A Comparative Study of the Anti-Platelet Effects of cis- and trans-Resveratrol

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Abstract

Although various biological activities of resveratrol have been extensively studied, most reports have focused on trans-resveratrol and little attention has been paid to the cis-isomer. In this study, the effect of cis-resveratrol on platelet activity was examined and compared with that of the trans-isomer. Treatment with cis-resveratrol resulted in inhibition of platelet aggregation induced by thrombin, collagen or ADP, which are representative aggregation-inducing agents, and the trans-isomer elicited the same effects. These effects were concentration-dependent in the range of 1-100 μ M. However, the potency of the cis-isomer was much lower than that of the trans-isomer; the IC_{50} values for the cis-isomer versus the trans-isomer were 31 ± 12 vs 15 ± 3 , 16 ± 3 vs 9 ± 4 , and 60 ± 15 vs 25 ± 6 μ M for thrombin-, collagen- and ADP-induced aggregation, respectively. These results indicate that cis-resveratrol has a less potent anti-platelet activity, compared with the trans-isomer, and raise the possibility that the biological activities of the cis-isomer may be different from those of the trans-isomer. It will be necessary to evaluate the activity of cis-resveratrol independently of the trans-isomer.

Key Words: Resveratrol, Platelets, Anti-platelet effect

INTRODUCTION

Resveratrol is a natural polyphenolic compound that is widely present in red wines and other dietary components such as peanuts, grapes and mulberries. It has been demonstrated to have various biological activities including free radical scavenging, anti-inflammation, vasorelaxation, induction of endothelial nitric oxide synthase, and anti-cancer activity (Zbikowska and Olas, 2000). The anti-platelet effect of resveratrol has also been extensively studied, which serves as one of the major activities related with cardiovascular protective effect (Olas and Wachowicz, 2005). Indeed, resveratrol is effective against platelet adhesion to collagen or fibrinogen and granular secretion, as well as the whole procedures of aggregation (Zbikowska et al., 1999; Olas et al., 2001b). Diverse biochemical functions have been suggested as mechanisms underlying the anti-aggregatory effect of resveratrol including the inhibition of eicosanoid synthesis and phosphoinositide hydrolysis (Zbikowska et al., 1999; Olas et al., 2005; Crescente et al., 2009), the suppression of reactive oxygen species (ROS) production (Olas et al., 1999; Olas et al., 2001a), the blocking of calcium signaling (Dobrydneva et al., 1999),

the stimulation of nitric oxide generation (Gresele et al., 2008), and the modulation of signaling molecules such as protein kinase C (Yang et al., 2008b), phospholipase C (Yang et al., 2008a), p38 MAPK (Shen et al., 2007).

Resveratrol exists as either the trans-isomer [(E)-resveratrol, 5-[(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol; CAS Number 501-36-0] or cis-isomer [(Z)-resveratrol, 5-[(Z)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol; CAS Number 61434-67-1] (Orallo, 2006) (Fig. 1). The term resveratrol has, however, generally referred to only the trans-isomer and little attention has been paid to cis-resveratrol. As a necessary consequence, most studies were done with trans-resveratrol or with an undefined mixture of both isomers, and little is known regarding the biological activities of cis-resveratrol (Orallo, 2006). This has been the case with studies described above reporting the anti-platelet effects of resveratrol. This may have been due to the absence, until recently, of commercially available cis-resveratrol (Leiro et al., 2004; Orallo, 2006). Now, however, a few chemical companies have started to produce cis-isomer.

There are only a limited number of reports that have examined the biological effects of cis- versus trans-resveratrol. For instance, cis-resveratrol exhibited slightly weaker effects in

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Fig. 1. Chemical structures of cis- and trans-resveratrol.

Fig. 2. Inhibition of thrombin-induced platelet aggregation by resveratrol. Platelets were treated with either cis- or trans-resveratrol for 3 min, and aggregation was elicited by 0.2 U/ml thrombin. Representative tracings of platelet aggregation are presented in (A) and the concentration-dependent inhibitory effect is plotted in (B). Values are means $\pm$ SE of 5-8 independent experiments. *Significantly different from the groups treated with trans-resveratrol ($p < 0.05$). c: cis-resveratrol, t: trans-resveratrol.

anti-cancer activity (Pettit *et al.*, 2002) and elevated cytosolic calcium in vascular myocytes more potently (Campos-Toimil *et al.*, 2005; Campos-Toimil *et al.*, 2007). Recently, Rius *et al.* (2010) tested the inhibitory effect of cis- and trans-resveratrol against angiotensin II (AngII)-mediated vascular inflammation and found only the trans-isomer to be effective, although it is not clear whether such a result is caused by a difference in potency or is due to different mechanisms (Rius *et al.*, 2010). Taken together, they allow us to hypothesize that the effect on platelet activity may also be different between cis- and trans-resveratrol. Hence, this study was designed and performed to investigate the effect of cis-resveratrol on platelet aggregation and to compare it with the effect of the trans-isomer.

MATERIALS AND METHODS

Materials

Both cis- and trans-resveratrol were purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). Collagen and ADP

were from Chrono-log Co. (Havertown, PA, USA) and thrombin was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals used were of the highest purity available and purchased from standard suppliers.

Animals

All animal experiments were conducted in accordance with protocols approved by the Ethics Committee of the Animal Service Center at Chonnam National University. Male Sprague-Dawley rats weighing 150-250 g were purchased from Daehan Biolink (Eumseong, Korea). Prior to experiments, animals were acclimated for 1 week in the laboratory animal facility and maintained at constant temperature and humidity with a 12-hr light/dark cycle. Food and water were provided *ad libitum*.

Preparation of platelets and measurement of platelet aggregation

A platelet aggregation study was performed using a 4-channel aggregometer (Chrono-log, Havertown, PA, USA) as de-

Fig. 3. Inhibitory effect of cis- and trans-resveratrol on platelet aggregation induced by collagen or ADP. Each isomer was incubated with platelets for 3 min and (A) collagen- or (B) ADP-induced aggregation was measured turbidimetrically. Concentrations of collagen and ADP used were 3 μ g/ml and 5 μ M, respectively. Values are means $\pm$ SE of (A) 3-6 or (B) 4-8 independent experiments. *Significantly different from the groups treated with trans-resveratrol ($p < 0.05$).

scribed previously (Lee *et al.*, 2002). In brief, blood was collected from the abdominal aorta of ether-anesthetized rats using acid-citrate-dextrose (1:6) as an anticoagulant. After centrifugation at 150 g for 15 min, platelet-rich plasma (PRP) was obtained from the supernatant, and platelets were isolated by further centrifugation at 1,500 g for 20 min. Platelets were washed once with washing buffer solution (138 mM NaCl, 2.8 mM KCl, 0.8 mM $MgCl_2$, 0.8 mM NaH_2PO_4 , 10 mM HEPES, 5 mM EDTA, pH 7.4), and were finally resuspended in suspension buffer solution (138 mM NaCl, 2.8 mM KCl, 0.8 mM $MgCl_2$, 0.8 mM NaH_2PO_4 , 10 mM HEPES, 5.6 mM dextrose, 1 mM $CaCl_2$, pH 7.4). The number of platelets was adjusted to 2×10^8 cells/ml. Platelets were incubated with the indicated concentrations of cis- or trans-resveratrol, and aggregation was induced with sub-maximal concentrations of thrombin, collagen or ADP.

Statistical analysis

The means and standard errors (SE) of the means were calculated for all experimental groups. The data were subjected to Mann-Whitney test to determine the significance of differences in the effects between cis- and trans-resveratrol. Statistical analysis was performed using SigmaStat software (Version 3.5, Systat Software, San Jose, CA, USA). In all cases, a p -value of < 0.05 was used to determine significance.

RESULTS

In order to test whether resveratrol isomers inhibited platelet aggregation, platelets were exposed to resveratrol for 3 min, and then aggregation was induced with platelet agonists: thrombin, collagen or ADP. As shown in Fig. 2 and 3, trans-resveratrol reduced aggregation by thrombin, collagen and ADP in a concentration-dependent manner, as previously reported (Zbikowska *et al.*, 1999; Olas and Wachowicz, 2005). Platelet aggregation could also be inhibited by cis-isomer, and the inhibition was concentration-dependent in the range of 1-100 μ M (Fig. 2, 3). However, significant difference was observed in potency between the two isomers, i.e. cis-resveratrol was much less effective than the trans-isomer. IC_{50} values of cis- versus trans-isomer were 31 ± 12 vs 15 ± 3 , 16 ± 3 vs 9 ± 4 , and 60 ± 15 vs 25 ± 6 for thrombin-, collagen- and ADP-induced aggregation, respectively (Table 1). These results indicate that cis-resveratrol is also capable of suppressing the

Table 1. IC_{50} of resveratrol for thrombin-, collagen- or ADP-induced platelet aggregation

	IC_{50} (μ M) ^a		
	Thrombin	Collagen	ADP
Cis-resveratrol	31 ± 12	16 ± 3	60 ± 15
Trans-resveratrol	15 ± 3	9 ± 4	25 ± 6

^aThe calculated concentration required for 50% inhibition of aggregation. Values are expressed as mean $\pm$ SE from 3-8 independent experiments.

response of platelets to proaggregatory stimuli. However, the clinical efficacy as well as the potency of the cis-isomer may not be equal to that of the trans-isomer.

DISCUSSION

Although extensive attention has been paid to the biological activities of resveratrol, most studies to date have focused on the trans-isomer. Hence, there have been only a limited number of reports comparing cis- and trans-resveratrol content in diets. Recently, a growing number of papers are reporting the quantitative significance of cis-resveratrol in diets (Vitrac *et al.*, 2005; Zamora-Ros *et al.*, 2008). The cis-isomer can be formed from trans-isomer by exposure to ultraviolet radiation (Stivala *et al.*, 2001). cis-Resveratrol is virtually not detected in grapes, but it is present in wines at variable concentrations, which has been ascribed to its production from trans-resveratrol by yeast isomerases during fermentation (Soleas *et al.*, 1997; Fremont, 2000; Cvejic *et al.*, 2010). Given these studies, it is obvious that cis-resveratrol, like the trans-isomer, is also available through the diet and is capable of exerting its clinical effects. It is, therefore, of consequence to characterize the biological activities of cis-resveratrol.

There are only a few comparative studies that reported differences in biological functions between cis- and trans-resveratrol (Orallo, 2006). Such differences have been observed both in vitro and in vivo, including its anti-cancer effect (Pettit *et al.*, 2002), calcium-elevating activity in vascular myocytes, and suppression of AngII-mediated vascular inflammation (Campos-Toimil *et al.*, 2005; Campos-Toimil *et al.*, 2007). There was

also a difference in metabolic rate through glucuronidation, i.e. glucuronidation occurred at a faster rate with cis-resveratrol (Aumont *et al.*, 2001). Most of the findings appear, to date, to be a quantitative difference rather than a qualitative one between the two isomers. Here, the results of our study provide the novel findings that the anti-platelet effect of cis-isomer is not as potent as that of the trans-isomer.

In this study, cis-resveratrol, like the trans-isomer, suppresses platelet aggregation induced by proaggregatory stimuli such as collagen, ADP and thrombin. However, the potency of the cis-isomer was significantly lower than that of the trans-isomer, the cis-isomer showing an approximately 2-fold higher IC₅₀. Bertelli *et al.* (1996) tested the anti-aggregatory effect of cis-resveratrol with collagen. Although cis-resveratrol was effective, as shown here, they did not find any difference between the two isomers, which is not consistent with the results of our study (Bertelli *et al.*, 1996). It might be due to the simplicity of their experiments. In their short paper, they compared only one concentration of resveratrol and did not test a broad concentration range, which might allow them to miss concentrations that do show a difference.

The anti-platelet effect of trans-resveratrol has been well demonstrated through extensive studies and diverse biological activities have been suggested as mechanisms (Olas and Wachowicz, 2005). However, it is complicated and still unclear as the direct target molecules have never been identified despite the fact that so many mechanisms are known to be involved. Accordingly, it may not be simple to elucidate the reason why the cis-isomer is less potent than the trans-isomer. Currently, the underlying mechanism remains elusive and it is not even clear whether both isomers share a common mode of action. Further study will be required to clarify the mechanisms underlying the anti-platelet effect of cis-resveratrol.

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