

ITS 2 DNA條碼應用於成茶分子鑑別之研究

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摘要

酒精性肝疾病(Alcoholic Liver Disease)及脂肪肝(Alcoholic Steatohepatitis)為慢性疾病，藉由酒精而引發酒精性肝損傷。因現代人生活習性關係，此問題在近年越來越受到重視。本研究目的以飼料添加組合乳酸菌方式來改善及降低酒精性肝損傷的發生。在C57BL/6N小鼠餵食流質性酒精飼料之條件下，探討此乳酸菌組合對於改善由酒精所引發的酒精性肝損傷之影響。本實驗將24隻C57BL/6N雄性小鼠分為空白組、酒精組及組合乳酸菌組。空白組以Lieber-DeCarli流質一般飼料自由取食；酒精組與組合乳酸菌組則以Lieber-DeCarli流質酒精飼料自由取食，試驗之乳酸菌樣品直接添加至流質飼料中。實驗共為期八週，在實驗期間記錄體重、採集血液分析GOT、GPT、三酸甘油脂及總膽固醇，並在第八週時犧牲，採集小鼠肝臟進行相關基因表現量、抗氧化酵素活性、肝臟中三酸甘油脂含量及組織切片等檢測。實驗結果顯示，組合乳酸菌組血清中GOT、GPT及三酸甘油脂的數值有下降之趨勢($P < 0.05$)，而總膽固醇方面則無顯著差異性($P > 0.05$)。肝臟中SREBP-1及TNF- α 基因相對表現量降低約4倍和4.3倍($P < 0.05$)。在肝臟組織中GSH、GPx及GSH Rd酵素都有顯著上升($P < 0.05$)，分別增加約6.8%、58.1%及22.7%；而catalase與SOD酵素活性則無顯著差異($P > 0.05$)。在肝臟中三酸甘油脂含量亦有明顯的降低($P < 0.05$)；在組織切片方面，組合乳酸菌組較酒精組有明顯減少肝臟中油滴的堆積。綜上所述，本實驗結果顯示在服用此組合乳酸菌之後，具有改善及降低因酒精所引起之酒精性脂肪肝及相關肝損傷。

關鍵詞：酒精性肝損傷、脂肪肝、三酸甘油脂、膽固醇、組合乳

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參考文獻

1. 行政院衛生署。2013。民國101年國人十大死亡原因統計報告。
2. 行政院衛生署。2010。健康食品護肝功能評估方法修正草案。
3. 曾秋隆。1994。曾氏獸醫血液學。藝軒圖書公司。台北。
4. 黃欣智、蔡銘鴻、陳邦基。2012。肝硬化的診治概要。內科學誌23:392-397。
5. 劉倚孝。2012。以C57BL/6N小鼠模式探討益生菌改善酒精性脂肪肝及肝損傷之研究。弘光科技大學碩士論文。台中。
6. Ahrens, H. 1991. Liver Function, Vol. 3 from the Measurement in Medicine Series. Biometrical Journal. 33:400-400.
7. Benchimol, E. I. and Mack, D. R. 2004. Probiotics in relapsing and chronic diarrhea. J Pediatr Hematol Oncol. 26:515-7.
8. Brown, A. C. and Valiere, A. 2004. Probiotics and medical nutrition therapy. Nutr Clin Care. 7:56-68.
9. Bruha, R., Dvorak, K. and Petryl, J. 2012. Alcoholic liver disease. World J Hepatol. 4:81-90.
10. Fischer, M., You, M., Matsumoto, M. and Crabb, D. W. 2003. Peroxisome proliferator-activated receptor alpha (PPAR α) agonist treatment reverses PPAR α dysfunction and abnormalities in hepatic lipid metabolism in ethanol-fed mice. J Biol Chem. 278:27997-8004.
11. Frei, B. and Mark, R. M. 1999. CAN ANTIOXIDANT VITAMINS MATERIALLY REDUCE OXIDATIVE DAMAGE IN HUMANS? Free Radical Biology & Medicine. 26:1034-1053.
12. Fungwe, T. V., Cagen, L., Wilcox, H. G. and Heimberg, M. 1992. Regulation of hepatic secretion of very low density lipoprotein by dietary cholesterol. J Lipid Res. 33:179-91.
13. Gressner, A. M. and Bachem, M. G. 1995. Molecular mechanisms of liver fibrogenesis--a homage to the role of activated fat-storing cells. Digestion. 56:335-46.
14. Higashikawa, F., Noda, M., Awaya, T., Nomura, K., Oku, H. and Sugiyama, M. 2010. Improvement of constipation and liver function by plant-derived lactic acid bacteria: a double-blind, randomized trial. Nutrition. 26:367-74.
15. Horton, J. D., Goldstein, J. L. and Brown, M. S. 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. Journal of Clinical Investigation. 109:1131-1125.
16. Iimuro, Y., Gallucci, R. M., Luster, M. I., Kono, H. and Thurman, R. G. 1997. Antibodies to tumor necrosis factor alfa attenuate hepatic necrosis and inflammation caused by chronic exposure to ethanol in the rat. Hepatology. 26:1530-7.
17. Ishak, K. G., Zimmerman, H. J. and Ray, M. B. 1991. Alcoholic liver disease: pathologic, pathogenetic and clinical aspects. Alcohol Clin Exp Res. 15:66-45.
18. Mate, J. M., Perez, G. C. and Castro, I. N. 1999. Antioxidant Enzymes and Human Diseases. Clinical Biochemistry. 32: 595-603.
19. Kershenobich Stalnikowitz, D. and Weissbrod, A. B. 2003. Liver fibrosis and inflammation. A review. Ann Hepatol. 2:159-63.
20. Kirpich, I. A., Solovieva, N. V., Leikhter, S. N., Shidakova, N. A., Lebedeva, O. V., Sidorov, P. I., Bazhukova, T. A., Soloviev, A. G., Barve, S. S., McClain, C. J. and Cave, M. 2008. Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: a pilot study. Alcohol. 42:675-82.
21. Landis, G. N. and Tower, J. 2005. Superoxide dismutase evolution and life span regulation. Mech Ageing Dev. 126:365-79.
22. Lash, L. H., and Jones, D. P. 1985. Distribution of oxidized and reduced forms of glutathione and cysteine in rat plasma. Arch Biochem Biophys. 240:583-92.
23. Lieber, C. S. and DeCarli, L. M. 1989. Liquid diet technique of ethanol administration: 1989 update. Alcohol Alcohol. 24:211-197.
24. Lin, S. C., Lin, Y. H., Chen, C. F., Chung, C. Y. and Hsu, S. H. 1997. The hepatoprotective and therapeutic effects of propolis ethanol extract on chronic alcohol-induced liver injuries. Am J Chin Med. 25:325-32.
25. Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 25:402-8.
26. Lu, Y., Wu, D., Wang, X., Ward, S. C. and Cederbaum, A. I. 2010. Chronic alcohol-induced liver injury and oxidant stress are decreased in cytochrome P4502E1 knockout mice and restored in humanized cytochrome P4502E1 knock-in mice. Free Radic Biol Med. 49:1406-16.
27. Maher, J. J. 1997. Exploring alcohol's effects on liver function. Alcohol Health Res World. 21:12-5.
28. Masella, R., Benedetto, R. D., Vari, R., Filesi, C. and Giovannini, C. 2005. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. J Nutr Biochem. 16:577-86.
29. Montalto, M., Nucera, G., Santoro, J., Curigliano, V., Vastola, M., Covino, M., Cuoco, L., Manna, R., Gasbarrini, A. and Gasbarrini, G. 2005. Effect of exogenous beta-galactosidase in patients with lactose malabsorption and intolerance: a crossover double-blind placebo-controlled study. Eur J Clin Nutr. 59:489-93.
30. Nanji, A. A., Khettry, U., Sadrzadeh, S. M. and Yamanaka, T. 1993. Severity of liver injury in experimental alcoholic liver disease. Correlation with plasma endotoxin, prostaglandin E2, leukotriene B4, and thromboxane B2. Am J Pathol. 142:367-73.
31. Niemela, O., Parkkila, S., Juvonen, R. O., Viitala, K., Gelboin, H. V. and Pasanen, M. 2000. Cytochromes P450 2A6, 2E1, and 3A and production of protein-aldehyde adducts in the liver of patients with alcoholic and non-alcoholic liver diseases. J Hepatol. 33:901-893.
32. Oneta, C. M. and Dufour, J. F. 2002. Non-alcoholic fatty liver disease: treatment options based on pathogenic considerations. Swiss Med Wkly. 132:505-493.
33. Parvez, S., Malik, K. A., Kang, S. A. and Kim, H. Y. 2006. Probiotics and their fermented food products are beneficial for health. J Appl Microbiol. 100:1171-85.
34. Pastore, A., Federici, G., Bertini, E. and Piemonte, F. 2003. Analysis of glutathione: implication in redox and detoxification. Clinica Chimica Acta. 333:39-19.
35. Peters, J. M., Cattley, R. C. and Gonzalez, F. J. 1997. Role of PPAR alpha in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643. Carcinogenesis. 18:2029-33.
36. Rao, R. K., Seth, A. and Sheth,

- P. 2004. Recent Advances in Alcoholic Liver Disease I. Role of intestinal permeability and endotoxemia in alcoholic liver disease. *Am J Physiol Gastrointest Liver Physiol.* 286:G881-4. 37. Reddy, G., Altaf, M., Naveena, B. J., Venkateshwar, M. and Kumar, E. V. 2008. Amylolytic bacterial lactic acid fermentation - a review. *Biotechnol Adv.* 26:34-22. 38. Sass, D. A. and Shaikh, O. S. 2006. Alcoholic hepatitis. *Clin Liver Dis.* 10:219-37
39. Sawada, H., Furushiro, M., Hirai, K., Motoike, M., Watanabe, T. and Yokokura, T. 1990. Purification and characterization of an antihypertensive compound from *Lactobacillus casei*. *Agric Biol Chem.* 54:3211-9. 40. Segawa, S., Hayashi, A., Nakakita, Y., Kaneda, H., Watari, J. and Yasui, H. 2008. Oral administration of heat-killed *Lactobacillus brevis* SBC8803 ameliorates the development of dermatitis and inhibits immunoglobulin E production in atopic dermatitis model NC/Nga mice. *Biol Pharm Bull.* 31:884-9. 41. Shimano, H., Shimomura, I., Hammer, R. E., Herz, J., Goldstein, J. L., Brown, M. S. and Horton, J. D. 1997. Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. *J Clin Invest.* 100:2115-24. 42. Thurman, R. G. 1998. II. Alcoholic liver injury involves activation of Kupffer cells by endotoxin. *Am J Physiol.* 275:G605-11. 43. Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M. and Mazur, M. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact.* 160:40-1. 44. Weltman, M. D., Farrell, G. C. and Liddle, C. 1996. Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. *Gastroenterology.* 111:1645-53. 45. Wendel, A. and Cikryt, P. 1980. The level and half-life of glutathione in human plasma. *FEBS Lett.* 120:209-11. 46. Woodcroft, K. J. and Novak, R. F. 1999. Insulin differentially affects xenobiotic-enhanced, cytochrome P-450 (CYP)2E1, CYP2B, CYP3A, and CYP4A expression in primary cultured rat hepatocytes. *J Pharmacol Exp Ther.* 289:1121-7. 47. Yahagi, N., Shimano, H., Hasty, A. H., Matsuzaka, T., Ide, T., Yoshikawa, T., Amemiya-Kudo, M., Tomita, S., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Osuga, J., Harada, K., Gotoda, T., Nagai, R., Ishibashi, S. and Yamada, N. 2002. Absence of sterol regulatory element-binding protein-1 (SREBP-1) ameliorates fatty livers but not obesity or insulin resistance in Lepob/Lepob mice. *J Biol Chem.* 277:19353-7. 48. Zakhari, S. 2006. Overview: how is alcohol metabolized by the body? *Alcohol Res Health.* 29:245-54.