Zagayko A and Briukhanova T, J Cell Biol Cell Metab 2015, 2: 006
DOI: 10.24966/CBCM-1943/100006

HSOA Journal of Cell Biology and Cell Metabolism

Research Type

Influence of 1-(4-Chlorophenyl)-N, N-Dimethyl-Alpha-(2-Methylpropyl) Ciclo Butanmethanamine (Sibutramine) to the Cytokine Profile of Blood Serum at Experimental Insulin Resistance in Rats

Andrey Zagayko* and Tatiana Briukhanova

Biochemistry Department, National University of Pharmacy, Ukraine

Abstract

Obesity is the serious problem of today health system because it is one of the trigger insulin resistance and IR-associated diseases. In this scientific paper the mechanisms of sibutramine therapeutic benefit realization in the course of insulin resistance were studied by examining its influence on cytokine profile, glucose profile, regulatory hormones content in the blood of animals. Oral administration of sibutramine for 3 weeks accompanied with normalization of such parameters as glucose, immunoreactive insulin, HOMA-IR index. The preparation increased the level of adiponectin and serotonin and decreased resistin content, IL-6, tumour necrosis factor α, cortisol. The described changes of the parameters can explain mechanism of sibutramine therapeutic benefit realization at the model of immune resistance.

Keywords: Cytokines; Insulin resistance; Sibutramine

Introduction

Nowadays obesity is one of the most significant problems of today health system. According to the World Health Organization data the rate of this abnormality distribution grows every year and according to the expert broadcast the number of overweight patients is to be 2.3 billion by the year of 2016 [1]. Obesity has strong correlation to Insulin Resistance syndrome (IR) development and other complications associated with cells desensitization to insulin action, such as metabolic syndrome, atherosclerosis, cardiovascular pathology and others [2]. Obesity formation is accompanied by a range of pathological changes in the lipolysis/lipogenesis processes and fatty tissue metabolic imbalance [3].

One of the key elements of IR development pathogenesis is lypotoxicity of Free Fatty Acids (FFA) that released actively from adipose tissue due to lipolysis activation and disbalance of humoral factors secreted by adipocytes [4]. Adipose tissue produces different groups of active molecules: adipokines (adiponectin, resistin, visfatin and others), pro-inflammatory cytokines (Tumor Necrosis Factor - TNF-α; TNF-β, neutrophil-activating factor - IL-6, IL-8), inflammatory markers (fibrinogen, C-reactive protein), complement component (C3, factor B and D), renin-angiotensin system components (angiotensinogen, angiotensin II), plasminogen activator inhibitor - 1 and others [5].

Under obesity conditions progressed adipokines secretion balance disturbance expresses by adiponectin secretion decrease plays an important role in IR development and atherogenic dyslipidemia [6]. According to the literature data adipose tissue mass has negative correlation with adiponectin content. This adipokine increases sensitivity of hepatocytes and myocytes to insulin by reduction of intracellular content of triacylglyceroles (increasing gene expression of fatty acid oxidation key enzymes) [7]. Depressed glucose production by liver cells blocks pre-adipocytes differentiation, expresses antiatherogenic action (decreases secretion of Very Low Density Lipoproteins - VLDL, decreases intensity of triacylglicerolemia and monocytes adhesion to aorta endothelin) and expresses anti-inflammatory action (reduces TNF-α production by macrophagocytes, blocks nuclear factor NF-κB activation).

Resistin is an adipokine that expresses opposite action of cells sensitivity to insulin. It neutralizes inhibitory effect of insulin to glucose production in the liver and depresses glucose absorption by skeletal muscles that lead to IR development. Resistin increases gene expression of gluconeogenesis enzymes in hepatocytes [8].

Pro-inflammatory cytokines production by adipocytes (IL-6, TNF-α and TNF-β) increases the obesity development. Their content have direct correlation with the quantity of adipose tissue. IL-6 realizes its inflammatory activity by acute phase proteins synthesis stimulation and activation of hypothalamo-pituitary-adrenal axis (that occurs due to the increase of cortisol secretion and hypercortisolemia), increase of VLDL secretion (atherogenic dyslipidemia formation), somatotropin level rising, and lipoprotein lipase activity decrease. Increase of IL-6 content in the blood leads to hyperglycaemia and strengths of glucose stimulating effect to insulin release that in complex leads to IR. Tumor necrosis factor blocks tyrosine kinase activity of insulin receptor and suppresses expression of glucose intracellular carriers in the muscles. Due to TNF-α possesses direct inhibitory effect to insulin receptors this cytokine can be considered as the powerful IR mediator formed in obesity conditions. Besides, TNF-α neutralizes adiponectin activity that exacerbate IR course. TNF-β mediates increase of pre-adipocytes' proliferation and disorder of adipose tissue morphology, its secretion

***Corresponding author:** Andrey Zagayko, Biochemistry Department, National University of Pharmacy, Ukraine, Tel: +38 0577063066; E-mail: andrey.zagayko@gmail.com

Citation: Zagayko A, Briukhanova T (2015) Influence of 1-(4-Chlorophenyl)-N, N-Dimethyl-Alpha-(2-Methylpropyl) Ciclo Butanmethanamine (Sibutramine) to the Cytokine Profile of Blood Serum at Experimental Insulin Resistance in Rats. J Cell Biol Cell Metab 2: 006.

Received: April 04, 2015; **Accepted:** July 09, 2015; **Published:** August 27, 2015

is increased under obesity and it inversely makes contribution to IR progression [9].

The increased production of inflammatory cytokines activates hypothalamo-pituitary-adrenal axis due to increase of glucocorticoids secretion [10].

According to the literature data increase of cortisol content in the blood plays one of the key roles in IR development. This hormone activates lipolysis processes in peripheral tissues and lipogenesis in the liver. Lipolysis intensification exhibits by increase of free fatty acids content. This effect can be explained both direct stimulating effect to lipolysis and indirect effect by strengthen of catecholamine secretion and their lipolytic action through cAMP - related mechanism. Developed under these conditions hypercortisolemia leads to abdominal obesity development by influence on cortisol-dependent lipoprotein lipase. This lipase is mainly localized at adipocytes capillary tube of the upper body. The big amount of released free fatty acids can activate different signal pathways in cells, including MARK-related protein kinases, by increase of reactive oxygen intermediate content and NF-κB activation.

Sibutramine is a medicine that effects on central mechanisms of obesity formation by noradrenaline and serotonin uptake inhibition and activation of β3-adrenoreceptors of brown adipose tissue that exhibits by strengthen of thermogenesis [11,12]. In the range of researches the efficiency of this medicine at obesity in adults and children under different by therapeutic durations conditions have been studied [3,13-15]. But the mechanism of this medicine therapeutic action to IR is still not clarified. In this investigation the supposed mechanisms of pharmacological efficiency of sibutramine in 3 weeks injections under the IR model in rats has been studied by examining the cytokines profile dynamics and individual hormone parameters.

Materials and Methods

In the experiments was used the male wistar rats weighting 160 g - 200 g, kept in the vivarium of the Central Research Laboratory of the National University of Pharmacy under the temperature of 22±1°C; residual humidity 50-60%, in the room with the changeable light conditions "day-night'. The experiments were carried out in accordance with European Convention for the protection of vertebrate animals used for experimental purposes and other scientific ones [16] and code of ethic of world medical association (Declaration of Helsinki, 1964).

The animals were divided into three experimental groups by 10 ones in each group according to the purposes of the experiment: intact control (healthy animals kept at standard diet of vivarium); control abnormality - animals with IR simulating by daily intraperitoneal injection of dexamethasone, dose 15 mg/kg, during 5 weeks and kept at fructose reached diet (60.3% - fructose, 18.3% - proteins, 5.2% - fat); sibutramine group - animals with IR simulating by daily intraperitoneal injection of dexamethasone, dose 1.5 mg/kg, during 5 weeks and kept at fructose reached diet (60.3% - fructose, 18.3% - protein, 5.2% - fat) and starting from the 4th week of dexamethasone injection add oral daily water suspension of sibutramine hydrochloride, in dose 10 mg/kg of animal weight for 3 weeks. At the end of the experiment, rats were decapitated under chloralose-urethane anesthesia, blood was collected to obtain blood serum.

Glucose concentration was determined by glucose oxidase test (Sigma, USA), Immunoreactive Insulin (IRI) content by radioassay *in vitro* using the standard reagent kit (Linco Research, USA). The parameter of IR index HOMA-IR was calculated by determination of glucose level and IRI in blood serum in the fasted state using HOMA algorithm (Homeostatic Model Assessment). Concentration of adiponectin and resistin was measured with the standard reagent kit Quintikine M Kit (R&D Systems Inc.). Cortisol content was determined with the standard reagent kit manufactured by Neogen Corporation (USA). Determination of serotonin, IL-6, TNF-α was carried out using reagent kits manufactured by Cusabio biotech Co., Ltd (China).

Statistical computation of the obtained data was carried out using the STATISTICA program (StatSoftInc., USA, version 6.0). The importance of intergroup differences was estimated by Mann-Whitney non-parametric test.

Results and Discussion

Long-term administration of dexamethasone small doses along with keeping the animals at high-caloric fructose-enriched diet was accompanied with formation of a range of pathological complication specific for obesity and IR syndrome. Hyperglycaemia and hyperinsulinemia occurred, the IR index increased in 1.69 times (see table 1). Sibutramine injection leads to normalization of the above mentioned parameters that hadn't been adequately differ from intact control. Such dynamics of the parameters can be explained by depressed influence of the preparation to the orexia expressed by body mass decrease and as the result of obesity correction as the factor of IR pathogenesis. Orexia regulation by sibutramine is carried out by noradrenergic and serotonergic mechanisms. Anorexigenic effect of the preparation may realize through influence on hormone synthesis and/or secretion that regulate saturation and energy balance (leptin, glucagon-like peptide-1, pancreatic peptide tyrosile-tyrosine and others).

Parameter/Group	Intact control	Model pathology	Sibutramine treated
Glucose, mmol/L	5.800±0.440	11.200±0.390*	9.360±0.360**
IRI, pmol/l	92.500±2.305	138.280±2.406*	115.800±3.205**
HOMA-IR	1.78	3.02	2.44

Table 1: Influence of sibutramine to the glucose content, IRI and changes of HOMA-IR index under experimental insulin resistance in rats.

* - changes are accurate regarding parameters to intact control (p≤0.05)

** - changes are accurate regarding parameters to model pathology (p≤0.05)

Therapeutic effect of sibutramine exhibits in positive effect to cytokine and hormone profiles. The preparation injection was accompanied by accurate increase of adiponectin level that in its turn reduced the rate of gluconeogenesis in the liver (decrease of hyperglycaemia intensity), activated carrier fatty acids and β-oxidation of fatty acid in hepatocytes and myocytes (decrease of free fatty acids lipotoxicity), increased cells sensitivity to insulin (hyperglycemia elimination and mediated elimination of hyperinsulinemia), blocked pre-adipocytes differentiation (eliminating TNF-β pathological effects). Besides, adiponectin level has negative correlation with Carnitin-Palmitoyl Transferase-1 activity (CPT-1) that participates in lipids hepatic metabolism (along with 5' Adenosine Monophosphate-activated Protein Kinase - AMPK). As can be seen from the above mentione information sibutramine eliminates activation of CPT-1 signaling pathway and

lipids oxidation. Sibutramine treatment accompanied by resistin level lowering that provokes IR development and progression. It acted as the additional mechanism of realization the therapeutic benefit of the preparation.

It is already known that the obesity is accompanied by inflammation that is mediated by pro-inflammatory cytokines and IL-6 and TNF-α have the leading roles among them. Activation of the above mentioned factors lead to pathological effects cascade: activation of hypothalamo-pituitary-adrenal axis (account for the increase of cortisol level), stimulation of leptin production (hyperleptinemia development and feeding behavior regulation disorder), oxidative stress development, VLDL secretion and liver cholesterol production increasing (atherogenic dyslipidemia), lipogenesis activity decreasing (by suppression of corresponding enzymes) and others. Sibutramine application has substantially normalized IL-6 and TNF-α level that in its turn exhibited in elimination of their negative effect.

Based on the data obtained during our experiment we can suggest that the sibutramine has the ability to eliminate inflammatory expression mediated by IL-6 and TNF-α influence under obesity. This fact hasn't been studied earlier. Besides feeding behavior correction (by hyperleptinemia elimination that was demonstrated in the range of the paper works) sibutramine had correlation with atherogenic dyslipidemia appearance that also agreed with the data of our experiments. The influence of sibutramine to lipids and lipoproteins metabolism was studied in these experiments.

The studied preparation decreases the cortisol content that has the positive effect to animals' state and leads to diminishing of IR activity. Cortisol stimulates lipolysis and releasing of FFA, increases their oxidation in the liver. The excess of acetyl CoA could be used in ketogenesis. In addition, cortisol increases lipolytic action of catecholamines via a cAMP-dependent mechanism. The administration of sibutramine has diminished these effects and accordingly diminished the IR activity. Positive influence on hypercortisolemia is mediated by several mechanisms: firstly, suppressive influence on pro-inflammatory cytokines production; secondly, decrease of hyperglycemia and hyperinsulinemia development due to obesity correction that makes conditions to decrease of contrinsular hormones (cortisol) secretion.

Glucocorticoids stimulate food consumption by neuropeptide Y influence that is the significant stimulant of feeding behavior [17]. So as sibutramine decreases the cortisol serum content we can speak about its indirect effect on neuropeptide Y activity, but this fact required more detailed study.

Besides neuropeptides it is also known that biogenic amines especially serotonin possesses expressed influence on feeding behavior regulation.

Interaction of 5-HT serotonine neurone interaction in the encephalon and food consumption control is demonstrated in the range of the paper works. So as sibutramine pharmacodynamics is based on neuronal uptake of noradrenaline the increase of this monoamine level in animals was characteristic.

Parameter	Intact control	Model pathology	Sibutramine treatment
Cortisol, ng/ml	335.67±14.56	571.95±19.75*	352.3±12.86**
Serotonin, ng/ml	23.65±1.34	12.87±1.24*	25.66±1.98**
Adiponectin, ng/ml	278.95±17.13	146.67±12.78*	256.45±11.98**
Resistin, ng/ml	104.55±9.08	171.44±12.13*	119.09±9.88**
IL-6, pg/ml	0.43±0.08	1.87±0.12*	0.66±0.03**
TNF-α, pg/ml	8.42±0.15	10.04±0.12*	8.87±0.11**

Table 2: Sibutramine influence on different regulatory hormones and cytokines level content at experimental insulin resistance in rats.
* - changes are accurate regarding parameters to intact control (p≤0.05)
** - changes are accurate regarding parameters to model pathology (p≤0.05)

Conclusion

Some mechanisms of sibutramine anorectic action under the model of experimental insulin resistance in rats due to examining the dynamics of cytokine profile and hormonal profile of the blood are studied in the present work. Medication administration during 3 weeks allow to minimize risks connected with cardiovascular complications have been presented at long-term sibutramine administration (more than 3 months). The results obtained in the experiment help to consider sibutramine not only as the medication for short-term therapy of the obesity and IR-related diseases and also as the comparator agent in the clinical and pre-clinical trials of new medicinal preparations that have influence on the food behavior for the investigation it mechanisms of action.

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