Cardiovascular Pharmacology

Genistein attenuates low temperature induced pulmonary hypertension in broiler chicks by modulating endothelial function

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A R T I C L E   I N F O
Article history:
Received 21 February 2010
Received in revised form 19 August 2010
Accepted 6 September 2010
Available online 18 September 2010

Keywords:
Genistein
Pulmonary hypertension
Pulmonary vascular remodeling
Nitric oxide
Broiler

A B S T R A C T
Pulmonary arterial hypertension is characterized by high pulmonary blood pressure, vascular remodeling and right ventricular hypertrophy. In the present study, we investigated whether genistein would prevent the development of low temperature-induced pulmonary hypertension in broilers. Hemodynamic parameters, vascular remodeling, the expression of endothelial nitric oxide and endothelin-1 content in lung tissue were evaluated. The results demonstrated that genistein significantly reduced pulmonary arterial hypertension and suppressed pulmonary arterial vascular remodeling without affecting broilers' performance. The beneficial effects appeared to be mediated by restoring endothelial function especially endothelial nitric oxide and endothelin-1, two critical vasoactive molecules that associated with the development of hypertension. Genistein supplementation might be a potential therapeutic strategy for the treatment of pulmonary hypertension.

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1. Introduction
Pulmonary arterial hypertension is a disease of small pulmonary arteries characterized by high pulmonary blood pressure, vascular remodeling and right ventricular hypertrophy (Runo and Loyd, 2003; Humbert et al., 2004). The pathogenesis of pulmonary arterial hypertension involves a complex and multi-factorial process in which endothelium-derived vasoactive molecules, such as nitric oxide (NO), prostacyclin, endothelin-1 (ET-1), serotonin, and thromboxane. These molecules have been increasingly recognized as critical factors (Siow et al., 2007) and potential therapeutic targets in treatment of pulmonary arterial hypertension (Budhiraja et al., 2004; Siow et al., 2007). Recent studies showed that impaired NO signaling plays an important role in maintaining the balance between endothelial mediators with opposing action on pulmonary vasculature during the development of hypertension (Demoncheaux et al., 2005). Restoration of endothelial NO synthase activity prevents or reverses this process in experimental pulmonary arterial hypertension in rats (Kanno et al., 2001; Abe et al., 2004).

Epidemiological studies and clinical data suggest that estrogens have cardiovascular protective effects by various mechanisms (Lissin and Cooke, 2000). However, an increased incidence of side effects limits their therapeutic potential (Armitage et al., 2003). Given the demonstrated risks of conventional estrogen therapy, the phytoestrogens, including genistein and daidzein, are currently receiving more attention because of their potential health benefits in preventing chronic diseases such as cardiovascular disease, obesity and osteoporosis (Satchell and Lydeking-Olsen, 2003; Altavilla et al., 2004; Park et al., 2005). In vitro and in vivo studies showed that genistein can lower blood pressure and alleviate oxidative stress in human subjects and experimental hypertensive rats (Hodgson et al., 1999; Rivas et al., 2002; Homma et al., 2006) which can be partly explained by the restoration of NO-mediated vasorelaxation (Mishra et al., 2000; Karamsetty et al., 2001; Walker et al., 2001; Squadrito et al., 2002). All these data suggest that genistein may be able to suppress pulmonary arterial hypertension by modulating NO-mediated signaling pathway.

In addition, pulmonary arterial hypertension is one of the most frequent signs in broiler’s ascites syndrome which is still one of the leading causes of death in poultry industry. However, the underlying mechanism is not fully known as yet. Therefore we hypothesize that genistein might prevent low temperature induced pulmonary hypertension through modulating endothelial function in broilers and explore the potential mechanisms that contribute to these protective effects. To this aim, broilers were bred under normal or low ambient temperature in the presence or absence of genistein. The hemodynamic parameters, vascular remodeling and the expression of endothelial NO synthase and endothelin-1 in lung tissue were examined.

2. Materials and methods

2.1. Animals
One day-old commercial male Arbor Acre broiler chicks were maintained in an environmental chamber with continuous lighting...
UV detection as previously described (Squadrito et al., 2002). Performance liquid chromatography (HPLC) system equipped with 2.4. Determination of plasma genistein levels in broiler ventricle weight ratio were measured respectively (Huchzermeyer, 1988). A polyethylene plastic catheter was pushed forward slowly to the right ventricle to measure of the right side of the neck. A polyethylene plastic catheter was right cardiac catheter as previously described (Yang et al., 2005).

Heart rate, pulmonary arterial systolic pressure, right ventricular hypertrophy, heart rate and saturation of hemoglobin with oxygen in abdominal cavity, right ventricular dilation, hydropericardium, and vascular congestion, as have been previously reported (Druyan et al., 2007).

2.3. Performance, pulmonary arterial pressure, right ventricular hypertrophy, heart rate and saturation of hemoglobin with oxygen in arterial blood

Feed consumption and body weight gain were recorded on days 21 and 42, respectively. The mean body weight gain and the ratio of feed to weight gain were calculated for each treatment. At 21, 35 and 42 days of age, heart rate, pulmonary arterial systolic and diastolic pressure (mm Hg) of broilers were measured using a right cardiac catheter as previously described (Yang et al., 2005). Briefly, birds were restrained in a dorsal position on the operating-table and locally anesthetized with 5% procaine chloride in the middle of the right side of the neck. A polyethylene plastic catheter was inserted into the jugular vein after the jugular vein was separated. The catheter was pushed forward slowly to the right ventricle to measure heart rate and then pulmonary artery for pulmonary arterial pressure determination. Pressure signals were sent to the host computer of RM-6000 type Polygraph (Nihon Kohden Ltd., Japan) through a catheter was pushed forward slowly to the right ventricle to measure pressure signals were sent to the host computer of RM-6000 type Polygraph (Nihon Kohden Ltd., Japan) through pressure sensors. After the in vivo measurements were completed, saturation of hemoglobin with oxygen in arterial blood and the right ventricle to total ventricle weight ratio were measured respectively (Huchzermeyer, 1988).

2.4. Determination of plasma genistein levels in broiler

Plasma genistein levels in broilers were measured by using a high-performance liquid chromatography (HPLC) system equipped with UV detection as previously described (Squadrito et al., 2002).

2.5. Histological examination

Segments with a thickness of 0.5 cm adjacent to the bronchi were removed from the lungs and fixed with 10% formaldehyde solution for more than 24 h and dehydrated in an ascending gradient of ethanol. After becoming transparent in dimethylbenzene, the lung tissues were embedded in paraffin and routinely processed into sections of 5 μm in depth followed by Weigert–van Gieson staining for elastin (Herget, 1991). Small pulmonary arterioles with an external diameter in the range of 20 to 50, 50 to 100 and 100 to 200 μm were studied using an automatic image analyzer (BHI2, Olympus, Japan) with the advanced software (Motic 3.0). Twelve average regions of cross section were chosen. The adventitia and the lumen diameters were measured respectively, following which the relative medial thickness (%) was recorded and analyzed. The relative medial thickness of pulmonary arterioles with different cut angles and conditions of either contractile or relaxation was computed from the above measurements according to the previous methods (Barth et al., 1993; Tan et al., 2005).

2.6. Measurement of lung eNOS activity

Pulmonary vascular eNOS activity was determined by measuring the calcium-dependent conversion of [3H]-arginine to [3H]-citrulline as previously reported (Fadel et al., 2000). Briefly, the lungs were quickly frozen in liquid nitrogen immediately after removal. Tissue was homogenized on ice in lysis buffer containing 1 mM leupeptin, 1 mM pepstatin A, 1 mM phenylmethylsulfonyl fluoride. Homogenates were incubated at 37 °C for 30 min in 50 mM Tris/HCl buffer (pH 7.4) in the presence of 1 mM NADPH, 10 μM l-valine and a mixture of unlabelled and 10 μM l-[3H]arginine (1 μCi/ml). The radioactivity was measured in the supernatant by a liquid scintillation counter. eNOS activity was expressed as picomoles of [3H]-l-citrulline produced per milligram protein in 30 min.

2.7. Western blot analysis of endothelial NO synthase expression

The expression of NO synthase in broiler lungs was analyzed by western blot as previously reported (Tan et al., 2007). Briefly, lung tissues were homogenized in lysis buffer containing 1 mM NaF, 1 mM protease inhibitor cocktail and centrifuged for 15 min at 12,000 g to remove cellular debris. The protein concentration was determined using a Bio-Rad protein assay kit. Equal amounts of protein were separated on SDS-page gels and transferred to PVDF membranes (Millipore). The membranes were blocked in a 5% skimmed milk solution at room temperature for 1 h, and then incubated with diluted anti-eNOS antibody.

2.8. Measurement of lung ET-1 and cGMP

Broiler lung tissues were separated and immediately frozen in liquid nitrogen. ET-1 was measured in lung homogenate using a radioimmunoassay kit as described previously (Naruse et al., 1989). Lung cGMP was determined as previously described (Takashima et al., 2006) using the Biotrad enzyme immunoassay system from Amersham Biosciences Corp (Buckinghamshire, UK) and results were expressed as femtomoles of cGMP per milligram of dry weight.

2.9. Statistical analysis

Comparisons between groups were performed using two-way ANOVA followed by the Duncan test. Differences were considered statistically significant at the level of P<0.05 and data are presented as means±S.E.M. The statistical analysis was performed with the software SPSS 11.0 for Windows (SPSS, Chicago, IL).

3. Results

3.1. Plasma genistein levels in broilers

The levels of genistein in plasma were determined and shown in Table 1. The plasma genistein is directly correlated with the genistein supplement in broiler diets. There is no difference between the plasma concentrations of genistein in broilers raised under low

Table 1. The plasma genistein is directly correlated with the genistein supplement in broiler diets. There is no difference between the plasma concentrations of genistein in broilers raised under low
temperature as compared with corresponding control raised under normal temperature (Table 1).

### 3.2. Growth rate and ascites incidence

To explore whether the genistein supplementation has any effect on the performance of broilers, body weight gain and the ratio of feed to weight gain were determined. Both body weight gain and the ratio of feed to weight gain in 0, 20, 50 mg/kg diet groups under low temperature were kept at a similar level as compared with that of corresponding control during the first growth stage (0 to 21 days) (Table 2). However, these two parameters under low temperature were significantly lower than those in control when comparing them as a whole experimental period (0 to 42 days) suggesting that the chicks under low temperature had less weight gain and slower growth rates during the second growth period. Moreover, high dose of genistein (50 mg/kg) significantly improved body weight gain and decreased feed to weight gain (0 to 42 days) compared with non-treatment group under low temperature, indicating a beneficial effect of genistein on the broilers’ performance.

The incidence of hydropericardium and mortality associated with ascites were confirmed using previous method (Druyan et al., 2007). As shown in Table 3, low temperature increased the mortality and incidence of hydropericardium in non-treatment group as compared with that of control under normal temperature (from 1/100 and 3/100 respectively under normal temperature to 27/100 and 21/100 respectively under low temperature) during the whole experimental periods (0–42 days). However, genistein supplementation markedly decreases the mortality associated with ascites from 27/100 to 6/100 and 21/100 to 4/100 under low temperature as compared with that of control under normal temperature.

### 3.3. Pulmonary arterial pressure and heart rate

As shown in Table 4, low temperature significantly increased mean pulmonary arterial pressure of broilers on 21, 35 and 42 days of age compared with that of control in non-treatment groups (P < 0.05 for three time points). The higher dose genistein treatment (50 mg/kg) kept pulmonary arterial pressure at the similar level compared with that of control under normal temperature.

Low temperature significantly increased heart rate of broilers on day 21 and decreased it on day 42 compared with corresponding time points of control in non-treatment groups (P < 0.05 for the two time points). Genistein supplementation under normal temperature did not affect heart rate at the three time points (Table 4).

### 3.4. The ratio of right ventricle to total ventricle weight and arterial hemoglobin oxygen saturation

The right: total ventricle weight ratio in broilers under low temperature condition without genistein supplementation on days 35 and 42 were significantly increased compared with corresponding non-treatment controls under normal temperature (P < 0.05), (Table 4). The supplementation of genistein at 50 mg/kg diet kept the ratio of right ventricle to total ventricle weight at the similar level compared with that of control under normal temperature at the three time points.

Low temperature significantly decreased the arterial hemoglobin oxygen saturation at the three time points compared with those of control (Table 4). Genistein supplementation, especially at high concentration (50 mg/kg diets), abrogated the decrease of the arterial hemoglobin oxygen saturation, and kept it at similar levels to those of control.

### 3.5. Pulmonary artery remodeling

As shown in Table 5, the relative medial thicknesses of pulmonary artery in the ranges of 20 ≤ d ≤ 50, 50 ≤ d ≤ 100 or 100 ≤ d ≤ 200 μm are significantly greater in the non-treatment group under low temperature than those under normal temperature on days 35 and 42, respectively (P < 0.05). Genistein supplementation suppressed low temperature induced vascular remodeling and maintained the thickness at similar levels of those in control under normal temperature. Fig. 1 showed the morphological change of pulmonary arteriole structure with pulmonary arteriole external diameter ranging from 20 to 50 μm under normal temperature, low temperature or low temperature treated with genistein (50 mg/kg diet).

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### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature</th>
<th>Normal temperature</th>
<th>Low temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genistein (mg/kg diets)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>20</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Serum</td>
<td>21 day</td>
<td>1.3 ± 0.32</td>
<td>5.0 ± 1.42</td>
</tr>
<tr>
<td>GEN</td>
<td>35 day</td>
<td>1.7 ± 0.30</td>
<td>6.1 ± 0.93</td>
</tr>
<tr>
<td>(mg/ml)</td>
<td>42 day</td>
<td>1.9 ± 0.41</td>
<td>8.8 ± 1.41</td>
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</table>

Data for plasma genistein levels were shown as means ± S.E.M. from ten birds. GEN = genistein.

### Table 2

<table>
<thead>
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<th>Treatment</th>
<th>Temperature</th>
<th>Normal temperature</th>
<th>Low temperature</th>
</tr>
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<tbody>
<tr>
<td>Genistein (mg/kg diets)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>20</td>
<td>50</td>
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</tr>
<tr>
<td>BWG</td>
<td>0–21 days</td>
<td>0.77 ± 0.043</td>
<td>0.77 ± 0.064</td>
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<tr>
<td></td>
<td>0–42 days</td>
<td>2.05 ± 0.064a</td>
<td>2.06 ± 0.085a</td>
</tr>
<tr>
<td>F/G</td>
<td>0–21 days</td>
<td>1.51 ± 0.079</td>
<td>1.51 ± 0.044</td>
</tr>
<tr>
<td></td>
<td>0–42 days</td>
<td>1.90 ± 0.035ab</td>
<td>1.90 ± 0.066ab</td>
</tr>
</tbody>
</table>

BWG = body weight gain; F/G = the ratio of feed to weight gain. Data were shown as means ± S.E.M. from ten birds. "a" Means in a row with no common superscript differ significantly (P < 0.05).
3.6. Effects of genistein on pulmonary vascular NOS activity and its protein expression

As shown in Fig. 2, low temperature decreased eNOS activity as compared with normal temperature control, this is in agreement with previous studies indicating that eNOS is associated with hypertension in various experimental systems (Chou et al., 1998; Safar et al., 2001). Genistein treatment (both low and high doses) significantly increased eNOS activity in pulmonary vascular tissue in broilers under low temperature. Genistein also increased eNOS activity in normal temperature groups; however, there was no significant difference in both dose treatments (Fig. 2A). Consistent with the eNOS activity, western blot analysis showed a markedly down-regulated eNOS level in low temperature induced hypertensive broilers which were largely restored by genistein treatment (Fig. 2B).

3.7. Lung ET-1 peptide levels and cGMP levels

In consistent with our previously study, ET-1 level was markedly increased in low temperature control as compared with the normal temperature control, suggesting that alteration of ET-1 is involved in the development of pulmonary hypertension. Genistein administration (higher and lower doses) significantly decreased the lung ET-1 peptide levels in broilers under low temperature compared with that of low temperature control (Fig. 3). Neither dose of genistein affects the ET-1 peptide level in the normal temperature counterparts. The cGMP levels in lung tissue were markedly lower compared with normal temperature control. However, genistein treatment significantly increased cGMP levels under low temperature (Fig. 4) which is in consistent with the eNOS activity and protein levels as demonstrated in Fig. 2. Genistein did not affect lung cGMP levels in normal temperature groups.

4. Discussion

We have previously shown that Ca2+ channel antagonist or ET-1 receptor antagonist can attenuate PH with ascsites in broilers. However, the side effects on the growth and the performance of broilers limited their application (Yang et al., 2005; Yang et al., 2007). This encouraged us to identify small molecules that could be of potential therapeutic interest for the treatment of pulmonary arterial hypertension, without negative effects.

In the present study, we provided evidence that low ambient temperature leads to a reduced eNOS and an increased endothelin-1

**Table 3**

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>Low temperature</th>
<th>Genistein (mg/kg diets)</th>
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<td></td>
<td></td>
<td>0</td>
<td>20</td>
<td>50</td>
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<tr>
<td></td>
<td></td>
<td>0</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>Mortality</td>
<td>of ascites</td>
<td>0–21 days</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0–42 days</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Incidence</td>
<td>of hydropericardium</td>
<td>0–21 days</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td>0–42 days</td>
<td>3</td>
<td>2</td>
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* Absolute numbers, initial number of chickens per group is 100.

**Table 4**

Mean pulmonary arterial pressure (mPAP), heart rate (HR), the ratio of the of right ventricle to the total ventricle weight (RV:TV, %) and saturation of hemoglobin with oxygen in arterial blood (SaO2) of broilers in the different groups and investigated intervals, respectively. Data are expressed as means ± S.E.M. from ten birds.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature</th>
<th>Normal</th>
<th>Low</th>
<th>Genistein (mg/kg diets)</th>
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<td>50</td>
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</table>

**Table 5**

Relative medial thickness of pulmonary arteriole with external diameters ranging from 20 to 50, 50 to 100 μm and 100 to 200 μm in different groups and time points were shown as means ± S.E.M. from ten birds.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature</th>
<th>Normal</th>
<th>Low</th>
<th>Genistein (mg/kg diets)</th>
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<td></td>
<td></td>
<td>0</td>
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<td>50</td>
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</table>

RMT = Relative medial thickness. **Means in a row with no common superscript differ significantly (P < 0.05).
levels in lung vascular arteries and induced pulmonary hypertension as characterized by an increased blood pressure (Table 4), vascular remodeling in broilers (Table 5 and Fig. 1) and an increased mortality incidence (Table 3), suggesting that impaired endothelial function is associated with the development of hypertension in our animal model. Genistein supplementation significantly reduced low temperature induced pulmonary arterial pressure (Table 4), inhibition of pulmonary arterial vascular remodeling (Fig. 1) and decrease the incidence of mortality in broilers by improving endothelial function involving enhanced eNOS activity (Fig. 2) and lung cGMP levels (Fig. 4) and decreased ET-1 content (Fig. 3) without decreasing broilers' performance.

Genistein is one of the main biologically active isoflavones in soy-derived products with various health benefits associated with chronic diseases. It has been reported that populations that consume a diet high in phytoestrogens have lower risks of hypertension or pulmonary hypertension implicated in cardiovascular disease (Lichtenstein, 1998; Mishra et al., 2000; Ambra et al., 2006; Homma et al., 2006; Cho et al., 2007; Si and Liu, 2008), however, the underlying mechanism is not fully understood. Moreover, the effects of genistein on broiler's vasculature function and its possible anti-hypertension mechanism remains unknown, even though pulmonary hypertension is commonly observed in broilers and causes huge economic loss in poultry industry. We hypothesized that genistein might reduce low temperature induced pulmonary hypertension by modulating vasoactive mediators in hypertensive broilers.

To test this hypothesis, we started with low temperature induced pulmonary model in broilers which had been reported to display the characteristics of pulmonary hypertension and the results are consistent with the previous study from other labs and ours (Olkowski and Classen, 1998; Wideman and Tackett, 2000; Tan et al., 2005; Yang et al., 2007). Supplementation of genistein markedly inhibited low temperature induced pulmonary hypertension and arrested the vascular remodeling (Table 4 and Fig. 1).
The imbalance of vasoactive mediators, such as NO and endothelin-1 (ET-1), has been recognized as a critical factor that contributes to endothelial dysfunction which consequently result in pulmonary arterial hypertension. To investigate whether these factors are involved and responsible for the beneficial effect of genistein against low temperature-induced pulmonary hypertension, we determined the eNOS activity, a critical vasodilator, in lung tissue of hypertensive broilers and found that eNOS activity and expression were significantly reduced in low temperature-induced hypertensive chicks, which were markedly restored by genistein, indicating improved endothelial function, suggesting that the beneficial effect is related, at least partly, to the modulation of eNOS activity. This observation along with the increased lung cGMP levels, indicated that eNOS-NO-cGMP axis was activated upon genistein treatment. This result is consistent with the previous studies showing that genistein up-regulates eNOS expression in hypertensive rats (Chou et al., 1998; Squadrito et al., 2000; Safar et al., 2001; Si and Liu, 2008).

It has been reported that ET-1, a potent vasoconstrictor and mitogenic peptide, plays an important role in the initiation and evolution of pulmonary hypertension in human and rats (Giaid et al., 1993; Celik and Karabiyikoglu, 1998). Our recent study also showed that ET-1 is elevated in low temperature-induced pulmonary hypertension in broilers which can be reversed by endothelin receptor antagonist BQ123 (Yang et al., 2005). In the present study, genistein treatment produced a significant decrease of lung ET-1 level in hypertensive broilers as compared to the low temperature control even though this effect is less than that of endothelin receptor antagonist (data not shown). This finding is in agreement with a recent study in postmenopausal women (Squadrito et al., 2002), showing that improved vascular function of genistein is associated with an increased ratio of nitric oxide to endothelin-1. However, in monocrotaline-induced pulmonary hypertensive rats, genistein treatment improves the down-regulation of expression of lung eNOS, consistent with our data, but did not affect the endothelin-1 levels in the lungs (Homma et al., 2006). This discrepancy may be due to the several important differences between studies, for example, species, methods used to induce hypertension, treatment periods and doses.

NO and ET-1 are endothelium-derived vasoactive factors that interact with one another (Lavalle et al., 2001). NO causes vasodilation and inhibits smooth muscle cell proliferation (Loscalzo, 1995). ET-1 causes potent vasoconstriction of the systemic and coronary vasculature through binding to endothelin receptors, increase monocyte adhesion and promote vascular smooth muscle cell proliferation, opposing the effects of NO (Mathew et al., 1996). Studies in porcine aorta indicated that NO inhibits the production of ET-1 by a cyclic GMP-dependent pathway (Boulanger and Luscher, 1990). On the other hand, endothelin-1 receptor B present in endothelial cells mediates the production of NO (de Nucci et al., 1988), thus constituting a feed-back regulatory loop to maintain a normal vascular tone. In the present study, genistein treatment activated eNOS-NO-cGMP signaling pathway (Figs. 2 and 4) and decreased levels of ET-1 (Fig. 3) in lung tissue. However, in vitro study using endothelial cells or smooth muscle cells in combination with eNOS or cGMP inhibitors as shown in previous study (Boulanger and Luscher, 1990), is required to test whether the deceased ET-1 is regulated by genistein or genistein activated eNOS-NO-cGMP.

The mechanism by which genistein increases the expression of eNOS and decreases ET-1 expression is unclear. There are at least two possibilities that might contribute to the activation of eNOS. The first one is that genistein, like estrogen, activates eNOS activity through classical actions of estrogen via transcriptional activation of estrogen-responsive genes involving estrogen receptors. However, it appears not the case in our system, because estrogen receptor antagonist ICI 182, 780 did not block genistein induced eNOS activity (data not shown). Similar results were also observed in human endothelial cells (Rathel et al., 2005). Secondly, it might mediated by inhibiting tyrosine kinase (Minchenko and Caro, 2000). However, it should be noted that this inhibitory property requires a much higher concentration of genistein (100 μM) (Makela et al., 1999) which is quite higher than the plasma level of genistein in our study (Table 1), thus excluding the possibility that genistein activated eNOS through the inhibition of tyrosine kinase.

Moreover, genistein has also been suggested to inhibit hydrogen peroxide production and increase the activity of antioxidant enzymes, such as catalase, manganese superoxide dismutase, glutathione peroxidase and glutathione reductase leading to a reduced oxidative stress and improved endothelial function and reduced blood pressure in vivo (Wei et al., 1995; Mahn et al., 2005). More study is required to test whether genistein is the main active component that is responsible for the activation of antioxidant genes, because in their study, soy protein rich diet was used which contains multiple phytoestrogens, including genistein and daidzein, instead of single molecule was used.

In summary, this is the first study providing evidence that supplementation of genistein significantly attenuated low temperature induced pulmonary hypertension and inhibited vascular remodeling in broilers without affecting the performance index. The beneficial effect of genistein appears to be mediated by restoring endothelial function, especially eNOS and ET-1, two critical vasoactive mediators.
mediators associated with the development of hypertension. Therefore, genistein might have therapeutic effects for the treatment of pulmonary hypertension.

Acknowledgements

This work was supported by the Yangtz River Scholar and Innovation Research Team Development Program (Project No. IRT0945) and by the grants from National Natural Science Foundation of China (No. 30700576) and State Key Laboratory of Animal Nutrition (Project No. 2004DA125184-0807).

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