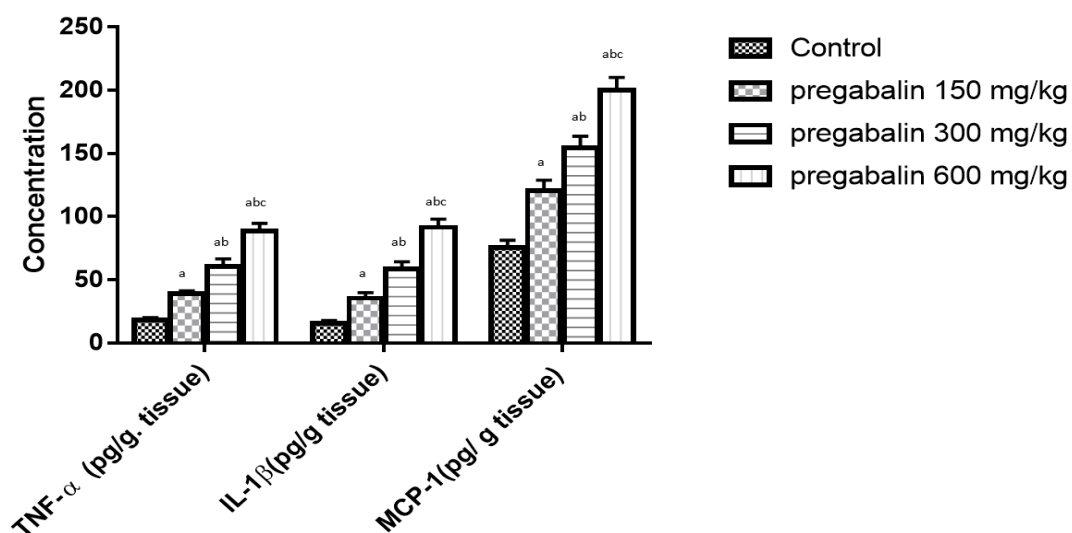


Fig.3. Effect of different doses of pregabalin on brain tissue Tumor Necrosis Factor -Alpha (TNF- α), Interleukin-1beta ,Monocyte Chemoattractant Protein-1(MCP-1) , (n=10, Mean \pm SEM) ^a significant from control group , ^b significant from pregabalin (150 mg/kg) , ^c significant from pregabalin (300 mg/kg).

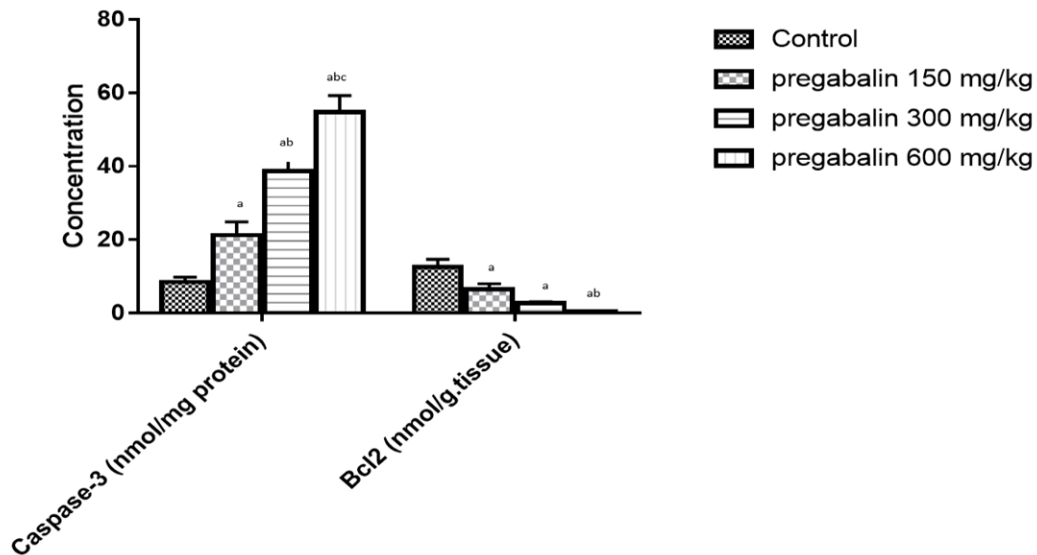


Fig.4. Effect of different doses of pregabalin on brain tissue Caspase-3, B-cell lymphoma2 (Bcl2) , (n=10, Mean \pm SEM) ^a significant from control group , ^b significant from pregabalin (150 mg/kg) , ^c significant from pregabalin (300 mg/kg).

Discussion:

Pregabalin misuse/abuse represents a growing trend that is causing significant patient harm. Multiple case reports of its abuse potential and dependence, including withdrawal symptoms, have been published[6,24]. Toxicity may occur after overdose or at prolonged therapeutic doses following accumulation resulting in adverse effects especially on the CNS. The current study was conducted to highlight the neurotoxic effect of long-term administration of overdoses of pregabalin and signal the biochemical aspects that might trigger these toxic effects.

The current results showed that prolonged administration of high doses of pregabalin resulted in a significant decline in dopamine and norepinephrine. These results were in the same line with the study of Taha et al.[16] which indicated a significant suppression in dopamine and norepinephrine following pregabalin administration in high doses. Pregabalin binds potently to the alpha-2-delta protein in the brain[25] which is associated with voltage-gated calcium channels. This potent binding has been shown to reduce depolarization-induced calcium influx at nerve terminals, with a consequential reduction in the release of several excitatory neurotransmitters, including dopamine, glutamate, noradrenaline, substance P, and calcitonin gene related peptide CGRP[26]. On the other hand, our data demonstrated a significant elevation in brain serotonin level after prolonged administration of pregabalin in high doses. Jellestad et al. [27] reported that Pregabalin with its serotonergic action has a liability to cause serotonin syndrome which is caused due to excess serotonin concentration in the central nervous system and/or peripheral nervous system leading to cognitive, autonomic and somatic effects. Gabapentin, an analogue of pregabalin, has been shown to increase serotonin levels in the CNS [28] via inducing alterations in central serotonin metabolism (which may be reflected by changes in peripheral serotonin levels), the interaction of serotonin with blood platelets reflects its central actions[29]. If gabapentin inhibited the central release of serotonin, it might also inhibit the efflux of the transmitter from blood platelets, thus rendering the transmitter less susceptible to degradation and increasing its availability after stimulation[28].

In the present study, the long term administration of pregabalin supra therapeutic doses instigated brain oxidative stress and provoked the levels of lipid peroxide. Also, significantly abrogated antioxidant defenses as brain SOD, GSH and CAT activities which scavenge free radicals and prevent their

injurious effects ,rendering brain tissues vulnerable to free radicals attack.These results were in agreement with the study of Kamel [30] who reported a significant elevation in brain lipid peroxidation associated with a reduction SOD and CAT following chronic oral pregabalin administration for 90 days. Also, Taha et al [16] demonstrated a significant induction in oxidative stress with prolonged high dose of pregabalin.The disturbance in oxidants/antioxidant balance could be partially attributed to the inhibition of CGRP induced by pregabalin where CGRP deletion is associated with enhanced oxidative stress and a loss of endogenous antioxidant expression[31]. Also, another study by Pen-Silva et al [32] revealed that increased serotonin increases oxidative stress in heart valves through an MAO-A-dependent mechanism. MAO-dependent generation of reactive oxygen species (ROS) may be important for the understanding of mitogenic actions of serotonin which includes 1-activation and translocation of mitogen-activated protein kinases [33] and the phosphatidylinositol 3-kinase pathway [34] 2-activation of cell cycle proteins [35] and 3- transactivation of other mitogenic receptors such as the platelet-derived growth factor receptor[36].

Inflammation is a defense mechanism that protects the body from the damage caused by the endogenous or exogenous stimuli[37]. ROS are reported to be centrally involved in the progression of many inflammatory diseases and present functions in signaling and mediation of the inflammation[38].In the present study treatment with overdose of pregabalin for long term induced an elevation in brain tissue inflammatory mediators (TNF- α , IL -1 β and MCP-1).It is known that oxidative stress increases the gene expression and synthesis of pro-inflammatory cytokines, mediated by activation of the transcription factor nuclear factor- κ B, activator protein 1 which translocates to the nucleus augmenting the expression of

pro-inflammatory genes such as IL-1 β , TNF- α [39]TNF - α in turn stimulates the production of ROS by sensitizing infiltrating leukocytes and MCP-1[40,41].

The current results indicated significant elevation in apoptotic markers following high doses of pregabalin as manifested by increased Caspase 3 associated with a decline in Bcl2 these results were in accordance with Taha et al[16]. Prolonged administration of high dose pregabalin has been reported to enhance the p38-MAPK/JNK/ERK signaling in the cerebral cortex[42]. Mitogen-activated protein kinase (MAPK) signaling pathways organize a great constitution network that regulates several physiological processes, like cell growth, differentiation, and apoptotic cell death. Due to the crucial importance of this signaling pathway, dysregulation of the MAPK signaling cascades is involved with oxidative stress and DNA damage[43]. Activation of p38 MAPK induces stimulation of the mitochondrial apoptosis pathway and regulates the equilibrium between BCL2 and BAX expression in mitochondria. Phosphorylation of p38 MAPKs promotes release of cytochrome c from mitochondria through inhibition of BCL2 and shifting of BCL2:BAX ratio, producing successive activation of apoptotic proteases caspase-9, and caspase-3 [16].

Conclusion:

Chronic abuse of high doses of pregabalin could induce neurotoxic effects via deregulating neurotransmitters release, instigating oxidative stress marks, depleting antioxidant defense, inducing inflammatory and apoptotic mediators. The PGB- induced neurotoxic effects was dose dependent. Further studies should address the extent of abuse which increases the liability towards adverse toxic effects.

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