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The Effect of a High Monosodium Glutamate Diet in Inducing Changes in Microbiota Diversity and Kidney Pathology in Hamsters Infected with *Opisthorchis viverrini*

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ABSTRACT

OBJECTIVE: To investigate the effect of a combination of a high dose of monosodium glutamate (MSG) and chronic *Opisthorchis viverrini* (*O. viverrini*) infection on kidney pathology and microbiota changes compared to either factor alone.

METHODS: Forty male golden hamsters were divided into four groups (10 hamsters per group): non-infected hamsters fed with standard diet (NC), *O. viverrini* infected hamsters fed with standard diet (OV), non-infected hamsters fed with high doses of MSG in drinking water (MS), and *O. viverrini* infected hamsters fed with high doses of MSG in drinking water (OM). After 8 months, fecal samples were collected, DNA extracted and subjected to 16S-rRNA sequencing analysis to determine microbial diversity. Kidneys were also collected for histopathological study.

RESULTS: Kidney histopathology showed tubular damages and tubular fibrosis were significantly prominent in the OM group, which showed higher pathology changes than in the OV group or MS groups. Next generation sequencing indicated that the levels of Firmicutes to Bacteroides ratio decreased in the OV group (0.28), MS group (0.43) and OM group (0.43) respectively when compared to control group (0.52). In genus levels, *Methanobrevibacter, Ruminococcus_1, Escherichia-Shigella, Bacteroides, Akkermansia* and *Oligella* were abundance in the OM group.

CONCLUSION: The changing of gut microbiota distribution and kidney pathology changes were more severe in the cases of *O. viverrini* infection together with MSG consumption. This study provides a first step towards focusing on diet and parasitic infections.

KEYWORDS: chronic infection, gut microbiota, monosodium glutamate, *Opisthorchis viverrini*

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INTRODUCTION

A major public health problem in many developing countries is parasitic infection. *Opisthorchis viverrini* (*O. viverrini*) infection is known to cause hepatobiliary disease and cholangiocarcinoma. Up to date, at least 6.71 million people are infected with *O. viverrini* in Thailand[1] and the highest prevalence is found in the northeastern region (18.6% and may be up to 85.6% of population in some areas). Moreover, kidney disease was observed in Syrian golden hamsters infected with *O. viverrini* experiment by showing a complete obsolesce of the glomeruli characterized by deposition of amyloid proteins, tubular atrophy, interstitial inflammation, and tubular fibrosis[2]. In human studies, many biomarkers of opisthorchiasis were represented including miR-192 in the urine in *O. viverrini* infected, periductal fibrosis and also cholangiocarcinoma (CCA) groups[3]. In chronic opisthorchiasis study found that urinary 8-oxodG is a biomarker of the progression of advanced periductal fibrosis and CCA[4]. Moreover, urinary excretion of microproteinuria were found in patients during *O. viverrini* infection[5] but the associations of *O. viverrini* infection and kidney disease is unclear.

Monosodium glutamate (MSG) is a sodium salt of glutamic acid and it is naturally present in the bodies of humans and animals; therefore, it would be present in a diet that is rich in protein-containing foods such as meat, vegetables and dairy products. MSG was used as a flavor enhancer, that increases the taste of food that usually added to Asian foods such as Japanese miso soup and processed meat[6]. Although the Food and Drug Administration has considered MSG as a food ingredient that is "generally recognized as safe," many people exhibit symptoms of allergies and other disorders[7-9]. Thus, many researchers are investigating whether MSG could be harmful[9-10]. According to the WHO suggested daily MSG consumption should not be more than 120 mg/kg/day[11]. The average daily intake of MSG in Japan and Korea in the 1990s was 1.20–1.70 g/day[12]. In addition, the average daily MSG intake in Khon Kaen, Thailand is 4.00 ± 2.20 g/day and can be as high as 14.00 g/d[13]. Daily MSG consumption has been associated with decreased pancreatic β-cell mass and increased bleeding and islet fibrosis[14]. Chronic oral intake of 4.00 mg/g body weight MSG increased kidney function and structural abnormalities, including glomeruli, tubular swelling, capillary congestion and microhemorrhages in the stromal areas of the kidney tubules[15], and there appears to be a risk of kidney stones[16].

In recent decades, several studies reported that the gut microbiota, a community of microorganisms including bacteria, yeasts, archaea and viruses plays the most important role in maintaining human health. In addition, the community of gut microbiota in the human body can be used to monitor disease and health[16]. The two major types of phyla are Firmicutes (such as *Roseburia* spp., *Lactobacillus* spp. and *Faecalibacteriums* spp.) and Bacteroides (such as *Bacteroides* spp. and *Akkermansia* spp.) found in the human gut microbiota, and they play crucial roles in health maintenance, including digestion of complex carbohydrates and fiber, preventing of inflammation, the synthesis of essential vitamins (vitamin B12 and vitamin K) and regulation of metabolism[17-19]. The expression between Firmicutes/Bacteroides ratio was used as a marker of several pathological conditions[19-20]. However, the relative distribution of the gut microbiome can be vary depending on age, gender, behavior and environmental factors. The most important factor affecting the community and types of gut microbiota is diet[21]. For example, a decrease in Bacteroides levels and an increase Firmicutes levels were observed in hamster fed a high fat diet[20,22]. The reduction in Bacteroides and Firmicutes was observed in MSG-fed hamsters. In addition, hamsters with chronic opisthorchiasis showed an increase in *Methanobrevibacter*, *Desulfovibrio*, *Akkermansia* and *Roseburia*, which is associated with metabolic syndrome[20,23]. Moreover, MSG consumption also affected levels of *Faecalibacterium*, *Megamonas*, *Blautia* and

Collinsella compared to healthy controls[24]. Taken together, we hypothesize that a combination of a high dose of MSG and chronic opisthorchiasis induces more severe of kidney pathology and microbiota changes than either factor alone.

In this study, we aim to investigate the effects of *O. viverrini* infection in combination with MSG intake using advanced sequencing technologies focusing on microbiota community balance and histopathological changes in the hamster model. This result could be a first step towards a focus on nutrition and parasitic infections that could lead to new prevention strategies for opisthorchiasis and opisthorchiasis-associated metabolic diseases.

METHODS

The animal experiment was conducted in the Animal Unit, Faculty of Medicine, Khon Kaen University, and was approved by the Animal Ethics Committee of Khon Kaen University, based on the Ethics for the Animal Experiment of the National Research Council of Thailand (IACUC-KKU-22/63). Forty male golden hamsters (*Mesocricetus auratus*) aged 6-8 weeks were randomly divided into 4 groups (ten hamsters per group): non-infected hamsters fed standard chow (NC), *O. viverrini*-infected hamsters fed with standard diet (OV), non-infected hamsters fed with high levels of monosodium glutamate (MSG concentration 4 mg/g-body weight) in drinking water (MS), and *O. viverrini*-infected hamsters fed with high levels of MSG in drinking water (OM) for 8 months. For *O. viverrini* infection, animals were infected with 50 viable *O. viverrini* metacercariae by oral inoculation as previously described[25]. The animals were kept under controlled environmental conditions and had access to free food and water consumption throughout the period. All hamsters were starved for 2 days prior to euthanasia. Fecal samples were collected individually from the colon and followed by transferred to a sterile tube and stored at -80°C until DNA extraction. Kidney tissues were collected and immediately placed in 10% buffered formalin for histopathological study.

For histopathological study, the kidney tissue was stained with hematoxylin and eosin[23]. The area of fibrosis was visualized by staining with picrosirius red kit. The sections were examined under a light microscope. Ten random tissue areas were selected for examination at a magnification of 2000. The percentage of positive fibrosis areas was analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA) and expressed as mean ± SD[26].

Fecal DNA was extracted from individual hamsters using the TIANamp Stool DNA Kit (Tiangen Biotech, Beijing). DNA samples in each group were then pooled and the measured concentrations using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and the extracted DNA samples were stored at -20°C. The assessment of the presence of gut microbiota profiles was performed according to the procedures previously reported by our research group[22].

The percentages of positive fibrosis areas were expressed as mean ± SD. To compare the significance of the differences between groups, one-way Analysis of Variance (ANOVA) with post-hoc correction (Fisher's Least Significant Difference) was performed using IBM SPSS Statistics version 26 (IBM Corporation, NY, USA).

RESULTS

The effects of *O. viverrini* infection and high consumption of monosodium glutamate on kidney pathologies were determined by H&E staining (figure 1A). In the control group, hamsters represented normal tubular cells and glomeruli, whereas tubular dilatation was obviously observed in the OM and OV groups. In addition, slightly tubular dilatation was visualized in the MS group. Kidney fibrosis was presented by picrosirius red staining (figure 1B). Accumulation of fibrosis was noted in the glomerulus and in the proximal and distal tubules in OM group. The percentage of fibrosis areas in the OM group was significantly highest positive on picrosirius red staining (3.54 ± 1.17), compared with the MS group (1.10 ± 0.38) ($p < 0.001$), the OV group (1.25 ± 0.19) ($p < 0.001$) and NC group (0.62 ± 0.24) ($p < 0.001$), as shown in Figure 1C.

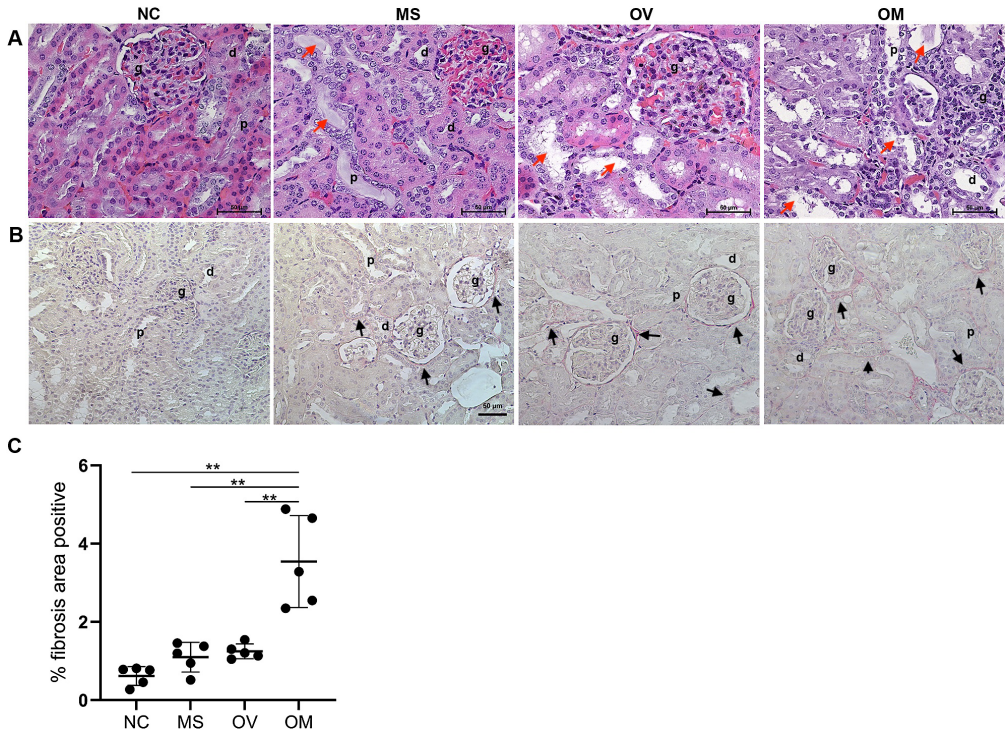

Figure 1 Histopathology and fibrosis expression in kidney tissues. A) histopathology of H&E staining kidney tissues, B) picrosirius red staining, C) percentage of fibrosis area positive (n = 5 per group). Scale bars 50 µm and magnification 400x.

Abbreviations: d, distal tubule; g, glomerulus; MS, hamsters fed with high monosodium glutamate; NC, non-infected control; OM, *Opisthorchis viverrini*-infected hamsters fed with high monosodium glutamate; OV, *Opisthorchis viverrini*-infected; p, proximal tubule

Black arrows mean fibrosis while red arrows mean tubular dilatation.

** $p < 0.001$

The effects of *O. viverrini* infection and high monosodium glutamate consumption on microbiota diversity. The alteration of microbial composition at the phylum level (figure 2A): Bacteroides and Firmicutes were most abundant in all experimental groups. The highest abundance of Euryarchaeota was recorded in the MS group, OV and OM compared to the normal control. The ratio of Firmicutes and Bacteroidetes was reduced in the OV group (0.28), the MS group (0.43) and the OM group (0.43) compared to the NC group (0.52). We further investigated the different changes at the genus level, as shown in Figure 2B and Table 1. The MS group showed high abundance of *Methanobrevibacter, Roseburia*, *Ruminococcus_1* and *Lachnospiraceae NK4A136*_group compared to the control group. The abundance of *Methanobrevibacter*, *Roseburia, Ruminococcaceae_UCG*-013, *Akkermansia*, and *Candidatus_Saccharimonas* was increased in the OV group. In addition, the levels of *Methanobrevibacter*, *Ruminococcus_1*, *Escherichia-Shigella*, *Bacteroides*, *Akkermansia* and *Oligella* were increased in the OM group compared to the control group.

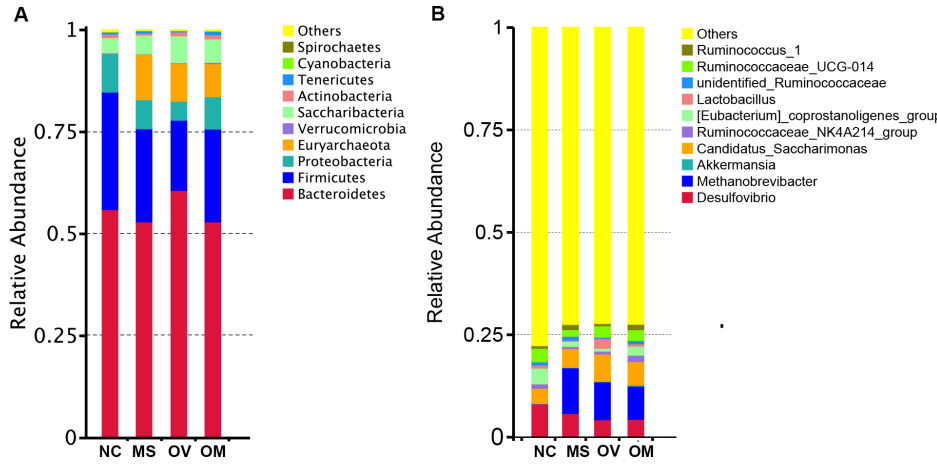

Figure 2 The effect of *Opisthorchis viverrini* infection and high monosodium glutamate consumption on microbiota diversity. A) At the level of phylum and B) At the level of genus.
Abbreviations: MS, hamsters fed with high monosodium glutamate; NC, non-infected control; OM, *Opisthorchis viverrini*-infected hamsters fed with high monosodium glutamate; OV, *Opisthorchis viverrini*-infected

Table 1 The relative abundance of microbial expression at genus level

Taxon	Groups			
	NC	MS	OV	OM
Ruminococcaceae_UCG-014	0.03287	0.01617	0.02754	0.02627
Oligella	0.00007	0.00007	0.00007	0.00014
Acinetobacter	0.00000	0.00000	0.00000	0.00004
Lachnospiraceae_NK4A136_group	0.00617	0.01326	0.00276	0.00365
Ruminiclostridium	0.00446	0.00392	0.00077	0.00185
Prevotellaceae_UCG-001	0.00408	0.00148	0.00130	0.00232
Enterorhabdus	0.00059	0.00037	0.00068	0.00077
Anaerotruncus	0.00465	0.00483	0.00160	0.00267
Ruminiclostridium_5	0.00463	0.00446	0.00262	0.00241
[Eubacterium]_coprostanoligenes_group	0.03859	0.01255	0.00702	0.02193
Candidatus_Saccharimonas	0.03736	0.04606	0.06624	0.05745
Paenalcaligenes	0.00000	0.00000	0.00000	0.00012
Lactobacillus	0.00750	0.00285	0.02374	0.00583
dgA-11_gut_group	0.00210	0.00020	0.00009	0.00023
Desulfovibrio	0.08123	0.05845	0.04303	0.04415
Akkermansia	0.00082	0.00075	0.00173	0.00267
Burkholderia-Paraburkholderia	0.00164	0.00116	0.00098	0.00059
Ruminococcaceae_NK4A214_group	0.01070	0.00522	0.00709	0.01535
Bacteroides	0.00349	0.00182	0.00061	0.00954
Ruminiclostridium_9	0.01405	0.01150	0.00337	0.00756
Tyzzerella	0.00734	0.00560	0.00146	0.00230
Escherichia-Shigella	0.00059	0.00052	0.00025	0.02848
Oscillibacter	0.00508	0.00389	0.00093	0.00257

Table 1 The relative abundance of microbial expression at genus level (continued)

Taxon	Groups			
	NC	MS	OV	OM
Prevotellaceae_UCG-003	0.01499	0.00175	0.00062	0.00517
Ruminococcus_1	0.00647	0.01266	0.00592	0.01332
Ruminococcaceae_UCG-013	0.00831	0.00809	0.01399	0.00829
Peptococcus	0.00242	0.00234	0.00111	0.00166
Anaerostipes	0.00000	0.00000	0.00000	0.00014
Allobaculum	0.01235	0.01119	0.01351	0.01718
Christensenellaceae_R-7_group	0.00276	0.00169	0.00267	0.00280
Ruminococcaceae_UCG-009	0.00774	0.00597	0.00442	0.00497
Unidentified_Ruminococcaceae	0.00793	0.00959	0.00330	0.00815
Roseburia	0.00070	0.01091	0.00128	0.00041
Helicobacter	0.00312	0.00216	0.00053	0.00299
Methanobrevibacter	0.00046	0.11141	0.09244	0.08107

Abbreviations: MS, hamsters fed with high doses of monosodium glutamate; NC, non-infected control; OM, *Opisthorchis viverrini*-infected hamsters fed with high doses of monosodium glutamate; OV, *Opisthorchis viverrini*-infected

DISCUSSION

This study confirmed that persistent infection with *O. viverrini* and consumption of MSG increased the severity of liver and kidney pathology and altered microbiota diversity in the hamster model using advanced sequencing technologies.

Prolong *O. viverrini* infection affects the liver and biliary ducts, leading to inflammation, fibrosis and cholangiocarcinoma. Several studies have reported the effects of chronic *O. viverrini* infection on pathological changes in the kidneys[2,20,23,27]. Infection with *O. viverrini* can trigger inflammatory responses in the hepatobiliary system and the inflammatory mediators may enter the blood circulation and contribute to kidney disfunction. In addition, *O. viverrini* -infected hamsters, histopathological changes of the kidneys were detected after 8 weeks of infection. Moreover, tubular atrophy, interstitial inflammation and tubular fibrosis were observed after 12 weeks of infection[2]. Moreover, *O. viverrini* antigens were observed in glomerular endothelial cells, mesangial cells, tubular cells and peritubular capillaries[27]. In addition, daily MSG consumption was associated with decreased pancreatic β-cell mass and increased hemorrhages and fibrosis[14]. Chronic oral intake of 4 mg/g body weight MSG resulted in decreased renal function and caused kidney pathology, including glomeruli, tubular swelling, capillary congestion and microhemorrhages in stromal areas of renal tubules[15], and there appears to be a risk of kidney stones in animal model[16]. No current information is available on the combination of hamsters infected with *O. viverrini* and fed with MSG. Our data showed that tubular dilatation and increased fibrosis of the glomerulus and the proximal and distal tubules were found in the OM group. This finding may suggest that prolonged consumption of MSG in *O. viverrini* infected hamsters leads to increased severity in kidney pathology.

It has been observed that infection with *O. viverrini* affects the microbiome of the host[28]. The alteration of bacteria can lead to an imbalance of bacteria that is associated with disease progression[29]. The severity of fibrosis has been found to be related to the dysbiosis of the microbiota caused by the infection[30]. *O. viverrini* infection led to changes in the microbiota components of the gastrointestinal tract, including biliary and had an impact on kidney tissue[31-32]. In addition, a previous study suggested that the increasing number of *Methalobrevibacter*

was related to kidney damage and kidney disease, and the change in the gut microbiome diversity was related to more pathogenic microorganisms with uremic toxin and renal fibrosis[20]. Our study found that the alteration of *Methanobrevibacter*, *Roseburi*, *Ruminococcaceae_UCG*-013, *Akkermansia*, *Candidatus_Saccharimonas* was identified in the feces of hamsters infected with *O. viverrini*. Fecal microbial communities showed numerous other differences at the genus level. In one study, it was also reported in previous studies that *O. viverrini* causes a change in Firmicutes by an increase in *Ruminococcaceae*, *Lacnospiracea* and *Lactobacillus*[30]. In addition, the production of *Methanobrevibacter*, *Akkermansia*, and *Burkholderia–Paraburkholderia* was relative higher in animals infected with *O. viverrini* than in the control group after 4 months[23]. The MSG diet increased the alteration of *Methanobrevibacter*, *Roseburia* and *Ruminococcus_1* after 4 months and also affected the gut microbiota diversity including the levels of *Faecalibacterium*, *Megamonas*, *Blautia* and *Collinsella*, which tended to be increased compared to non-treated controls[24]. The presence of microbial communities was consider as one of many different factors driving changes. Infection of *O. viverrini* in the bile ducts and liver causes tissue damage and obstruction leading to infection by microorganisms from the intestine, resulting to cholangitis and CCA[30]. The increasing of pathogenic bacteria communities may affect the host immune responses, metabolism and susceptibility to other infection. The detection of microbial presence-associated with *O. viverrini* infection may help to predict disease progression and guide treatment decisions.

The combination of *O. viverrini* infection and MSG intake resulted in the alteration of the major phyla of the gut microbiome and the ratio of Firmicutes to Bacteroides. This study showed that a combination of *O. viverrini* infection and MSG intake are the factors that cause the diversity of the bacterial community and the increase of the bacteria associated with metabolic syndrome, including *Methanobrevibacter*, which is associated with obesity[33], and *Akkermansia*[22]. In addition, *Methanobrevibacter* can produce methane (0.35 L/day) and be excreated in feces. Previous studies have found that methane is associated with inflammation, colon cancer[34-35], type I diabetis[36], coronary artery and heart disease[37]. Although there is no evidence of *Methanobrevibacter*-related kidney disease, previous study suggest that the imbalance of the gut microbiome leads to an increase in uremic toxin (trimethylamine *N*-oxide metabolite) and oxidative stress, inflammation and kidney disease[38]. We suggest that *Methanobrevibacter* proliferation might be involved in the inflammatory response to diet and parasite-induced kidney pathologies. However, in this experiment, the relative abundance of gut microbiota profiles was analyzed from pooled samples and we did not evaluate the gut microbiota before exposing it to the experiment. Therefore, a statistical analysis should be performed in the further work for comparison, as the abundant of some genera varies. This could affect on the abundance of some bacteria in our result, which is one of the limitations in our study.

CONCLUSION

Prolonged combination of *O. viverrini* infection and daily high dose of MSG consumption leads to pathological changes such as fibrosis and tubular dilatation and kidney damages. This study shows that *O. viverrini* infection and MSG affect both pathological changes and the diversity of the gut microbiome. However, our study has limitations in the data related to the use of pooled samples to observe microbiota changes. A more comprehensive understanding of the taxon presented would require an individual sample approach. However, this observation provides a first step towards understanding the severity of feeding behavior and parasitic infections.

CONFLICT OF INTEREST

There are no potential conflicts of interest.

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DATA AVAILABILITY STATEMENT

All data generated or analyzed in this study are included in this article. Future questions may be directed to the corresponding author.

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