1,3,5,8-Tetrahydroxyxanthone regulates ANGPTL3–LPL pathway to lessen the ketosis in mice

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A B S T R A C T

Ketosis is a metabolic disorder closely associated with both lipid and carbohydrate metabolism. Recent studies show that angiopoietin-like protein 3 (ANGPTL3) contributes to the development of metabolic disorder. The objective of this study was to explore the inhibitory effect of 1,3,5,8-tetrahydroxyxanthone (Xan), a naturally occurring flavonoid compound, on ketosis and the mechanisms involved in this regulation. After 4 weeks, Xan (10 or 30 mg/kg, intragastrically) treatment decreased plasma total ketone bodies, malondialdehyde, 8-isoprostane, triglyceride, total cholesterol levels, and hepatic ANGPTL3 expression concomitantly with increased plasma glucose concentration and adipose lipoprotein lipase (LPL) expression in ketosis murine. The present results suggest that Xan regulates ANGPTL3–LPL pathway to lessen the ketosis in mice.

1. Introduction

Ketosis is the accumulation of excessive amounts of ketone bodies in body tissues and fluids, occurring when fatty acids are incompletely metabolized. Ketones comprise acetone, acetoacetate, and beta-hydroxybutyrate (BHB). Very high levels of ketone can be toxic, making the blood more acid, and may damage organs such as the liver and kidneys. Commonly, ketoacidosis is diabetic ketoacidosis (DKA), resulting from elevated fat metabolism because of a shortage of insulin, which is related primarily to type 1 diabetes, and may lead to a diabetic coma if left untreated (Kitabchi et al., 2006).

It is well known that ketosis is a metabolic disorder closely associated with both lipid and carbohydrate metabolism (Loor et al., 2007). Angiopoietin-like (ANGPTL) proteins have been found to contain a coiled-coil domain and a fibrinogen-like domain similar to those identified in angiopoietins. Recent studies show that ANGPTL3 can suppress lipoprotein lipase (LPL) and contribute to metabolic disorder of both lipid and carbohydrate metabolism (Robciuc et al., 2010). Therefore, regulation of ANGPTL3–LPL pathway may be useful pharmacologic targets for treatment of ketosis.

Xanthone is a polyphenol derivative in plants. Previous investigations have shown that some polyphenolic compounds can regulate both lipid and carbohydrate metabolism (Baba et al., 2007; Pal et al., 2003; Peng et al., 2011). Our recent studies also have shown that 3,4,5,6-tetrahydroxyxanthone can regulate lipid metabolism by inhibiting ANGPTL3 expression and increasing LPL expression (Xiao et al., 2008). As mentioned above, regulation of ANGPTL3–LPL pathway may be a useful pharmacologic target for treatment of ketosis. Therefore, we hypothesized that xanthone can regulate ANGPTL3–LPL pathway to lessen the ketosis. There is close association between the oxidative stress level and clinical ketosis in diabetic patients, cows with subclinical ketosis, water buffalo, ewes with pregnancy toxemia (Al-Qudah, 2011; Youssef et al., 2010). It has been reported that Vitamin E can attenuate the damage induced by ketosis (Sahoo et al., 2009). Therefore, antioxidant agents may prevent ketosis by reducing oxidative stress. As a polyphenol derivative, xanthone has a strong antioxidant property (Jiang et al., 2003, 2004). Previous findings have suggested that oxidative stress may induce the expression of ANGPTL3 and inhibit the expression of LPL (Moon et al., 2007; Miida et al., 2008; Casanovas et al., 2009). Based on antioxidant properties of xanthone, it is probable that xanthone can regulate ANGPTL3–LPL pathway to lessen the ketosis as a result of inhibiting oxidative stress.

Previous investigation has reported that acetone administration can create the model of ketosis (Stadler et al., 2008). The objective of this study was to explore the inhibitory effect of 1,3,5,8-tetrahydroxyxanthone (Xan), a naturally occurring polyphenol...
compound, on ketosis and the mechanisms involved in this regulation. In the present study, therefore, we tested whether Xan could regulate ANGPTL3–LPL pathway to lessen the ketosis in a murine model of longer-term ketosis.

2. Materials and methods

2.1. Reagents

Xan (purity: 98.0%) (Fig. 1) was obtained from Shenzhen Gamart Technology Development Co. Ltd. (Shenzhen, China). Antibody was purchased from Santa Cruz Biotechnology (USA). Other reagents were purchased from Sinopharm Chemical Reagent (Shanghai, China).

2.2. Animals

C57BL/6J male mice weighing 20–22 g were purchased from the Laboratory Animal Center, Faculty of Veterinary Medicine, Hu-nan Agricultural University. Animals were housed under the same conditions and were fed with chow and water ad libitum. All animals received humane care adhered to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 86–23, revised 1986). In addition, animal research in our methods section was approved and granted by Hu-nan Agricultural University ethics review board.

2.3. Experimental protocols

Forty mice were divided randomly into four groups (n = 10): C57BL/6J control, untreated ketosis murine, two groups of Xan-treated (10 or 30 mg/kg per day, intragastrically) ketosis murine. In the ketosis murine, mice were given 2% acetone dissolved in their drinking water for 3 weeks ad libitum (Stadler et al., 2008). Xan was dissolved in a vehicle including 0.5% saline carboxymethyl cellulose and 10% gum acacia. In the control group and model group, mice were given with vehicle of Xan (30 mg/kg per day). After 4 weeks, all mice were fasted overnight before being euthanized. Blood was collected from the carotid artery after anesthesia with pentobarbital (80 μg/kg IP) according to the previous methods (Chen et al., 2003). Plasma was prepared via centrifugation at 2500 g for 20 min at 4°C and stored at –70°C for measurement of blood metabolic parameters. The liver and adipose were harvested for the analysis of the expressions of ANGPTL3 and LPL.

2.4. Measurement of blood metabolic parameters

Fasting blood samples obtained from the artery added were added into precooled tubes containing EDTA (final concentration 4 mmol/L) and centrifuged at 2500g for 20 min at 4°C. Plasma total cholesterol (TC), triglyceride (TG), glucose, and total ketone bodies levels were measured by enzymatic method (bioMerieux, Lyon, France) utilizing an automated analyzer according to the manufacturer’s recommendations. The content of thiobarbituric acid reac-

tive substances (TBARS), reflecting the level of lipid peroxide, was determined spectrophotometrically using previously described methods (Papazzo et al., 2011) and expressed as the amount of malondialdehyde (MDA). Plasma 8-isoprostane concentration was measured using an 8-isoprostane competitive enzyme immunoassay (EIA; Cayman Chemicals, Ann Arbor, MI), following the protocol provided for determination of total (free and esterified) 8-isoprostane using a previously described method (Papazzo et al., 2011).

2.5. Expressions of ANGPTL3 and LPL mRNA

Real-time PCR was performed to quantify ANGPTL3 and LPL mRNA expressions. Briefly, total RNA was extracted from liver or adipose using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and reverse-transcribed. The following primer pairs were used (all shown 5′→3′): ANGPTL3 (forward), ACCACACAGCTGATGTTTCTC- TAC (reverse), CCACCCAGAAGGTCACCAG (Ge et al., 2005); LPL (forward), ACTGCCCTCAGATGCCCCTA (reverse), TTGTGGTGCGTTGCCATCTCTC (Ranganathan et al., 2006); GAPDH (forward), ACT- CCACTCAGCGGAAATTC (reverse), TCTCCATGCTTGGAAGAACA (Vetrone et al., 2009). The specificity of the primers was checked prior to using them. PCR was carried out under the following conditions: 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C plus 40 s at 60°C.

2.6. Analysis of protein expression of ANGPTL3 and LPL

Samples were taken from tissues (ANGPTL3 in liver, LPL in adipose tissue) and homogenized in the lysis buffer (10 mM NaH2PO4, pH 6.8, 1 mM EDTA, 10 mM β-mercaptoethanol, 0.25% Triton X-100 and 0.02% NaN3) using an electric homogenizer. Homogenates were sonicated further for 5 min on ice. The homogenate was centrifuged at 10,000g for 10 min at 4°C, and the supernatant was isolated. The protein concentration was measured by the protein assay (Bio-Rad). The proteins were separated by 12.5% SDS–PAGE and transferred into PVDF membrane using the Phast System (Pharmacia). The Western blot analyses were carried out according to methods described previously (Ono et al., 2003; Wiersma et al., 2009).

2.7. Statistical analyses

Results are expressed as means ± S.D. Differences between groups were evaluated by ANOVA and the unpaired Student’s t-test for multiple comparisons. P < 0.05 was chosen as statistically significant.

3. Results

3.1. Plasma MDA and 8-isoprostane levels

As biomarkers of oxidative stress, MDA and 8-isoprostane productions were determined after 4 weeks of treatment with Xan. Plasma MDA and 8-isoprostane levels were higher in the model mice than those in the control group. Xan (10 or 30 mg/kg) treatment prevented the elevated levels of MDA and 8-isoprostane in mice (P < 0.05 or P < 0.01; Fig. 2).

3.2. Hepatic ANPTL3 expression

In order to investigate the effect of Xan on ANPTL3 (a liver specific protein), hepatic ANPTL3 mRNA and protein expressions were analyzed after 4 weeks of administration with Xan. The expression of ANPTL3 in ketosis murine was clearly augmented compared
with that of control group \((P < 0.01)\). Xan (10 or 30 mg/kg) treatment resulted in clear decrease in ANPTL3 expression of ketosis murine \((P < 0.05\) or \(P < 0.01)\) (Fig. 3).

### 3.3. Adipose LPL expression

The expression of LPL (EC 3.1.1.34), a key enzyme to the hydrolysis of TG-rich lipoproteins, was down-regulated in ketosis murine compared with that in control group \((P < 0.01)\). LPL expression was up-regulated in ketosis murine consuming Xan (10 or 30 mg/kg) following 4 weeks of treatment \((P < 0.05\) or \(P < 0.01\), respectively) (Fig. 4).

### 3.4. Plasma lipids levels

The levels of plasma lipids (TG and TC) were significantly increased in the ketosis murine compared with the control group \((P < 0.01)\). Plasma TG and TC levels decreased significantly \((n = 10, P < 0.05\) or \(P < 0.01)\) following supplementation in Xan (10 or 30 mg/kg) groups (Fig. 5).

### 3.5. Plasma glucose concentration

Plasma glucose concentration in the model group were decreased significantly in the ketosis murine \((P < 0.01)\). Xan (10 or 30 mg/kg) treatment for 4 weeks markedly increased plasma glucose concentration in ketosis murine \((n = 10, P < 0.05\) or \(P < 0.01)\) (Fig. 6).

### 3.6. Plasma total ketone bodies concentration

As expected, 2% acetone administration successfully created a model of ketosis in mice. Plasma ketone bodies concentration in the model group were elevated significantly in the ketosis murine \((P < 0.01)\). Xan (10 or 30 mg/kg) treatment for 4 weeks markedly suppressed the elevation of plasma ketone bodies concentration in ketosis murine \((n = 10, P < 0.05\) or \(P < 0.01)\) (Fig. 7).

### 4. Discussion

Ketosis is a condition of elevated levels of ketone bodies in the blood (Kitabchi et al., 2006). It is well known that ketosis is related to a metabolic disorder of both lipid and carbohydrate metabolism.
Recent studies have reported that elevated expression of ANGPTL3 contributes to metabolic disorder of carbohydrate metabolism and lipid metabolism (Robciuc et al., 2010). Seven angiopoietin-like proteins (ANGPTLs; angiopoietin-related proteins) 1–7 have been found (Oike et al., 2003, 2004). Recently, ANGPTL-family members have been indicated to be regulators of both lipid and carbohydrate metabolism (Hato et al., 2008). The importance of ANGPTL3 for lipid metabolism was first ascertained by means of the genetic analysis of a mutant strain of obese mice with low plasma lipid levels. Administration of recombinant ANGPTL3 to ANGPTL3-knock-out mice as well as wild type mice elevated the plasma levels of non-esterified fatty acid, TG and TC (Koishi et al., 2002). ANGPTL3 suppresses LPL thereby lessening very-low-density-lipoprotein-TG clearance (Shimizugawa et al., 2002; Shimamura et al., 2003). ANGPTL3 also decreases high-density lipoprotein by inhibiting endothelial lipase (Shimamura et al., 2007). The N-terminal coiled-coil domains of ANGPTL3 and ANGPTL4 are important for the suppression of LPL by changing LPL from an active dimer into catalytically inactive monomers (Ono et al., 2003). ANGPTL3 is also elevated in both insulin-deficient and -resistant diabetic states (Inukai et al., 2004). The results of the present study showed that plasma total ketone bodies level were increased concomitantly with elevated TG and TC levels, decreased glucose concentration, increased ANGPTL3 expression and inhibited LPL expression in ketosis murine. These findings suggest regulation of ANGPTL3–LPL pathway may be a useful pharmacologic target for treatment of ketosis.

There are many evidences suggest oxidative stress is involved in the development of ketosis. Youssef et al. reported that oxidative...
stress level was associated with clinical ketosis in water buffalo. There was a negative correlation between glucose and BHB. However, there was a positive correlation between MDA and BHA. These results indicate that hyperketonemia in buffalo is related to an increase of oxidative stress levels (Youssef et al., 2010). Al-Qudah et al. reported that serum concentrations of BHB and TBARS, markers of lipid peroxidation, were markedly higher in ewes with pregnancy toxemia when compared with concentrations in healthy pregnant and nonpregnant ewes. In ewes with pregnancy toxemia, a strong positive correlation was found between concentrations of BHB and TBARS (Al-Qudah, 2011). Moreover, on the one hand, an association between hyperketonemia and the products of lipid peroxidation has also been demonstrated. Al-Qudah et al. suggested that oxidative stress in ewes affected by pregnancy toxemia and ketosis is a risk factor in the development of lipid peroxidation (Al-Qudah, 2011); on the other hand, ketone bodies can produce superoxide radicals and stimulate oxidative stress and cellular dysfunction, as noted in cows with subclinical ketosis or in diabetic patients (Al-Qudah, 2011). Stadler et al. also revealed that acetone administration, a model of ketosis, can cause protein oxidation and lipid peroxidation through a free radical-dependent mechanism driven mainly (Stadler et al., 2008). Furthermore, it has been shown that pharmacological interventions that improve oxidative stress can decrease the damage of ketosis (Sahoo et al., 2009). The results of the present study showed that plasma total ketone bodies level were increased concomitantly with elevated MDA and 8-isoprostane levels in ketosis murine. Therefore; further studies need to be done on the efficacy of antioxidants as an ancillary treatment to abolish the damage caused by ketosis.

5. Conclusion

In summary, we have studied the effect of Xan on the mechanisms that regulate the state of ketosis in mice. The primary targets of the compound are ANGPTL3 and LPL. Our findings revealed that Xan regulates ANGPTL3–LPL pathway to lessen the ketosis in mice. The results of the present work complement and sharpen the understanding of the efficacy of antioxidants as an ancillary treatment to abolish the damage caused by ketosis.

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**References**


