Effect of Ultraviolet Blood Irradiation in Kidney Function of Diabetes Mellitus Model

Gareeballah Osman Adam¹,², Byung-Yong Park³, Shang-Jin Kim¹, Kyung-Min Choi⁴, Jin-Shang Kim¹, Hyung-Sub Kang¹, Gi-Beum Kim⁴,⁵

¹Department of Pharmacology, College of Veterinary Medicine, Chonbuk National University, Iksan Campus, 79 Gobong-ro, Iksan-si, Jeollabuk-do 54596 Republic of Korea
²Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, Sudan University of Science and Technology, P.O. Box No. 204, Hilat Kuku, Khartoum, Sudan
³Department of Veterinary Anatomy, College of Veterinary Medicine, Chonbuk National University, Iksan Campus, 79 Gobong-ro, Iksan-si, Jeollabuk-do 54596 Republic of Korea
⁴Department of R&D Planning, Institute of Jinan Red Ginseng, Jinan, Jeonbuk, 55442 Republic of Korea
⁵Department of Biochemistry, Chonbuk National University Medical School, Chonbuk National University, 567 Baekje-daero, deokjin-gu, Jeonju-si, Jeollabuk-do 54896 Republic of Korea

Abstract. The purpose of this study is to evaluate the effects of ultraviolet blood irradiation on the blood when a low dose of ultraviolet C (UV-C) is directly irradiated to the blood in a diabetic rabbit model and to evaluate the effects on treatment for diabetes. Type 1 diabetes was induced by intravenous (IV) injection of alloxan monohydrate 110 mg/kg into New Zealand white rabbits weighing 2-2.5 Kg. The ultraviolet blood irradiation (UBI) treatment was performed a total of 8 times. We evaluated the effects of the UBI treatment on diabetes through hematological analysis before and after UBI treatment were performed. Our results indicate that the reduced body weight is increased and blood glucose levels are significantly reduced after the UBI treatment is performed when compared to those prior to the UBI treatment. In addition, CRE, BUN and UA levels indicating renal functions are significantly reduced when compared to those prior to the UBI treatment. When the UBI treatment is performed in a diabetic rabbit model, our results indicate that blood glucose levels are reduced. Hematological analysis demonstrates that the UBI treatment is effective to alleviate the diabetes.

Keywords: ultraviolet blood irradiation, Type 1 diabetes rabbit model, blood glucose level, creatinine (CRE), blood urea nitrogen (BUN), uric acid (UA)

1 Introduction

Once it enters the 20th century, the most innovative invention in medical history is various antibiotics such as penicillin, steroids and vaccines. The invention of antibiotics and steroids is very effective in treatments for acute inflammatory diseases, but there are some side effects such as occurrence of resistant bacteria against antibiotics and adverse effects of steroid hormones. With regard to current medicine
of the 21st century, advanced professional therapeutic methods are being developed to treat incurable diseases and rare diseases using genetic analysis and stem cells. However, there are still many diseases which cannot be treated by above professional therapies. In addition, there are new diseases, expression of antibiotic resistant bacteria and various syndromes which cannot be identified nor treated by the modern medicine [1-5].

As the medication and genetic therapies reach their limits, new therapeutic methods are being highlighted. As a new therapeutic method, the therapy using UV light was studied and applied in the clinical trials in the US and Western countries until the 1950s. Once antibiotics such as penicillin and steroid hormone agents were invented, the therapeutic methods using UV light were not studied any longer in the US and Western countries. However, it has taken the place as a field of medical technology through active research and clinical trials in Germany and Soviet Union (Russia) [6, 7]. As antibiotics or steroid hormone agents reach their limits in infectious diseases and chronic autoimmune diseases since 1980’s and 1990’s, the therapeutic methods using UV light are being highlighted. Researchers are actively conducting the research on the new therapeutic method in Germany, US, Russia and China and are trying to develop it as a new clinical therapeutic method [7-11]. Research on this therapy is actively being conducted in the world but the fundamental mechanism for the therapy has not been understood. Many clinical studies on the therapy have been conducted and thousands of research papers have been published [12-18].

Diabetes is a metabolic disease with high blood glucose levels caused by the metabolic disorder resulting from defects of hormones such as insulin, glucagon or glucocorticoids involved in the metabolism of glucose or by abnormal reactions in the pathway [19]. Because the diabetes is characterized by high blood glucose levels, which leads to a wide range of malfunctions in the metabolic control over carbohydrates, proteins, fats and electrolytes, it is closely associated with increases in various chronic degenerative diseases [6]. Diabetes emerges as the disease causing social problems, because it has a very high prevalence as a typical chronic metabolic disease. Diabetes goes beyond endemic limits and is approaching epidemic proportions globally [19, 20]. The incidence of diabetes is very low in Korea when compared to that in Western countries. However, the incidence is gradually increasing in Korea due to recent changes in diet, environment and lifestyle [21, 22]. According to the study of Huh et al., the maximum insulin secretion of Korean people is about half as high as that of Westerners. Reductions in insulin secretion have started at a lower blood glucose level in the serum insulin response curve. It has been reported that Korean people do not overcome weak insulin resistance and develop diabetes, because congenital or acquired insulin secretion of Korean people is lower than that of Westerners [23, 24]. It has been known that secondary complications such as diabetic retinopathy, neuropathy and nephrosis are caused when high blood glucose levels are sustained in diabetic patients. Thus, it is important to control blood glucose levels when diabetic patients are treated [25].

Thus, in this study, we would like to evaluate the effects of ultraviolet blood irradiation on the diabetes by using physical methods with UV light rather than drug therapy such as insulin injection in order to get over diabetes causing serious problems.
2 Materials and Methodology

2.1 Design of Ultraviolet Blood Irradiation Device

In this study, we have produced the ultraviolet blood irradiation (UBI) device to identify effects of ultraviolet blood irradiation on the blood in a diabetic animal model. Fig. 1 shows a simple drawing and photo of the UBI device. G4T5 TUV 4W Germicidal Fluorescent Light Bulb (Philips, USA) with the wavelength of 260 nm is used as the UV lamp. A circular quartz crystal cuvette is produced in length of 150 nm, thickness of 1 mm, and inner diameter of 4 mm. Both ends of the cuvette can be connected to syringes.

Fig. 1. Schematic photographic of the ultraviolet blood irradiation device.

2.2 Experimental Animals

Adult male twelve New Zealand white rabbits of body weighing 2 - 2.5 kg were used in the study. All the rabbits were kept in cages with wide square mesh at the bottom to avoid coprophagy and maintained under controlled conditions of humidity, temperature (22±2 °C) and 12 hours light and dark cycle. Food and water were provided ad libitum. They were fasted for 18 hours prior to the experiment, allowing free access to water only. The experimental protocols were approved by the Institutional Animal Ethics Committee. All experimental protocols (CBU2013-0010) were approved by the Committee on the Care of Laboratory Animal Resources, Chonbuk National University and were conducted in accordance with the Guide for the Care and Use of Laboratory [26].

2.3 Diabetes Procedure for Injecting Alloxan Monohydrate

The 12 rabbits weighing between 2 to 2.5 kg were made diabetic by injecting intravenously 110 mg/kg body weight of alloxan monohydrate (A7413, Aldrich) [27]. Before giving alloxan, the normal blood glucose levels of all rabbits were estimated. After 2 hours of alloxan injection the 5% Dextrose injected to the all-diabetic rabbits intraperitoneally to prevent a hypoglycemic condition of rabbits with alloxan. After 72 hours of alloxan injection, the blood glucose levels of all surviving rabbits were determined by the glucose oxidase method.
2.4 Ultraviolet Blood Irradiation Treatments

It is confirmed that the diabetes is induced by measuring blood glucose levels in rabbits at 72 hours after alloxan is injected. The blood is collected from diabetic rabbits after 1 week. The UBI treatment is performed to the blood. For the UBI treatment, UV is irradiated to the blood collected through autotransfusion and the blood is transfused back to the original rabbit. Anticoagulation Sodium Citrate Solution (BOIN ACDA SOLN, SBD Co., Ltd.) is used to prevent coagulation of the blood when it is collected. 10 ml blood is collected from the vein by using 20 ml syringe. UV light with the intensity of 10.290 J/cm² is irradiated to the blood which passes at a constant flow rate using the syringe pump in the UBI device. After the UBI treatment is performed, the blood is transfused back to the original rabbit. The UBI treatment is performed a total of 8 times. Rabbits are stably raised in a laboratory animal breeding facility. Food and water are sufficiently supplied.

2.5 Biochemical Analysis

Blood was collected from the ear marginal vein. Blood collection, storage, and measurement were performed as previously described [28]. A Nova Stat Profile® pHOx® Ultraanalyzer (NOVA Biomedical Corp., Waltham, MA, USA) was used to measure the levels of lactate, pH, HCO₃⁻, hemoglobin, and hematocrit, in freshly collected whole blood. After clotting, blood serum was separated by centrifugation at 3000 rpm for 20 min. The levels of glucose (Glu), creatinine (CRE), blood urea nitrogen (BUN), and uric acid (UA) were analyzed using a Model 7020 auto analyzer (Hitachi, Tokyo, Japan).

3 Results

3.1 Effects of the UBI Treatment on Body Weight and Glucose Levels

Fig. 2 shows changes in body weight and blood glucose levels of diabetic rabbits before and after the ultraviolet blood irradiation (UBI) treatment is performed. As the results, body weight does not decrease on day 3 after alloxan injection. However, the body weight of diabetic rabbits tends to decrease while the UBI treatment is performed 8 times. It still decreases in 8 weeks after the UBI treatment is discontinued. However, it tends to increase over 8 weeks after the UBI treatment is discontinued. In addition, glucose (Glu) levels are changed as follows: Glu level is 600 ± 74.73 mg/dl prior to the UBI treatment over 3 days after alloxan injection. Glu level is 471.1 ± 102.5 mg/dl while the UBI treatment is performed 8 times. The Glu level significantly decreases after the UBI treatment is performed. Glu level is 433.3 ± 118.3 mg/dl over 8 weeks after the UBI treatment is discontinued. It significantly decreases when compared to that prior to the UBI treatment.
Fig. 2. Effects of ultraviolet blood irradiation treatments on the body weight (a), whole blood concentration and serum levels of glucose (Glu) (b) in alloxan-induced diabetic rabbits. Data are reported as means ± SEMs (n = 12). *: p < 0.05; **: p < 0.01; and ***: p < 0.001, Bonferroni post hoc test following one-way ANOVA versus the BD (Before alloxan-induced diabetic); #: p < 0.05; ##: p < 0.01; and ###: p < 0.001, Bonferroni post hoc test following one-way ANOVA versus NUBI (Before ultraviolet blood irradiation treatments procedure). BD; Before alloxan-induced diabetes, NUBI; Before ultraviolet blood irradiation treatments procedure, 8th; 8th times treatments procedure of ultraviolet blood irradiation, A8; After 8th times treatments procedure 8 weeks later, A10; After 8th times treatments procedure 10 weeks later.

3.2 Organ Harvest

Fig. 3 shows the conditions of organs in diabetic rabbits that do not undergo the UBI treatment and undergo the UBI treatment after diabetes is induced. The stomach and bladder become abnormally small in the diabetic rabbit that do not undergo the UBI treatment. The pancreas is substantially damaged. In addition, left renal becomes abnormally swollen. While it is taken out, a large amount of urine is leaked. After swollen left renal is excised, it is reduced as small as right renal. When the inside of the left renal is observed by naked eyes, many tissues are damaged when compared to those in the right renal. Thus, it seems to be unable to play its roles. However, when organs of rabbits undergoing the UBI treatment are observed by naked eyes, their conditions are much better than those in rabbits that do not undergo the UBI treatment.

Fig. 3. Photos of the organ without UBI treatments procedure rabbit (a) and with 8th times UBI treatments procedure rabbit (b).
3.3 Effect of the UBI Treatment on the Renal Function by Serum Metabolic Enzymes Analysis

Fig. 4 shows results of renal function tests such as serum creatinine (CRE), blood urea nitrogen (BUN) and uric acid (UA) to examine the efficacy of UBI treatment on the diabetes in a diabetic rabbit model. Prior to alloxan injection, CRE, BUN and UA levels are 0.925 ± 0.097, 27.03 ± 3.75 and 2.675 ± 0.496 mg/dl, respectively. The levels are significantly increased when compared to those prior to alloxan injection. However, in the diabetic rabbit model undergoing the UBI 8 times treatments, CRE, BUN and UA levels are 1.025 ± 0.198, 34.73 ± 7.76 and 3.042 ± 0.629 mg/dl, respectively. The levels are significantly decreased when compared to those before the UBI treatment is performed. CRE, BUN and UA levels are 0.958 ± 0.173, 33.31 ± 4.11 and 3.075 ± 0.245 mg/dl, respectively, over 8 weeks after the UBI treatment is discontinued. The levels are significantly decreased when compared to those before the UBI treatment is performed.

Fig. 4. Effects of ultraviolet blood irradiation treatments on the renal function by serum analysis in alloxan-induced diabetic rabbits. CRE, creatinine (a); BUN, blood urea nitrogen (b); UA, uric acid (c). Data are reported as means ± SEMs (n = 12). *: p < 0.05; **: p < 0.01; and ***: p < 0.001, Bonferroni post hoc test following one-way ANOVA versus the BD (Before alloxan-induced diabetic); #: p < 0.05; ##: p < 0.01; and ###: p < 0.001, Bonferroni post hoc test following one-way ANOVA versus NUBI (Before ultraviolet blood irradiation treatments procedure). BD: before alloxan-induced diabetes, NUBI: Before ultraviolet blood irradiation treatments procedure, 8th: 8th times treatments procedure of ultraviolet blood irradiation, A8: After 8th times treatments procedure 8 weeks later.

4 Discussion

Treatments for patients using the UBI treatment can be applied to surgical diseases such as infections in the lung or pleura, pancreatitis, bone myositis, sepsis and thrombosis, and medical diseases such as hypertensive cardiovascular diseases, pneumonia, and asthma [29]. In addition, it is being used to treat gynecological infectious diseases, diseases in the endocrine system such as diabetes and thyroid disease, or neurological diseases such as stroke, and brain injury [30]. It has been reported that the UBI treatment has various effects and almost no adverse events. However, high dose of UV radiation may have some side effects such as skin rash or redness, blisters, destruction and necrosis of blood cells, and reduction of oxygen transfer capability. During the treatments using the UBI device, no side effects have been reported [31].
Since 1943, alloxan has been widely used to induce diabetes by selectively blocking secretion of insulin, because it has a strong oxidizing power which destroys cells in the pancreas and reduces the area of cells [32]. Cooperstein and Watkins [33] claimed the mechanism of alloxan to induce diabetes as follows: Alloxan is bound to glucose in the cellular membrane of β-cell or is present around the glucose. Thus, it is bound to sulphydryl protein which may play the important role for the general functions of the β-cell, which increases the penetration into the cellular membrane. Due to the increased penetration, the β-cells are destroyed and the diabetes is induced [34]. After all, compounds with a strong oxidizing power such as alloxan induce type 1 diabetes which is insulin-dependent diabetes mellitus characterized by blocking the production and secretion of insulin in the β-cell.

The most important function of β-cells is to synthesize, store and secret insulin which is the hormone to lower the blood glucose levels. When the blood glucose level is high, the β-cell secretes and produces insulin at the same time. The cellular membrane of the β-cell has voltage-gated calcium channels and ATP-sensitive potassium channels. As the ATP-sensitive potassium channel usually opens, potassium diffuses out of the cell depending on the concentration gradient across the membrane. If the glucose level is high outside the cell, the glucose is imported through Glucose Transporter 2 (GLUT2). Because the β-cell phosphorylates the glucose using the glucokinase, the metabolism occurs only in the conditions with physiological glucose levels or higher glucose levels. ATP is produced through the metabolism of glucose, the ATP/ADP ratio is increased. When the ATP/ADP ratio is high, the ATP-sensitive potassium channel is closed and thus potassium cannot be diffuse. Since the potassium is accumulated inside the cells, the membrane potential becomes more positive and thus the closed voltage-gated calcium channel opens. It results in calcium influx along the concentration gradient of calcium. Vesicles containing insulin are transported to the edge of the cells and then insulin is secreted through the fusion with cell membrane (exocytosis) [35]. β-cells promote the metabolism of glucose through secretion of insulin and thus produce ATP. However, damaged β-cells increase Glu levels in the blood. If the diabetes is not properly treated, it develops into diabetes complications such as diabetic neuropathy [36]. If patients with type 1 diabetes are not properly treated, it develops into overt diabetic neuropathy in 80% of patients. In the case of patients with type 2 diabetes, it develops into overt diabetic neuropathy in 20–40% of patients after 15 years [37]. Various mechanisms including metabolic factors such as high blood glucose levels, hematological factors and genetic predisposing factors are involved in the process of development into the diabetic neuropathy [38, 39]. In general, blood glucose levels are determined by supply of glucose from the outside and changes in using glucose in cells. Even if the rate of using glucose is drastically increased, blood glucose levels are relatively strictly controlled within a certain range. It does not only prevent various acute or chronic complications caused by hyperglycemia or hypoglycemia, but also minimizes the effects on organs which use glucose as a major energy source [40]. Various hormones and factors in the nervous system are involved in such control over glucose levels. In the renal, homeostasis of glucose is regulated through a variety of mechanisms including production of glucose, use of glucose and renal re-absorption due to the effects of these factors [41].

Creatinine (CRE) is an energy that is used for muscle contraction. When creatine is
inefficiently dehydrated in the muscles, it is secreted into the blood as a final metabolite and then excreted in the renal [42, 43]. Some of CRE is excreted to the renal tubule and is not re-absorbed after it is filtered in glomerulus of the renal. Thus, blood CRE levels are being used as an indicator of renal functions. Increases in CRE levels represent substantial renal defects. Results of experiments indicate that blood CRE level is high in a diabetic rabbit model but the blood CRE level tends to decrease in a diabetic rabbit model undergoing the UBI treatment.

In normal people, proteins are metabolized and most of them are finally excreted as urea in the renal. Urea is excreted through the renal as the final product of protein and amino acid metabolisms. BUN is blood urea nitrogen and it is mostly filtered and excreted in glomerulus of the renal. However, some of BUN is re-absorbed depending on the water contents in the body, which is affected by amounts of protein intake, amounts of urine and gastrointestinal bleeding. Because blood urea level is high when the renal function is poor, blood BUN level is one of indicators evaluating renal functions. The results of experiments indicate that blood BUN level is high in a diabetic rabbit model but BUN level tends to decrease in the diabetic rabbit model undergoing the UBI treatment. In addition, purine is one of building blocks of nucleic acids (DNA and RNA) included in the nucleus of the cell and it is broken down into UA as a final product in the body. It is mainly filtered in the renal and excreted in the urine. If the UA level is elevated in the blood because UA is excessively produced or is not properly excreted, urate is accumulated in the joint and surrounding soft tissues. Renal functions decline and the abilities to filter the blood are reduced. Thus, gout occurs with increasing UA levels. However, in this study, our results indicate that UA levels are increased in a diabetic rabbit model induced by alloxan injection but increased UA levels do not cause gout.

5 Conclusions

The results of experiments confirm that the β-cell is damaged by alloxan injection. Since insulin is not released due to damage of β-cell, blood Glu levels are elevated. However, blood Glu level is reduced in the diabetic rabbits undergoing the UBI treatment. The reason why Glu levels are reduced is that the UBI treatment promotes the re-esterification process by facilitating the metabolism of glucose, which increases glycerol 3-phosphate produced from glucose. In addition, the renal plays an important role in the metabolism of glucose. As shown in the results of experiments, the renal tissue is observed by naked eyes and many tissues of the renal are damaged. Thus, it does not properly play roles. In addition, results of hematological analysis indicate that CRE and UA levels indicating renal functions are high. However, as the renal is not damaged in a diabetic rabbit model undergoing the UBI treatment, it can properly play roles. In addition, CRE and UA levels indicating renal function are also low. Therefore, as the UBI treatment can lower the blood Glu levels and prevent the damage of the renal, liver and pancreas, it is found that the UBI treatment is very effective in the diabetes.
References