

EFFECT OF GRANISETRON ON EXPERIMENTAL MODEL OF DIABETES INDUCED NEUROPATHIC PAIN PERCEPTION IN RATS

NITHYA S*, SUMAN RAY

Department of Pharmacology, School of Pharmaceutical Sciences, Vels University (VISTAS), Velan Nagar, P.V. Vaithyalingam Road, Pallavaram, Chennai 600117. Email: sermugapandian.nithya@gmail.com

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ABSTRACT

The aim of present study was to study the effect of chronic treatment of (6 weeks) of Granisetron (1mg/ml & 10mg/ml) an adenosine agonist on blood glucose level and in prevention of diabetic neuropathy. Evaluation of diabetic neuropathy was performed after 6weeks of single injection of Streptozotocin (70mg/kg, i.v) in rats. Blood glucose level, glycated haemoglobin, grip strength, pain sensitivity and threshold in diabetic rats were measured at the end of 6th week. The results of the present study indicate that the 6 weeks treatment of Granisetron demonstrates hypoglycemic effect; it markedly decreases the blood glucose level in the diabetic rats. There is also decrease in grip strength in diabetic rats indicates to induction of neuropathy or nerve damage. Granisetron increase the grip strength of diabetic rats. There was also found loss of pain perception in diabetes rats which measured using hot plate and tail flick method. Granisetron increases the licking time and withdrawal latency in hot plate and tail flick respectively in a dose dependent manner. This indicates the presence of pain perception and prevention of nerve damage demonstrates its protective effect in diabetic neuropathy. Our study concludes the chronic treatment of Granietron significantly protects from the development of diabetic neuropathy.

Keywords: Diabetic neuropathy, Diabetes mellitus, Pain perception and Granisetron (1 & 10mg/ml).

INTRODUCTION

Diabetic neuropathy (DN) is a peripheral nerve disorder caused by diabetes which leads to cause a significant morbidity [1,3].The risk of developing diabetic peripheral neuropathy (DPN) increases with duration of the disease and degree of glycemic control, and other contributing factors such as hypertension, dyslipidaemia, smoking, body mass index and hyperinsulinaemia. The symptoms of diabetic neuropathy are often slight at first but can occasionally flare up suddenly and affect specific nerves so the affected individual will develop double vision, drooping eyelids, or weakness and atrophy of the muscles. Nerve damage caused by diabetes generally occurs over a period of years and may lead to problems with the digestive tract and sexual organs, which can cause indigestion, diarrhoea or constipation, dizziness, bladder infections, and impotence [2,5].

The main risk factor for diabetic neuropathy is hyperglycemia. It is important to note that people with diabetes are more likely to develop symptoms relating to peripheral neuropathy as the excess glucose in the blood results in a condition known as Glucojasinogen. This condition is affiliated with erectile dysfunction and epigastric tenderness which in turn results in lack of blood flow to the peripheral intrapetene nerves which govern the movement of the arms and legs. The progression of neuropathy is dependent on the degree of glycemic control in both Type 1 and Type 2 diabetes. Duration of diabetes, age, cigarette smoking, hypertension, height and hyperlipidemia are also risk factors for diabetic neuropathy[4,6,7].

The pathogenesis of DPN is believed to be multifactorial with hyperglycemia being the primary risk factor. Suggested theories that postulate the aetiopathogenesis of DN include abnormalities of protein glycation, sorbitol accumulation, polyol pathway flux, protein Kinase C activation, advanced glycation end products, receptor for advanced glycation end products, a decrease in neuronal nitric oxide synthesis protein, and micro vascular hypoxia, resulting in oxidative stress[1,4,8].

Since DN is not clearly understood, it is hard to make a definitive course of treatment[9]. Drugs that have been used in the management of DN include tricyclic antidepressants and selective serotonin reuptake inhibitors[9,10]. Many pharmacological options are available to treat DPN but still it is difficult for patients to obtain complete relief because of poor glycemic control. Prevention through strict glycemic control remains the mainstay of therapeutic

intervention because effective disease modifying therapies are yet not available. In reported case study adenosine receptor activation ameliorates type 1 diabetes[11,12,22].Granisetron being an adenosine agonist was aim at to evaluate the influence of chronic treatment of Granisetron (1mg/ml & 10mg/ml) on blood glucose level and on progression neuropathy in STZ-induced 6 week diabetic rats.

MATERIALS AND METHODS

Glucose estimation kit (Hi-Media Lab, Mumbai), Streptozotocin (Gift sample from Nicholas Piramal Pvt. Ltd. Mumbai), Glycosylated Hb Kit. All the reagent and chemicals used in present study were of analytical grade. Granisetron (Sun Pharmaceuticals Gujarat, India) was prepared in saline

Male Wister rat weighing 175 -200 gm were used. The animals were fed with standard diet had free access to water under well ventilated condition of 12 h day light cycle. The animals were adapted to laboratory condition for 7 days prior to the experiments. The studies were performed with the approval of Institutional Animal ethics committee (IAEC) Experimental design:

Experimental model of diabetes was induced by i.v. injection of Streptozotocin (70 mg/kg) in Wister rat (180-220gm) and the chronic treatment of Granisetron (1mg& 10mg/ml) was started after stabilization blood glucose level from day 13th of Streptozotocin injection. Effect of Granisetron on blood glucose level, glycosylated haemoglobin and diabetic neuropathic pain were evaluated after 6th week. Induction of diabetic neuropathy was evaluated by grip strength[13,14] and the neuropathic pain perception by threshold by tail flick and hot plate method[13-15].

Measurement of dn by behavioural status

A grip strength determination was used for evaluating neuromuscular strength [13,14]. The grip strength of animals was measured by simply hanging of animals with their Fore limb on fine metal wire which was held at two end of pole. The time taken to hold the metal wire to fall on the surface was considered for the muscle strength determination. The animals whose muscle or nerves get damage or weak it get fall soon on the floor. The force achieved (in terms of time) by the animal for staying in hanging stage was recorded.

Evaluation of the effect of diabetic neuropathy on pain sensitivity and pain threshold were done by Hot Plate and Tail Flick

methods[12,14,15]. The rats were placed on the hot plate (55-58°) and the time until either licking or jumping occurs was recorded by a stop watch. A cut off time of 10 s was kept to avoid damage to the paw of the animal.

Evaluation of pain threshold in diabetic rats was determined by withdraw latency in tail flick test[12,14,15]. Tail of each diabetic rat was exposed to radiant heat which was given by placing the hot water in glass. The intensity of radiant heat (55-58°) was adjusted to obtained withdrawal latency of not more than 6 second in both diabetic and non-diabetic rats. The tail flick latency is the time interval taken by rat to flick its tail after exposure to a source of radiant heat. Cut of time was fixed at 10 s.

Statistical methods

Blood glucose level was analyzed by ANOVA followed by Dunnet test. Data of grip strength, pain sensitivity and threshold and the glycosylated haemoglobin were analyzed by student unpaired t test. The significant difference was compared at $P < 0.05$. (Graph Pad prism version 5.0)

RESULTS

In developed countries diabetes is the leading known cause for the neuropathy pain and it is the most common complication and greatest source of morbidity and mortality in diabetes patients. It is estimated that the prevalence of neuropathy in diabetic patients is approximately 20%. DN is implicated in 50-75% of non-traumatic amputations. Generally, the largest cases of neuropathy in patients (referred to as idiopathic in origin) are of unknown causes. Other known causes genetic factor of implication. Damaging chemical agents such as chemotherapy drugs, and HIV. Since diabetic neuropathy is not clearly understood, it is hard to make a definitive course of treatment [1,16].

Various classes of antidepressant agents that help in regulation and treatment of depressive emotions and neuropathic pain by sustaining balanced level of two neurotransmitters serotonin and nor epinephrine. Serotonin and nor epinephrine are implicated in modulating descending inhibitory pain pathways in the central nervous system, and are known to help in regulating emotions as well as sensitivity to pain [1,17].

Table 1: Effect of chronic treatment of Granisetron on blood glucose regulation in Diabetic rats

Treatment	Fasting serum glucose concentration (mg/dl)			
	0 Day	15 Days	30 Days	42 Days
Vehicle	98	98	98	98
Diabetes	360	365	370	390***
Granisetron(1mg/ml)	400	380	324	310*
Granisetron(10mg/ml)	400	370	315	300**
Glibenclamide(5mg/kg)	410	350	290	275***

Values are given for six rats in each. Values are statistically significant at $p < 0.005$ (student unpaired t test). *Diabetics control rat were compared with normal values in rats. **Drugs treated groups of rats were compared with diabetic control.

Table 2: Effect of Granisetron on Grip Strength after 6 weeks in Diabetic Rats

Treatment	Time (sec)
Vehicle	35
Diabetes	15*
Granisetron(1mg/ml)	28*
Granisetron(10mg/ml)	31**
Glibenclamide(5mg/kg)	34***

Values are given for six rats in each. Values are statistically significant at $p < 0.005$ (student unpaired t test). *Diabetics control rat were compared with normal values in rats. **Drugs treated groups of rats were compared with diabetic control.

Table 3: Effect of Granisetron on pain sensation using Hot plate method

Treatment	Time (sec)
Vehicle	3.8
Diabetes	3.8*
Granisetron(1mg/ml)	4.2*
Granisetron(10mg/ml)	4.8**
Glibenclamide(5mg/kg)	5.7***

Values are given for six rats in each. Values are statistically significant at $p < 0.005$ (student unpaired t test). *Diabetics control rat were compared with normal values in rats. **Drugs treated groups of rats were compared with diabetic control.

Table 4: Effect of Granisetron on Pain Sensation using Tail Flick Method

Treatment	Time (sec)
Vehicle	2*
Diabetes	3.8*
Granisetron (1mg/ml)	4.2*
Granisetron (10mg/ml)	4.9**
Glibenclamide(5mg/kg)	5.2***

Values are given for six rats in each. Values are statistically significant at $p < 0.005$ (student unpaired t test). *Diabetics control rat were compared with normal values in rats. **Drugs treated groups of rats were compared with diabetic control.

Table 5: Level of Glycosylated haemoglobin in STZ induced Diabetic rats

Treatment	Glycosylated % Hb
Diabetic control	17.24
Granisetron (1mg/ml)	0.68*
Granisetron (10 mg/ml)	12.58**
Granisetron (5 mg/ml)	13.5**

Values are given in mean \pm SD for groups of six rats in each. Values are statistically significant at $p < 0.05$ (student unpaired t test). *Diabetic control rat were compared with normal values in rats. **Drug treated groups of rats were compared with diabetic control

DISCUSSION

In diabetes there is loss of pain perception and it is thought due to nerve damage and induction of peripheral neuropathy [18,19]. Painful diabetic neuropathy significantly affects the quality of life; so far no ideal drug has been available for its management. In the absence of curative therapy, the main aim of the management is to provide symptomatic pain control. In reported case study adenosine receptor activation ameliorates type 1 diabetes [11,12,22]. Granisetron being an adenosine agonist was aimed to evaluate the influenced by DN. Chronic treatment of granisetron (1mg/ml & 10mg/ml) on blood glucose level and on progression neuropathy in STZ-induced 6 week diabetic rats.

Results of chronic treatments with granisetron demonstrates to significant decreases in the blood glucose level at 30th and 42nd day while the more significant ($P < 0.01$) effect observed only on 42 day (fig. 1). The observed effect of granisetron was comparable to standard hypoglycemic agent glibenclamide.

In the present study we measured the Glycosylated hemoglobin in diabetes and were treated with granisetron. In STZ-induced diabetic rats, increased ($P < 0.05$) levels of glycosylated hemoglobin were found. The observed increase in the level of glycosylated hemoglobin in diabetic control group rats might be due to the presence of excessive amounts of blood glucose. During diabetes, the excess of glucose present in blood reacts with the hemoglobin to form HbA1c which has been found to be increased over a long period of time in diabetes mellitus [16]. There is an evidence that glycation may itself induce the generation of oxygen derived free radicals in diabetic condition which may be the leading cause of development of diabetic neurological complications like neuropathic pain, depression [18-20].

The result of the present study indicates that the level of glycosylated haemoglobin significantly ($P < 0.05$) decreases after chronic treatments with granisetron (Table 1). The main risk factor for DN is hyperglycemia. People with diabetes are more likely to develop symptoms relating to peripheral neuropathy as the excess glucose in the blood results in a condition known as Glucojasinogen [20]. The risk of developing DPN increases with duration of the disease and degree of glycemic control. The symptoms of diabetic neuropathy are often slight at first but can occasionally flare up suddenly and affect specific nerves so that an affected individual will develop double vision, drooping eyelids, or weakness, atrophy of the muscles and nerve damage [2,5].

In the present study induction of diabetic neuropathy was evaluated in terms of muscle and nerve strength by measuring grip strength in 6 week STZ-induced diabetic rats. The diabetic animals demonstrates to significant ($P < 0.05$) decreased the grip strength as compared to normal rats indicating muscle weakness and induction of neuropathy, whereas chronic treatment with granisetron at both the doses indicate to raise in dose dependent way ($P < 0.05$) the grip strength in diabetic rats (fig. 2). Thus the results of the present study demonstrate the protective effect of granisetron on grip strength in diabetic condition.

Diabetic condition has been reported to cause a decrease in the antinociceptive effect of drugs like morphine [18,19]. Previously it is reported that granisetron has antinociceptive effect [21] while the results of the present study demonstrated that the antinociceptive effect of granisetron is attenuated in diabetic animals which was similar to that reported for morphine in diabetic animals earlier

[18,19]. The decreased antinociceptive effect in diabetes may be due to development of DN characterized by neurodegeneration resulting in the loss of pain perception [18,20].

CONCLUSION

In the present study the pain threshold measured by hot plate and tail flick method of analgesia, indicated to significant ($P < 0.05$) decreased in the foot withdrawal and flicking latency (time interval taken by rats to withdrawal its legs or flick its tail after exposure to source of radiant heat) in both hot plate and tail flick test respectively in diabetic rats (figs 3 and 4). These results indicated a loss of pain perception in diabetic rats in both hot plate and tail flick method, which could be attributed to nerve damage resulting due to the development of DN in 6-weeks diabetic rats [18]. While chronic treatment with granisetron at both the doses caused an increase ($P < 0.05$) in the latency time dose dependently in both models respectively. The increase in latency time indicate presence of pain perception in animals thus it conclude that granisetron protect from the nerve damage in the diabetic animals.

Thus from the results it indicates that chronic treatment of granisetron prevent progression of diabetic neuropathy. Hence, it could be helpful in treating the diabetic patient having the complication like diabetic neuropathy.

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