

Study of Pharmacological Properties of Plaferon LB on the Experimental Model of Insulin-dependent Diabetes Mellitus

Marika Gamkrelidze, Rusudan Rukhadze, Ivane Datunashvili

The experimental Institute of Morphology, Tbilisi, Georgia

ABSTRACT

Insulin-dependent diabetes mellitus (IDDM) is characterized by selective destruction of insulin-producing islet beta cells. Reactive free radicals act as intermediates in cytokine-induced destruction of beta-cells. Present study investigated effect of the preparation plaferon LB on morphological changes in pancreatic islets caused by experimental diabetes in rats. Study of H&E sections revealed marked histological changes in pancreatic tissue from the control diabetic group animals, whereas in animals from the plaferon LB treated group signs of periductal necrosis have not been found; atrophied areas occurred, though they were rare; fat infiltration has not been observed. This experiment has manifested that plaferon LB significantly reduces pancreatic tissue lesions induced by alloxan. Due to its ability to inhibit the synthesis of proinflammatory cytokines and the production of free radicals plaferon LB acts as a protector of insulin-producing beta cells and contributes to the regulation of blood glucose level.

KEYWORDS: *insulin-dependent diabetes mellitus, islet beta cells, plaferon LB*

D iabetes is recognized as one of the leading causes of morbidity and mortality in the world. About 3% of the world's population suffers from this disease.

Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease caused by infiltration of pancreatic islets by mononuclear cells of immune system, followed by selective destruction of insulin-producing islet beta cells. This autoimmune destruction results in insulin deficiency and hyperglycemia. By the time of clinical manifestation of IDDM more than three fourths of beta cells are destroyed and chronic inflammatory infiltration - so called insulite - is formed in the islets of Langerhans.

Reactive free radicals act as intermediates in cytokine-induced destruction of beta-cells [1]. Oxygen and nitrogen free radicals initiate lipid peroxidation of intracellular membranes. It results in widespread injury, including disaggregation of ribosomes, followed by decreased protein synthesis. Failure of the cell to synthesize the apoprotein moiety of lipoproteins causes accumulation of intracellular lipids (fatty change). Plasma membrane damage, caused by products of lipid peroxidation, results in cellular swelling and massive influx of calcium, with resultant mitochondrial damage, denaturation of cell proteins and cell death. Beta cell death is thought to occur by apoptosis, and later in severe cases by necrosis [2].

Characteristic morphological changes in pancreatic tissue in insulin-dependent diabetes are the following: sclerosis and hyalinosis of the Langerhans islets; regenerative hypertrophy of remained islets; sclerosis and lipomatosis of the skeleton of pancreas. The remained islets contain cells with enlarged nucleus, certain number of degranulated beta cells and chronic inflammatory infiltration [3,4].

Plaferon LB is a domestically produced preparation obtained from human placenta. It has wide spectrum of pharmacological effect including immunomodulating, anti-inflammatory and antioxidative properties.

It has been discovered that plaferon LB inhibits synthesis of proinflammatory cytokines such as IL-1, INF-g, TNF- α . According to the present concept, IL-1 (produced by macrophages) and INF-g (produced by T cells) activate NO-synthase generating NO free radical which is one of the most important mediators of beta-cell destruction [6]. Previous research provided evidences that plaferon LB

suppresses production of free radicals thereby reducing damage of beta cells and having positive effect on the level of blood glucose [5].

Present study aims to investigate an effect of plaferon LB on morphological changes in pancreatic islets caused by experimental diabetes in rats.

Research materials and methods: The study has been performed on mature white rats, 180-200 g of weight. Diabetes was induced by intraperitoneal injection with alloxan at a single dose of 150 mg/kg. Experimental animals were divided into three groups: the I group - the plaferon LB treated group (10 animals), the II group - the control diabetic group (10 animals), the III group - plaferon LB treated intact group (10). After diabetes was induced, animals from the I group had been treated with plaferon LB for 8 days (daily intramuscular injections at a dose of 0,25mg/kg), whereas animals from the II group received only physiological solution administered intramuscularly (at a daily dose of 0,25mg/kg). Intact rats from the III group had been treated with plaferon LB for 8 days (intramuscular injections at a dose of 0,25 mg/kg).

Blood glucose levels were determined using the standard indicators of Medi-test, and have been measured several times during this period: before alloxan administration, on day 2 and day 10 after injection of alloxan.

Rats were sacrificed on day 10 after injection of alloxan. Pancreas was removed, fixed in 12% neutral-buffered formalin and then embedded in paraffin. Serial sections (5mm thick) were stained with hematoxylin and eosin (H&E). Preparations were examined in light microscope for histopathological analysis.

Results and discussion: As it is presented in the table, on day 2 after alloxan administration blood glucose level in the II - control diabetic group was increased by 72 %. On day 10 after injection blood glucose level in animals from the II group was maintained at the same level, whereas in the I group - the plaferon LB treated group - it was dropped by 16 %. Also as there was demonstrated in our study Plaferon LB does not have any effect on blood glucose level in the intact rats.

Study of H&E sections revealed marked histological changes in pancreatic tissue from the II - control diabetic group animals. Pancreatic tissue structure in its exocrine part was generally maintained. Excessive development of

connective tissue and fat infiltration was observed. There were places of periductal necrosis. Blood vessels were dilated.

Most part of Langerhans islets have undergone sclerosis and hyalinosis; regenerative hypertrophy of remained islets has been observed.

In animals from the II group - plaferon LB treated group signs of periductal necrosis have not been found. Atrophied areas occurred, though they were rare. Fat infiltration has not been observed.

Morphological picture of pancreatic samples taken from the III - the plaferon LB treated intact group have not revealed any alterations.

There has been manifested in this experiment that plaferon LB significantly reduces pancreatic tissue lesions induced by alloxan. Due to its ability to inhibit the synthesis of proinflammatory cytokines and the production of free radicals plaferon LB acts as a protector of insulin-producing beta cells and contributes to the regulation of blood glucose level; thereby plaferon LB can also be considered as a remedy for prevention of late severe complications of diabetes mellitus.

Animal groups	N	Initial	On day 2 after alloxan injection	On day 10 after alloxan injection
Control diabetic group	10	60,45±1,18	103,94±4,82	102,6±12,31
Plaferon LB treated group	10	60,45±1,18	103,94±4,2	84,5±3,5*

* - p < 0,01

Tab.1 Variation of blood glucose level in alloxan -induced diabetic rats under the influence of plaferon LB.

REFERENCES:

1. D.L. Eizirik, T. Mandrup-Poulsen. A choice of death - the signal-transduction of immune-mediated beta-cell apoptosis. *Diabetologia* 44: 2115-2133, 2001
2. Radicals and oxidative stress in diabetes. *West IC. Diabetic medicine* 17, 171-180, 2000
3. The Pathogenesis of Insulin-Dependent Diabetes Mellitus. Mark A. Atkinson, Noel K. Maclaren. *The New England Journal of Medicine*. Vol. 331:1428-1436, Nov 24, 1994. Number 21
4. Histopathological and immunohistochemical analysis of the endocrine and exocrine pancreas in twelve cattle with insulin-dependent diabetes mellitus (IDDM). Taniyama H, Hirayama K, Kagava Y, Kurosava T, Tajima M, Yoshin T, Furuoka H. *J Vet Med Sci/ 1999 jul;61 (7):803-10.*
5. Коррекция окислительного стресса при аллоксановом диабете с помощью плаферона ЛБ. Гамкрелидзе М.М., Датунашвили В.Т., Папава М.Б. Саникидзе Т.В., Бахуташвили В.И. *Georgian Medical News* N2 (107) Февраль 2004.
6. Cytokines and their roles in pancreatic islet b-cell destruction and insulin-dependent diabetes mellitus. Rabinovitch A, Suarez-Pinzon W.L. *Biochemical Pharmacology*, Vol.55, pp. 1139-1149, 1998

Исследование фармакологических свойств плаферона ЛБ на экспериментальной модели инсулин-зависимого сахарного диабета

Мари́ка Гамкрели́дзе, Русудан Рухадзе, Иван Датунашвили

Институт экспериментальной морфологии, Тбилиси, Грузия

РЕЗЮМЕ

Инсулинзависимый сахарный диабет - аутоиммунное заболевание, причиной которого является инфильтрация панкреатических островков мононуклеарными клетками иммунной системы с последующей селективной деструкцией бета-клеток, секретирующих инсулин. Характерные морфологические изменения при ИЗСД включают: склероз и гиалиноз островков Лангерганса, регенерационную гипертрофию сохранившихся островков, склероз и липоматоз стромы поджелудочной железы. Исследовалось влияние препарата плаферон ЛБ на морфологические изменения панкреатических островков вследствие аллоксанового диабета и установлено, что отечественный препарат плаферон ЛБ, благодаря своим иммуномодулирующим, противовоспалительным и антиоксидативным свойствам, значительно уменьшает аллоксаниндуцированное повреждение и атрофирование панкреатических островков, предупреждает жировую инфильтрацию и развитие перидуктального некроза, способствует регулированию уровня глюкозы в крови. Поэтому плаферон ЛБ можно также рассматривать как средство для предотвращения дальнейших тяжелых осложнений при ИЗСД.

КЛЮЧЕВЫЕ СЛОВА: ИЗСД, деструкция бета-клеток, плаферон ЛБ