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Effect of Alcohol on Insulin Secretion and Viability of Human Pancreatic Islets

Утицај алкохола на инсулинску секрецију и вијабилност хуманих острваца панкреаса

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SUMMARY

Introduction/Objective There are controversy data in the literature on the topic of effects of alcohol on insulin secretion, apoptosis and necrosis of the endocrine and exocrine pancreas.

The goal of this research was to determine how alcohol affects the insulin secretion and viability of human adult pancreatic islets in vitro during seven days incubation.

Methods Human pancreatic tissue was digested with Collagenase XI, using a non-automated method. Cultures were incubated in RPMI medium containing alcohol (10 μl of alcohol in 100 ml of medium). Secretory capacity (SI) and viability of the islets were determined on the first, third and seventh day of cultivation.

Results Analysis of the viability of the islets showed that there wasn't significant difference between the control and the test group. In test group viability of the cultures declined with the time of incubation. Insulin secretory capacity (SI) of the test group was higher compared to the control group, by 50% and 25% on the first and third day of cultivation respectively. On the seventh day insulin secretion was reduced by 25%. The difference was not statistically significant ($p>0.05$). In the test group, significant decline in insulin secretion was found on the third and seventh day of incubation ($p\leq0.05$).

Conclusion Alcohol can increase or decrease insulin secretion of islets cultures, which may result in an inadequate response of pancreatic β-cells to blood glucose, leading to insulin resistance, and increased risk of developing type 2 diabetes.

Keywords: alcohol; insulin secretion; viability; insulin resistance; type 2 diabetes

САЖЕТАК

Увод/Циљ У литератури постоје контрадикторни подаци на тему ефеката алкохола на лучење инсулина, апоптозе и некрозе ендокриног и егзокриног панкреаса.

Циљ овог истраживања је да се утврди како алкохол утиче на лучење инсулина и одрживост острваца адултног хуманог панкреаса ин витро током седам дана инкубације.

Методе Ткиво хуманог панкреаса је разложено колагенезом XI, користећи неаутоматизовану методу. Културе су биле инкубиране у RPMI раствору који садржи алкохол (10 μl етанола у 100 ml раствора). Секреторни капацитет и одрживост острваца су одређивани првог, трећег и седмог дана култивације.

Резултати Анализа одрживости острваца показала је да не постоји значајна разлика између контолне и тестиране групе. У тестираној групи одрживост култура опала је са временом инкубације. Капацитет инсулинске секреције тестиране групе био је већи у односу на контролну групу, за 50% првог и 25% трећег дана култивације. Седмог дана инсулинска секреција се смањила за 25%. Разлика није била статистички значајна ($p>0,05$). У тестираној групи откривен је значајан пад инсулинске секреције трећег и седмог дана инкубације ($p\leq0,05$).

Закључак Алкохол може повећати или смањити инсулинску секрецију културе острваца, што може довести до неадекватног одзива β-ћелија панкреаса на глукозу у крви, што даље доводи до инсулинске резистенције, и повећане могућности за развијање дијабетеса типа 2.

Кључне речи: алкохол; инсулинска резистенција; инсулинска секреција; одрживост; дијабетес тип 2

INTRODUCTION

Alcohol consumption is a part of the tradition and customs of many communities worldwide. It is customary to take a glass of drink as an aperitif and during or after meals wine or beer. Alcohol is taken primarily for good mood or for better digestion. The question is how and to what extent the amount of consumed alcohol affects insulin secretion of human adult pancreatic islets. Perfusion model of the rat pancreas and measure of basal insulin secretion showed that ethanol reduced glucose-induced insulin secretion by means of dose related effect [1].

Dembele et al. [2] examined the effects of ethanol and fatty acids on β-cell (cell line from rat) metabolism and survival. It was observed that both substances generate cellular oxidative stress, and affect mitochondrial function. Ethanol causes β-cell death by apoptosis while fatty acids cause cell death by necrosis predominantly. Pancreatic β-cell dysfunction is a prerequisite for the development

of type 2 diabetes Alcoholism is a risk factor and ethanol increases oxidative stress in beta-cells. Cells in a such state increase expression of PHB (poly3-hydroxybutyric acid) synthase genes, to protect them from the harmful effects of ethanol [3].

Studies in rats in vivo showed that long term alcohol feeding suppresses apoptosis in the pancreas, however, increases the sensitivity of acinar cells to endotoxin-induced injury that may cause pancreatitis in alcoholics [4]. Alcohol causes reactive hypoglycemia by attenuating the release of counter regulatory hormones, redistribution of pancreatic blood flow and direct stimulation of insulin secretion. Signaling of ethanol-induced insulin secretion from rat insulin-secreting cell lines (INS-1 and INS-1E) bypasses calcium and protein kinase C (PKC) involving steps. An extra pool of secretory vesicles not available for glucose is exploited for exocytose after ethanol stimulation [5]. Study of an in-vitro isolated rat pancreas perfusion system showed that ethanol decreases glucose-stimulated insulin secretion. Second phase secretion (30-60 min) was inhibited at both low (100 mg/dl) and high (1000 mg/dl) alcohol concentration [6]. Several studies have shown that ethanol causes insulin resistance in the liver and skeletal muscle by interfering with insulin signaling [7, 8]. One study [9], examining the influence of alcohol, ethanol concentration 20 or 80 mM for 24 or 48 hours in B-cell lines and isolated murine islets showed that chronic exposure to ethanol causes B-cell dysfunction by reducing not only insulin secretion but also B-cell insulin content by interfering with muscarinic signaling and PKC activation but not the K-ATP channels. Light-to-moderate alcohol consumption (for women <28, 28-64, >64 g/week) for healthy women was associated with enhanced insulin sensitivity, reduced basal insulin secretion rate and lower fasting plasma glucagon concentration, providing consistent mechanisms for the reduced risk of type 2 diabetes [10]. Data for the effect of alcohol consumption on insulin secretion are conflicting; some studies show the absence of an association in men [11, 12]. The final product of ethanol oxidation is acetate, which is converted to acetyl-CoA in peripheral tissue. Acetate may modulate fat oxidation and decrease lipolysis. Therefore, an increase in skeletal muscle oxidative capacity after alcohol intake has been suggested even though evidence is limited in humans [13, 14].

The aim of the present study is to examine effect of ethanol on viability and insulin secretion of human adult pancreatic islets in the culture during short-term incubation period (7 days) to solve the controversy existed in the literature.

METHODS

Human adult pancreatic tissue was procured from the Institute for Gastrointestinal Diseases,Clinical Center of Serbia, Belgrade. Tissue samples were collected from live donors, after total or subtotal pancreatectomy for cysts or tumors [15]. In tumors, healthy tissue was obtained near the line of the resection. Histopathological analysis of tissue samples showed no tumor cells. All procedures were performed in accordance with the rules of Ethical Committee of the Medical Faculty in Belgrade. Written consent was obtained from all patients.

Pancreatic tissue was transported in physiological solution from the Institute for Gastrointestinal Diseases to the Laboratory for Cells Culture in the Clinic for Endocrinology, Diabetes and Metabolic Diseases. The material was kept in the refrigerator at 4°C (cold ischaemia). Biometric data of the material (tissue weight, cold and warm ischaemia) are given in Table 1.

Table 1. Biometric data of samples.

Parameters	Tissue weight (g) mean ± SE	Warm ischemia (min.) mean ± SE	Cold ischemia (min.) mean ± SE
Control group	5.03±0.57	117.2±17.14	70.2±28.33
Group with alcohol	2.26±0.48	128.63±20.06	112.13±35.70
Statistical significance (p≤0.05)	0.195	0.061	0.1

Digestion of pancreatic tissue

For pancreatic tissue digestion we used Collagenase XI – product number C7657, Sigma Aldrich(activity >1200 collagen digestion units per mg solid; 2-5 FALGPA hydrolysis units per mg solid). This enzyme preparation also contained clostripain, nonspecific neutral protease and tryptic activities. Isolation of pancreatic islets was performed in aseptic conditions in a laminar flow hood by non-automatic method [16]. Tissue was transferred to Hanks solution (Sigma-Aldrich) and mechanically chopped. This material was collected with the pipette and put in test tubes containing the Collagenase solution (5 mg/ml concentration). The duration of incubation was 30 min at 37°C with occasional mechanical stirring. After incubation, the content of the test tube was centrifuged at 400 g for 10 min at 15°C. Supernatant was decanted and the remaining islets were rinsed several times with Hanks solution to eliminate excess of lipids and collagenase. After rinsing, islets were resuspended in the final RPMI 1640 medium (Sigma-Aldrich) supplemented with 0.1% L-glutamine, 5.5 mM glucose, 25 mM hepes, 100 U / ml penicillin, 100μg/ml streptomycin and 10% fetal calf serum (FCS , Sigma). The islets were incubated in plastic flasks (Falcon 3013, volume 50 cc) at 37˚ C in a 5% CO_2 and 95% humidity atmosphere for 7 days. RPMI medium was changed every 24 hours of incubation. Warm ischaemia time is the time measured from the beginning of the mechanical mincing of the tissue, including the isolation procedure, to the moment when the islets were placed in the culture medium. A total of 20 cultures were incubated at standard medium (control group) and 27 cultures were incubated at medium with ethanol (test group) in standard conditions for 7 days. In the test group certain concentration of ethanol was added to culture medium using Widmark formula. It was taken in consideration that a man of average weight 75 kg drinks 2 glasses (2×0.5dl) of 40% strong drink per day which would make his blood alcohol concentration 0.62 promils. The authors have chosen this formula so the influence of alcohol in vitro researches would be closest to in vivo conditions. In order to achieve specified concentrations, 10 μl of pure ethanol was added in 100 ml RPMI medium to give 0.00165 M (0.16 mM) solution of alcohol. Ethanol was added to the medium during incubation every 24 hours when replacing RPMI medium, and before the start of glukose stimulation.

Determination of the viability of isolated human adult islets

Viability of the islets was determined by dithizone (DTZ) staining on first, third and seventh day after their isolation.

Preparation of the Dithizone solution. Fifty milligrams of DTZ was dissolved in 10 ml of DMSO and 10 ml of Hanks solution. The solution was sterilized by passage through nylon filters of 0.20 μm pore size. Samples (1 ml of each culture) were stained with 0.2 ml of DTZ solution and placed in an incubator at 37°C in a 5% CO_2 and 95% humidity atmosphere for 30 min. Stained islets were rinsed in Hanks solution and resuspended in 1 ml of RPMI medium. Islets viability was determined using a stereo-light microscope and special counting micro-chambers [17].

Determination of the functional capacity and insulin secretion

To determine preservation of the functional capacity of the isolated islets, glucose-stimulated insulin secretion was measured on the first, third and seventh day of cultivation [18, 19]. A static glucose stimulation assay was performed. Samples (approximately 1000-2000 islets per culture) were incubated for 1 h in low glucose (2.8 mM/L RPMI) medium with ethanol (10 μl ethanol in 100 ml RPMI) then 1 h in high glucose (20 mM/L RPMI) medium and 1 h in low glucose medium again. After each step of stimulation, cultures were centrifuged at 400G, for 10 min at 15°C. Supernatant was decanted and stored at -18°C for insulin quantification. Insulin content was determined by radioimmunoassay (RIA INSULIN PEG). Sensitivity of the assay is 0.60 mIU/L and detection range 0.6-300 mIU/L. Relative insulin release was expressed as a stimulation index (SI) and calculated as the ratio of insulin release during high glucose stimulation to insulin release during low glucose stimulation.

Statistical analysis

All results are expressed as mean±SE. P value of less than 0.05 was considered to be statistically significant. For statistical analysis of the data has been used "Analysis of variance" (ANOVA, Fisher).

RESULTS

Islet Viability

The percentage of viable islets in the cultures was determined on the first, third and seventh day after isolation. Viability of the islets was determined by DTZ staining and results are presented in Table 2. Immediately after isolation (day 1) percentage of viable islets incubated in normal medium,

Table 2. Percentage of colored islets by culture, viability of the human adult pancreatic islets in the culture, for control group and group incubated with alcohol during seven days of cultivation.

Parameters	Time of cultivation % of viability (mean ± SE)			Comparison by days of incubation (p)		
	1st day	3rd day	7th day	1st and 3rd day	1st and 7th day	3rd and 7th day
Control group	51.60±2.16	58.00±6.49	40.0±6.27	0.376	0.119	0.081
Test group with ethanol	64.70±5.99	54.30±4.58	37..80±5.64	0.216	0.011*	0.053
p	0.090	0.654	0.800			

without alcohol (control group) and in medium containing alcohol (test group) was 51.58±2.16 and 64.68±5.99% respectively and the difference was not statistically significant (p=0.090). On the third day of cultivation, numbers of distinctly stained islets in control and test groups were similar (57.99±6.49 and 54.23±4.58% respectively, p=0.654). On the seventh day of incubation, viability was higher in the control group (39.99±6.27%) compared to the test group (37.79±5.64%) and the difference was statistically no significant (p=0.800).

Comparison of viability by days of incubation (first and third, first and seventh, third and seventh) in control group showed no statistically significant difference (p>0.05). In the test group there is statistically significant difference between first and seventh day (p=0.011).

Insulin secretion

To determine the functional capacity of the isolated islets, a static glucose test was performed on the first, third and seventh day of cultivation. Stimulation index (SI) values calculated for both groups and days of cultivation are presented in Table 3. On the first day of stimulation insulin secretion (SI) for control group was 0.60±0.13 and for test group SI was 1.22±0.27. The difference

Table 3. Insulin secretion (SI) in control group and cultures with ethanol (test group) on the first, third and seventh day of incubation.

Parameters	Time of cultivation Stimulation index (mean ± SE)			Comparison by days of incubation (p)		
	1st day	3rd day	7th day	1st and 3rd day	1st and 7th day	3rd and 7th day
Control group	0.60±0.13	0.80±0.16	0.80±0.11	0.616	0.506	0.966
Test group with ethanol	1.20±0.27	1.00±0.13	0.60±0.16	0.626	0.058	0.028*
p	0.257	0.215	0.547			

was not statistically significant (p=0.257). On the third day of stimulation SI value for control group was 0.80±0.16 and for test group was 1.01±0.13 (p=0.215). On the seventh day of stimulation, SI for control group was 0.80±0.11 and for test group SI=0.60±0.16 (p=0.547).

Comparison of functional capacity by days of incubation (first and third, first and seventh, third and seventh) in the control group showed no statistically significant difference (p>0.05). In the test group, there is a marginal statistical significance between first and seventh day (p=0.058). Between third and seventh day of cultivation there is a statistically significant difference (p=0.028), while between first and third day there is no statistically significant difference (p=0.626).

DISCUSSION

The percentage of distinctly stained cells in the cultures containing ethanol (test group) on the first day of incubation was higher by 25% compared to control group (medium without ethanol), but on the third and seventh day of incubation, the percentage of stained cells was lower in test group by 6.38 and 5.5% respectively. Since the difference is not statistically significant, we can conclude there is no difference in cell viability between the control and the test group for all days of incubation (first, third and seventh). Viability in the control group showed no statistical significance by days of

incubation. However, in the test group, viability was the highest on the first day of cultivation and declined on the third and seventh day by 16% and 42%, respectively. The difference was statistically significant (p=0.011). These results are acceptable because islet viability is determined by Dithizon (DTZ) which binds to zinc in insulin. On the first day of incubation, B cells contain the highest concentration of insulin. In test group alcohol increases the permeability of the membrane and facilitates degranulation and the release of insulin, so depot of insulin is gradually reduced by the length of incubation (from the first to the seventh day).

Insulin secretory capacity (SI) was higher in the test group compared to control group by 50% and 25% on the first and third day of incubation respectively, while on the seventh day SI was lower by 25% in the the test group. There is no statistically significant difference in insulin secretory capacity between control and test groups. However, in the test group there is a decline in insulin secretion during cultivation by 17% and 50% on the third and seventh day compared to the first day (p=0.058). There is also statistically significant decline in insulin secretion by 40% between third and seventh day (p=0.028).

Similar results are presented in the paper [9], where insulin secretion in cell culture increases after 24 h incubation with lower alcohol concentration (20 mM). However, higher concentration of alcohol (80 mM) has the opposite effect. After 48 h incubation, both concentration of alcohol reduce the level of insulin secretion compared to the control group. Results of other authors [1, 6], confirm that ethanol reduces insulin secretion.It should be emphasized that reduction in the number of stimulated beta cells during incubation due to the effects of natural processes of apoptosis and necrosis does not affect the obtained values of insulin secretion, which are expressed in the form of secretory index (SI). SI represents the ratio between the values of insulin secretion after stimulation with high glucose concentration and values after low glucose stimulation. Thus secretory capacity does not depend of the total number of stimulated cells. Although in laboratories worldwide there is a practice to associate SI values with a strict number of stimulated cells [19].

Some authors try to explain how alcohol causes increased or decreased insulin secretion in response to glucose stimulation. Stimulation of insulin secretion with diacylglycerol is explained by the release of Ca from endoplasmic reticulum [20]. Response to high glucose stimulation decreases due to reduced levels of cAMP caused by reduced levels of ATP [2, 3, 21].

In standard conditions glucose-stimulated insulin secretion increases oxygen consumption and ATP. This is associated with potassium (K +)-induced membrane depolarization, leading to rapid entry of Ca2 + ions into the cell through voltage-dependent channels. Fusion of secretory granules containing insulin with the cell membrane depends of calcium ions. Cyclic AMP emphasizes action of glucose and amino acids. Cyclic AMP stimulates release of Ca2 + ions from intracellular organelles or may activate a kinase that phosphorylates one of the components of micro-filaments-tubules system, so this structure becomes contractile and sensitive to Ca2 +ions. If the Na + outside the cell is replaced with other monovalent cation, then the effects of glucose and other secretagogues are

reduced. Na^+ concentration may regulate the intracellular Ca^{2+} using a co-transportation system [21]. It is possible that the effect of certain concentrations of ethanol somehow bypasses the dependence of Ca- release from the synthesis of cAMP.

Higher SI values in test group (SI higher by 50% on the first day of incubation) mean increased insulin secretion of pancreatic islets in response to high glucose stimulation (see methods and materials). Probably alcohol consumed immediately before or during ingestion, can have a protective effect in type 2 diabetes because increased insulin release reduces the harmful effects of postprandial hyperglycemia. It is known that hyperglycemia has a toxic effect on B cells because it increases the percentage of their apoptosis [22]. The body is struggling to maintain a normal blood sugar level, so a chronic state of hypoglycemia occurred after chronic alcohol consumption, especially if alcohol is taken without food, can lead to insulin resistance, which exists in the pathogenesis of diabetes type 2 [23].Acute alcohol consumption increases insulin secretory capacity of islets, which could lead to hypoglycemia in patients with normal glycemic control. In vivo researches had shown that ethanol increased insulin secretion during glucose tolerance test [24, 25].

Symptoms of hypoglycemia are very similar to the symptoms of alcohol condition. But alcohol consumption after meals may improve insulin secretion thereby reducing potential harmful effects of increased concentrations of blood glucose. Consumption of alcohol without food increases insulin secretion and leads to discharge depot of insulin, which can lead to insulin resistance. The effect of alcohol mainly depends on the administered dose in the body. Adverse effects are manifested in the increased consumption of alcohol causing accumulation of nitric oxide (NO) in the organism that has a detrimental effect on the whole body. Increased NO synthesis under the influence of inducible nitric oxide synthase (iNOS) is associated with various cytotoxic damages [26]. Larger amounts of NO produced in macrophages that infiltrate the endocrine pancreas tissue can lead to damage of B cells and increased apoptosis of these cells [27]. In the initiating of apoptosis particular importance has accumulation of calcium ions in the cytosol [26].Also, long-term alcohol consumption could lead to beta cell sensitivity to the presence of endotoxin in the blood thereby increasing the percentage of necrosis of pancreatic islets [2, 4]. It is known that the presence of bacteria in the pancreas can also affect insulin secretion and the development of diabetes [28] and chronic alcohol consumption would only intesify this effect.

Our experiment showed that insulin secretion decline during cultivation, especially on the seventh day (Table 3). Inadequate response to glucose stimulation may lead to hyperglycemia and insulin resistance that are prerequisite for development of diabetes type 2. Similar results were obtained by other authors [29-32]. In addition to the impact on carbohydrate metabolism, experiments on rats showed considerable impact of alcohol on lipid metabolism. These results demonstrated that visceral fat is more susceptible to alcohol toxicity compared to subcutaneous fat, and disruption of adipose lipogenesis contributes to the pathogenesis of alcoholic lipodystrophy [33].

CONCLUSION

Results suggesting a protective role of alcohol consumption in the development of type 2 diabetes [34, 35, 36], should be interpreted with caution. To determine the true impact of alcohol on insulin secretion, results obtained on animal models and animal cell lines should be taken with reserve,because alcohol consumption is characteristic of humans and there is difference in body metabolism. Considering authors express alcohol concentration in different units, perhaps there is a need for standardization.

Based on the presented results we can conclude that alcohol concentration 0.165mM slightly increases insulin secretion in the culture on the first and third day of incubation. Longer cultivation, the greater decline in insulin secretion in the test group compared to control group. The effect of alcohol on insulin secretion is most noticeable in the test group, clearly showing the long-term effects of alcohol, which causes a decline in insulin secretion.

Alcohol can increase or decrease insulin secretion of human pancreatic islets, which may result in an inadequate response of pancreatic B-cells to blood glucose, leading to insulin resistance, and increased risk of developing type 2 diabetes.

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