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Histopathological Analysis of Liver Tissue in Monosodium Glutamate-Induced Obese Rats.

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ABSTRACT

The aim of our study was to investigate effects of Monosodium glutamate (MSG) neonatal treatment on metabolic profile and liver morpho-functional state in rats at 16-weeks of age. We included 40 newborn Wistar male rats, divided into 2 groups of 20 animals each: intact and MSG treated. Newborns rats of intact group were administered with saline subcutaneously (s.c.) in the volume of 8 µl/g at 2-10th postnatal days. Newborns rats of MSG-group received a solution of MSG (4.0 mg/g of body weight) s.c. at 2-10th postnatal days. Within 4 months after birth, rats had a normal diet. The parameter of obesity was obtained by Lee index for each male rat at month 4 of life (120 days). Liver tissue was fixed in 10% formalin, dehydrated and imbedded in paraffin wax. Paraffin sections of 5 µm were cut and stained with hematoxylin and eosin. We suggest that neonatal MSG treatment in 16 weeks rats are associated with previously described clusters of metabolic syndrome such as visceral obesity, dyslipidemia and impairment in insulin sensitivity, but also lead to the development of typical for NAFLD histopathological changes which can be recognized as a novel animal model for NAFLD/NASH.

Keywords: histopathology, liver, MSG, obese

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INTRODUCTION

Today the prevalence of obesity continues to increase that provides challenge to scientists [1]. Overweight and obesity cause a number of diseases, namely, cardiovascular diseases, type 2 diabetes, dyslipidemia, premature death, non-alcoholic fatty liver disease (NAFLD) as well as different types of cancer [2]. Approximately 1.7 billion people in the world suffer from being overweight, most notably in developed countries [3]. The lack and inefficiency of drugs for obesity treatment cause the search and examination of new anti-obesity compounds [4, 5]. Increased portion sizes in commercially marketed food items, inexpensive food sources such as fast food, increased availability of vending machines with energy-dense items, increased use of high fructose corn syrup, and less physical education in schools.

Well known flavor enhancer – monosodium glutamate (MSG), E 621 is widely used in food industries [6]. In 1968, a report appeared in the New England Journal of Medicine, describing a constellation of symptoms in patients who dined in one of the growing number of Chinese restaurants. The symptoms of the so-called «Chinese restaurant syndrome» included numbness, radiating to the back, arms, and neck; weakness; and palpitations. Later reports included other symptoms, such as tightness, flushing, tearing, dizziness, syncope, and facial pressure [7]. But the mechanism of genesis «Chinese restaurant syndrome» is still unclear. Although the evaluations conducted by the U.S. Food and Drug Administration and some other organizations concluded that MSG was a safe food ingredient for the general population, none of them answered the question: is MSG consumption healthy?

In our previous work we have shown that the injection of monosodium glutamate during 30 days to rats in doses 15 and 30 mg/kg (1 and 2 gram of MSG on average statistical person) evoked pancreatitis [8, 9] gastric mucosa (GM) lesions, strengthened the stress action on GM and obesity [10]. To take into account the data of literature about pharmacological blockade of central glutamate receptors results in an attenuation of stress-induced responses of several hormones and mediators, such as adrenocorticotropic hormone, prolactin, and catecholamines [11, 12] we concluded that excitement of glutamate receptors against the background of long-term injection of MSG increases the stress action on GM via enhancement of stress-induced responses of adrenocorticotropic hormone and catecholamines. So the long-term excessive consumption of MSG can lead to gastritis and ulcer disease of stomach. Secondly, the maximum daily dose of MSG, as well as other food additives must be reviewed with regard to their effect on GM [13].

Today role of MSG in the development of obesity in people who abuse with “fast food” is contradictory. Some scientists believe that this food additive causes metabolic disorders and weight gain [14], but others claim that use of MSG, even in large doses, does not harmful for health [15]. The aim of our study was to investigate effects of MSG neonatal treatment on metabolic profile and liver morpho-functional state in rats at 16-weeks of age.

METHODS

We included 40 newborn Wistar male rats, divided into 2 groups of 20 animals each: intact and MSG treated. Newborns rats of intact group were administered with saline subcutaneously (s.c.) in the volume of 8 µl/g at 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} postnatal days. Newborns rats of MSG-group received a solution of MSG (4.0 mg/g of body weight) s.c. at 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} postnatal days [16, 17]. Within 4 months after birth, rats had a normal diet.

The parameter of obesity was obtained by Lee index for each male rat at month 4 of life (120 days). This index was defined as the cube root of body weight (g) x 10 / naso-anal length (cm), for which a value equal to or less than 0.300 was classified as normal. Rats presenting values higher than 0.300 were classified as obese and included in this experiment.

Liver tissue was fixed in 10% formalin, dehydrated and imbedded in paraffin wax. Paraffin sections of 5 µm were cut and stained with hematoxylin and eosin. The specimens were examined under a XS-4130 MICROmed microscope. To assess morphological changes in the liver we used NAS (NAFLD activity score), which included histological features and has been defined as unweighted sum of scores for steatosis (0-3), lobular inflammation (0- 3) and ballooning (0-2). According to NAS scores ≥5 are considered as non-alcoholic steatohepatitis (NASH), while those with a NAS.

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Statistical analysis performed by using SPSS-20 software. All data in this study were expressed as mean ± standard deviation (M± SEM) or %. Data distribution was analyzed using the Kolmogorov-Smirnov normality test. Statistical comparisons between groups were conducted using Student's *t* test (for continuous variables) and χ^2 test for categorical variables. The difference between groups was defined to be statistically significant when a p-value was less than 0.05.

RESULTS

4-month rats injected with MSG neonatally were characterized by the significant changes in body parameters. We observed the increase of body mass of MSG rats by 11.2 % ($p<0.05$), at the same time their nose-to-anus length was extremely reduced from 24.2±0.3 to 21.6±0.3 cm, or by 16.0 % ($p<0.01$). Such body changes were resulted in the significant raise of body mass index (BMI) and Lee's index by 39.0% ($p<0.001$) and by 16.0% ($p<0.001$) in obese rats. As we consider Lee's index = 0.03 as critical level for determination whether or not obesity develops, we have found the incidence of obesity in MSG-group in 100% cases. All this suggests the stunted growth of MSG-rats and the accumulation of fat that caused the raise of rat weight. Moreover, the analysis of adipose tissue deposition showed the prevalence of visceral adiposity. The mass of visceral adipose tissue of MSG-obese rats has enlarged by 7.3 times ($p<0.001$) as compared with intact rats (Tabl.1).

Table 1. Anthropometric parameters of experimental male rats

	Intact rats (n=20)	MSG- rats (n=20)	p
Weight	284,16±8,65	316,08±9,69	<0,05
Body mass index	0,49±0,02	0,67±0,02	<0,001
Naso-anal lehgth	24,2±0,31	21,6±0,29	<0,01
Lee index	0,27±0,005	0,32±0,004	<0,001
Visceral adipose tissue	2,42±0,22	17,83±1,70	<0,001

Data are presented as the M ± SEM.

Neonatal subcutaneous injection of MSG led to the development of visceral obesity (Fig.1) and NAFLD as consequence of metabolic disorder in 4-month rats.

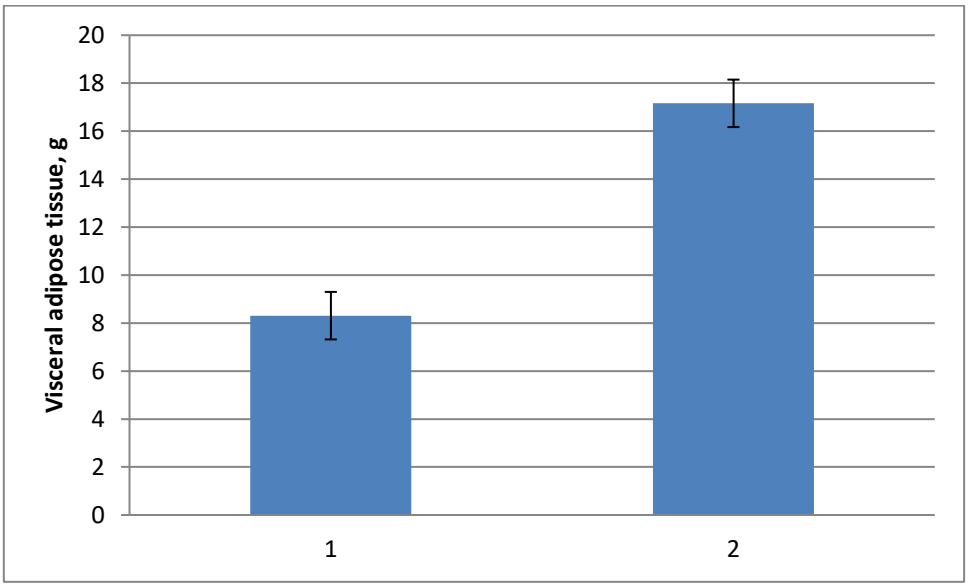


Fig. 1. Visceral adipose tissue (1 - Intact rats, 2 - MSG- rats). Data are expressed as mean ± SE. p< 0.001 (Student's t-test).

Histological analysis of liver micropreparations confirmed the development of NAFLD in rats. It was registered evidence of steatosis, lobular inflammation and ballooning degeneration in liver tissue in 4-month rats treated with MSG neonatally (Fig. 2). The degree of steatosis was increased by 9 times ($p<0.001$) in MSG-rats as compared with intact rats. Intact rats did not display inflammation in liver, at the same time lobular inflammation in MSG-group reached 1.20±0.17 points (Table 2). Similar results were recorded for ballooning

degeneration (Table 2). Total NAS in MSG-rats were higher by 16.6 times ($p<0.001$) compared with values of intact rats. NASH were not detected in intact rats, however, MSG-obese rats has NASH with prevalence 33.3% (Table 2).

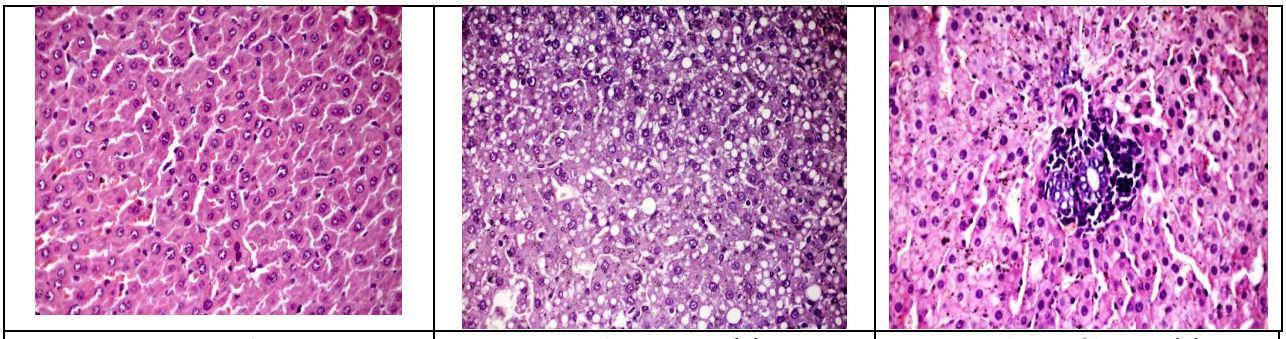
The signs of hepatic fat dystrophy and microvascular disorders in rats of second group, which were received monosodium glutamate in neonatal period, were found. The fat vacuoles of various size in hepatocytes were observed. Predominantly the medium droplet dystrophy were observed, however the foci of small and big droplet fat dystrophy occured (Fig. 2-A). Also, the disorders of microcirculation such as expansion of intralobular viens, stasis of red blood cells in vien and arteries, sludge of red blood cells with microthrombosis were found. The desquamation of endothelium in some vessels were observed.

The enlargement of lymph spaces (gaps between liver parenchyma and connective tissue), focal infiltration of lymphoid cells, spread of limfoid cells to the liver parenchyma and the formation of limfoid knots were found. The focal moderate limfoid cell infiltration was also observed in fibrous tissue of the portal tracts. Also, the presence of macrophages in the infiltrates was noted (Fig. 2 B).

Table 2. Morphological changes of the rats liver tissue in the conditions of MSG-obesity

Parameters	Intact rats (n=20)	MSG- rats (n=20)
Steatosis (0-3)	0.20±0.13	1.80±0.17*
Lobular inflammation (0-2)	0.0±0.0	1.20±0.17*
Ballooning degeneration (0-2)	0.0±0.0	0.27±0.11*
Total NAS (0-8)	0.20±0.13	3.33±0.36*
Prevalence of NASH, %	—	33,3

Data are presented as the M ± SEM. * - Significant differences in $p<0.001$.



Control | **Fatty hepatocytes (A)** | **Lymphatic infiltration (B)**

Fig. 2. Light microscopic micrographs of the liver tissue of MSG-group rats stained with hematoxylin and eosin, ×400.

DISCUSSION

NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) that can have different degrees of fibrosis and progress to liver cirrhosis and hepatocellular carcinoma [18]. NAFLD is the most common liver disorder in Western countries, affecting 17-46% of adults, with differences according to the diagnostic method, age, sex and ethnicity [19].

Animal models of NAFLD/NASH are classified into genetic models, nutritional models, and combination models of genetic and nutritional factors. Genetic models include animals deficient in leptin and/or its receptors; animals with over-expression of Abcb11; and animals that lack peroxisome proliferator activated receptor-alpha (PPARα). Nutritional (dietary) approach: over-nutrition, overfeeding of unsaturated fat (high-fat diet - HFD), and a methionine and choline deficient (MCD) diet. A combination of genetic factors with others such as nutritional factors, oxidative stress, and drugs [20].

An ideal animal model of NAFLD/NASH should not only reproduce the established pathology of the human disease, but also reenact the context within which a disease develops and progresses. Accordingly, the liver of

the animal model of NAFLD/NASH should show steatosis, intralobular inflammation, hepatocellular ballooning, and, ideally, perisinusoidal fibrosis in zone 3 and susceptibility to liver tumors. Furthermore, the animal should show metabolic abnormalities such as obesity, insulin resistance, fasting hyperglycemia, dyslipidemia, and altered adipokine profile [21].

To date, there is no single animal model that displays the range of histopathologic and pathophysiologic features associated with NAFLD/NASH [22-24]. The currently available animal models are associated with various drawbacks in that they do not or only partially reflect the real picture of human NASH in terms of etiology, pathogenesis, and disease mechanisms [20, 22, 24, 25] Those models that are claimed to mimic all of the major characteristics of human NAFLD/NASH (obesity, IR, fibrosis, steatosis, and steatohepatitis) are genetically modified animal models fed either HFD or MCD diets [20, 22].

Animal models support a causative association between obesity and neonatal or maternal administration of high doses of MSG [26]. MSG acts on immature neurological mechanisms that regulate food intake and energy expenditure by ablating cells in the arcuate nucleus of the hypothalamus and destroying circumventricular neurons. MSG altered the production of orexigenic and anorexigenic molecules as proopiomelanocortin, cocaine- and amphetamine-regulated transcript and neuropeptide Y [27]. Neonatal neurotoxity effect of MSG leading to the development of neurochemical, endocrine, metabolic and behavioural abnormalities in adulthood including hypophagia, obesity, hypoactivity, delayed puberty, and elevated plasma corticosterone levels [28]. Furthermore, stunted growth, increased adipose tissue stores, and a marked increase in plasma triglycerides [29], insulin [30] and fasting glucose levels [31] have been noted in rats.

Our study also mentioned that neonatal treatment with MSG associated with the development of typical histopathologic NAFLD changes in 16-weeks rats. This results in agreement with previously reported, that find in the livers of MSG mice, moderate centrilobular microvesicular steatosis, ballooning degeneration with Mallory bodies, and scattered infiltration of neutrophils and lymphocytes were observed [32]. Lazarin Mde O. et al. postulated that the enhanced glucose-6-phosphate dehydrogenase activity observed in the livers of MSG-obese rats could be associated with liver fat accumulation and likely plays a central role in the mitochondrial defence against oxidative stress [33].

CONCLUSION

In conclusion our results suggest that neonatal MSG treatment in 16 weeks rats are associated with previously described clusters of metabolic syndrome such as visceral obesity, dyslipidemia and impairment in insulin sensitivity, but also lead to the development of typical for NAFLD histopathological changes which can be recognized as a novel animal model for NAFLD/NASH.

Abbreviations

HFD - high-fat diet; GM - gastric mucosa; MSG – monosodium glutamate; NAFLD - non-alcoholic fatty liver disease; NASH – non-alcoholic steatohepatitis; MCD - methionine and choline deficient (MCD) diet.

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