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Effect of globin digest on the liver injury and hepatic gene expression profile in galactosamine-induced liver injury in SD rats

Kaori Yamamoto [1], Yuka Sasakawa *,[1], Fumiko Nakaoka, Mayumi Nakao, Miki Nakamura, Akari Kominami, Michiyo Abe, Chizuko Fukuhama, Kyoichi Kagawa

MG Pharma Inc., 7-7-25, Saito-Asagi, Ibaraki, Osaka 567-0085, Japan

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ABSTRACT

Aims: We investigated the effect of globin digest (GD) on the liver injury and hepatic gene expression profile in galactosamine (GalN)-induced liver injury.
Main methods: The effect of GD on the liver injury was examined by measuring the activities of serum transferases and hepatic antioxidant enzymes, histopathological analysis, gene expression profile, and proteins of the peroxisome proliferator-activated receptor alpha (PPARα) and met proto-oncogene (c-Met) in SD rats at 24 h after GalN administration. The effect of GD on the expression of PPARα and its target gene in AML-12 mouse hepatocytes was also examined.
Key findings: GD suppressed the elevated activities of serum transferases in GalN-induced liver injury in SD rats. The thiobarbituric acid reactive substance content in GalN-injured liver was a decreasing tendency by GD. GD suppressed the increased oxidized glutathione content, and increased the decreased protein, reduced glutathione contents, and catalase activity in GalN-injured liver. GD may improve the antioxidant defense system and protein synthesis in GalN-injured liver. GD suppressed the elevated expression of the genes related to the inflammation, and decreased the histopathological grade value of inflammatory cell infiltration in GalN-injured liver. GD increased the expression of PPARα protein in GalN-injured liver, and also increased the expression of PPARα and its target gene in AML-12 hepatocytes. The total and phosphorylated c-Met proteins in GalN-injured liver were the increasing tendencies by GD.
Significance: These findings indicate that GD has the hepatoprotective effect on GalN-induced liver injury in SD rats.

Introduction

Globin digest (GD), an oligopeptide mixture derived from edible globin proteins, improved hyperlipidemia in humans and other mammals (Kagawa et al., 1996, 1998, 1999, 2008; Kamei et al., 2001; Inagaki et al., 2002; Okuda et al., 2004). Clinical studies of GD have shown that the postprandial serum triglyceride (TG) and chylomicron levels were suppressed (Kagawa et al., 1998, 1999, 2008). It has also been shown that GD improved hyperglycemia in humans and mice (Haibin, 2008; Tong et al., 2008; Sasakawa et al., 2008; Nakaoka et al., 2010).

Because GD has improvement effects on metabolic disorders, the effect of GD on the liver injury, a metabolic disorder (review: Chuang et al., 2004), and hepatic gene expression profile in galactosamine (GalN)-induced liver injury in SD rats was examined. GalN is a hepatotoxin frequently used to induce an experimental liver injury (Keppler et al., 1968; review: Decker and Keppler, 1972). GalN mainly causes liver injury via the generation of free radicals and depletion of uridine triphosphate (UTP) nucleotides (Sinha et al., 2007). High dose of GalN is known to cause hepatic necrosis by UTP depletion (Keppler et al., 1970), leading to inhibition of RNA synthesis (review: Plaa, 1991) and protein synthesis (Koff et al., 1971; Shinozuka et al., 1973), and induction of apoptosis in the liver of the rats and mice (Tsutsui et al., 1997; Muntané et al., 1998; Sun et al., 2003; El-Mofty et al., 1975). It has been reported that endotoxemia caused by GalN plays an important role in the progress of GalN-induced liver injury (Tsubomizu et al., 1986; Iwaki et al., 1988). The hepatocyte degeneration induced by GalN itself may be followed by severe liver damage due to hepatocytotoxic factors in endotoxemia (Iwaki et al., 1988). It has also been shown in GalN-treated rats that hepatic levels of antioxidant such as reduced glutathione (GSH) and hepatic activities of antioxidant enzymes such as superoxide dismutase (SOD) (an enzyme to scavenge O_2^-), catalase (CAT) (an enzyme to decompose H_2O_2), and glutathione peroxidase (Gpx) (an enzyme to metabolize both H_2O_2 and lipid hydroperoxides using GSH as a cosubstrate) were reduced during liver injury development (Hu and Chen, 1992; Seçkin et al., 1993; Sun et al., 2003; Ohta et al., 2004). In the present study, we attempted to clarify the effect of GD on the

* Corresponding author. Tel.: +81 72 643 0171; fax: +81 72 643 0121.
E-mail address: y-sasakawa@mgpharma.co.jp (Y. Sasakawa).
[1] These authors contributed equally to this work.

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antioxidant status during GalN-induced liver injury by measuring antioxidants such as GSH and oxidized glutathione (GSSG) and antioxidant enzymes such as SOD, CAT, Gpx, and glutathione reductase (GR) (an enzyme to reduce GSSG to GSH) (review: Mannervik, 1987; review: Meister, 1988).

GD increased hepatic CAT activity as an approximate indicator of peroxisome proliferation in high-fat diet-fed mice (Matsutaka et al., 1992). Because it has been reported that the peroxisome proliferator-activated receptor alpha (PPARα) ligands increased the CAT activity in thioacetamide (TAA)-injured liver of the rats (Toyama et al., 2004), the effect of GD on the expression of PPARα protein in GalN-injured liver, and PPARα and its target gene, fatty acid transport protein (FATP), in AML-12 mouse hepatocytes was examined.

Materials and methods

Materials

GD (MG Pharma, Osaka, Japan) was used for the examination. GD is produced from the enzymatic hydrolysis of globin protein in bovine or swine hemoglobin (Kagawa et al., 1991). The digest is an oligopeptide mixture that consists of 3–5 amino acid residues. GD is composed of more than 91% proteins and less than 8% free amino acids, and the molecular weights of the peptides contained range from 100 to 1500 u. GD was dissolved in distilled water and administered orally to rats at 2 g/kg. Uridine (213-00771, Wako, Osaka, Japan) was dissolved in distilled water and administered orally to rats at 1 g/kg. The administering capacity of GD and uridine was 10 ml/kg. GD dissolved in distilled water at 200 mg/ml was used at doses of 0.1 mg/ml, 1 mg/ml, and 10 mg/ml for in vitro examination. Fenofibric acid (Toronto Research Chemicals, Ontario, Canada) was dissolved in dimethyl sulfoxide to 100 mM and used at a dose of 200 μM for in vitro examination.

Animals and treatments

This study conformed to the ethical guidelines for animal experimentation of MG Pharma Inc. in accordance with the Declaration of Helsinki. SD strain male rats (age, 7 weeks; weight, 271–298 g) (Japan SLC, Shizuoka, Japan) were used. The rats were housed in an air-conditioned room (23 ± 2 °C, 50 ± 10% RH) with a 12-h light and dark cycle (7:00–19:00 light hours). The rats were kept in an experimental animal room for 7 days with free access to food and water. Rats with pelage in good condition and weights of 271–298 g were used for the experiments.

GalN-induced liver injury

SD rats were divided into the following four groups at random, with three rats per group: vehicle control, GalN control, 2 g/kg GD, and 1 g/kg uridine. Uridine was used as the positive control. Uridine could reverse UTP deficiency and the inhibition of RNA and protein synthesis after GalN administration (Farber et al., 1973). D-galactosamine hydrochloride (GalN) (079-02054, Wako, Osaka, Japan) was dissolved into saline and administered intraperitoneally to rats of the GalN control, GD, and uridine groups at 300 mg/kg. Saline was administered intraperitoneally to rats of the vehicle control group. The administering capacity of GalN and saline was 10 ml/kg. Distilled water was administered orally to rats of the vehicle control group twice, at 1 h before saline and at 8 h after saline administration. Distilled water was administered orally to rats of the GalN control group twice, at 1 h before GalN and at 8 h after GalN administration. Two g/kg GD or 1 g/kg uridine was administered orally to rats twice, at 1 h before GalN administration

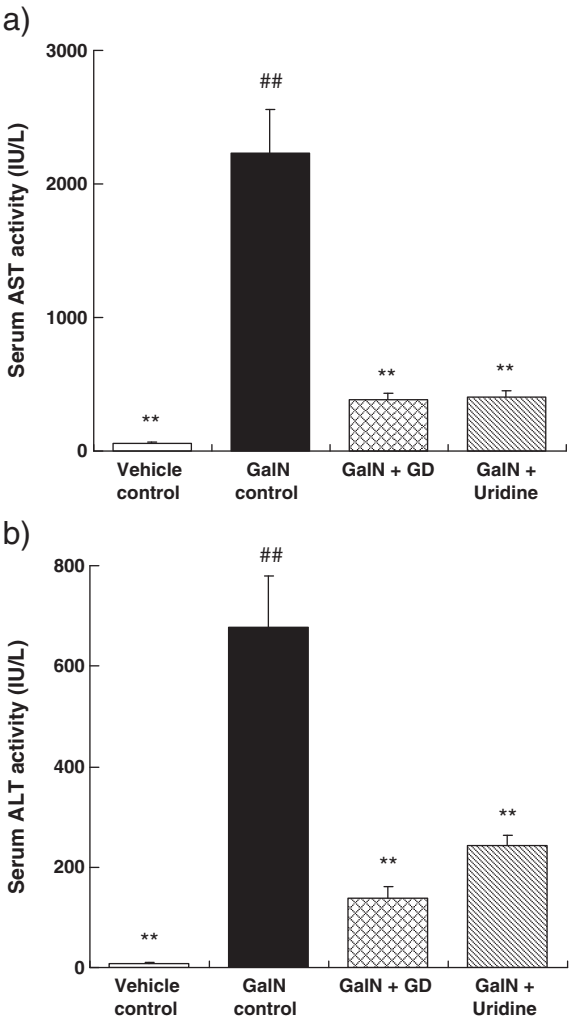

Fig. 1. Effect of GD on the serum transferase activities in GalN-induced liver injury in SD rats. The values are the means of three rats, with standard errors represented by vertical bars. The levels of serum activities of AST (a) and ALT (b) at 24 h after GalN administration were measured. Significant difference was seen between the vehicle control values ($^{\#\#}$ $p<0.01$) and the GalN control values (** $p<0.01$) by ANOVA and Ryan's method.

Table 1
Effect of GD on the body weight, liver weight, hepatic TG, T-CHO, and protein contents in GalN-induced liver injury in SD rats.

Group	n	Body weight (g)		Liver weight (g)	TG	T-CHO	Protein
		Before GalN	24 h after GalN	24 h after GalN	(mg/g liver wet weight)		
Vehicle control	3	278 ± 2	257 ± 1	7.6 ± 0.1**	7.1 ± 0.3**	2.9 ± 0.0*	253.7 ± 6.8**
GalN control	3	285 ± 4	262 ± 4	10.4 ± 0.5$^{\#\#}$	40.8 ± 4.7$^{\#\#}$	4.0 ± 0.3$^{\#}$	199.3 ± 10.7$^{\#\#}$
GalN + 2 g/kg GD	3	293 ± 3	269 ± 5	10.2 ± 0.2$^{\#\#}$	34.7 ± 3.3$^{\#\#}$	3.8 ± 0.4	238.0 ± 3.1**
GalN + 1 g/kg Uridine	3	288 ± 5	260 ± 3	10.1 ± 0.2$^{\#\#}$	46.0 ± 8.3$^{\#\#}$	5.6 ± 0.3**	240.7 ± 4.4**

The values are the means of three rats, with standard errors. The body weight before GalN administration and at 24 h after GalN administration, liver weight, hepatic TG, T-CHO, and protein contents at 24 h after GalN administration were measured. Significant difference was seen between the vehicle control values ($^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$) and the GalN control values (* $p<0.05$, ** $p<0.01$) by ANOVA and Ryan's method.