様式Ｃ－１９

科学研究費助成事業（科学研究費補助金）研究成果報告書

平成２４年６月１日現在

機関番号：16201
研究種目：基盤研究（Ｃ）
研究期間：2009～2011
課題番号：21591751
研究課題名（和文）　希少糖の肝臓虚血再灌流障害保護作用及び肝切除後の再生促進効果の解析
研究課題名（英文）　Effect of rare sugars on ischemia-reperfusion injury of hepatectomized rats.
研究代表者
Ｍ・Ａ　ＨＯＳＳＡＩＮ（HOSSAIN MOHAMMAD AKRAM）
香川大学・希少糖研究センター・研究員
研究者番号：20294754

研究成果の概要（和文）：
希少糖の肝臓虚血再灌流障害保護作用及び肝切除後の再生促進効果の解析の研究を実施した。７０％肝切除後の肝臓を 30 分間虚血に暴露した。100mg/kg 体重の割合で D-アロースを静脈内投与したところ、再還流後 60 分まで血流が改善し、白血球の浸潤が抑制された。また残存肝における肝細胞の増殖が促進された。次に肝臓の虚血の原因のひとつである、肥満や動脈硬化を招来するラットである OLETF ラットを用いて、抗酸化作用を有する別の希少糖 D-プシコースの働きを解析した。D-プシコースは体重減少をおこし、動脈硬化を改善した。肝臓の脂肪毒性を改善するものとして注目された。

研究成果の概要（英文）：Studies were undertaken to examine the effect of rare sugars D-allose and D-psicose.

1) Initially we performed the study on the effect of D-allose against ischemia-reperfusion injury of hepatectomized rats. 70% of rat livers were resected under 30 min total ischemia. Intravenous 100mg/kg body weight D-allose was infused 30 min before inducing ischemia. Blood flow in the remnant liver was improved by D-allose till 60 min reperfusion. D-allose improved the remnant liver function and survival through the suppression of neutrophil enzyme MPO and stimulation of DNA labeling index and proliferating cell nuclear antigens (PCNA).
2) Next, we examined the effect of D- in lowering blood glucose and abdominal fat by using a T2DM model OLETF rats. Treated rats were fed with or without 5% D-psicose in drinking water for 13 weeks. D-psicose significantly reduced body weight and abdominal fat mass. Oral glucose tolerance test (OGTT) showed reduced blood glucose and insulin levels suggesting improvement of insulin resistance. Oil-red-O staining revealed reduced lipid accumulation in the liver. D-psicose also protected the pathological changes in pancreatic islets. These data demonstrate that D-psicose controls blood glucose levels by reducing lipotoxicity in liver and by preserving pancreatic b-cell function.

交付決定額

（金額単位：円）

	直接経費	間接経費	合　計
2009 年度	1,900,000	570,000	2,470,000
2010 年度	1,000,000	300,000	1,300,000
2011 年度	700,000	210,000	910,000
年度			

年度			
総　計	3,600,000	1,080,000	4,680,000

研究分野：
科研費の分科・細目：外科系臨床医学、消化器外科学
キーワード：(1) Rare sugar D-psicose (2) Type 2 diabetes mellitus (T2DM) (3) OLETF rats (4) Insulin resistance (5) Adiposity (6) Pancreatic b-cells

１．研究開始当初の背景

Background of D-allose study: In liver surgery, during resection induction of ischemia is inevitable to reduce bleeding as well as to have a clear operation field. During this time ischemic injury is caused and after operation when blood is reintroduced to the remnant liver reperfusion injury occurs. During reperfusion ROS from the activated neutrophils becomes the main causative agents of injury. Moreover, disturbed microcirculation by the addition of activated neutrophils on vascular endothelium aggravates the injury. If suppression of neutrophils either by their activation or by participation through reducing the number of neutrophils is possible the neutrophil-induced IRI might be reduced. **Background of D-psicose study:** Obesity has emerged as one of the serious life-style related health problems all over the world. It is the result of an energy imbalance caused by an increased ratio of caloric intake to energy expenditure, and is associated with excess fat accumulation and high blood sugar levels, followed by type 2 diabetes mellitus. It is possible to stop T2DM or to keep control with life-style modification or food-additive simple medications which has neither caloric value nor relative side-effects. Since rare sugar D-psicose has been reported as a zero-calorie sweet sugar and a strong the reducer of elevated blood sugar and excess fat accumulation by several studies on rats and healthy human volunteers, the present study was furnished.

２．研究の目的

Since D-allose has been shown to reduce the number of segmented neutrophils we designed our study aiming at minimize IRI through the suppression of neutrophils and improvement of microcirculation in the remnant liver after operation. Background of D-psicose study: Obesity has emerged as one of the serious life-style related health problems all over the world. It is the result of an energy imbalance caused by an increased ratio of caloric intake to energy expenditure, and is associated with excess fat accumulation and high blood sugar levels, followed by type 2 diabetes mellitus. It is possible to stop T2DM or to keep control with life-style modification or food-additive simple medications which has neither caloric value nor relative side-effects. Since rare sugar D-psicose has been reported as a zero-calorie sweet sugar and a strong the reducer of elevated blood sugar and excess fat accumulation by several studies on rats and healthy human volunteers, the present study was furnished.

The aforesaid effects of D-psicose have been studied on normal rats and borderline diabetic healthy humans. So, the purpose of our study was to evaluate these effects on type 2 diabetic rat model, OLETF rats.

３．研究の方法

Rat liver was subjected to 30 min total ischemia by occluding the blood supply to the middle and left lobes and the non-ischemic lobes were resected. Treated rats were injected D-allose (200mg/kg body weight) 20 min prior to inducing ischemia. Control group was given normal saline. Peripheral tissue blood flow of the liver was measured by laser Doppler tissue flow meter. After 60 min reperfusion venous blood was drawn to measure serum levels of liver enzymes and BrdU was injected intraperitoneally before sacrifice at each time point. On sacrifice liver tissue was fixed in paraffin and stained with respective antibodies. Some animals were followed up for 1 week to observe survival.

４．研究成果

Tissue blood flow: There was no difference of liver tissue blood flow between the two groups in the pre-ischemic period. On reperfusion, blood flow was significantly

improved in the D-allose treated group compared with control group (Fig. A). It is clear from this finding that postischemic microcirculatory stasis of blood flow is caused by the adherence of neutrophils to the microvascular endothelium as well as plugging of liver sinusoids by neutrophils causes the obstruction of blood flow and D-allose improved blood flow through decreasing neutrophils in the circulation or suppressing neutrophil activation during reperfusion.

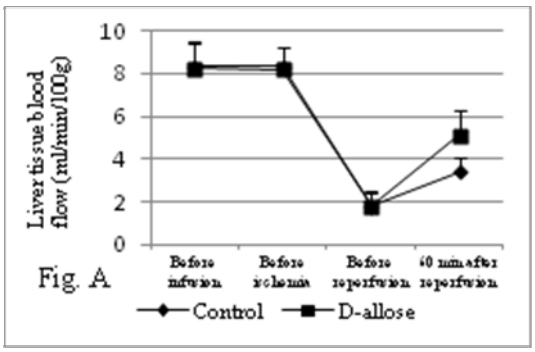


Fig. A

BrdU incorporation: DNA synthesis by regenerating hepatocytes in the remnant liver is shown in Fig. B. The labeling index was peaked at 12h postoperatively. This finding indicates that D-allose effectively accelerate hepatocute DNA synthesis and regeneration in hepatectomized IRI rats.

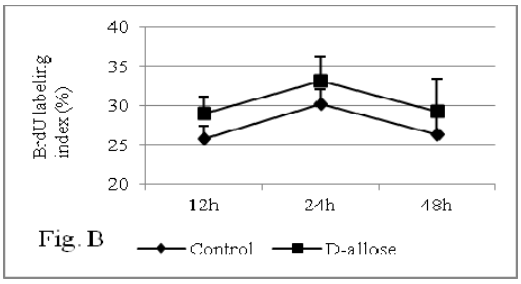


Fig. B

Physical appearance of the diabetic non-treated control rats was bulky than other rats. At the study end-point D-psicose treatment reduced the body weight significantly than the non-treated control rats (Fig-1).

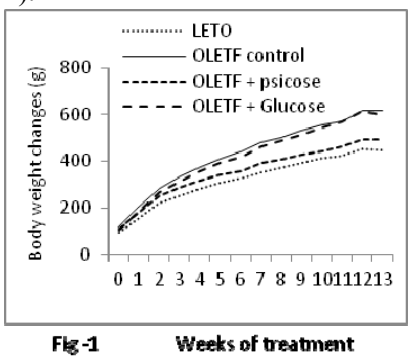


Fig -1 Weeks of treatment

Periodical blood glucose levels remained constant within the normal physiological levels in the D-psicose treated OLETF rats, where the levels elevated severely in the control rats (Fig-2).

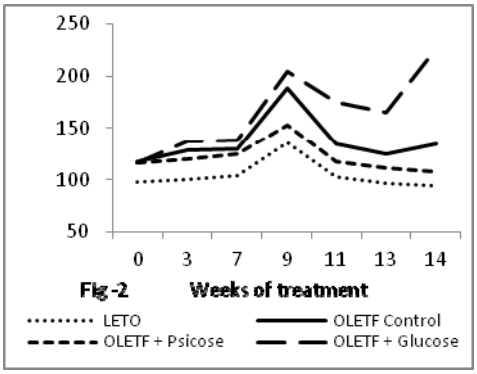


Fig -2 Weeks of treatment

Blood glucose levels in all diabetic OLETF rats were similar at 15 min of OGTT. The values were significantly lower from 30 t0 90 min in D-psicose rats compared with control and D-glucose rats (Fig-3), which means marked improvement of insulin resistance in the treated rats by the rapid movement of gluocse into the cells.

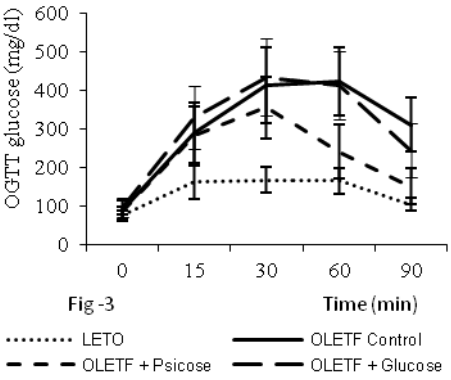
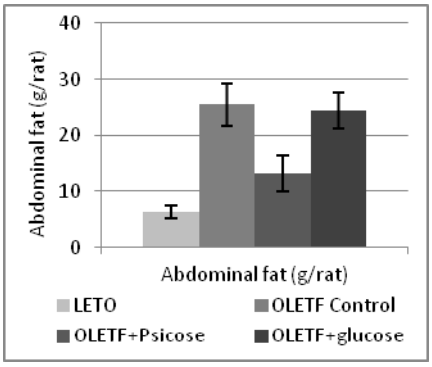


Fig -3

The total amount of epididymal and reirenal fat deposition accumulation in the abdomen was significantly reduced in the D-psicose-treated rats (Fig. 4).



Reduced amount of fat in the liver also evaluated by Oil-red-O staining. Histological analysis indicated that the control OLETF rats accumulated large-sized droplets of lipid evaluated by the microscopic size of adipocyte outlet, where the D-psicose treated rats showed smaller lipid droplets.

There was striking differences of pancreas morphology by HE and other staining. Pancreas histology revealed islet hypertrophy with disorganized and irregular shape and the islets were seperated into clusters. In some control rats the islets were shown expanded into the adjacent endocrine tissues, which suggest islet hypertrophy. Entensive fibrosis and fatty degeneration were also marked in the hypertrophied islets of control rats, where the above-mentioned features less prominet or absent in the D-psicose treated rats. In the psicose-treated rats the islets were orgaized with small and round shapes, minimum fibrosis and less fatty deposition. So, D-psicose prevented the pancreas from diabetes-induced injuries.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計１　件）

①. Mohammad AHossain, Shigeru Kitagaki, Daisuke Nakano, Akira Nishiyama, Yasunobu Funamoto,Toru Matsunaga, Ikuko Tsukamoto, Fuminori Yamaguchi, Kazuyo Kamitori, Youyi Dong, Yuko Hirata, Koji Murao, Yukiyasu Toyoda, Masaaki Tokuda Rare sugar D-psicose improves insulin sensitivity and glucose tolerance in type 2 diabetes Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Biophysical and Biochemical research Communications 2011:405:7-12.

〔学会発表〕（計５　件）

①. Mohammad A Hossain, Yuko Hirata, Kazuo Kamitori, Youyi Dong, Fuminori Yamaguchi, Masaaki Tokuda. Rare sugar D-psicose protects pancreas b-islets and thus improves insulin resistance in OLETF rats. The 5th Symposium of International Society of Rare Sugars, November 09-12, Takamatsu, Kagawa, Japan

②. Mohammad A Hossain, Yuko Hirata, Kazuo Kamitori, Youyi Dong, Fuminori Yamaguchi, Masaaki Tokuda. Rare sugar D-psicose protects pancreas b-islets and thus improves insulin resistance in OLETF rats. Japanese Society of Physiology; Chugoku-Shikoku Regional Meeting, Oct 22-23, 2011, Hirashima University, Hiroshima, Japan (Oral)

③. Mohammad A Hossain, Yuko Hirata, Kazuo Kamitori, Youyi Dong, Fuminori Yamaguchi, Masaaki Tokuda. D-psicose protects pancreas □-islets and thus improves insulin resistance in OLETF Rats. The 32nd Annual Meeting of Japan Society for the Study of Obesity, Sept 23-24, 2011, Awajishima, Japan (Poster)

④. Mohammad A Hossain, Yuko Hirata, Kazuo Kamitori, Youyi Dong, Fuminori Yamaguchi, Masaaki Tokuda. Rare Sugar D-Psicose Ameliorates Glucose Intolerance in Type-2 Diabetes OLETF Rat Model. Federations of Asian and Oceanian Physiological Society Congress 2011, , September 11-14, 2011, Taipei, Taiwan (Poster)

⑤. Mohammad A Hossain, Kitagaki S, Nishiyama A, Toyoda Y, Yamaguchi F, Kamitori K, Dong Y, Hirata Y, Tokuda M. Rare sugar D-psicose ameliorates glucose intolerance in type-2 diabetes rat model. 3rd. International Conference on Drug Discovery and Therapy, February 07-11, Dubai, UAE (Poster)

〔図書〕（計　件）

〔産業財産権〕
○出願状況（計０　件）

名称：
発明者：
権利者：
種類：
番号：
出願年月日：
国内外の別：

○取得状況（計０件）

名称：
発明者：

権利者：
種類：
番号：
取得年月日：
国内外の別：

〔その他〕
ホームページ等

６．研究組織
(1)研究代表者
M・A HOSSAIN (HOSSAIN MOHAMMAD AKRAM)
香川大学・希少糖研究センター・研究員
研究者番号：20294754

(2)研究分担者
徳田　雅明 (TOKUDA MASAAKI)
香川大学・医学部・教授
研究者番号：10163974

(3)連携研究者
（　　）

研究者番号：