Horticulture Innovation Australia

Final Report

Protective effects of white button mushroom (*Agaricus bisporus)* against non-alcoholic fatty liver disease

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Media Summary

Nonalcoholic fatty liver disease (NAFLD) describes the abnormal accumulation of fat in the liver of people who drink little or no alcohol, generally called "**fatty liver**". NAFLD patients show clinical symptoms such as fatigue, pain in the upper right abdomen and weight loss. NAFLD can progress to liver failure, where the liver no longer functions adequately. Estrogen (female hormone) provides a protective effect against the development of fatty liver in women. Therefore, postmenopausal women have a higher risk of developing fatty liver. However, currently, there is no specific drug to prevent/treat this liver disease.

Our study findings suggest that WBM is protective against fatty liver in a model of postmenopausal women, ovariectomized (OVX) mice. The lab results indicate that white button mushroom (WBM) can prevent fatty liver by both an androgen-dependent process (through the reduction of the levels and activity of protein to produce androgen) and androgen-independent processes (such as fat synthesis pathway). We did not see significant changes in the blood levels of liver enzyme in postmenopausal women who take WBM. Since the mice were fed with WBM diet for three months, it is thought to be difficult to see differences for a mushroom intake of only 85 days in postmenopausal women.

Technical Summary

Nonalcoholic fatty liver disease (NAFLD) includes various hepatic pathologies ranging from hepatic steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. Since estrogen provides a protective effect on the development of NAFLD in women, postmenopausal women have a higher risk of developing NAFLD. Hepatic steatosis is an early stage of fatty liver disease and can further develop to the aggressive stages (nonalcoholic steatohepatitis, fibrosis and cirrhosis). Currently, there is no specific drug to prevent/treat these liver diseases.

Recent research from our laboratory has revealed that androgen can promote NAFLD in female mice through the induction of the expression of SREBP1. To study a detailed mechanism for the inhibition of fatty acid synthesis pathway by white button mushrooms (WBM), we have started experiments using Huh7 and PLC5 human liver cell lines. Also, an attempt was also made to verify the effect of WBM on NAFLD by examining human blood samples from the mushroom clinical trial of postmenopausal women free of breast cancer.

Pathological examination of liver tissue showed less fat accumulation in the livers of OVX mice on WBM diet; moreover, these animals had improved glucose clearance ability. There were no significant differences in accumulation of fat in the livers between the mice fed with the WBM diet (30 g/kg high fat diet) and control mice suggesting that at this low level, the protective effect of WBM against NAFLD is not obvious.

Our *in vitro* experiments have found that WBM treatment suppresses the levels of both 5-alpha reductase and SREBP1 in the absence of androgen. These results suggest that WBM can prevent hepatic steatosis by both androgen-dependent mechanisms (through the reduction of the levels and activity of 5-alpha reductase) and androgen-independent mechanisms (through the suppression of LXR activity, as indicated in our PLoS One paper [2011;6(10):e26654)).

Introduction

Nonalcoholic fatty liver disease (NAFLD) includes various hepatic pathologies ranging from hepatic steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. Estrogen provides a protective effect on the development of NAFLD in women. Therefore, postmenopausal women have a higher risk of developing NAFLD. Hepatic steatosis is an early stage of fatty liver disease and can further develop to the aggressive stages (nonalcoholic steatohepatitis, fibrosis and cirrhosis). Currently, there is no specific drug to prevent/treat these liver diseases.

Materials & Methods

As a mouse model of postmenopausal women, seven-week old, female C57B1/6J mice were ovariectomized (OVX,). Control mice (sham) were subjected to sham operation survival surgery. Mice were then divided into three dietary groups, with eight mice per group: high fat diet (HFD) or HFD+WBM (30 or 120 g/kg HFD). The diets were continued for 3 months.

Recent research from our laboratory has revealed that androgen can promote NAFLD in female mice through the induction of the expression of SREBP1. To study a detailed mechanism for the inhibition of fatty acid synthesis pathway by WBM, we have started experiments using Huh7 and PLC5 human liver cell lines.

An attempt was also made to verify the effect of WBM on NAFLD by examining human blood samples from the mushroom clinical trial of postmenopausal women free of breast cancer. We measured the blood levels of liver enzymes (AST and ALT) and Bilirubin as a liver functional marker to evaluate WBM effects using patient's paired samples before and after mushroom intake.

Results

Pathological examination of liver tissue showed less fat accumulation in the livers of OVX mice on WBM diet; moreover, these animals had improved glucose clearance ability. This effect was only observed in the mice fed with HFD+WBM (120 g/kg HFD). There were no significant differences in accumulation of fat in the livers between the mice fed with the WBM diet (30 g/kg HFD) and control mice. These results indicate that at this low level, the protective effect of WBM against NAFLD is not obvious.

Our *in vitro* experiments have found that WBM treatment suppresses the levels of both 5-alpha reductase and SREBP1 in the absence of androgen. These results suggest that WBM can prevent hepatic steatosis by both androgen-dependent mechanisms (through the reduction of the levels and activity of 5-alpha reductase) and androgen-independent mechanisms (through the suppression of LXR activity, as indicated in our PLoS One paper [2011;6(10):e26654,. PLoS is an Open Access Journal]).

WBM has an anti-aromatase activity (shown in our Cancer Research 2006 paper). Aromatase is the enzyme making estrogen. Estrogen has protective effects in liver. The side effects for liver in patients ingesting WBM were a concern, however our results showed that WBM intake did not cause liver side effects in postmenopausal women in clinical trial in City of Hope. We did not see significant improvement in liver enzyme of

patients; however the liver enzyme levels (base line) in those patients were within the normal range. The mice were fed with WBM diet for three months. In humans, the patients were given WBM for only 85 days and limited number of patient samples were analyzed, thus preventing definitive results from such analyses.

Discussion

If this effect could be demonstrated in humans, postmenopausal women would have a natural option for avoiding liver steatosis and its associated conditions. This prevention of the early stage of the disease leads to a reduction of the risk of NAFLD. Furthermore, at the same time WBM would suppress aromatase to decrease risk of breast cancer, as reported previously from our laboratory.

This effect was only observed in the mice fed with HFD+WBM (120 g/kg HFD), not with HFD+WBM (30 g/kg HFD). The next logical experiment will be to check the effect of WBM at 30 g/kg in ovariectomized mice that are fed a regular diet. *In vitro* mechanistic experimentation showed that mushroom has multiple ways to prevent liver steatosis. Clinical samples results suggested that WBM did not increase the steatosis, even though WBM has anti-estrogen effects. We need to increase the duration of the study to get definitive information on the beneficial effects of WBM against liver steatosis in human population.

Technology Transfer

Dr. Chen has presented the findings regarding the protective effects of mushrooms against fatty liver to a number of visitor groups to City of Hope, to Chinese Cancer Survivors associated with the American Cancer Society, to a Chinese group in Guangzhou, China, and to researchers at the National Taiwan University College of Medicine. Dr. Chen also gave a keynote talk on his mushroom research in August 2012 at the 18th ISMSC.

Recommendations

To further explain the effect of WBM against fatty liver, it is worthwhile to check the effect of WBM at 30 g/kg in ovariectomized mice that are fed a regular diet. Although the protective effect of WBM against liver steatosis is demonstrated in our animal studies, it is important to perform additional experiments to generate data that will allow us to make appropriate recommendations on the dosage of WBM for postmenopausal women to prevent fatty liver.

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