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Effectiveness of metformin in combination with intranasal insulin for the treatment of metabolic and hormonal disturbances in adult male rats with metabolic syndrome induced by impaired breastfeeding

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ABSTRACT

BACKGROUND: Limiting or temporally stopping breastfeeding can lead to the development of metabolic syndrome in adulthood, which requires the development of approaches for its prevention and correction. One such approach is treatment with metformin or intranasal insulin. Since the targets of these agents differ and may complement each other, it has been suggested that their combined use could be effective.

AIM: To study the effect of a four-week co-administration of metformin (orally, 120 mg/kg/day) and intranasal insulin (1.2 IU/kg/day) in male rats with metabolic syndrome, induced by breastfeeding disruption on postnatal days 19–21, on their metabolic and hormonal parameters.

MATERIALS AND METHODS: The study treatment was compared with monotherapy using the same drugs.

RESULTS: It was found that adult male rats with disrupted breastfeeding developed obesity, dyslipidemia, hyperleptinemia, impaired glucose tolerance, and a reduction in the number of β -cells and the area of pancreatic islets, which are characteristic of metabolic syndrome. Long-term treatment with metformin and its combination with intranasal insulin partially or fully normalized body weight, abdominal fat, and metabolic and hormonal parameters, with the restorative effect of combination treatment on such parameters as body weight, fat mass, glucose tolerance, and blood glycated hemoglobin levels being more pronounced than with metformin alone.

CONCLUSIONS: The results of the study support the use of a combination of metformin and intranasal insulin to normalize metabolic and hormonal parameters in metabolic syndrome induced by breastfeeding disruption in early days of life.

Keywords: metformin; intranasal insulin; metabolic syndrome; hyperleptinemia; glucose tolerance; breastfeeding.

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Эффективность комбинации метформина и интраназального инсулина для коррекции метаболических и гормональных нарушений у взрослых самцов крыс с метаболическим синдромом, вызванным нарушением грудного вскармливания

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АННОТАЦИЯ

Актуальность. Ограничение или краткосрочное прекращение грудного вскармливания может приводить к развитию метаболического синдрома во взрослом возрасте, что требует разработки подходов для его предотвращения и коррекции. Одним из них является лечение метформином или интраназально вводимым инсулином. Поскольку мишени этих препаратов различаются и они могут взаимодополнять друг друга, то было высказано предположение об эффективности их комбинированного использования.

Цель — изучение влияния четырехнедельного совместного введения метформина (перорально, в дозе 120 мг/кг × сут) и интраназально вводимого инсулина (в дозе 1,2 МЕ/кг × сут) самцам крыс с метаболическим синдромом, обусловленным прерыванием грудного вскармливания в 19–21-й дни постнатального развития, на их метаболические и гормональные показатели.

Материалы и методы. Исследование проводили в сравнении с монотерапией теми же препаратами.

Результаты. Установлено, что у взрослых самцов крыс с нарушением грудного вскармливания развиваются ожирение, дислипидемия и гиперлептинемия, нарушается толерантность к глюкозе, снижаются число β -клеток и площадь панкреатических островков, что характерно для метаболического синдрома. Длительное лечение с помощью метформина и его комбинации с интраназально вводимым инсулином частично или полностью нормализовало массу тела и абдоминального жира, метаболические и гормональные показатели, причем восстанавливающий эффект комбинированной терапии в отношении таких показателей, как масса тела и жировой ткани, толерантность к глюкозе и уровень гликированного гемоглобина в крови, был более выражен, чем таковой монотерапии метформином.

Заключение. Результаты исследования свидетельствуют в пользу перспективности применения комбинации метформина и интраназально вводимого инсулина для нормализации метаболических и гормональных нарушений при метаболическом синдроме, вызванном дефицитом грудного вскармливания в раннем онтогенезе.

Ключевые слова: метформин; интраназальный инсулин; метаболический синдром; гиперлептинемия; глюкозотолерантность; грудное вскармливание.

Как цитировать

Деркач К.В., Иванцов А.О., Басова Н.Е., Шпаков А.О. Эффективность комбинации метформина и интраназального инсулина для коррекции метаболических и гормональных нарушений у взрослых самцов крыс с метаболическим синдромом, вызванным нарушением грудного вскармливания // Обзоры по клинической фармакологии и лекарственной терапии. 2024. Т. 22, № 3. С. 289–300. DOI: <https://doi.org/10.17816/RCF626249>

BACKGROUND

Eating disorders in early childhood, including insufficient or, on the contrary, excessive food intake, as well as replacement of breast milk with artificial formula, causes functional changes in the formation of endocrine and other body systems, which in adulthood can lead to metabolic and hormonal disorders, including obesity and metabolic syndrome (MS). Accordingly, one of the most important tasks of modern medicine is to study the mechanisms of development of metabolic, hormonal and endocrine dysfunctions caused by nutritional disorders at an early age, as well as the search for effective ways to correct them in adulthood.

A suitable model to investigate the relationship between early postnatal eating disorder and delayed metabolic and hormonal disorders is the cessation of breastfeeding in rats from Days 19 to 21 of postnatal development by bromocriptine- or methylglyoxal-induced suppression of lactation in lactating females [1, 2]. As a result, mature animals develop obesity and some signs of MS, as demonstrated previously both by us [3, 4] and other authors [1, 2].

One of the reasons for metabolic and hormonal disorders in adulthood caused by the cessation of breastfeeding is impaired insulin signaling in the brain, which can be at least partially remedied by administering intranasal insulin (INI) to rats, immediately after a fasting period in the early postnatal period [4]. However, from a practical point of view, the use of INI therapy at an early age is difficult to realize, especially given the absence of clear signs of metabolic and hormonal disorders that develop significantly later. In this regard, it is noteworthy that INI appears to be ineffective at a later age without significantly affecting the pattern of neonatally programmed disorders [4]. Therefore, it is important to find effective ways to correct already developed MS, for which metformin (MF) therapy can be used, which, as we have shown, restores a number of metabolic and hormonal parameters in adult male rats with restricted early breastfeeding [3]. There are numerous evidences of high efficacy of MF in the clinical settings for the correction of MS and type 2 diabetes mellitus (T2DM), making it one of the most widely used pharmacological agents for this purpose [5–7]. However, the use of MF has a number of limitations due to serious side effects when using its relatively high doses [8, 9]. Therefore, the actual task is to reduce the dose of MF by using its combinations with other drug products that enhance its therapeutic effect. A combination of MF and INI may be promising. This combination allows, on the one hand, normalizing the body weight, improving the metabolic status and increasing the systemic insulin sensitivity (when using MF), and on the other hand, normalizing the activity of the brain insulin system reduced in various forms of MS,

thus preventing neurodegenerative processes in the brain and improving central insulin regulation of physiological functions (when using INI). To date, under experimental conditions, the combination of MF and INI has only been used to correct abnormalities in diet-induced DM2 [10] and contraceptive-induced MS [11], and to attenuate insulin resistance (IR) induced by dexamethasone treatment of rats [12].

The aim is to study the effect of long-term MF therapy in combination with INI for the correction of metabolic and hormonal parameters disturbed in adult male rats with MS caused by short-term interruption of breastfeeding, and to perform this study in comparison with MF and INI monotherapy at the same doses and of the same duration.

MATERIALS AND METHODS

For experiments on MS induction and its treatment with MF and INI, male Wistar rats were used jointly and separately and kept in standard vivarium conditions with free access to water and dry food. All procedures were performed in full compliance with the requirements of the Bioethics Committee of the Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences (Protocol No. 2-1/2023 of January 26, 2023), as well as with the European Communities Council Directive (1986, 86/609/EEC) and the Guide for the Care and Use of Laboratory Animals.

The MS model induced by interruption of breastfeeding in rats, denoted as the “neonatal” model, was induced according to our previously described methodology [3, 4]. Male rats were deprived of both breastfeeding and other food sources for three days, from Days 19 to 21 of postnatal development, followed by transfer to standard dry food on Day 22. Breastfeeding was interrupted by treating lactating females with bromocriptine mesylate, which was administered daily, for 3 days, orally through a tube, at a daily dose of 10 mg/kg. As a result, lactation ceased, and instead of breast milk, females began to secrete colostrum, which did not contain the nutrients, hormonal agents and micronutrients, which are contained in breast milk and required for the full growth and development of rats. The control group consisted of male immature rats with normal breastfeeding regimen fed by females receiving placebo instead of bromocriptine mesylate. Subsequently, male immature rats were kept on a standard diet until six months of age, when some of the animals began to develop signs of MS. The body weight and postprandial blood glucose levels were assessed to select animals with severe MS. Glucose concentration was measured using test strips and a Life Scan glucometer (Johnson & Johnson, Denmark). Animals with a body weight that was increased by at least 8% compared to the control rats and with a postprandial blood glucose

level of at least 6.0 mmol/L were classified as animals with MS, randomly assigning them to experimental groups.

The following groups (6 rats in each group) were formed: control (C), where animals received 0.9% sodium chloride instead of the drug products; untreated MS (MS), MS with 4-week treatment with MF (orally, 120 mg/kg $\times$ day; MS-M), MS with 4-week treatment with INI (1.2 IU/kg $\times$ day; MS-I), and MS with combined treatment with MF and INI at the same doses (MS-MI). For intranasal administration, insulin was dissolved at the required concentration in 0.1 M Na-citrate buffer (pH 4.5) and, turning the rat on its back, the solution was injected into both nostrils (approximately 10–20 μ L into each nostril) as previously described [13]. MF was dissolved in water and administered by gastric tube. Glucose tolerance was assessed 3 days before the end of the experiment using the intraperitoneal glucose tolerance test (IpGTT). During IpGTT, rats were intraperitoneally injected with 40% glucose solution at a total dose of 2 g/kg and blood glucose levels were assessed for 120 min after glucose load. Blood insulin and leptin levels were also measured before glucose load and 120 min after it using ELISA kits Rat Insulin ELISA (Mercodia, Sweden) and ELISA for Leptin, Rat (Cloud-Clone Corp., USA). Blood was withdrawn from the tail vein under local anesthesia using 3 mg/kg lidocaine solution.

Before the end of the experiment, the level of free fatty acids was measured in rat blood (in the fasted state) using NEFA FS kit (DiaSys, Germany), glycated hemoglobin content using Multi Test HbA1c System kit (Polymer Technology Systems, USA), triglycerides and total cholesterol concentration using Triglycerides multiCare-in and Cholesterol multiCare-in test strips (Biochemical Systems International, Italy). Rats were then anesthetized (chloral hydrate, 400 mg/kg, intraperitoneally), decapitated, pancreatic tissue was harvested to evaluate the morphology of pancreatic islets, and abdominal fat mass was estimated.

Pancreatic tissue samples were fixed in formalin (10% final concentration) dissolved in 50 mM Na-phosphate buffer, pH 7.4, then used for histologic analysis. Histochemical staining of pancreatic islets with hematoxylin and eosin was performed using Thermo Scientific Lab Vision Autostainer 720 (Thermo Fisher Scientific, USA). Morphometric evaluation of images was performed using AxioVision software (Carl Zeiss, Germany). The number of pancreatic islets, their diameter (in microns) were estimated, and the product of these parameters was used to quantify the area of islets.

Statistical analysis was performed using the IBM SPSS Statistics 26 program package (IBM, USA). Normality of distribution was tested using the Shapiro-Wilk test and Levene's test for equality of variances was used. Intergroup comparisons were performed using one-factor

analysis of variance with Tukey's test. Differences were considered significant at $p < 0.05$. Results were presented as the mean and error of the mean ($M \pm SEM$).

RESULTS

Adult male rats with the neonatal MS model had significantly increased total body weight and abdominal fat mass. Triglycerides and total cholesterol levels were also elevated, and there was a trend toward higher levels of free fatty acids, indicating signs of dyslipidemia. According to the IpGTT results, glucose tolerance was impaired in the MS group, as indicated by increased glucose concentrations 120 min after glucose load and increased AUC_{0–120} values (integrated area under the glucose curve in IpGTT) (Table 1, Figure 1). Along with this, there was an increase in glycated hemoglobin in the blood and a tendency to increased fasting glucose levels, although the differences with controls in this case were not significant.

In addition to metabolic disorders in rats of the MS group, changes in hormonal parameters were noted. They had significantly elevated basal and glucose-stimulated levels of leptin, which is produced by white fat adipocytes and regulates eating behavior and peripheral energy metabolism, evidencing the development of leptin resistance. Rather unexpectedly, baseline insulin levels in rats with MS did not differ from the controls, and a significant increase was detected only 120 min after glucose load. At the same time, the insulin resistance index, calculated as the product of baseline insulin and glucose concentrations, in the MS group did not differ from the control, indicating the absence of obvious signs of insulin resistance in this MS model (Table 2).

To elucidate possible reasons for the lack of baseline hyperinsulinemia in rats with MS, the morphology of pancreatic islets was examined using histologic analysis. The total number of islets was reduced in the MS group and the product of the number of islets by their size (diameter) was reduced by 42%, allowing estimating their total area (Table 3). The data obtained indicate that the number of insulin-producing pancreatic cells in rats with the neonatal model of MS is reduced, which we suggest is the reason for the near-normal baseline insulin levels.

Next, we studied the effects of four weeks of combination therapy of MF (orally, 120 mg/kg $\times$ day) and INI (1.2 IU/kg $\times$ day) on the metabolic and hormonal disorders we identified in MS rats. The study included comparison with monotherapy with the same drug products at the same doses. MF monotherapy normalized body weight, lipid metabolism parameters (levels of triglycerides, cholesterol and free fatty acids), restored glucose sensitivity, which is illustrated by normalization of blood glucose level 120 min after glucose load

Table 1. Body weight and abdominal fat mass, lipid content, glycated hemoglobin levels, as well as AUC₀₋₁₂₀ values for glucose curves in adult male rats with the “neonatal” model of metabolic syndrome and the effects of treatment with metformin, intranasal insulin, and their combination on these parameters, $M \pm SEM$

Таблица 1. Масса тела и абдоминального жира, содержание липидов, гликированного гемоглобина, а также значение AUC₀₋₁₂₀ для глюкозных кривых у взрослых самцов крыс с «неонатальной» моделью метаболического синдрома и влияние на эти показатели лечения метформином и интраназальным инсулином и их комбинацией, $M \pm SEM$

Parameter	Groups				
	C (n = 6)	MS (n = 6)	MS-M (n = 6)	MS-I (n = 6)	MS-MI (n = 6)
Body weight, g	401 ± 7	461 ± 10*	429 ± 9	456 ± 12*	412 ± 10**.#
Abdominal fat mass, g	6.9 ± 0.4	11.0 ± 0.7*	7.9 ± 0.7	9.3 ± 0.8*	7.0 ± 0.3**.#
Triglycerides, mM	2.15 ± 0.10	3.34 ± 0.15*	2.53 ± 0.08**	2.94 ± 0.09*	2.46 ± 0.12**.#
Total cholesterol, mM	4.52 ± 0.09	5.66 ± 0.21*	4.65 ± 0.19**	4.75 ± 0.25**	4.62 ± 0.11**
Free fatty acids, mM	3.92 ± 0.24	4.75 ± 0.35	4.32 ± 0.19	4.05 ± 0.25	4.15 ± 0.26
HbA1c, %	4.53 ± 0.21	5.43 ± 0.16*	4.75 ± 0.29	4.45 ± 0.06**	4.33 ± 0.15**
Glucose (0 min), mM	4.05 ± 0.15	4.75 ± 0.29	4.27 ± 0.17	4.37 ± 0.29	4.23 ± 0.20
Glucose (120 min), mM	4.52 ± 0.17	5.73 ± 0.22*	4.72 ± 0.18**	4.88 ± 0.32	4.23 ± 0.12**
AUC ₀₋₁₂₀ , c.u.	1126 ± 70	1420 ± 37*	1225 ± 52	1249 ± 51	1063 ± 36**.#

Note. C, control; MS, untreated metabolic syndrome; MS-M, MS with 4-week metformin treatment; MS-M, MS with 4-week intranasal insulin treatment; MS-MI, MS with combined metformin and intranasal insulin treatment; AUC₀₋₁₂₀, area under the curve “glucose concentration, mmol/l — time, min” (in arbitrary units) in the intraperitoneal glucose tolerance test. Differences with the control (*) and MS group (**), as well as between the MS-M and MS-MI groups (#) or between the MS-I and MS-MI groups (##) are statistically significant at $p < 0.05$.

Примечание. C — контроль; MS — метаболический синдром без лечения; MS-M — MS с 4-недельным лечением метформином; MS-I — MS с 4-недельным лечением интраназальным инсулином; MS-MI — MS с совместным лечением метформином и интраназальным инсулином; AUC₀₋₁₂₀ — площадь под кривой «концентрация глюкозы, ммоль/л — время, мин» (в условных единицах) в интраперитонеальном глюкозотолерантном тесте. Различия с контролем (*) и группой MS (**), а также между группами MS-M и MS-MI (#) или между группами MS-I и MS-MI (##) статистически значимы при $p < 0,05$.

Table 2. Baseline and glucose-stimulated levels of leptin and insulin in the blood of male rats with the “neonatal” model of metabolic syndrome and the effects of treatment with metformin, intranasal insulin, and their combination on these parameters, $M \pm SEM$

Таблица 2. Базовые и стимулированные глюкозой уровни лептина и инсулина в крови самцов крыс с «неонатальной» моделью метаболического синдрома и влияние на них лечения метформином и интраназальным инсулином и их комбинацией, $M \pm SEM$

Parameter	Groups				
	C (n = 6)	MS (n = 6)	MS-M (n = 6)	MS-I (n = 6)	MS-MI (n = 6)
Insulin (0 min), ng/mL	0.51 ± 0.07	0.48 ± 0.07	0.58 ± 0.09	0.39 ± 0.06	0.53 ± 0.06
Insulin (120 min), ng/mL	0.70 ± 0.09	1.42 ± 0.18*	0.82 ± 0.14**	0.88 ± 0.12**	0.80 ± 0.10**
Leptin (0 min), ng/mL	1.14 ± 0.14	2.43 ± 0.21*	1.51 ± 0.18**	1.91 ± 0.23*	1.37 ± 0.18**
Leptin (120 min), ng/mL	1.50 ± 0.17	3.28 ± 0.29*	1.99 ± 0.18**	2.36 ± 0.20**	1.74 ± 0.19**
Insulin resistance index, c.u.	2.05 ± 0.29	2.38 ± 0.47	2.51 ± 0.43	1.70 ± 0.29	2.20 ± 0.18

Note. Presented are the values of blood insulin and leptin concentrations before (0) and 120 minutes (120) after glucose load. Insulin resistance is calculated as the product of the baseline blood glucose and insulin concentrations. C, control; MS, untreated metabolic syndrome; MS-M, MS with 4-week metformin treatment; MS-I, MS with 4-week intranasal insulin treatment; MS-MI, MS with combined metformin and intranasal insulin treatment. Differences between the C (*) and MS (**) groups are statistically significant at $p < 0.05$.

Примечание. Представлены значения концентраций инсулина и лептина в крови до (0) и через 120 мин (120) после глюкозной нагрузки. Инсулиновая резистентность рассчитана как произведение базовых концентраций глюкозы и инсулина в крови. C — контроль; MS — метаболический синдром без лечения; MS-M — MS с 4-недельным лечением метформином; MS-I — MS с 4-недельным лечением интраназальным инсулином; MS-MI — MS с совместным лечением метформином и интраназальным инсулином. Различия с группами C (*) и MS (**) статистически значимы при $p < 0,05$.

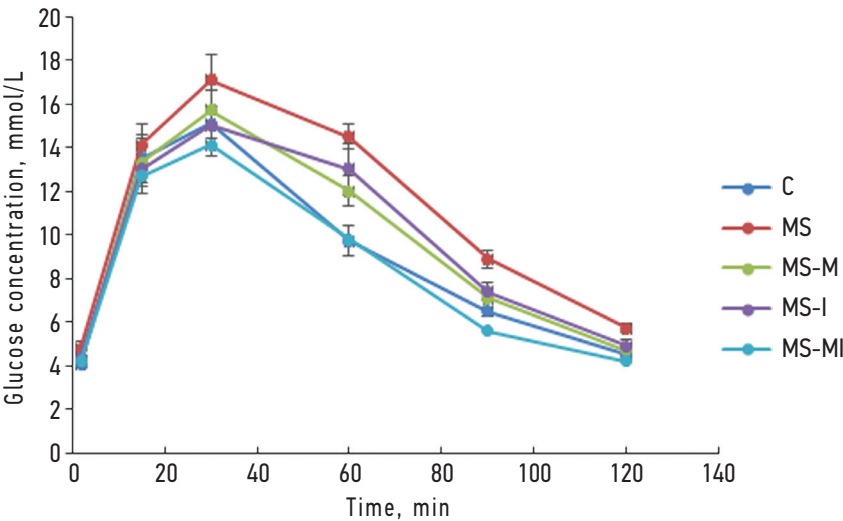


Figure. Blood glucose concentration in male rats at 120 minutes after glucose load in the intraperitoneal glucose tolerance test. C, control; MS, untreated metabolic syndrome; MS-M, MS with 4-week metformin treatment; MS-I, MS with 4-week intranasal insulin treatment; MS-MI, MS with combined metformin and intranasal insulin treatment

Рисунок. Концентрация глюкозы в крови самцов крыс в течение 120 мин после нагрузки глюкозой в интраперитонеальном глюкозотолерантном тесте. С — контроль; MS — метаболический синдром без лечения; MS-M — MS с 4-недельным лечением метформином; MS-I — MS с 4-недельным лечением интраназальным инсулином; MS-MI — MS с совместным лечением метформином и интраназальным

Table 3. Number and diameter of pancreatic islets in the pancreas of rats with the “neonatal” model of metabolic syndrome, and the effects of treatment with metformin, intranasal insulin, and their combination on these parameters, $M \pm SEM$

Таблица 3. Число и диаметр панкреатических островков в поджелудочной железе крыс с «неонатальной» моделью метаболического синдрома и влияние на эти показатели лечения метформином и интраназальным инсулином и их комбинаций, $M \pm SEM$

Group	Number of islets*	Diameter of islets, μm	Product of the number of islets by their diameter, $\times 10^3$
C (n = 6)	43 ± 4	182 ± 9	7.7 ± 0.5
MS (n = 6)	$27 \pm 5^{**}$	168 ± 8	$4.3 \pm 0.6^{**}$
MS-M (n = 6)	39 ± 3	194 ± 9	$7.4 \pm 0.4^{\#}$
MS-I (n = 6)	34 ± 4	185 ± 20	$5.9 \pm 0.3^{**}$
MS-MI (n = 6)	38 ± 3	197 ± 9	$7.5 \pm 0.4^{\#}$

Note. C, control; MS, untreated metabolic syndrome; MS-M, MS with 4-week metformin treatment; MS-I, MS with 4-week intranasal insulin treatment; MS-MI, MS with combined metformin and intranasal insulin treatment. *The number of islets is calculated for one of the sections with the maximum area of the pancreas. Differences between the C (**) and MS (#) groups are statistically significant at $p < 0.05$.

Примечание. С — контроль; MS — метаболический синдром без лечения; MS-M — MS с 4-недельным лечением метформином; MS-I — MS с 4-недельным лечением интраназальным инсулином; MS-MI — MS с совместным лечением метформином и интраназальным инсулином. *Число островков рассчитано для одного из срезов с максимальной площадью поджелудочной железы. Различия с группами С (**) и MS (#) статистически значимы при $p < 0,05$.

and reduction of AUC_{0-120} (Table 1, Figure 1). MF also reduced baseline and glucose-stimulated leptin levels elevated in MS, indicating increased tissue sensitivity to leptin (Table 2). The total number and area of pancreatic islets were restored in the MS-M group (Table 3). The baseline insulin level did not change significantly, being close to its normal values, and the glucose-stimulated insulin level (120 min after glucose load) did not differ from that in the control group (Table 2). INI monotherapy had no significant effect on the evaluated metabolic and hormonal parameters (Tables 1–3). Thus, in the

MS-I group, body and adipose tissue weight, triglyceride and leptin levels differed significantly from the control values. The exceptions were total cholesterol, glycated hemoglobin, and insulin levels 120 min after the load, which were significantly decreased in INI-treated compared with untreated MS rats and did not differ from the control (Tables 1, 2). The MS-I group also showed partial recovery of the number of pancreatic islets, but their total area, although increased by 37% compared to the MS group, remained lower than that in the control (Table 3).

The combined use of MF and INI demonstrated a more pronounced restorative effect than MF monotherapy on a number of parameters. The MS-MI group did not differ significantly from the C group in any of the evaluated parameters (Tables 1–3). Thus, in contrast to MF monotherapy, body and abdominal fat weight, glycated hemoglobin level and AUC_{0-120} for the glucose curve in IpGTT were significantly reduced in the MS-MI group. Moreover, the AUC_{0-120} in the MS-MI group was significantly lower than in the MS-M group, indicating a more pronounced restorative potential of the combination therapy with respect to glucose tolerance. In terms of pancreatic islet area recovery, combination therapy and monotherapy with MF were comparable.

DISCUSSION

We showed that at 6–7 months of age male rats that were subjected to a three-day interruption of breastfeeding at an early age (P19–P21) showed distinct signs of MS, including increased body weight and adipose tissue mass, impaired glucose tolerance and insulin and leptin responses to glucose load, increased triglyceride and total cholesterol levels, and decreased number of insulin-producing β -cells and total pancreatic islet area. These changes are generally consistent with the metabolic and hormonal disorders that we and other authors previously identified in adult rats with interrupted breastfeeding [1–4, 14, 15]. At the same time, such disorders begin to be detected already in three-month-old animals [2] and further aggravate, being most pronounced at 6–10 months of age [1, 3, 4].

One of the approaches to correct these disorders in neonatal MS is the course administration of MF, which is characterized by high efficacy in metabolic disorders, i.e. DM2 and MS, accompanied by obesity, dyslipidemia and impaired sensitivity to insulin and leptin [5, 7, 16]. We previously showed that 4-week MF treatment of 9-month-old MS male rats induced by interrupted breastfeeding resulted in recovery of a number of their metabolic and hormonal parameters [3]. Moreover, a high daily dose of MF (250 mg/kg) was most effective in reducing body weight and restoring metabolic parameters, normalizing lipid status and leptin levels, while a relatively low dose (125 mg/kg $\times$ day) had a positive effect on thyroid and gonadal axis parameters but was less effective in normalizing body weight and metabolic parameters [3]. In this regard, it should be taken into account that long-term therapy with high doses of MF may lead to a number of adverse effects, such as gastrointestinal dysfunction, including microbiome changes, and lactoacidosis [8, 9, 17]. Therefore, great expectations are assigned to the development of approaches to potentiate the restorative effect of MF without increasing its dose, which is possible when using combination therapy.

We previously showed that INI is effective in a number of metabolic disorders [18], including MS caused by restricted breastfeeding [4], which are characterized by functional abnormalities in the brain insulin signaling system. One of the reasons for these abnormalities is the increased activity of negative regulators of insulin signaling in neurons and glial cells, leading to central insulin resistance [19–21]. Another reason is the impaired transport of insulin across the blood-brain barrier, which reduces the efficiency of its delivery to the brain from the circulation and is due to disturbances in the insulin-transport mechanism and structural organization of the blood-brain barrier [18, 19, 22]. The intranasal administration of insulin directly to the brain normalizes insulin signaling in the CNS and thereby ensures normal regulation of the physiological processes dependent on it. However, according to our data, in rats with interrupted breastfeeding INI was effective only at an early age, preventing metabolic disorders in adulthood, but was ineffective in treating already developed neonatal MS in adult animals [4]. The low efficacy of INI monotherapy in adult animals with neonatal MS was confirmed in the present study.

The studied combination therapy with MF and INI was superior to MF monotherapy in a number of parameters, as indicated by the following data. Combination therapy led to recovery of the full range of evaluated metabolic and hormonal parameters, which changed in the conditions of neonatal MS. These parameters were not only significantly different from those in MS rats, but also did not differ from those in the control group. The AUC_{0-120} demonstrating glucose tolerance was significantly lower in the combination treatment group than in the MF monotherapy group, indicating that the restorative effect of the combination of MF and INI on glucose homeostasis was more effective compared with MF therapy. This was also demonstrated by more pronounced decrease in glycated hemoglobin in the MS-MI group. Moreover, in contrast to MF monotherapy, combination therapy resulted in significant reductions in body weight and abdominal fat mass, which we suggest is due to the anorexigenic effect of INI.

Previously, we obtained data on the effectiveness of combined administration of MF and INI in the treatment of male rats with DM2 induced by high-fat diet and low-dose streptozotocin [10]. However, DM2 differs significantly from the neonatal MS both in pathogenesis and in the nature and severity of metabolic and hormonal disorders. There are studies of the effectiveness of combined use of MF and INI for treatment of female rats with a MS model caused by long-term administration of oral contraceptives, i.e. ethinylestradiol and levonorgestrel [11], as well as male rats with insulin resistance and hyperglycemia induced by dexamethasone, although this model of metabolic disorders by etiology and pathogenesis has little

in common with MS and is not accompanied by obesity [12]. Treatment of MS induced by contraceptive treatment, administered for one week with a medium dose of MF (150 mg/kg $\times$ day) and a quite high dose of INI (2 IU/rat $\times$ day), reduced glucose and glycated hemoglobin levels, normalized lipid levels, catalase, a marker of oxidative stress, and expression of tumor necrosis factor- α , an inflammation marker. Together, these and our data indicate the prospects for the combined use of MF and INI for the treatment of various forms of MS and DM2 in terms of etiology and pathogenesis, including for the treatment of MS, in the pathogenesis of which eating disorders in early ontogenesis play the key role.

The INI-induced potentiation of the restorative effect of MF on metabolic and hormonal disorders is due to the complementarity of the effects and targets of MF and INI. MF easily penetrates into brain tissue through the blood-brain barrier and improves metabolic processes both in the brain (by acting on neurons) and in the periphery (through central mechanisms). This is largely due to its restorative effect on carbohydrate and lipid metabolism disturbed in MS and DM2, as well as on the nervous system functions not only in experimental conditions, but also in the clinical settings [23–26]. The main mechanisms of action of MF in the brain are activation of AMP-activated protein kinase, a key energy sensor of cells [27], as well as normalization of reactive oxygen species production and activity of pro-inflammatory and apoptotic factors elevated in DM2, MS and associated neurodegenerative diseases [28–30].

There is evidence that the metabolism-restoring antioxidant and anti-inflammatory effects of MF lead to increased tissue sensitivity to insulin and leptin not only in the periphery but also in the brain [31–33]. As a consequence, the stimulating effect of INI on the brain insulin system under MF treatment is increased, and this accounts for the more pronounced effect of INI under combination therapy on neuronal survival and functional activity, as well as on peripheral metabolism. This, in turn, contributes to a more effective effect of MF on target tissues, including the brain. Here the mechanism of potentiating effect of insulin on leptin signaling pathways in the CNS, and vice versa, the effect of leptin on insulin signaling [34–36] is reproduced, first demonstrated twenty years ago [37, 38]. In this regard, it is noteworthy that a number of authors suggest the use of co-administration of leptin and insulin in patients with forms of diabetes and other metabolic disorders that are characterized by reduced leptin levels [36, 39].

CONCLUSION

Thus, it was shown for the first time that adult male rats with interrupted breastfeeding develop metabolic

and hormonal disorders characteristic of MS, as well as reduced pancreatic islet area. Their long-term treatment with MF and its combination with INI partially or completely restores these parameters, with combination therapy being superior to MF monotherapy in terms of the effectiveness of restoring a number of parameters (body weight and adipose tissue mass, glycated hemoglobin level, glucose tolerance assessed by IpGTT). This is in favor of the prospect of using the combination of MF and INI to normalize metabolic and hormonal disorders in MS developed due to breastfeeding deficiency in early ontogenesis.

ADDITIONAL INFO

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