Antihyperglycaemic effect of laser acupuncture treatment at BL20 in diabetic rats

Jorge Cornejo-Garrido,[1] Flavia Becerril-Chávez,[2] Gabriel Carlín-Vargas,[2] Juan Manuel Ordoñez-Rodríguez,[2] María del Carmen Abrajan-González,[2] Rosario de la Cruz-Ramírez,[2] Cynthia Ordaz-Pichardo[1]

[1]Laboratorio de Biología Celular y Productos Naturales, Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional, México, Distrito Federal, México
[2]Departamento de Acupuntura, Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional, México, Distrito Federal, México

Correspondence to
Cynthia Ordaz-Pichardo, Laboratorio de Biología Celular y Productos Naturales, Edificio de Posgrado, Primer Piso, Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional, Guillermo Massieu Helguera No. 239, C.P., Delegación Gustavo A. Madero, México, DF 07320, México; dra_cynthia@hotmail.com

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ABSTRACT

Objective To investigate the antihyperglycaemic activity of laser acupuncture stimulation at 650 and 980 nm at BL20 in streptozotocin (STZ)-induced diabetic rats.

Methods Seventy healthy adult male albino Wistar rats weighing 250±50 g were divided into seven groups of 10 animals each. Groups I–III comprised healthy control rats which were untreated (I) or stimulated with laser acupuncture at 650 nm (II) and 980 nm (III), respectively. Groups IV–VII underwent induction of diabetes with a single intraperitoneal administration of STZ at 50 mg/kg. Animals with blood glucose levels of ≥200 mg/dL on the fifth day were used for the experiments and were left untreated (group IV), treated with glibenclamide (group V) or stimulated with laser acupuncture at 650 nm (group VI) and 980 nm (group VII), respectively. Laser acupuncture was applied at BL20 on alternate days for a total of 12 sessions over a 28-day period.

Results After 28 days of treatment, STZ-induced diabetic rats stimulated with laser acupuncture at 650 and 980 nm had significantly lower glucose levels compared with untreated diabetic rats (242.0±65.0 and 129.8±33.2 vs 376.5±10.0 mg/dL, both p≤0.05). Treatment at 980 nm also attenuated the increase in glucose between day 1 and day 28 compared with the glibenclamide-treated diabetic group (41.5±19.6 mg/dL vs 164.1±13.7 g/dL, p<0.05). Laser acupuncture treatment did not affect the blood count or biochemical profile and was not associated with any morphological changes in the pancreas, liver, kidney or spleen.

Conclusions Stimulation with laser acupuncture at 650 and 980 nm at BL20 in STZ-induced diabetic rats has antihyperglycaemic activity. The results support further evaluation of laser acupuncture as an alternative or complementary treatment for the control of hyperglycaemia.

INTRODUCTION

Diabetes mellitus is an aetiologically and clinically heterogeneous group of chronic disorders characterised by hyperglycaemia and disturbances in the metabolism of carbohydrates, fats and proteins. Diabetes can occur when the pancreas does not produce sufficient insulin (a hormone that regulates blood sugar) or when the body cannot effectively use the insulin it produces.

There are two main types of diabetes.

Type 1 diabetes involves the autoimmune destruction of pancreatic β-cells and patients therefore have a complete deficiency of insulin. Patients with this type of diabetes, which can be immunologically mediated or idiopathic, are rarely obese. Type 2 diabetes can vary between a state of insulin resistance with relative insulin deficiency and a predominantly secretory defect with insulin resistance. Unlike type 1 diabetes, most patients suffer from obesity, which itself causes some degree of insulin resistance. Patients who are not categorised as obese tend to accumulate body fat in the abdominal region.[1] Uncontrolled diabetes produces hyperglycaemia and can cause serious damage to many of the body systems, especially the heart, blood vessels, eyes, kidneys and nerves.[2] It is estimated that 347 million people worldwide have diabetes, which is responsible for approximately 3.4 million deaths annually. More than 80% of deaths caused by diabetes occur in developing countries.[2]

The treatment of diabetes involves lowering blood glucose. Patients with type 1 diabetes require insulin while those with type 2 diabetes can often be treated with hypoglycaemic medications but may additionally require insulin. Management also involves blood pressure control, screening and treatment for retinopathy, blood lipid control and screening for early signs of kidney disease. These measures should be complemented by a healthy diet, regular physical activity, maintenance of normal body weight and avoidance of tobacco use.[2]

The hypoglycaemic drugs currently used to treat diabetes are expensive and are associated with undesirable side effects. There is growing interest in alternative therapies, which may offer an economical and effective treatment choice without side effects.[3] Acupuncture is an important component of Traditional Chinese Medicine (TCM) and has been used for thousands of years.[4] However, despite this long history and tradition, it is not a static discipline. One of the most recent technological developments has been the application of laser to acupuncture points. Laser acupuncture is defined as the stimulation of traditional acupuncture points with low-intensity, non-thermal laser irradiation[5] and uses different wavelengths in the green, yellow, orange, red and infrared regions of the electromagnetic spectrum.[6]

Classical acupuncture therapy and electroacupuncture have been used for treatment of diabetes and its complications over recent decades.[3] Experimental studies have demonstrated that acupuncture can treat various metabolic disorders such as hyperglycaemia, obesity, hyperphagia, hyperlipidaemia, inflammation, altered activity of the sympathetic nervous system and insulin signalling defects.[3,7] Stimulation of the BL20 acupuncture point is indicated in TCM to treat diabetes and its complications.[8–10] BL20 is located 1.5 cun (two finger breadths) lateral to the dorsal midline, at the level of the lower border of the spinous process of the eleventh thoracic vertebra (T11). The aim of this study was to evaluate the antihyperglycaemic effect of laser acupuncture (650 and 980 nm) at BL20 and the associated impact on haematological, biochemical and histopathological parameters in streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

STZ-induced diabetic rats

Healthy adult male albino Wistar rats weighing 250 ±50 g were obtained from the biotery of the Facultad de Estudios Superiores Iztcala-UNAM. The animals were housed at the biotery of the Escuela Nacional de Medicina y Homeopatía-Instituto Politécnico Nacional (IPN) and kept in acrylic cages at 24±2°C under 12 h light/dark cycles and fed with rodent diet and water *ad libitum*. Diabetes was induced by a single administration of STZ from Sigma Chemical Co. (St Louis, Missouri, USA) at 50 mg/kg intraperitoneally.[11] After 48 h and 5 days, glucose concentrations were measured in blood samples obtained by tail vein puncture after 10 h of fasting using a glucometer (Optium Xceed; Abbott Laboratories, Abbott Park, Illinois, USA). Animals that had a blood glucose level of ≥200 mg/dL on the fifth day were used for the experiments. All of the animal procedures were performed according to the Mexican Official Standards (NOM-033-ZOO-1995 and NOM-062-ZOO-1999)[12,13] and were approved by the Ethical Animal Committee of the Escuela Nacional de Medicina y Homeopatía-IPN act ENMH-CB-057, which complies with international rules and policies.

Laser acupuncture treatment (650 and 980 nm) at BL20

Stimulation with laser acupuncture at BL20 was applied every other day up to a total of 12 sessions over 28 days under the following conditions: wavelength 650 or 980 nm (7 and 50 mW output, respectively), duration 30 s bilaterally, energy output 2.49 J and frequency 3000 Hz (Acupoint 2045D, 50 mW output, Acupoint, Mexico).

Laser acupuncture was applied by FB-C, GC-V and JMO-R (professors and doctors who have specialised in acupuncture for more than 10 years) and by MdCA-G and RdlC-R (students specialising in acupuncture during the development of this research).

The treatment was started on the seventh day after STZ administration. The animals were divided into seven groups of 10 animals each. Group I was an untreated healthy control group. Groups II and III were healthy control groups stimulated with laser acupuncture at 650 and 980 nm, respectively. Groups IV and V were STZ-induced diabetic groups that were left untreated and treated with the reference drug (glibenclamide, 1 mg/kg daily by an intragastric route), respectively. Groups VI and VII were STZ-induced diabetic groups stimulated with laser acupuncture at 650 and 980 nm, respectively.

Blood glucose concentrations were measured in all the animals after 10 h of fasting once a week using a glucometer in blood samples obtained by tail vein puncture. Changes in the body weight of the animals between the first day (before the beginning of the experiment) and the 28th day (after treatment) were recorded.

Full blood count and biochemical profile

After 28 days of treatment and 10 h of fasting, animals were humanely killed according to the International Standards and NOM-033-ZOO-1995 as previously described.[12] Two blood samples were obtained from each rat by cardiac puncture. The first sample was mixed with anticoagulant (EDTA) and processed in a Beckman Coulter AC-T instrument prior to determination of the following blood parameters: haemoglobin level plus leucocyte, granulocyte, lymphocyte and monocyte counts. The second sample was collected without anticoagulant and was centrifuged at 570 g for 10 min at 4°C and processed in a Cobas Mira Roche instrument. The resultant serum was analysed and the following biochemical parameters were measured: cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) plus direct, indirect and total bilirubin.

Morphology and histopathological analyses

A complete dissection was performed and pancreas, liver, spleen and kidney tissues were macroscopically examined. Samples (0.5–1 cm) of each organ were fixed in 10% neutral buffered formalin (J T Baker) with phosphate buffered saline. After the usual processes of dehydration, clearing and infiltration, the organs were embedded in paraffin and sectioned into 6–10 μm slices with a microtome. The tissues were stained with haematoxylin and eosin and slides were prepared and examined microscopically and photographed using an achromatic plane objective (10×, 40× and 100×).

Statistical analyses

Results are expressed as mean±SEM. Statistical significance was determined by one-way analysis of variance followed by a Newman–Keuls *post hoc* multiple comparison test. GraphPad Prism Version 5.0 (GraphPad Software) was used for statistical analysis. A p value <0.05 was considered to be statistically significant.

RESULTS

Antihyperglycaemic activity of laser acupuncture treatment (650 and 980 nm) at BL20

Table 1 shows glucose levels at days 1 and 28 by experimental group as well as the mean difference between the two time points for each individual animal. As expected, there were no statistically significant differences in baseline glucose levels between the seven groups prior to administration of STZ and/or treatment with laser acupuncture or glibenclamide. Glucose levels of the healthy untreated rats and healthy controls stimulated with laser acupuncture (groups I–III) remained <100 mg/dL throughout the experiment and did not differ significantly at day 28 relative to day 1. By contrast, glucose levels in the untreated diabetic group (group IV) were significantly elevated at day 28 (p<0.01), indicating successful induction of hyperglycaemia by STZ. Compared to baseline, levels were similarly elevated in groups V and VI (diabetic rats treated with glibenclamide and 650 nm laser acupuncture, respectively, p<0.05) but not in group VII (STZ plus 980 nm laser acupuncture). Consequently, when the overall changes in glucose level from day 1 to day 28 were analysed, untreated diabetic rats had a significantly greater increase in glucose than the healthy control groups. This increase appeared to be mitigated in all three treated diabetic groups as the increase in glucose was smaller (p<0.001) than for untreated diabetic rats. This effect was most pronounced in group VII (diabetic rats receiving 980 nm laser acupuncture), which did not differ significantly from the healthy controls with respect to the overall glucose changes.

Table 1 Glucose level (mg/dL) of experimental rats

Group	Animal status	Day 1	Day 28	Change from day 1 to day 28
I	Control	79.0±1.1	83.5±9.5	4.5±5.5
II	Control+laser 650 nm	68.5±1.5	99.0±3.0	30.5±1.9
III	Control+laser 980 nm	69.0±3.4	80.0±11.0	11.0±6.6
IV	Diabetic	93.5±2.9	376.5±10.0*	283.0±6.0*
V	Diabetic +glibenclamide	91.5±4.8	255.6±23.2*†	164.1±13.7*†
VI	Diabetic+laser 650 nm	83.7±2.8	242.0±65.0*†	158.4±37.6*†
VII	Diabetic+laser 980 nm	88.3±7.0	129.8±33.2†	41.5±19.6†‡

Data are mean±SEM.
Each group comprises 10 rats. Glucose levels were measured before induction of diabetes mellitus with streptozocin and at the 28th day after treatment.
*Significantly different from control groups I–III (p<0.05).
†Significantly different from the untreated diabetic group IV (p<0.05).
‡Significantly different from the diabetic+glibenclamide group V (p<0.05).

Body weight

Rats in all three healthy control groups gained weight over the 28-day period of the experiment (table 2). This body weight gain was reduced significantly (p<0.01) in untreated diabetic rats (group IV) compared with healthy non-diabetic rats (groups I–III). In the diabetic group treated with glibenclamide (group V), a slight recovery of the weight gain was

Table 2 Body weight (g) of experimental rats

Group	Animal status	Day 1	Day 28	Day 28 (weight gain)
I	Control	309.8±11.8	380.4±12.3	70.6±11.2
II	Control+laser 650 nm	309.0±9.4	413.6±11.0	104.6±14.8
III	Control+laser 980 nm	283.8±17.9	373.6±6.7	89.8±23.2
IV	Diabetic	316.3±10.3	321.8±16.4	9.3±12.1*
V	Diabetic +glibenclamide	284.4±8.2	331.8±12.8	47.4±6.6
VI	Diabetic+laser 650 nm	352.8±10.4	376.1±16.7	21.8±11.7*
VII	Diabetic+laser 980 nm	362.9±9.8	390.0±12.9	27.1±15.0*

Data are mean±SEM.
Each group comprised 10 rats. Body weight was measured before induction of diabetes mellitus with streptozocin and at the 28th day after treatment.
*Significantly different from control groups I–III *(*p<0.05).

Cornejo-Garrido J, *et al. Acupunct Med* 2014;**32**:486–494. doi:10.1136/acupmed-2014-010573

Table 3 Haemoglobin levels and blood count of experimental rats

Day 28 after treatment

Group	Animal status	Leucocyte ($\times10^3$/mL)	Granulocyte (%)	Lymphocyte (%)	Monocyte (%)	Haemoglobin (g/dL)
I	Control	10.5±0.6	76.8±1.0	15.2±0.8	7.9±0.3	16.9±0.6
II	Control+laser 650 nm	10.0±0.4	79.6±1.7	13.0±1.8	7.3±1.7	16.2±0.2
III	Control+laser 980 nm	10.2±1.8	78.3±0.7	13.2±1.9	8.4±1.4	16.5±0.2
IV	Diabetic	6.6±0.7*	79.4±3.8	13.2±2.1	7.2±2.1	17.2±0.3
V	Diabetic+glibenclamide	9.0±0.6	71.9±6.1	15.9±2.7	12.2±3.4	17.9±0.1
VI	Diabetic+laser 650 nm	9.5±0.4	76.8±2.7	13.4±2.5	9.8±0.9	17.2±0.5
VII	Diabetic+laser 980 nm	7.5±1.3	81.7±3.5	10.4±2.2	7.9±1.3	16.9±0.2

Data are mean±SEM.
Each group comprised 10 rats.
*Significantly different from the untreated control group I (p<0.05).

observed as the body weight gain did not differ significantly from healthy controls in this group (p>0.05). In the diabetic groups treated with laser acupuncture at 650 and 980 nm (groups VI and VII), no such recovery of weight gain was observed.

Full blood count and biochemical profile

Values for the blood counts (leucocytes, granulocytes, lymphocytes and monocytes) and haemoglobin level of diabetic animals treated with glibenclamide or laser acupuncture at 650 and 980 nm (groups V–VII) were not significantly different from those of healthy rats (groups I–III), as illustrated in table 3. However, untreated diabetic rats had lower leucocyte counts than untreated healthy control animals (p<0.05). This STZ-induced reduction in leucocytes appeared to be attenuated by glibenclamide and laser acupuncture treatment, as the levels did not differ between groups I–III and groups V–VI (p>0.05).

Table 4 shows the lipid profiles (cholesterol, triglycerides, HDL, LDL and VLDL) by experimental group. Diabetic animals treated with laser acupuncture at 650 and 980 nm presented no statistically significant differences from the healthy control groups. The diabetic group treated with glibenclamide exhibited elevated levels of VLDL compared with the untreated healthy group only (p<0.05).

Similarly, the kidney (urea and creatinine) and liver (AST, ALT, direct bilirubin, indirect bilirubin and total bilirubin) profiles of diabetic animals treated with laser acupuncture at 650 and 980 nm did not show any statistically significant differences compared with the healthy groups (table 5). The untreated diabetic group showed a significant increase in the ALT enzyme level (p<0.05) relative to the healthy rats (groups I–III). Finally, the diabetic group treated with glibenclamide had higher levels of direct bilirubin (p<0.05) and total bilirubin (p<0.01) than the healthy control groups I–III.

Morphology and histopathological analyses

Tissue sections from the pancreas, liver, spleen and kidneys of rats treated with laser acupuncture treatment (650 nm and 980 nm) at BL20 were examined. Histological analysis of the pancreas from healthy groups and diabetic animals treated with laser (650 nm and 980 nm) or glibenclamide showed normal lobes, intralobular and extralobular ducts,

Table 4 Lipid profile of experimental rats

28th day after treatment

Group	Animal status	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
I	Control	57.0±1.4	88.8±15.1	11.2±0.3	27.5±3.6	18.0±3.2
II	Control+laser 650 nm	58.3±7.1	146.7±38.8	11.7±1.2	17.3±13.0	30.0±8.1
III	Control+laser 980 nm	58.5±3.7	102.3±14.2	12.0±1.2	26.7±5.2	21.3±2.0
IV	Diabetic	70.9±4.0	155.0±30.7	14.4±0.8	26.6±7.3	30.8±4.0
V	Diabetic+glibenclamide	68.2±3.8	199.5±30.6	13.7±0.7	14.3±2.4	38.6±2.2*
VI	Diabetic+laser 650 nm	54.8±2.6	136.5±20.8	10.8±0.5	16.5±2.6	26.8±2.7
VII	Diabetic+laser 980 nm	76.2±9.8	139.0±12.0	15.0±1.1	32.5±4.5	28.5±4.4

Data are mean±SEM.
Each group comprised 10 rats.
*Significantly different from the healthy control group I (p<0.05).
HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

Table 5 Kidney and liver profile of experimental rats

28th day after treatment								
Group	Animal status	Urea (mg/dL)	Creatinine (mg/dL)	AST (U/L)	ALT (U/L)	DB (mg/dL)	IB (mg/dL)	TB (mg/dL)
I	Control	56.7±3.3	0.75±0.11	141.3±10.2	59.0±2.9	0.22±0.04	0.03±0.01	0.25±0.03
II	Control+laser 650 nm	55.7±3.2	0.68±0.04	156.3±18.4	86.0±6.6	0.35±0.10	0.11±0.07	0.46±0.16
III	Control+laser 980 nm	45.2±6.7	0.65±0.09	131.5±22.8	73.8±10.0	0.22±0.02	0.06±0.01	0.27±0.03
IV	Diabetic	71.5±7.6	0.78±0.05	354.9±78.7	289.1±74.3*	0.34±0.05	0.10±0.03	0.44±0.05
V	Diabetic+glibenclamide	52.2±2.6	0.47±0.08	218.5±34.8	140.3±22.6	0.74±0.14*	0.06±0.02	0.80±0.14*
VI	Diabetic+laser 650 nm	78.0±13.2	0.53±0.07	158.0±31.1	102.0±28.2	0.27±0.08	0.14±0.01	0.42±0.08
VII	Diabetic+laser 980 nm	66.2±11.0	0.58±0.08	217.0±76.3	143.8±58.1	0.35±0.05	0.10±0.04	0.42±0.02

Dara are mean±SEM.
Each group comprised 10 rats.
*Significantly different from control groups I–III (p<0.05).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; IB, indirect bilirubin; TB, total bilirubin.

connective tissue, arteries, veins, acinar cells, zymogen granules, centroacinar cells and islets of Langerhans. By contrast, samples from the untreated and glibenclamide-treated diabetic groups were characterised by reductions in the size of the islets of Langerhans and alterations in nuclear architecture with karyolysis and acidophilia. In addition, areas of necrosis and hypervascularised pancreatic tissue were observed. However, in the diabetic animals treated with laser at 650 nm there was no reduction in the size of the islets of Langerhans and the damage appeared reduced. In the case of diabetic animals treated with laser at 980 nm, cellular multiplications in the islets of Langerhans preserving nuclear architecture were found (figure 1).

Liver sections from healthy groups and diabetic animals treated with either laser acupuncture (650 nm and 980 nm) or glibenclamide showed normal hepatic architecture of Glisson's capsule, lobules, central vein, portal vein, portal artery, bile duct, hepatocytes, Küpfer cells and hepatic cords. By contrast, in the untreated diabetic animals, areas of necrosis and hypervascularity were found (figure 1).

Splenic sections from all seven experimental groups exhibited normal architecture of capsule, white pulp, red pulp, central artery, lymphoid follicles, splenic sinusoids, blood vessels and splenic cords (figure 1).

Kidney sections from all seven experimental groups showed a normal renal cortex with normal appearances of the capsule, glomerulus, urinary pole, intracapsular space, vascular pole, macula densa, distal tubule, proximal tubule, afferent artery, efferent artery and loop of Henle. However, a reduction in glomerular size was noted in the untreated diabetic group and diabetic groups treated with glibenclamide and laser acupuncture at 650 nm. By contrast, this glomerular atrophy appeared to be attenuated in the diabetic group treated with laser acupuncture at 980 nm (figure 1).

DISCUSSION

Antihyperglycaemic activity of laser acupuncture treatment (650 and 980 nm) at BL20

Acupuncture and electroacupuncture stimulation have been evaluated in view of their ability to lower blood glucose in hyperglycaemic humans and animal models at several acupuncture points.[14] However, this is the first study that describes the antihyperglycaemic action of laser acupuncture at BL20 in an animal model.

In the present study, the STZ-induced diabetic group stimulated with laser acupuncture at 650 nm exhibited a 45% reduction in the elevation of glucose levels observed in untreated diabetic rats. Furthermore, the STZ-induced diabetic group treated with laser acupuncture at 980 nm showed an 85% reduction relative to no treatment and 75% relative to diabetic rats treated with glibenclamide. It is noteworthy that the variance in day 28 glucose levels in the diabetic animals treated with glibenclamide or laser acupuncture was greater than untreated diabetic or healthy control rats, suggesting a variable response to treatment.

Previous studies have demonstrated that stimulation at BL20 in combination with other acupuncture points can reduce glucose levels and help reduce complications related to diabetes. Acupuncture stimulation at *Yishu*, BL13 and BL20 every other day for 12 weeks decreases fasting blood glucose to levels similar to that achieved with the reference drug (glibenclamide) and significantly reduces the fasting levels of insulin and leptin as well as insulin resistance.[15] Electroacupuncture applied at a frequency of 5 Hz at BL23, BL20, ST36 and SP6 significantly reduces the fasting level of plasma glucose and the blood glucose load (measured by the glucose tolerance test (GTT)) in patients with impaired glucose tolerance.[10] [16] Electroacupuncture at ST36, ST25 and BL20 has an effect on obesity in rats, which may be related to upregulation of obestatin expression in the hypothalamus.[17] With classical acupuncture, it has been

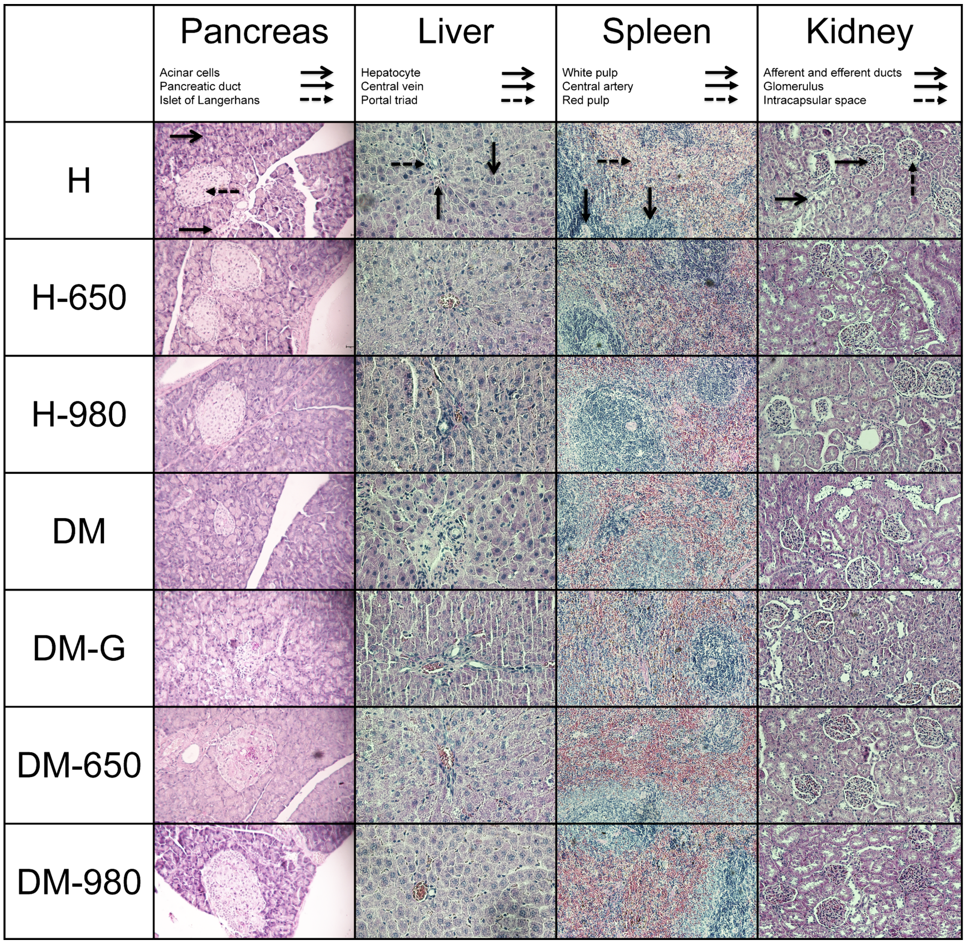

Figure 1 Histopathological analysis of the pancreas (20×), liver (20×), spleen (10×) and kidney (10×) in healthy control (H) and streptozocin (STZ)-induced diabetic (DM) rats that were either left untreated or given glibenclamide 1 mg/kg (G) or laser acupuncture at 650 (laser 650 nm) or 980 (laser 980 nm). The architecture of all organs was preserved after treatment with laser acupuncture at 650 or 980 nm in healthy control and STZ-induced diabetic rats.

reported that stimulation at BL18, BL20 and ST36 can improve and postpone the clinical symptoms of diabetic nephropathy.[18]

Acupuncture at other points have shown similar results—notably, acupuncture at TE5 in patients with cerebral infarction increases glucose metabolism locally in the cerebrum.[19] Stimulation of the auricular cavum concha for 30 min daily for 3 months significantly decreases the fasting level of plasma glucose and the blood glucose load measured by GTT.[20] Auricular electroacupuncture stimulation of the ipsilateral *Yi-Dan* and *Er-Shenmen* points in rats can reduce diabetic cellular injury, suppress tumour necrosis factor α expression and reduce intercellular adhesion molecule 1 (ICAM-1) and soluble vascular cell adhesion molecule 1 (sVCAM-1) concentrations in cerebral microvascular endotheliocytes.[21] In addition, acupuncture stimulation five times per week for 4–5 weeks in the abdominal and hind limb muscle of rats had a positive effect on the oral GTT.[22]

Despite numerous clinical and preclinical studies demonstrating that laser acupuncture is effective in treating various diseases, its mechanism of action is still not fully understood.[23] It is widely accepted that the effect begins with stimulation of the nervous system[24] and, in the case of the acupuncture point ST36, the hypoglycaemic effect appears to be mediated by cholinergic nerves as it is blocked by atropine. This cholinergic stimulation results in the release of proteins involved in the insulin signalling cascade, such as insulin-like growth factor and β-endorphin.[25] Previously it has been reported that treatment by electroacupuncture at CV12 induces secretion of endogenous β-endorphin which, in turn, stimulates the secretion of pancreatic insulin and glucagon causing the blood glucose lowering effect.[26]

In other studies of electroacupuncture it has been observed that regulation of fasting blood glucose and insulin levels is due to increased expression of sirtuin 1 (SIRT1) in skeletal muscle and peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α).[27] SIRT1 increases insulin sensitivity by repression of inflammation and is associated directly or indirectly with insulin signalling, whereas PCG-1α is a metabolic coactivator that interacts with transcription factors to induce mitochondrial biogenesis and respiration. The proposed mechanism suggests the activation of endothelial nitric oxide synthase (eNOS) by electroacupuncture stimulation. eNOS stimulates the expression of SIRT1 which, by deacetylation, produces PGC-1α which is required to activate genes associated with mitochondrial fatty acid oxidation in response to energy demands. The resultant increase in expression of mitochondrial genes is responsible for the regulation of blood glucose and insulin levels.[27]

The technique of laser acupuncture has many advantages over traditional acupuncture or electroacupuncture. A major advantage is its non-invasiveness, as the patient does not feel the acupuncture point stimulation, and treatments have low costs and a low risk of collateral damage from a combination of laser acupuncture with other medical treatments.

There are a few studies that have directly compared laser acupuncture with traditional acupuncture or electroacupuncture. Jiang *et al*[28] found that classical acupuncture at BL18, BL20 and BL23 has a hypoglycaemic effect when applied preventively and therapeutically. El-Kharbotly *et al*[29] recently reported that electroacupuncture and laser acupuncture are equally effective in the treatment of cervical spondylosis pain, while Cunha *et al*[30] found that laser acupuncture but not traditional acupuncture improves the revascularisation index in patients with peripheral arterial disease.

Laser acupuncture has been used successfully in the treatment of many conditions including diabetic foot ulcers, pain, hypertension and overweight/obesity. However, direct comparisons with traditional acupuncture or electroacupuncture are problematic because acupuncture points, timings and conditions of laser treatment differ widely.[5 31 32]

Currently, the exact mechanism of action by which 980 nm laser acupuncture at point BL20 induces an antihyperglycaemic effect remains unknown.

Body weight

Relative loss of body weight in STZ-induced diabetes occurs due to increased catabolism of fatty acids and proteins leading to muscle wasting.[33 34] In this experiment, body weight gain was significantly lower in untreated diabetic rats and a non-significant recovery of weight was observed in the diabetic groups treated with laser acupuncture at 650 and 980 nm.

This observation could be due to more effective control of the hyperglycaemic condition of the diabetic animals because it has been observed that a decrease in blood glucose levels may improve body weight in STZ-induced diabetic rats.[35 36] As this behaviour relates more to type 1 diabetes, it would be interesting to evaluate the effect of laser acupuncture treatment in models that are closer to models of type 2 diabetes, such as *db*/*db* or *ob*/*ob* mice, or repeated low-dose STZ.[37 38]

Full blood count and biochemical profile

There were no significant differences in blood counts between healthy control and treated diabetic groups, but leucocyte counts were decreased in the untreated diabetic animals. This decrease was restored in the diabetic groups treated with glibenclamide and laser acupuncture at 650 and 980 nm.

The lipid profile results were not significantly different between healthy control groups and diabetic animals treated with laser acupuncture at 650 and 980 nm. However, VLDL levels were significantly elevated in diabetic rats treated with glibenclamide. This effect has been reported previously by our group.[39]

The hepatic and renal profiles of untreated diabetic animals revealed elevated ALT levels, while diabetic animals treated with glibenclamide had significantly higher levels of total bilirubin and direct bilirubin. The increase in the concentration of the transferase enzymes involves processes of hepatic and myocardial necrosis, and similar values have been described extensively in the STZ-induced rat model of diabetes.[40–42] Laser treatment at 650 and 980 nm reverses these elevated ALT levels, suggesting clinical recovery.

Morphology and histopathological analyses

No obvious damage as a result of the application of the laser was observed in any of the organs analysed. By contrast, laser acupuncture treatment appears to reverse or prevent reductions in the size of the islets of Langerhans and the appearance of areas of necrosis and hypervascularisation of the pancreatic and liver tissues. Prevention of pancreatic islet damage has been reported following traditional acupuncture at points BL18, BL20 and BL23.[28]

The hepatic damage observed is related to the elevated ALT enzyme levels found in the untreated diabetic group. Normal renal function in healthy control groups was confirmed by physiological urea and creatinine values.

In conclusion, stimulation with laser acupuncture at 650 nm and 980 nm at BL20 in STZ-induced diabetic rats has an antihyperglycaemic effect. Treatment did not cause any alteration in the blood count and lipid profile, reversed the increased ALT levels caused by STZ administration, and was not associated with any morphological changes in the pancreas, liver, kidney or spleen. Treatment prevented the appearance of areas of necrosis and hypervascularisation of pancreatic and hepatic tissue. Treatment also prevented the reduction in the size of the islets of Langerhans (650 nm only) and induced cellular multiplications in them (980 nm only). To our knowledge, this is the first study to assess the antihyperglycaemic activity of laser acupuncture and the first to report this activity by stimulation at BL20 in an animal model. Further evaluation of laser acupuncture as an alternative or complementary treatment for the control of hyperglycaemia is warranted.

Summary points

- We evaluated the effect of laser acupuncture at 650 and 980 nm at point BL20 on hyperglycaemia in a rat model of diabetes induced by streptozotocin (STZ).
- After 28 days of treatment STZ-induced diabetic rats receiving laser acupuncture had significantly lower glucose levels relative to untreated diabetic controls.
- Laser acupuncture may have a role in the treatment for diabetes but further research (including randomised controlled trials in human subjects) are required.

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Contributors JC-G and CO-P established the model of STZ induced diabetic rats, supervised all experiments, performed the statistical analysis and drafting the article. FB-C and GC-V as the acupuncture teachers, taught to locate BL20. JMO-R as acupuncture teacher specialist in laser acupuncture, taught to apply the laser at BL20. MdcA-G and RdIC-R as students specialising in acupuncture were responsible for applying all treatments.

Competing interests MdCA-G and RdlC-R, who were students specialising in acupuncture during the development of this research, were recipients of student fellowships from CONACyT 358989 and 359516, respectively.

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AIM

Antihyperglycaemic effect of laser acupuncture treatment at BL20 in diabetic rats

Jorge Cornejo-Garrido, Flavia Becerril-Chávez, Gabriel Carlín-Vargas, Juan Manuel Ordoñez-Rodríguez, María del Carmen Abrajan-González, Rosario de la Cruz-Ramírez and Cynthia Ordaz-Pichardo

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