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EFFECT OF LASER AT *PISHU* POINT ON AMOUNT AND FUNCTION OF PANCREATIC β CELLS (*RATTUS NORVEGICUS*) INJECTED BY STREPTOZOTOCIN

Abdurachman
Department of Anatomy and Histology
Airlangga University School of Medicine
Surabaya

ABSTRACT

This study has an objective to obtain experimental laboratory proof on the effect of laser at pishu *point (BL-20) in order to increase the number of ß pancreatic cells, enlarge the width of Langerhans' islets and improve the function of ß cells. The increase of ß cell number and functions was examined using immunohistochemical method to insulin inside the cells. Normal insulin function in the cells was observed from fasting blood glucose. This was a laboratory experimental study using randomized the posttest-only control group design. This study was carried out in Experimental Animal Unit, Department of Biochemistry, Airlangga University School of Medicine. A number of 22 rats were divided into two groups. Control group comprised DM rats due to STZ induction, and treatment group comprised STZ-induced DM rats receiving laser at* pishu *point. Statistical analyses were independent t-test and Spearman's correlation test. It was found that the percentage of pancreatic ß cells and the width of Langerhans' islets were significantly higher in treatment group ($p < 0.05$), while fasting blood glucose level was significantly lower in experimental group ($p < 0.05$). Furthermore, a strong correlation ($p < 0.5$) was found between the increase of ß cells count, the increased width of Langerhans' islets and the reduction of fasting blood glucose level. It indicated that the effect of laser at* pishu *point in STZ-induced DM rats increase the number as well as the function of ß cells.*

Keywords: *Laser, DM, STZ, ß cells*

Correspondence : Abdurachman, Department of Anatomy and Histology, Airlangga University School of Medicine, Jl. Mayjend Mustopo 47, Surabaya, phone 62-31-5020251 ext. 148

INTRODUCTION

Acupuncture has become a part of international medication system. Therefore, the proof of its advantage should be demonstrated through experimental laboratory research. At the same time the cases of diabetes mellitus (DM) is increasing from year to year. This study was carried out to combine two efforts, i.e., to prove the efficacy of Acupuncture using experimental laboratory research and to support the efforts to overcome DM cases.

MATERIALS AND METHODS

Research Type and Design

The study was carried out at the laboratory to the experimental animals, which were divided into two groups, treatment and control group. In addition to control group, some variables were also controlled in order to maintain the validity of causative effect of the independent variable on the dependent variable. We performed randomization (Hartono 2002) by dividing evenly the samples into two groups, control and treatment. This study was not conducted to only one animal, but to several animals (replication). This type of study was included in laboratory experimental study. The results were examined at the end of experiment. Therefore, this study used the posttest-only control group design (Tjokronegoro & Sudarsono 1999).

Twenty-two rats were injected with STZ of 60 mg/kg BW (Thulesen et al. 1997; Mahay et al. 2004). After 48 hours, blood glucose samples were taken from all (Varanil et al. 2002; Shafei et al. 2002). Rats were regarded as DM if their fasting blood glucose = 300 mg/dl (Jang et al. 2003). Rats with blood glucose of less than 300 mg/dl were excluded. Rats with DM were divided further using randomization.

Rats in treatment group were therapied once for two days for six-times therapy, while those in control group did not receive therapy. At the end of therapy period, all rats in both groups were sacrificed. Fasting blood glucose was examined. The pancreas was removed, fixed, and immunohistochemically examined.

Procedures

This study involved male Wistar strain white rats with bodyweight (BW) of 140-170 grams. Each was kept in a cage with temperature of 25 ± 2^0C, evenly exposed to daylight and night. Daylight started from 6 a.m. to 6 p.m., and night started from 6 p.m. to 6 a.m.

After being rendered DM, 22 animals were divided randomly into two groups. Control group comprised STZ-induced DM rats without laser therapy, while the treatment group comprised DM rats with laser therapy.

To become DM, rats were injected with STZ intraperitoneally using STZ dose of 60 mg/Kg BW (Thulesen et al. 1997; Mahay et al. 2004) in citrate buffer solution 0.1 M (Guz et al. 2001) with pH = 4.5. Such level of pH was used to prevent the inactivation of STZ (Shafei et al. 2002). The STZ was produced by Merck Tbk, Chemical Division, Catalogue No. 572201-1GM Batch No. B56981. Fasting blood glucose level examination was carried out 48 hours after STZ injection (Varanil et al. 2002; Syafei et al. 2002), using Johnson-Johnson One-Touch Strip test (LifeScan Inc. a Johnson & Johnson company Milpitas, CA 95035 U.S.A. No. SMC4212QT). In this experiment, rats with blood glucose of more than 300 mg/dl were included in DM criteria (Jang et al. 2003).

In treatment group, laser therapy was given once for two days, six times consecutively for 12 days (Hou 1993) starting from the finding that the rats was DM. Experimental rats were placed within square plastic box with height of 15 cm, width 33 cm, and length 40 cm. The box was covered with wire gauze, so that they could freely obtain oxygen. The wire gauze was also used to provide drink into the box. At the bottom of the box, a sufficient layer of rice husk was placed to absorb the urine of the animals. Between the husk and those rats, we placed another layer of wire gauze with the same size as that of cover to prevent direct contact between the rats and the rice husk, so that there was no contact between rats and their urine that damped the husk. The height of the lower wire gauze from the bottom of plastic box was 3 cm. The husk was changed each morning.

For laser therapy, we used laser semiconductor Al Ga In P with wavelength of 650 nm. Laser probe was attached to the rats' body. Laser was pointed perpendicularly at *pishu* point bilaterally until each point reached energy of 0.3 joule. Fasting blood glucose was examined at day 13 after the starting of the therapy. Blood samples were taken by excising the end of rats' tail using scalpel. Rats' pancreas was removed immediately after sacrifice.

Data Processing and Analysis

Data of the results were compared between those from control group and those from treatment group using independent-samples t-test. To test correlation between one and other variables, we used Pearson's correlation test.

RESULTS

Early fasting blood glucose (FBG) Description

Treatment group showed that early fasting blood glucose was minimally 367 mg/dl, maximally 560 mg/dl, mean 470.63 mg/dl with standard deviation of 61.88 mg/dl. Control group showed minimal fasting blood glucose 376 mg/dl, maximal 556 mg/dl, mean 467.72 mg/dl and standard deviation 59.41 mg/dl.

Table 1. Early FBG Description

Groups	n	$\bar{X}$	SD	Minimum	Maximum	*t-test* (independent 2 samples)
Treatment	11	470.64	61.884	367	560	$t = 0.112$
Control	11	467.73	59.416	376	556	$p = 0.912$

Above table shows that the mean of early FBG was almost similar between treatment (470.63 mg/dl) and control groups (467.72 mg/dl). Independent two-sample statistical t-test on early FBG revealed significant difference between treatment and control (p = 0.912). This indicated that the status of early FBG in treatment group was not different from that in control group.

Final FBG Description

In treatment group it was found that the change of FBG was minimally 97 mg/dl, maximally 413 mg/dl, mean 263.63 mg/dl and standard deviation 91.51 mg/dl. Control group showed the minimal FBG was 287 mg/dl, maximal 599 mg/dl, mean 395.09 and standard deviation 87.26 mg/dl.

Table 2. Final FBG Description

Groups	n	$\bar{X}$	SD	Minimum	Maximum	*t-test* (independent 2 samples)
Treatment	11	263.63	91.51	97	413	t = -3.448
Control	11	395.09	87.26	287	599	p = 0.003

Results showed that mean FBG in treatment group (263.63 mg/dl) was normal, while mean FBG in control group was still more than 300 mg/dl (395.09 mg/dl). Independent two-sample t-test on final FBG revealed significant difference between treatment and control (p = 0.003). This indicated that final fasting blood glucose in treatment group was different from that in control group.

Description of ß pancreatic cell percentage

In treatment group, it was found that the percentage of ß cells was minimally 0%, maximally 79.2%, mean 29.96% with standard deviation 21.42%. In control group, the minimal percentage was 0%, maximal 60.7%, mean 12.3% and standard deviation 15.77%.

Table 3. Description of ß pancreatic cell

Groups	n	$\bar{X}$	SD	Minimum	Maximum	*t-test* (independent 2 samples)
Treatment	11	29.96	21.42	0	79.2	t = 3.813
Control	11	12.3	15.77	0	60.7	p = 0.000

Results showed the difference of ß cell percentage in treatment group was twice higher than that in control group. It can be seen from the mean of ß cell percentage in treatment group (29.96%) as compared to that in control group (12.3%), which was higher in treatment group with a difference of 17.66%. Independent two-sample t-test on the percentage of ß cell showed significant difference between treatment and control (p = 0.000). These results showed that the percentage of ß cell in treatment group was different from that in control group.

Description of the size of Langerhans' islet

Table 4. Description of the size of Langerhans' islet

Groups	n	$\bar{X}$	SD	Minimum	Maximum	*t-test* (independent 2 samples)
Treatment	11	18364.69	10351.56	5023	49875	t = 6.264
Control	11	5881.69	4888.24	1864	24941	p = 0.000

Results showed that there was threefold difference in the size of Langerhans' islet in treatment group as compared to that in control group. This was indicated by the mean of the islet's size in treatment group, which was 18364.69, as compared to that in control group of 5881.69. Independent two-sample t-test on the size of Langerhans' islet revealed significant difference between treatment and control groups ($p = 0.000$). This indicated that the size of Langerhans' islet in treatment group was different from that in control group.

Correlation between Variables

The results of Pearson's correlation test were as follows:

Table 5. Results of Pearson's correlation test between the variables FBG and ß cell percentage, between FBG and Langerhans' islet cell size and between ß cell percentage and Langerhans' islet cell size

	Final FBG	ß cell percentage	Langerhans' islet size
Final FBG	r = 1	r = -.604**	r = -.459*
	P = -	p = 0.003	p = 0.032
ß cell percentage	r = -.604**	r = 1	r = 0.253*
	p = 0.003	p = -	p = 0.040
Langerhans' islet size	r = -.459*	r = 0.253	r = 1
	p = 0.032	p = 0.40	p = -

** = Significant correlation, if $p < .01$
* = Significant correlation, if $p < .05$

Above table shows that:

1. There was correlation between the variable of fasting blood glucose and ß cell percentage ($r = -0.604$; $p < 0.00$). Negative correlation indicated that the higher the ß cell percentage, the higher the insulin produced, and the lower the FBG level.
2. There was correlation between fasting blood glucose and Langerhans' islet size ($r = -0.459$; $p < 0.05$). Negative correlation showed that the higher the FBG level, the smaller the size of Langerhans' islet.
3. There was correlation between ß cell percentage and the size of Langerhans' islet ($r = 0.253$; $p < 0.05$), indicating that the higher the ß cell percentage, the larger the size of Langerhans' islet.
4. Among these correlations, the strongest ($p < 0.00$) was found between fasting blood glucose and ß cell percentage.

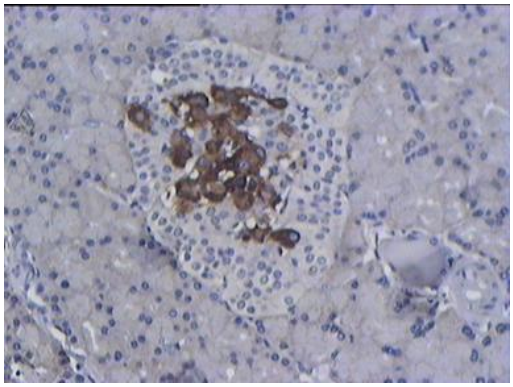
Microscopic Examination

The results of microscopic examination in this study were obtained from pancreatic tissue using immunohistochemistry.

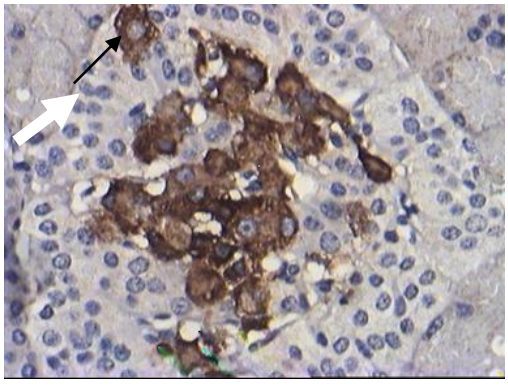
In Figure 1 (B) ß cell formation (black arrow) looks brown, as the result of immunohistochemical staining using monoclonal antibody against insulin. This indicated that the existing ß cells are positively producing insulin. The outermost border of Langerhans' islet in treatment group is also apparent in Figure 1 (B) (white arrow). The border describes the size of the islet.

1. Figure 2 obviously shows that ß cell count is far lower than that in treatment group.
2. It can be also seen that staining to insulin hormone is less strong, as the stained area is not as large as that in treatment group.
3. Regarding the size of Langerhans' islet, apparently the size in treatment group is larger than that in control group.

Both figures were taken using WinFast PVR program and had been magnified 200x and 400X by means of Olympus microscope. Langerhans' islet observation was carried out three times in each preparation.



A



B

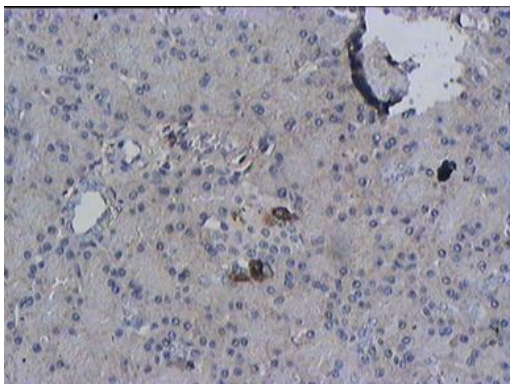
Figure 1. Using immunohistochemical reaction it can be seen that (A) insulin provides positive reaction towards monoclonal antibody against insulin. White arrow is the outermost border of Langerhans' islet, while the black arrow indicates ß pancreatic cells (B) of treatment group. A: magnification 200X B: magnification 400X

DISCUSSION

The effect of Toxicant STZ

Glucose stimulates insulin secretion through a number of regulating steps starting from transport into β cells by glucose transporter 2 (GLUT2). STZ enters β pancreatic cells also through GLUT2, resulting in DNA alkylation (Thulesen 1997). Therefore, STZ has a high affinity towards ß cells (Kroncke et al. 1995).

STZ injected intraperitoneally to rats' body with the dose of 60 mg/Kg BW may result in toxicity, particularly in ß cells of pancreatic Langerhans' islet. STZ is a compound known to have a strong potentiality to induce direct alkylation in DNA through methylation mechanism. It is also highly genotoxic. STZ may cause the breaking of DNA chain, induces untimely DNA synthesis, chromosome aberration and cell death. Some studies found the role of STZ as free-radical donor that causes fatal damage in DNA and chromosome (Bolzan & Bianchi 2002). DNA damage results in the activation of poly ADP-ribosylation, an important diabetogenic process induced by STZ compound, more important than its effect to damage DNA chain. Poly ADP-ribosylation results in the reduction of intracellular NAD^+ and ATP level.



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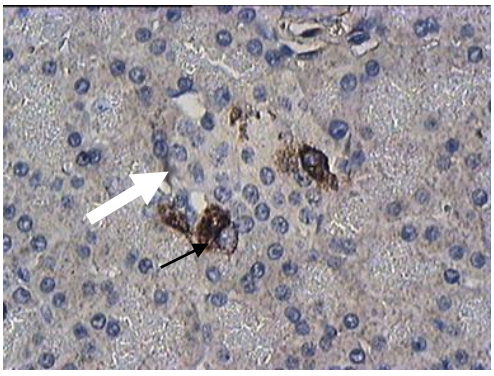

B

Figure 2. Using immunohistochemistry, it is evident that (A) insulin provides positive reaction to monoclonal antibody against insulin. White arrow is the outermost border of Langerhans' islet, while the black arrow is the ß pancreatic cells (B) of control group. A: magnification 200X B: magnification 400X

The increased ATP dephosphorilation after STZ administration provide resource for the enzyme xanthine oxidase, so that radical superoxide groups are formed.

In consequence, the compound hydrogen peroxide and hydroxyl radical groups are also formed. Subsequently, STZ also releases toxicant, the nitrogen oxide (NO). NO acts to inhibit the activity of enzyme aconitase, so that it also participates in the damage of DNA chain. Taken together, the action of STZ in the damage of ß pancreatic cells through necrosis mechanism (Szkudelski 2001).

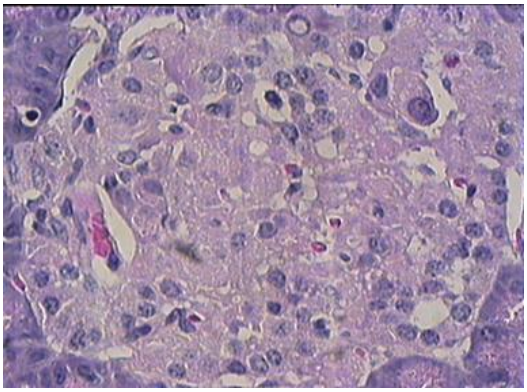

Figure 3. The picture of Langerhans' islet using routine Haematoxylin and Eosin (H&E) staining, magnification 400X in treatment group. It is very difficult to differentiate ß cell from other cell types.

The extent of ß pancreatic cells damage may reach more than 90% (Allison J, 1998), resulting in hyperglycemia in the rats' body. It was evident by the increase of rats' fasting blood glucose until reaching a level equal to or more than 300 mg/dl (Jang et al, 2003). Such condition meets the criteria of type 1 diabetes mellitus (DM) (Harrison's 2001).

Change of ß Cells Count and Function

The damage of ß cells in the pancreas may reduce their count and function. Morphologically, the reduction can be observed by using anatomic microscopic staining. One particular method in anatomic microscopic staining is immunohistochemistry, which is highly specific. In addition to disclose specifically the morphology of ß cells, which may also determine the cell count, it has also capacity to reflect the function of ß cells. This is because this method displays specific color of the insulin, while insulin is the marker of ß cells normal function. Halban (2001) wrote that the characteristic of ß cells is that it can exclusively produce insulin. In normal condition, this function cannot be taken over by other cells in an organism's body. Grupe (1995) stated that extracellular glucose level might directly illustrate the function of ß cells metabolism.

In line with obtained data in this study, the effect of treatment on ß cells percentage can be seen in Table 5.3. The results of statistical test showed that treatment provided significant effect ($p < 0.05$) on ß cells percentage. The increase of ß cells count of more than twice (29.96% compared to 12.3%) is directly detected in relations to the cells' function as insulin producer.

However, observable insulin increase in ß cells cannot be used as justification that the hormone has functioned normally (Oi et al. 1997). One of the functions is the insulin's role in reducing blood glucose level. Therefore, to ascertain that ß pancreatic cells-produced insulin has had the appropriate function, it is necessary to carry out the measurement of blood glucose as parameter. The measurement should be done in fasting to eliminate the effect of food intake.

As can be seen in the results of statistical test in Table 2, it is apparent that blood glucose in treatment group is significantly ($p < 0.05$) lower than FBG in control group. In this case, the FBG reduction is comparable to the increase of ß pancreatic cells that reaches 42%.

Change of Langerhans' Islet Size

Logically, ß cells damage resulting from necrosis that reaches more than 90% may certainly reduce the entire mass of ß cells. The following consequence is that the space occupied by ß cells in Langerhans' islet will be remarkably lessened. The whole size of the islet will also reduce. The reduction of the size will have remarkable effect, as ß pancreatic cells occupy almost 80% of the whole size of the islet (Eisenbarth 2002).

In this study, although the treatment of laser to point BL-20 could increase ß pancreatic cells count, it should have been examined whether the increase was from the remaining ß cells (less than 10%) or from other source (pancreatic duct). If the increasing ß cells count was from the remaining ß cells, which was the result of mitotic process due to stimulation, with the assumption that the each existing ß cells duplicated, it can be expected that the increase of Langerhans' islet would be no more than twice of the remaining ß cells, since cellular change towards mature ß cells required at least 11 days (Zulewski 2001), so that the observed increase of Langerhans' islet size would be no more than 30%.

The effect of therapeutic improvement in this study, which was conducted to the treatment groups, can be seen in Figure 1A and 1B, (compared to that in control groups in Figures 2A and 2B). In control group we can

see that the Langerhans' islet is smaller. After conducting size measurement using ImageTool both in treatment and control group, we found significant difference between both groups ($p < 0.05$). The results of statistical test can be seen in Table 4. The increase was more than three times (>300%). This confirmed the presumption that the newly formed ß cells were positively the results of stem cell proliferation in pancreatic duct.

Change of Wave Profile

Damaged cells in pancreatic organs, in addition to present the change of ß cells count and function, also present the change of wave profile. As ß cells belong to cells that arrange pancreatic organ, the change of wave profile would logically affect the wave profile of the pancreas as a whole. Sagiran has proved this in 2001. Using toxic agent, alloxan, he damaged male Wistar rats' ß pancreatic cells and found significant difference in the profile of electric power profile of pancreatic acupuncture point between treatment and control group. This reflects the difference of electric power profile between pancreas with damaged ß cells and normal pancreas. The study by Sagiran was inspired by the findings of Suhariningsih two years earlier (1999). She identified the difference in electric power profile of hepatic acupuncture point between hepatic cirrhosis patients and normal individuals.

In quantum mechanics, an ill organ produces wave deformation from its normal wave profile. The deformation will affect the meridian wave profile of the organ. The change can be detected at the appropriate acupuncture point of the organ (Rakovic 2001).

Biophysically, each molecule, both comprising three atoms, such as water (H_2O), or more, such as glucose ($C_6H_{12}O_6$), has their own wave profile. Each atom has typical wave profile, and so does compound, cell, organ, and even organ system.

Wirya (1998) proposed that the typical wave profile is the total of movement in whole particles, either particles that compose cells, organs, or tissues in human body. Therefore, each organ in the body has its own typical wave profile for themselves. Any deformation from the typical wave profile and each change of its frequency may expose information that indicates the abnormality of the organ.

A wave profile of an organ can be regarded as healthy if it is within the value of normal wave frequency. Anytime the wave profile of an organ deviates from the normal value, it can be assumed that the organ will be or has been ill. Oschman (2001) stated that pathological change of an organ's wave profile precedes pathological change of anatomic-microscopic morphology of the organ.

If an organ's wave profile has been identified as having pathological change, the change should be immediately inhibited, anticipated, and even reversed into normal by providing appropriate wave as required (Köhler 1997). The required wave is compatible with the existing lack of wave, which can be observed quantitatively in the frequency or wavelength of the abnormal wave (Wirya 1988).

Therapy to recover deformed wave profile of an organ can be provided through the acupuncture point appropriate to the organ (Rakovic 2001). In acupuncture, the point BL-20 is regarded as the chi source of the pancreas (Yanfu et al. 2002). From this point, the appropriate wave is transmitted towards pancreatic organ through meridian communication pathway (Wirya 1998). Reaching the pancreas, the wave is processed as information in order to develop cooperation at intercellular (Kim & Hebrok 2001), subcellular, and nuclear level to overcome the abnormality. The information is translated, for example, into molecular reactions, so that the organ can carry out some steps of recovery mechanism (Oschman 2001).

ß Pancreatic Cell Recovery

After stimulation (photon) reaches the pancreas, obtained information is also received by ß cells-precursor stem cells within pancreatic duct. The cells in this duct are normally highly sensitive to proper stimulation (Zulewski 2001). Receptors in cell surface are the main site of action of low-frequency electromagnetic field. In these receptors cellular response is triggered by various electromagnetic signals, and one of which is photon. Membrane protein that is closely related with receptors, such as adenylate cyclase and protein G, combined one molecular event at cellular surface with influx of a large amount of calcium ion (Oschman 2001). One of the effects of calcium ion increase in cellular cytoplasma is the redifferentiation of stem cell through mitosis and proliferation. For this purpose, free calcium ion in the cell will be bound with other second messenger, and so will the specific protein receptor, the calcium binding protein. The binding of calcium ion with phospholipase C (PLC) result in the phosporilation of phosphatidylinositol diphosphate (IP_2) to become phosphatidylinositol triphosphate (IP_3). Subsequently, IP_3 stimulates the release of intracellular calcium ion. The increase of intracellular calcium ion will form a calsineurin complex that will activate NFκß. The following process is the insertion of NFκß into the

nucleus to stimulate the DNA to perform replication, differentiation, and proliferation.

Replication, differentiation, and proliferation occur in pancreatic duct epithelium since ß cells in the islet of Langerhans actually come from this epithelium. Anatomically, the pancreatic duct epithelium develops from entodermal layer of embryonic tissue. The newly produced ß cells are precisely similar to those that have been necrotized, so that, both morphologically and physiologically, the new ß cells are the same as those damaged due to the administration of toxicant STZ. Taken together, the existing ß pancreatic cells have increased their count as well as their function.

Correlation between Variables

In this study correlation between some variables should be proven. The reason was that although there has been an increase of ß cell count, it was not necessarily that the increase was significant (reaching 300%) for the Langerhans' islet size. Such increase could also result from the mitosis of remaining ß cells, so that the obtainable increase of the cells would only be maximally 30%.

Similarly, the increase of Langerhans' islet cell did not necessarily indicate the increase of ß cell count. It could also possibly result from the hypertrophy of the remaining ß cells. If this was so, it could be expected that the size of Langerhans' islet would not be 30% or more.

The increase of insulin as seen in immunohistochemical staining also did not ensure its increasingly normal function. Such increase could also result from the hypertrophy of existing ß cells, so that it was necessary to conduct correlation test between the variables of ß cell count, Langerhans' islet size and ß cell function.

To prove the presence of correlation between Langerhans' islet cell and the increase of ß cells percentage, we conducted statistical test whose results can be seen in Table 5. From the table we see that there was positive correlation with $r = 0.464$, $p < 0.05$. The value proved that if ß cell count increases, the size of Langerhans' islet will also increase that may reach more than 300%.

In contrast, correlation test between Langerhans' islet cell and the reduction of FBG level (see Table 5), the correlation coefficient was negative ($r = -0.464$; $p < 0.05$). This negative value indicates that the larger the size of Langerhans' islet due to the increase of ß cell count, the lower the reduction of FBG level. This result was in accordance to the result of correlation test between Langerhans' islet cell and the increase of ß cell count. The increasing ß cell count indicated the increase of insulin and Langerhans islet cell. The final outcome was the reduction of FGB.

The results of correlation test between fasting blood glucose reduction and the increase of insulin can be seen in Table 5. The table shows that insulin increase correlates with the reduction of FGB ($r = -0.607$; $p < 0.01$). The correlation coefficient was negative, indicating that the increase of insulin is followed with FBG reduction. These data implicate that the produced insulin did have normal function, which was to reduce FBG level. Overall outcome of these correlation tests suggested that there was a change from DM into normal, which resulted from the increase of ß cell count and function. This confirmed the presumption that laser directed to point BL-20 will increase ß cell count and function through the stimulation of stem cells in pancreatic duct to perform proliferation and differentiation to become normal ß cells.

Furthermore, in Table 5.4 we can see that the size of Langerhans' islet in treatment group was three times larger than that in control group (18364.69 píxel^2 compared to 5881.69 píxel^2), while the increase of ß cell percentage, as seen in Table 5.3, in treatment group was more than twice (29.96% compared to 12.3%). Logically, the increase of ß cell percentage should have been in line with the increase of Langerhans' islet size. Moreover, if we notice the result of final fasting blood glucose, the FBG of treatment group increased only 42% of that in control group.

Some of the increase in those variables showed incompatibility between the size of Langerhans' islet, the percentage of ß cells and final FBG value. This might be possible due to following reasons:

1. ß pancreatic cell count in this study was counted based on their function as insulin producer, so that the ß cells that had not yet produced insulin, i.e., the immature ß cells (Larsen 2002, Moore 2003, Sadler 2004) were not counted. Consequently, these cells were overlooked.
2. Some insulin in ß cells was not active yet, so that the increase of insulin in ß cells had not shown its active function in reducing fasting blood glucose.
3. Laser treatment to point BL-20, although it was only given for 12 days (Hou 1993), had shown a significant increase. However, the increase of ß cell function in general remained less optimum.
4. ß cell count in one Langerhans islet reached 80% (Eisenbarth 2002). If in type 1 DM there were at least less than 10% of normal ß cell (Harrison's 2001), the observable size of Langerhans islet

would at least be 30% only. Then, if necrotic ß cells due to the effect of STZ could only be recovered a half from total remaining ß cells, the size of Langerhans islet would change into approximately 70% (based on the assumption that each cell in the Langerhans islet had almost similar size). This size had been twice larger than the size in the case of type 1 DM. Furthermore, if in this study we found more than three-times larger size (Table 4), this increase should have been in line with the increase of ß cell count of almost 80%.

CONCLUSION

It can be concluded from this study that the variable of fasting blood glucose (FBG) is highly important, as it may reflect the function as well as the count of ß cell. As compared to control group, in treatment group, with the increase of FBG to 42%, the increase of ß cell count will be more than twice higher and the enlargement of Langerhans' islet cell will be more than three-times.

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