Neuroprotective Effect of Pyridoxine Hydrochloride in Models of Mononeuropathy in Rats

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Summary

We have evaluated the neuroprotective actions of Pyridoxine hydrochloride 100mg/kg (PYR) by using partial sciatic nerve ligation (PSNL) and sciatic nerve crush injury (SNCI) models in wistar rats. The parameters used were thermal hyperalgesia (TH), motor function test (MFT) and motor nerve conduction velocity (MNCV). A steady improvement in TH was seen in PYR treated animals on day 21st (10.01±0.23) and day 14th (10.36±0.2 s) in the models of PSNL and SNCI respectively. There was a reduction in pain which was observed behaviorally in MFT in both the models and also a steady improvement in MNCV of PYR treated animals which improved on 15th day (30.39 ± 1.78m/s) and 30th (40.59± 2.54m/s) indicating that PYR has a considerable neuroprotective action in PSNL and SNCI in rats.

Keywords: Pyridoxine hydrochloride, Partial sciatic nerve ligation , Sciatic nerve crush injury.

Shortened Tittle: Pyridoxine Hydrochloride in mononeuropathy….

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Introduction

It is now very well known that events like nerve ligation directly causes mechanical injury to the nerve and symptoms produced in such animals resemble the symptoms produced in causalgia in humans. The animal model mimics both hyperalgesia (increased response to noxious stimuli) and allodynia (pain response to low threshold stimulus) [1.2.3.4]. Furthermore, it is also reported that extent of nerve trapped in the ligature also affects the effects elicited post surgery and most importantly the pain so produced post operatively is like burning sensation similar to burning sensation in causalgic humans [3,5,6]. Direct mechanical injury or ischemia or both can cause acute endothelial injury that can result in endothelial edema, agranulocyte plug or microvascular thrombosis. These factors interrupt the reflow and can cause continuous fiber injury. Moreover, endoneural edema may develop due to microvascular compression. Toxic substances released from neutrophils and macrophages after injury can impair tissue protection in normal conditions and permit the accumulation of free oxygen radicals which increase tissue destruction and cause tissue damage [5]. It has been reported that diets deficient in Pyridoxine (Vitamin B6) could cause peripheral neuropathy [7]. Vitamin B6 supplementation is recommended for patients suffering from Carpel tunnel syndrome [8]. Also in a study it was shown that Vitamin B6 (33mg/kg and 100 mg/kg) was shown to reduce thermal hyperalgesia in a model of mononeuropathy caused by loose ligation of sciatic nerve [9]. The objective of the study was to evaluate anti-hyperalgesic and neuroprotective effect of pyridoxine hydrochloride (100mg/kg) (PYR) after sciatic nerve lesion produced by partial sciatic nerve ligation [10] and sciatic nerve crush injury [11] in rats.

Material and Methods

Animals: Forty eight adult female Wistar rats (175-225g) were obtained from National Toxicology Centre, Pune, India. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70%. A 12:12 light : dark cycle was followed. All animals had free access to water and standard pellet laboratory animal diet. All the experimental procedure used in this study were reviewed and approved by Institutional Animal Care and Use Committee of Poona College of Pharmacy, Pune, India [12]

Preparation of the drug solution PYR :- Solution was prepared by dissolving pyridoxine hydrochloride (Himedia) in distilled water. The drug solution was stored in air tight bottles at room temperature in a cool and dry place away from moisture and sunlight. The solutions were freshly prepared for daily administration.

The volume of drug solution were calculated based upon the body weight of the animal.

Mononeuropathy caused by Partial Sciatic Nerve Ligation (PSNL) in Rats.

The method of Seltzer [10] was followed. The animals were divided into Control (vehicle treated), Sham(vehicle treated), Untreated, PYR treated.

Preparation of animals:-Under ether anesthesia and aseptic conditions the right sciatic nerve was exposed at high thigh level. In sham operated animals the nerve was left intact and the wound was closed with 2 muscle sutures and 3-4 skin sutures.

In experimental animals the sciatic nerve underwent partial injury. The dorsum of the nerve was carefully freed from surrounding connective tissues at a site near the trochanter just distil to the point at which the posterior biceps semitendinosus nerve branches off the common sciatic nerve. Using an iris forceps the nerve was fixed in its place by pinching the epineurium on its dorsal aspect, taking care not to press the nerve against underlying structures. A 4-0 silk suture was inserted into the nerve with 3/8 curved, reverse- cutting mini needle, and tightly ligated so that the dorsal 1/3 – ½ of the nerve thickness was trapped in the ligature. The wound was then closed as in sham operated rats.

In all rats the left leg and sciatic nerve were untouched. The animals were allowed to recover after surgery and from day 2 onwards PYR (100mg/kg) treatment once daily, p.o. was started and continued for 30 days daily. The observations were recorded daily in the morning (between 10 am to 12 pm) and doses were administrated immediately afterwards The effect of PYR was studied on the following parameters.

Thermal hyperalgesia (TH):- This was assessed using Ugo Basile Hot Plate instrument (at 55±1° C) [13]. Antinociceptive effects were determined according to the latency (in seconds) of limb withdrawal to the noxious thermal stimulation (Ugo Basile hot plate, Versace, Italy). Cut off value of planter test was set to 22 s to prevent limb injury. The pain threshold were tested on day 0,2,4,6,8,10,12,14, 21 and 28.

Motor function test (MFT) :- Motor function was monitored by observation of spontaneous gait and hind paw posture [14]. Each animal was placed in a plastic box with plexiglass walls and allowed to habituate for at least 5 minutes before the observation period. One animal at a time was observed for 15 min (3 x 300s). Different positions of the lesioned hind paw were rated according to a numerical scale described by Attal [14]. The readings were observed on day 0, 2, 15 and 30.

Mononeuropathy caused by Sciatic Nerve Crush Injury (SNCI) in Rats.

The method of Bishofs [11] was followed. The animals were divided into Control (vehicle treated), Sham(vehicle treated), Untreated, PYR treated.

Preparation of animals:- Under ether anesthesia and aseptic conditions the right sciatic nerve was exposed at high thigh level. In sham operated animals the nerve was left intact and the wound was closed with 2 muscle suture and 3-4 skin sutures.

In experimental animals the sciatic nerve underwent crush injury. The dorsum of the nerve was carefully freed from surrounding connective tissues at a site near the trochanter just distil to the point at which the posterior biceps semitendinosus nerve branches off the common sciatic nerve. Using an irish forceps the nerve was fixed in its place by pinching the epineurium on its dorsal aspect, taking care not to press the nerve against underlying structures. A forcep was used to crush the nerve twice for a period of 30 s with an interval of 60 s in between. The wound was then closed as in sham operated rats. In all rats the left leg and sciatic nerve were untouched.

The effect of PYR was studied on the following parameters.

Thermal hyperalgesia (TH):- TH was assessed as described earlier on day 0,2.7.14.21 and 28.

Motor function test (MFT) :- MFT was assessed as described earlier on day 0,2,15 and 30.

Motor Nerve Conduction Velocity (MNCV) :- The experiment was performed on the same group of rats on day 15^{th} and 30^{th} day. Rats were anesthetized using thiopental sodium (50 mg/kg, i.p) for electrophysiological recording. MNCV was recorded by stimulating the sciatic and tibial nerves at sciatic and tibial notch respectively by a 0.1 ms square wave pulse delivered through a pair of monopolar needle electrodes (1.0 – 1.5 mA, 2.0 mV/D) through a stimulator. Responses were recorded from the indigital plantar muscles using Students Biopac data acquisition system (Santa Barbara, CA, USA).

Statistical Analysis

Data was expressed as mean ± SEM of 6 animals in each group. To determine the statistical significance, ANOVA followed by Tukey-Kramer test (Instat/ Graphpad) was used. Differences between means were considered statistically significant if $p < 0.001$. For motor function test Kruskal-Wallis test followed by Dunn was used.

Results

Effect of PYR on TH in PSNL model in rats.

A steady improvement in TH was seen in PYR(100mg/kg) treated animals. The latency (sec) on day 21^{st} (10.01±0.23) was similar to that observed on day 0 (10.00±0.36) indicating complete recovery from algesia induced by heat.

Table 1. TH in PSNL model

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 21	Day 28
Normal	10.01 ±0.31	10.36 ±0.42	10.5 ±0.24	10.0 ±0.32	9.61 ±0.16	10.72 ±0.08	10.68 ±0.16	10.65 ±0.16	10.98 ±0.09	10.21 ±0.12
Sham	9.23 ±0.24	7.67± 0.32	10.0 ±0.26	9.65 ±0.17	9.03 ±0.18	10.25 ±0.14	10.48 ±0.14	10.54 ±0.25	10.61 ±0.08	10.43 ±0.11
Untreated	10.4 ±0.26	3.24± 0.13	3.2± 0.09	3.55± 0.12	3.64± 0.12	3.28± 0.09	3.82± 0.13	3.81± 0.16	3.21± 0.09	3.52± 0.21
PYR	10.00 ±0.56	4.53± 0.17	7.01± $0.12^{\#}$	8.27± $0.13^{\#}$	8.68± $0.11^{\#}$	9.23± $0.2^{\#}$	9.98± $0.11^{\#}$	9.91± $0.14^{\#}$	10.01 $\pm0.23^{\#}$	10.89 $\pm0.34^{\#}$

Data expressed in Mean ± S.E.M. ANOVA followed by Tukey test.. p <0.001 = #. Data compared with untreated. n=6

Effect of PYR on MFT in PSNL model in rats.

The pain scale observed in PYR(100mg/kg) treated animals improved indicating the reduction in pain perception which was observed behaviorally. Whereas in untreated group of animals no reduction in pain scale was observed implicating neuropathic pain.

Table 2. MFT in PSNL model

	Day 0	Day 2	Day 15	Day 30
Normal	0.0	0.0	0.0	0.0
Sham	0.0	0.271 ± 0.05	0.087 ± 0.027	0.099 ± 0.02
Untreated	0.0	2.92 ± 0.08	2.98 ± 0.09	3.03 ± 0.074
PYR	0.0	2.87 ± 0.078	$1.13 \pm 0.021^{\#}$	$1.021 \pm 0.012^{\#}$

Data expressed in Mean ± S.E.M. Kruskal Wallis followed by Dunn. p <0.001 = #. Data compared with untreated. n=6

Effect of PYR on TH in SNCI model in rats.

A steady improvement in TH was seen in PYR(100mg/kg) treated animals. The latency (sec) on day 14st (10.36±0.23) was similar to that observed on day 0 (12.41±0.33) indicating complete recovery from algesia induced by heat.

Table 3. TH in SNCI model

.	Day 0	Day 2	Day 7	Day 14	Day 21	Day 28
Normal	11.87	11.6	11.3	10.95	10.98	11.48
	±0.48	±0.49	±0.307	±0.33	±0.31	±0.20
Sham	11.91	9.95	10.75	11.4	11.3	11.38
	±0.32	±0.22	±0.23	±0.37	±0.20	±0.34
Untreated	11.92	4.97	5.26	5.23	4.95	4.78
	±0.20	±0.19	±0.11	±0.06	±0.09	±0.19
PYR	12.41	5.18	7.41	10.36	11.01	11.9
	±0.33	±0.16	±0.16$^{\#}$	±0.2$^{\#}$	±0.19$^{\#}$	±0.23$^{\#}$

Data expressed in Mean ± S.E.M. ANOVA followed by Tukey test. p <0.001 = #. Data compared with untreated. n=6

Effect of PYR on MFT in SNCI model in rats.

The pain scale observed in PYR treated animals improved indicating the reduction in pain perception which was observed behaviorally. Whereas in untreated group of animals no reduction in pain scale was observed implicating neuropathic pain.

Table 4. MFT in SNCI model

	Day 0	Day 2	Day 15	Day 30
Normal	0.0	0.0	0.0	0.0
Sham	0.0	0.271 ± 0.05	0.087 ± 0.027	0.099 ± 0.02
Untreated	0.0	2.92 ± 0.08	3.16 ± 0.09	3.03 ± 0.074
PYR	0.0	2.97 ± 0.09	1.40 ± 0.026$^{\#}$	1.20 ± 0.038$^{\#}$

Data expressed in Mean ± S.E.M. Kruskal Wallis followed by dunn. p <0.001 = #. Data compared with untreated n=6

Effect of PYR on MNCV in a model of mononeuropathy produced by SNCI in rats

MNCV of PYR treated animals improved on 15th day (30.39 ± 1.78m/s) and 30th (40.59± 2.54m/s)but was not was brought back to normal, whereas MNCV of untreated animals plummeted drastically (15.29 ±0.71m/s, 13.87±0.58m/s on day 15th and 30th respectively) indicating the presence of neuropathic pain.

Table 5. MNCV in SNCI model

Group	15th day MNCV (m/s)	30th day MNCV (m/s)
Normal	52.75 ± 2.68	53.01 ± 3.99
Sham	47.19 ± 3.85	47.86 ± 2.59
Untreated	15.29 ± 0.71	13.87 ± 0.58
PYR	30.39 ± 1.78$^{\#}$	40.59 ± 2.54$^{\#}$

Data expressed in Mean ± S.E.M. ANOVA followed by Tukey test. p <0.001 = #. Data compared with untreated. n=6

Discussion

Patients with peripheral nerve injuries occasionally experience chronic pain. This phenomenon is classified as neuropathic pain. Recently several animal models of neuropathic pain have been evaluated and there appear to be some similarities between these models and the clinical features of human patients. [10,15]. The majority of currently used neuropathic pain models share alterations in hind limb cutaneous sensory thresholds following partial injury to a peripheral (usually sciatic) nerve as a common feature. In particular, demonstration of hyperalgesia to noxious thermal stimuli and allodynia to cold and mechanical stimuli are used as outcome measures. Three most commonly used models are the chronic constriction injury (CCI) of sciatic nerves, the partial sciatic nerve ligation model (PSNL), and the spinal nerve ligation model (SNL)[4]. Nerve crush might be considered an extreme version of compressive nerve injury with an enhanced degree of vasa nervorum disruption [16]. Three factors are thought to cause hyperalgesia in the sciatic nerve ligation model, first is the ectopic

discharge generated from injured axons, second is release of cytokines from the inflammatory cells around the injured nerve and third is plastic changes in the sensory pathways to the spinal cord and brain [15].

There is evidence that severe peripheral nerve ischemia from vascular ligation damages endothelium, resulting in swelling, luminal narrowing and no re-flow. Although compression may result in temporary circulatory arrest it is unclear wheather this insult permanently injures nerve micro vessels. Comprehensive ischemia if maintained long enough, might induce no re-flow, as in the ligation experiments [16].

In a serious trauma like crush, a short period of localized total or subtotal ischemia is followed by evident increase in endoneural fluid pressure and impairment of the normal capillary blood flow in the endoneurium. These events results in the release of the endogenous chemical mediators, increase in vascular permeability and impairment of blood nerve barrier. Endothelial and intraneural edema with inflammatory response follows this process [5]. The peripheral nerve responds to trauma by an inflammatory reaction with increased vascular permeability and intraneural edema, local ischemia in the tissue causes metabolic impairment, which in turn allows the production of the toxic oxygen metabolites such as superoxide anion, hydrogen peroxide and hydroxyl radicals by the polymorphoneuclear leucocytes that infiltrate the lesion site. Free radicals and cytokines which are responsible for cell damage are released from neutrophills [6].

Results of the present study indicated that in PSNL and SNCI models of mononeuropathy, PYR (100mg/kg, O.D, 30 days) gradually reduced the pain latency(sec) produced by thermal stimulus [Table 1,3] . The onset was observed after 4 days of treatment in PSNL and after 7 days in SNCI model. Further continuation of treatment resulted in restoration of latency to heat stimuli to pre surgery period. The present study was further extrapolated to MFT, a behavioral parameter of assessing mononeuropathy in PSNL and SNCI. PYR (100mg/kg, O.D, 30 days) treatment improved stance and pain endurance caused by nerve injury. After 15 days of treatment the pain scale was not significantly different compared to that of day 0, indicating amelioration in pain [Table 2,4]. MNCV in untreated group was reduced while PYR(100mg/kg, O.D, 30 days) increased the conduction velocity [Table 5]. Reduction in MNCV is an indication of

neuropathic pain, while restoration of passage of impulse indicate an increase in conduction velocity.

PYR has established therapeutic uses in the treatment of neuropathy produced by antituberculosis drugs, alcohol induced neuropathy and pellagra syndromes. Studies have shown that diets totally deficient with PYR would result in peripheral neuropathy [7] but if overdose of PYR is given might result in neuropathy induced by PYR toxicity [8]. Wang [9] reported that Pyridoxine (33mg/kg and 100mg/kg) inhibits thermal hyperalgesia in another model of mononeuropathy caused by chronic constriction injury.

Our investigation suggest that pyridoxine hydrochloride (100mg/kg, O.D, 30days) is effective against TH in PSNL and SNCI models of mononeuropathy in rats which opens a possibility of exploring the potential of pyridoxine hydrochloride in the treatment of heat or crush injuries

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