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Studies on the lipid-regulating mechanism of alisol-based compounds on lipoprotein lipase

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ABSTRACT

Studies on the lipid-regulating effects of alisol compounds are reported that include alisol B, alisol A 24-acetate (24A), alisol A and an alisol B - 24A - alisol A mixture (content ratio = 1:1:1). The effects on the activity of lipoprotein lipase (LPL), a key lipid-modulating enzyme, were studied to investigate the molecular mechanism of lipid-regulating activity of alisols. The effects of alisols on regulating blood lipids and the activities of LPL were determined using a reagent kit method. The structure of LPL was obtained by homology modeling and the interactive mechanism of alisol monomers and the mixture with LPL was investigated by molecular simulation. The alisol monomer and mixture were shown to regulate blood lipids, suggesting that alisols may decrease the level of triglyceride (TG) by improving the activity of LPL. The order of intensity was: mixture > alisol A > alisol B > 24A, indicating that alisols of alismatis rhizoma feature a synergistic effect on LPL. The N- and C-terminus of LPL both represented the catalytic active domains of this lipid-regulating effect. Cys306, Gln129 and Ser166 were the key amino acid residues resulting in the lipid-regulating effect of the alisol monomer while Ser166 and Arg18 were found to be responsible for the lipid-regulating effect of the mixture. The C-terminus of LPL was indirectly involved in the enzymatic process. A folded side chain of alisols or the parent ring was found to bind somewhat weaker to LPL than an open side chain or parent ring. The hydroxyl groups on the C14-, C22-, C28-, C30- and C31-terminus in the side chain, the ring ether structure in C23-position, and the acetyl group in C29-position represented the key sites for the lipid-regulating action of alisols. Meanwhile, the C30-site hydroxyl group played an important role in the synergistic effect of the alisol mixture.

1. Introduction

Hyperlipidemia represents a major disease state, generating serious health concerns for the general public. The treatment of hyperlipidemia involving western medicine, although effective, exhibits various side effects and is often prone to recurrence [1–3]. Due to the low costs of traditional chinese medicine, high hyperlipidemia efficacy, and negligible side effects, the development of lipid-lowering drugs involving traditional chinese medicine has become one of the most studied area in the field of lipid-modulating drug research [4–9]. Alismatis rhizoma is the rhizome of *Alisma orientale* (Sam.) Juzep., which belongs to the alismataceae family. It is commonly used for lipid-lowering medicine in the clinical treatment of hyperlipidemia [10–13], in which triterpenoids represent the main lipid-regulating active ingredients, including alisol B, alisol A 24-acetate (24A) and alisol A [14,15]. However, studies on the molecular mechanism of alismatis rhizoma are generally not thorough enough to allow for comprehensive conclusions, resulting in a lack of guidance of the clinical medication mechanism and often leading to the limited development and utilization of alismatis rhizoma in lipid-regulating treatments. Hyperlipidemia mainly refers to high total cholesterol (TC) levels, high triglyceride (TG) levels, high low-density lipoprotein cholesterol (LDL-C) levels, and low high-density lipoprotein cholesterol (HDL-C) levels in the serum. Here, lipoprotein lipase (LPL) is closely associated with high TG. LPL represents a classical key lipid metabolitic enzyme where the rate-limiting enzyme of TG degradation reaction plays a key regulatory role in TG metabolism [16–19]. Importantly, the decrease of LPL activity may lead to hydrolysis disorder of TG and eventually cause increased TG levels [20,21].

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Table 1
Each groups of mice blood lipid levels ($\bar{x} \pm s$, $n = 11$).

Group	TC/ng·mL^{-1}	TG/ng·mL^{-1}	HDL-C/ng·mL^{-1}	LDL-C/ng·mL^{-1}
Control	8.31 ± 1.16	9.42 ± 1.23	68.87 ± 5.61	14.22 ± 4.57
Model	29.85 ± 1.47▲▲	30.27 ± 4.16▲▲	17.92 ± 3.85▲▲	51.71 ± 3.17▲▲
Positive	9.67 ± 1.64**	11.13 ± 1.33**	45.19 ± 5.05**	21.11 ± 4.37**
Alisol B high	19.11 ± 1.39**	17.36 ± 1.54**	33.76 ± 5.74**	29.43 ± 4.50**
Alisol B medium	25.38 ± 4.06**	26.68 ± 4.31**	28.19 ± 5.19**	31.92 ± 5.29**
Alisol B low	27.09 ± 1.71*	27.32 ± 2.15**	27.13 ± 4.14**	34.10 ± 5.91**
24A high	19.74 ± 5.23**	17.94 ± 3.82**	30.62 ± 3.82**	31.39 ± 4.01**
24A medium	26.33 ± 4.79**	26.82 ± 3.50**	27.47 ± 3.67**	32.57 ± 3.72**
24A low	27.15 ± 2.01*	27.79 ± 2.61*	25.39 ± 5.03**	34.94 ± 4.63**
Alisol A high	17.39 ± 2.29**	16.17 ± 1.21**	34.23 ± 4.81**	28.57 ± 2.63*
Alisol A medium	23.14 ± 3.53**	22.05 ± 2.59**	29.42 ± 6.12**	30.75 ± 2.51*
Alisol A low	24.32 ± 2.13**	24.47 ± 3.08**	27.85 ± 3.42**	33.14 ± 4.72**
Mixture	17.12 ± 3.91**	15.33 ± 5.19**	41.15 ± 4.38**	27.83 ± 3.27**

Note: compared to the control group, ▲▲$P < 0.01$, ▲$P < 0.05$; compared to the model group, **$P < 0.01$, *$P < 0.05$.

Therefore, this study investigated the lipid-regulating effects of alisol B, 24A, alisol A and an alisol B-24A-alisol A mixture (content ratio 1:1:1). Furthermore, we investigated the effects on LPL activity and the interaction with LPL, the key enzyme to reduce TG levels. Moreover, data on the binding energy, binding site, binding force, key binding groups, key amino acid residues of LPL, etc., were obtained and the binding mode of the interaction between alisol and LPL is discussed. Finally, the lipid regulating mechanism was explored from the perspective of LPL, potentially providing a reference for the clinical use of alismatis rhizoma.

2. Results and discussion

2.1. Determination of blood lipid levels in each group

The results of blood lipid levels in each group after 3 weeks of continuous administration are shown in Table 1. Compared with the control group, the model group exhibited significantly increased serum levels of TG, TC and LDL-C. However, significantly decreased serum levels of HDL-C were observed, suggesting that the hyperlipidemia model was established successfully ($P < 0.01$). Compared with the model group, the levels of TG, TC and LDL-C in serum were significantly decreased and HDL-C levels increased in each administration group ($P < 0.05$, $P < 0.01$). Taken in concert, the results showed that the order of the strength of each group was: mixture > alisol A > alisol B > 24A.

2.2. Determination of LPL activity

The determination results of LPL activity in each group are shown in Table 2. Compared with the control group, the LPL activity of the model group was found to be significantly decreased ($P < 0.01$). Compared with the model group, each administration group significantly enhanced the activity of LPL ($P < 0.01$). The order of intensity was: mixture > alisol A > alisol B > 24A.

Alisol monomers and the mixture were found to decrease the TG, TC and LDL-C levels and increase the HDL-C level in the serum, indicating that these compounds may regulate blood lipids. The order of intensity was: mixture > alisol A > alisol B > 24A. The alisol monomer and the mixture could promote LPL activity and the strength of this action was as follows: mixture > alisol A > alisol B > 24A. The latter finding illustrated that the alisol monomer and the mixture could regulate blood lipid levels, further suggesting that alisol compounds may decrease the level of TG by improving the activity of LPL. The mixture exhibited the strongest effect on the activity of LPL, indicating that alisols of alismatis rhizoma also feature a synergistic effect on LPL.

Table 2
Effect of Alisols on LPL activity ($\bar{x} \pm s$, $n = 11$).

Group	LPL/U·L^{-1}
Control	730.39 ± 40.21
Model	550.68 ± 50.75▲▲
Positive	724.89 ± 80.13**
Alisol B high	683.14 ± 71.23**
Alisol B medium	660.04 ± 73.20**
Alisol B low	638.97 ± 40.25**
24A high	677.55 ± 63.10**
24A medium	653.24 ± 51.93**
24A low	635.21 ± 53.78**
Alisol A high	687.19 ± 81.11**
Alisol A medium	671.95 ± 56.07**
Alisol A low	641.92 ± 73.21**
Mixture	693.37 ± 52.32**

Note: compared to the control group, ▲▲$P < 0.01$, ▲$P < 0.05$; compared to the model group, **$P < 0.01$, *$P < 0.05$.

2.3. Homology models

The LPL sequence was analyzed by a BLAST Search module in DS2.5 to obtain the highly homologous protein 2PPL_A. The equality of the two sequences was 68.2%, and both shared a 78% sequence identity [22]. Therefore, the sequence could be used as a template for the LPL protein structure. A minimization module was utilized for energy minimization of the predicted structures. The steepest descent and conjugate gradient was adopted for structure optimization until the energy gradient was basically stable [23]. In addition, the optimized structure was further subjected to analysis by a Ramachandran plot and Profile-3D for investigating the compatibility betweeen 3D structure and amino acid sequence with rationality verification [24]. As shown in Fig. 1, the model was evaluated by a Ramachandran plot and it was calculated that the number of amino acid residues in the unacceptable region accounted for only 2.2% of the total amino acid residues, indicating that the model was suitable [25]. The best 3D structure model of LPL is shown in Fig. 2. This structure was used for molecular simulation with alisols.

2.4. Molecular simulation results for alisol and LPL

LPL, a 60 ku glycoprotein, consists of 448 amino acid residues. Its secondary structure has not yet been elucidated and may contain two domains: the N-terminal domain (NTD) and the smaller C-terminal domain (CTD) [26]. There is a near-spherical domain at the N-terminus (1–312 residues), consisting of a beta-linked structure, which regulates the binding of LPL to the substrate. This renders LPL more suitable for hydrolysis of TG and determines the specificity of LPL. The C-terminus