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To Study the Effect of Liraglutide on Formalin Induced Tonic Pain in Obese and Diabetic Rabbits

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ABSTRACT: To study the effect of Liraglutide on formalin induced tonic pain in obese and diabetic rabbits. Rabbits were divided into three categories i.e. normal, obese and diabetic. All the three categories were further subdivided into Liraglutide and Vehicle treatment groups. Rabbits were made obese by giving HFD (High fat diet) for 10 weeks. Diabetes was induced by giving Dithizone 5mg/kg i.p. Tonic pain was induced with 5% formalin given subcutaneously. Liraglutide or vehicle was given for 3 weeks subcutaneously. It was observed that obese rabbits showed a significantly lesser pain score as compared to normal rabbits while diabetic rabbits showed a significantly higher pain scores. Liraglutide was then given to both the group of rabbits and it was observed that Liraglutide, though produced a significant fall in body weight but decrease in tonic pain scores was not significant in obese rabbits, but in diabetic rabbits a significant decrease in pain scores was observed..

KEYWORDS: Obesity; Diabetes; Liraglutide; Tonic Pain.

INTRODUCTION

Approximately, 150 million people worldwide are affected by type-2 diabetes mellitus (T2DM) and this figure is expected to double in the next 20 years [1]. Diabetes mellitus is a chronic metabolic disorder that results from defect in insulin secretion and/or insulin action or both.

There is evidence that nutrient induced secretion of the glucose regulating incretin hormone or glucagon-like peptide-1 (GLP-1) is impaired in diabetes mellitus [2]. The potential of GLP-1 as a therapy target for type 2 diabetes has gained increasing attention in recent years. Liraglutide, a human GLP-1 analogue, has recently been approved for the treatment of type 2 diabetes.

Diabetic patients have lower pain tolerance than do normal subjects. Hyperalgesia of diabetes has been noted in several studies on different experimental hyperglycemic models [3,4]. In streptozotocin-induced diabetes models thermal hyperalgesia was observed using tail flick test in adult rats [5,6]. Sensitization of peripheral nociceptors has been demonstrated in experimental diabetic rats [7]. There is considerable evidence that suggests modulation of responsiveness to nociceptive stimuli and antinociceptive effects of morphine in diabetes associated with hyperglycemia in animals [8-13].

The effect of liraglutide on nociceptive behavior in diabetes have been least studied. However, some case reports related to the incidence of acute pancreatitis leading to pain by liraglutide have been cited in literature [14-15]. It is important to find out the effect of liraglutide on pain sensitivity in diabetic subjects. The present study therefore, was taken up to find out the effect of liraglutide on formalin induced tonic pain in type 2 diabetes.

MATERIALS AND METHODS

The study was conducted in the Department of Pharmacology in collaboration with the Department of Biochemistry, Pt. BhagwatDayal Sharma, Post Graduate Institute of Medical Sciences, Rohtak. Approval for the study was obtained from the Institutional Animal Ethical Committee.

Animals

The study was conducted in New Zealand white adult rabbits weighing approximately 1.5-2 kg, of either sex, which were housed individually in standard cages at natural light/dark condition and room temperature maintained at 26±2°C. The animals were obtained from Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar. They were provided with food and water ad libitum. The animals were acclimatized to the laboratory conditions for at least 7 days prior to the experiments. Animals were fed with standard rabbit's chow having composition of all dietary elements appropriate for maintaining normal rabbit [16]. After a week of acclimatization period experiments were performed between 9 am to 2 pm to maintain uniformity and avoid diurnal variations. Care was taken to minimize sufferings and pain to the animals. All procedures followed the guidelines of the National Institute of Health, 1996 (Guide for the care and use of laboratory animals).

Experimental diabetes

To induce diabetes, the rabbits were given single injection of dithizone 5mg/kg body weight intraperitonealy. Seventy-two hours were allowed for full development of diabetes [17]. Rabbits showing blood glucose levels ≥250 mg/dl were considered diabetic.

Induction of Tonic Pain

After the animal got adapted to the test environment, 0.1 ml of 5% formalin was injected subcutaneously using fine needle into the center of planter surface of the paw of right hind limb [18]. The recording of pain responses in the freely moving animal was immediately started after the injection. Quantification of the pain produced by the formalin injection was done on the basis of observable alteration in the behavior of the animal [19]. Formalin produced biphasic nociceptive responses [20]. The pain intensity was rated according to the standard numerical pain rating scale from 0-3 in decreasing order [21].

0- Normal weight bearing on injected paw
1- Limping during locomotion or resting the paw lightly on the floor or normal grooming
2- Elevation of injected paw
3- Licking or biting the injected paw

Pain was quantified by measuring the amount of time spent by the rabbit in each of four behavioral categories during each time block of 300 sec (5 min) for a period of 60 min [22]. The mean numerical ratings were calculated following the standard procedures [21, 23].

Pain score= ∑ (respective weight x duration(s) in each behavior) /300

Experimental design and protocol

The rabbits were divided into two groups each having 6 rabbits

A. Normal rabbits
B. Diabetic rabbits
C. Obese rabbits

Rabbits of group A, B and C were further subdivided into two groups. One group received vehicle of inj. Liraglutide and other group received inj. Liraglutide 7μg/kg/daily s.c. daily for 3 weeks.

Rabbits of all groups were exposed to formalin test at different time intervals to evaluate pain scores after administration of the treatment. Pain responses were noted immediately after the subcutaneous injection of formalin in planter surface of right hind paw and weekly during treatment and at the end of the treatment.

Statistical analysis

The data of individual rabbits were collected from various study groups and were expressed in terms of mean ±SEM. The statistical significance was assessed by using paired t-test for evaluation of pain in diabetic and obese rabbits, before and after induction of diabetes and obesity. Intergroup analysis was performed by using unpaired t-test. Intra-group analysis after treatment with liraglutide or vehicle was done using repeated measures analysis of variance (RM-ANOVA) with Bonferroni's correction and intergroup analysis between treatment groups was done using one way analysis of variance. P value <0.05 was considered significant. All statistical calculations were performed using SPSS software package, version 20.

RESULTS

1. Formalin induced tonic pain scores:

Table 1 shows pain scores in diabetes, obese and normal rabbits. The pain score in diabetic rabbits was 2.26±0.05 which was found to be significantly higher ($p<0.01$) as compared to normal (1.79±0.06) and obese rabbits (1.64±0.02).However, obese rabbits showed significantly lower pain scores ($p <0.05$) compared to diabetic rabbits.

2. Effects of Liraglutide on pain scores in diabetes

Table 2 shows the changes in pain scores in normal (1.76±0.08), diabetic (1.93±0.09) and obese (1.64±0.02)

rabbitsafter treatment with liraglutide. Liraglutide decreased the pain scores significantly ($p<0.05$) in diabetic rabbits as compared to the effect of liraglutide given in normal rabbits. In obese rabbits, thoughliraglutide decreased the pain scores but, the decrease was not significant.

Table 1 Formalin induced pain scores

Category (n=6)	Pain Scores
Normal	1.79±0.06
Diabetic	2.26±0.05#
Obese	1.64±0.02*

All values are expressed as mean ± SEM

Comparison of pain scores of diabetic with normal rabbits ($p <0.01$)

* Comparison of pain scores of obese rabbits with normal rabbits ($p < 0.05$)

Table 2. Effect of Liraglutide on pain scores

Category (n=6)	Pain Scores	
	Before Liraglutide	After 3wks liraglutide (7µg/kg/day, s.c.)
Normal	1.80±0.06	1.76±0.08
Diabetic	2.25±0.09	1.93±0.09*
Obese	1.64±0.04	1.56±0.02¥

* Comparison of tonic pain scores of diabetic rabbits before and after liraglutide treatment ($p < 0.05$)

¥ Comparison of tonic pain scores between obese rabbits after liraglutide treatment ($p < 0.001$)

DISCUSSION

Liraglutide is a novel human GLP-1 analogue approved for treatment of type 2 diabetes mellitus. In our study, liraglutide significantly controlled dithizone induced-diabetes with hyperglycemia in rabbits. Tonic pain has been characterized as pain arising from inflammation. The present study demonstrated higher tonic pain scores in diabetic rabbits compared to obese rabbits. These were similar to the findings of other studies where diabetes with acute hyperglycemia have been shown to increase pain sensitivity [12, 24] but contrast reports have also been shown in some studies [11, 25]. The exact mechanism by which hyperglycemia increases pain sensitivity is not known, but several suggestions have been made, for example, the decreased pain thresholds have been attributed to hyperinsulinemia induced by hyperglycemia. Insulin is known to stimulate the Na^+/K^+ATPase in cell membranes [26] and this could change nerve function directly by stimulation of ATPase on nerve tissues or indirectly by universal changes in ion distribution. Another suggested mechanism is that a hyperglycemic state induces overproduction of intracellular sorbitol in tissues [27], which increases intracellular osmotic pressure that modulates several ionic conductance and increased Ca^{2+}-influx and membrane depolarization [28], both of which are known to increase pain sensitivity. Also contributing to decreased pain threshold in the hyperglycemic state is the level of endogenous opioid peptides (β endorphins). It has been established by Basbaun and Field [29] that increases in β endorphin levels in the cerebrospinal fluids ameliorates pain perception. Forman et al [24], noted that plasma pituitary and hypothalamic levels of the endogenous opioid peptides were reduced in female rats eight weeks following the induction diabetes using streptozotocin. These reductions in the β endorphin levels were related to the hyperalgesia observed in the streptozotocin diabetic rats. The present study showed increased pain threshold in diabetic rabbits treated with liraglutide possibly due to stringent control of hyperglycemia. Nothing has been established about the exact role of liraglutide on tonic pain in type 2 diabetes and requires further studies in this regard.

In our study decreased response to formalin induced tonic pain was seen in obese rabbits. Obesity has been implicated in the alteration of pain response by modulating the opioid system in both human [30, 31] and animals [32-34]. An elevated level of serum β-endorphin has been documented in the obese women exhibiting positive correlation with body weight [35, 36] and this elevation of serum β-endorphin ameliorates pain perception. Furthermore, another study conducted by Zahorska-Markiewicz B et al [33] demonstrated that though obesity reduced pain perception due to elevated serum β-endorphin levels but a weight reducing treatment did not change the pain sensitivity in obese women because altered metabolic responses persist even after reduction of body weight [37]. Similarly, in our study liraglutide treatment showed some reduction in pain scores but the reduction was not statistically significant which could be due to the fact that increased serum β-endorphin levels probably persisted even after treatment with liraglutide.

So, our findings of tonic pain perception are in agreement with those of Zahorska-Markiewicz B et al [33]. In our study also obese rabbits showed lesser tonic pain scores as compared to normal rabbits and liraglutide treatment though decreased the body weight but did not alter the tonic pain scores.

REFERENCES

1. Nasser, K.A., Gruber, A., Thomson, G.A. The emerging pandemic of obesity and diabetes: Are we doing enough to prevent a disaster? Int. J. Clin. Pract., 2006; 60: 1093-7.
2. Vilsboll, T., Krarup, T., Deacon, C.F., Madsbad, S., Holst, J.J. Reduced post prandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. Diabetes, 2001; 50: 609–13.
3. Wuarin, B.L., Zahnd, G.R., Kaufmann, F., Burklen, L., Adler, J. Hyperalgesia in spontaneous and experimental animal models of diabetic neuropathy. Diabetologia, 1987; 30(8): 653-8.
4. Courteix, C., Bardin, M., Fialip, J., Lavarenne, J., Eschalier, A. Daily insulin treatment relieves long-term hyperalgesia in streptozotocin diabetic rats. Neuroreport, 1996; 7: 1922-4.
5. Lee, J.H., MacCarty, R.C. Glycemic control of pain threshold in diabetic and control rats. Physiol. Behav., 1990; 47: 225-30.
6. Cameron, N.E., Jack, A.M., Cotter, M.A. Effect of alpha-lipoic acid on vascular responses and nociception in diabetic rats. Free Radic. Biol. Med., 2001; 31: 125-35.
7. Fuchs, D., Birklein, F., Rech, P.W., Sauer, S.K. Sensitized peripheral nociception in experimental diabetes of the rat. Pain, 2010; 151: 496-505.
8. Ellenberg, M. Diabetic neuropathy: clinical aspects. Metabolism, 1976; 25: 1627-67.
9. Mayne, N. Neuropathy in the diabetic and non-diabetic populations. Lancet, 1965; 2: 1313-6.
10. Morley, J.E., Lowenthal, M.N., Asvet, M.S., Kopelowitz, W., Klein, C., Kokoris, N. Problems experienced in a diabetic clinic for blacks. S. Afr. Med. J., 1977; 52: 215-8.
11. Levine, A.S., Morley, J.E., Wicox, G., Brown, D.M., Handwerger, B.S. Tail pinch behavior and analgesia in diabetic mice. Physiol. Behav., 1982; 28: 39-43.
12. Morley, G.K., Mooradian, A.D., Levine, A.S., Morley, J.E. Mechanism of pain in diabetic peripheral neuropathy. Effect of glucose on pain perception in humans. Am. J. Med., 1984; 77: 79-82.
13. Takeshita, N., Yamaguchi, I. Insulin attenuates formalin-induced nociceptive response in mice through a mechanism that is deranged by diabetes mellitus. J. Pharmac. Exper. Therap., 1997; 281: 315-21.
14. Knezerich, E., Canic, T., Kershaw, S., Drincic, A. Liraglutide- associated acute pancreatitis. Am. J. Health System Pharmacy, 2012; 69: 386-9.
15. Lee, P.H., Stockton, M.D., Franks, A.S. Acute Pancreatitis Associated with Liraglutide. Am. Pharmacother., 2011; 45(4): 714.
16. Harkness, J.E. Rabbit husbandry and medicine. Vet. Clin. North AM. Small Anim. Pract., 1987; 17: 1019-44.
17. Monago, C.C., Onwuka, F., Osaro, E. Effect of combined therapy of diabenese and nicotinic acid on liver enzymes in rabbits with dithizone induced diabetes. J. Exp. Pharmacol., 2010; 2: 145-53.
18. Kolesnikov, Y., Cristea, M., Oksman, G., Torosjan, A., Wilson, R. Evaluation of the tail formalin test in mice as a new model to assess local analgesic effects. Brain Res., 2004; 1029: 217–23.
19. Dubuisson, D., Dennis, S.G. The Formalin test: A quantitative study of the analgesic effect of morphin, meperidine and brain stem stimulation in rat and cat. Pain, 1977; 4: 161–74.
20. Sawynok, J., Liu, X.J. The formalin test: Characteristics and usefulness of the model. Reviews in Analgesia, 2004; 7: 145-63.
21. Coderre, T.J., Fundytus, M.E., Mckenna, J.E., Dalal, S., Melzack, R. The Formalin test: A validation of the weighted- scores method of behavioral pain rating. Pain, 1993; 54: 43-50.
22. Sinha, R., Dhungel, S., Sinha, M., Paudel, B.H., Bhattacharya, N., Mandal, M.B. Obesity attenuates formalin- induced tonic pain in British Angora rabbits. Indian J. Physiol. Pharmacol., 2009; 53(1): 83–7.
23. Aloisi, A.M., Lupo, C., Carli, G. Effects of formalin induced pain on exploratory behaviour in rabbits. Neuroreport, 1993; 4: 739–42.
24. Forman, L.J., Estilows, S., Lewis, M., Vasilenko, P. Streptozotocin alters immunoreactive β -endorphin levels and pain perception after 8 weeks in rats. Diabetes, 1986; 35: 1309-13.
25. Chan, P.C., Lin, M.T., Shian, L.R. Alterations in physiologic functions and in monoamine content in streptozotocin diabetic rats. Diabetes, 1986; 35: 481-5.
26. Clausen, T., Kohan, P.G. The effect of insulin on the transport of sodium and potassium in rat soleus muscle. J. Physiol., 1997; 265: 19-42.
27. Malone, J.I., Lowitts, S. Measurement of tissue sorbitol in diabetes mellitus: enzyme method versus- gas liquid chromatography. Metabolism, 1992; 41: 224-7.
28. Viana, F., Pera, E.D.L., Schmidt, R.F., Belmonte, C. Swelling activated calcium signaling in cultured mouse primary sensory neurons. Eur. J. Neurosci., 2001; 13: 722-34.
29. Basbaum, A., Fields, H.L. Endogenous pain control system: brainstem spinal pathways and endorphin circuitry. Ann. Rev. Neurosci., 1984; 7: 309-38.
30. Cozzolino, D., Sessa, G., Salvatore, T., Sasso, F.C., Giugliano, D., Lefebvre, P.J. et al. The involvement of the opioid system in human obesity: A study in normal weight relatives of obese people. J. Clin. Endocrinol. Metab., 1996; 81: 713–8.

31. Stewart, W.F., Ricci, J.A., Chee, E., Morganstein, D., Lipton, R. Lost productive time and cost due to common pain conditions in the U.S. workforce. JAMA, 2003; 290: 2443–54.
32. Zahorska-Markiewicz, B., Zych, P., Kucio, C. Pain sensitivity in obesity. Acta Physiol. Pol., 1988; 39: 183–7.
33. Zahorska-Markiewicz, B., Kucio, C., Pyszkowska, J. Obesity and pain. Hum. Nutr. Clin. Nutr., 1983; 37: 307–10.
34. Bair, M.J., Robinson, R.L., Katon, W., Kroenke, K. Depression and pain comorbidity: A literature review. Arch. Intern. Med., 2003; 163: 2433–45.
35. Ramzan, I., Wonga, B.K., Corcorana, G.B. Pain sensitivity in dietary-induced obese rats. Physiol. Behav., 1993; 54: 433–5.
36. Pradalier, A., Willer, J.C., Boureau, F., Dry, J. Relationship between pain and obesity: A electrophysiological study. Physiol. Behav., 1981; 27: 961–4.
37. Basbaum, A., Fields, H.L. Endogenous pain control system: brainstem spinal pathways and endorphin circuitry. Ann. Rev. Neurosci., 1984; 7: 309-38.

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