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LETTER TO THE EDITOR

Stress-induced PAI-1 expression is suppressed by pitavastatin in vivo

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Thromboembolism, including myocardial infarction, cerebral infarction, and pulmonary embolism, is frequently induced by a variety of stressors. Indeed, mental, and physical stressors decrease fibrinolytic activity [1] and contribute to the occurrence of thrombotic complications. We have already reported that plasminogen activator inhibitor-1 (PAI-1) expression is dramatically induced by restraint (immobilization) stress, a typical physicopsychological stress [2], with maximal induction in the adipose tissue in vivo, a change contributing to the development of tissue thrombosis [3]. PAI-1 regulates fibrinolysis by inhibiting plasminogen activation and elevated levels of plasma PAI-1 are observed in a variety of thrombotic conditions. In obese humans, increased plasma PAI-1 levels correlated with the amounts of visceral fat, suggesting that adipose tissue is the primary source of PAI-1 in this condition [4]. Statins, 3-hydroxy-methylglutaryl coenzyme A reductase inhibitors, have been widely used for the prevention of cardiovascular diseases primarily with their lowering serum cholesterol levels. Statins also exert pleiotropic and beneficial effects on the coagulation and fibrinolytic systems [5], which are regarded to be independent of cholesterol-lowering action.

The study described below demonstrated that pitavastatin attenuated the upregulation of PAI-1 gene in restraint-stressed mice. Twelve to sixteen-month-old male C57BL/6 J mice were administered orally 10 mg/kg/day of pitavastatin or atorvastatin for 3 weeks before the animals were received restraint stress. The dosage of agents we used is regarded to be much excess in comparison with clinical dose because rodents metabolize statins more rapidly than humans. Restraint stress, plasma collection, RNA extraction and quantitative RT-PCR assay were performed, as described previously [3]. PAI-1 antigen levels in plasma were quantified by a sandwich ELISA, as described previously [6]. All procedures were carried out according to the protocol approved by the Animal Care and Use Committee of Nagoya University. Twenty hours of restraint stress to mice caused a substantial induction of PAI-1 antigen in plasma and of PAI-1 mRNA in the liver and adipose tissues, which have been regarded as major sources of PAI-1 [3]. PAI-1 antigen in plasma was dramatically elevated after a 20 h-restraint stress, but this increase attenuated by 40% in mice pretreated with pitavastatin (Fig. 1, left panel). Free PAI-1 activity measured by t-PA binding assay was also elevated by stress and its increase was attenuated by pretreatment with pitavastatin in parallel with PAI-1 antigen level (not shown). Although t-PA antigen levels measured by ELISA were elevated after restraint stress, the degree of elevation (by 2-fold, not shown) was much smaller than PAI-1 induction (by 7-fold), showing that a prothrombotic state was induced by restraint stress. Pitavastatin also suppressed the induction of PAI-1 mRNA by restraint stress in the liver and adipose tissues about 60% of the control (i.e., pitavastatin naive) mice (Fig. 1, middle and right panels), while atorvastatin did not (not shown). As plasma cholesterol levels were not affected by statins in these mice (not shown), pitavastatin may suppress the upregulation of PAI-1 gene independent of its cholesterol-lowering action in restraint-stressed mice. It has been reported that statins reduce the PAI-1 expression by

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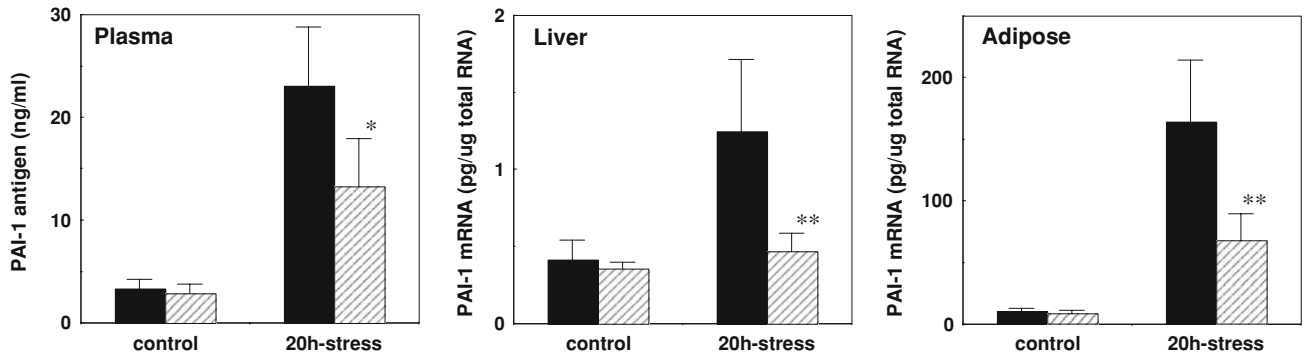

Fig. 1 Twelve to sixteen-month-old mice were administered pitavastatin (10 mg/kg/day) for 3 weeks ($n = 6$, respectively), followed by 20-h-restraint stress. As a control group, non-stressed mice and 20-h-stressed mice without pitavastatin administration were prepared ($n = 6$, respectively). The plasma was collected and measured for PAI-1 antigen (ng/ml) by ELISA assay. Liver and adipose tissue were harvested and analyzed for PAI-1 mRNA (pg/μg total tissue RNA) by competitive RT-PCR. *Closed bars* control (pitavastatin naive) group, *hatched bars* pitavastatin-treated group. The data are presented as the mean and SD. $^*P < 0.05$, $^{**}P < 0.02$ (statistically analyzed by one-way ANOVA)

suppressing the formation of geranylgeranylated proteins required for the proper synthesis of PAI-1 [7], and this may be one of the mechanisms by which pitavastatin attenuates the PAI-1 induction in restraint-stressed mice.

Several differences are observed in the pleiotropic effects of statins. Pitavastatin may more strongly suppress the molecular responses against stress insults, which include the induction of cytokine-induced nuclear factor-κB (NF-κB) and the production of oxidative stress markers in the ischemic model, in comparison with atorvastatin [8, 9]. The expression of PAI-1 gene is upregulated by oxidative stress markers (e.g., 4-hydroxynonenal and 8-hydroxy-2′-deoxyguanosine) [10] and NF-κB, both of which could be induced by stress-related inflammatory cytokines (e.g., TNF-α). Taken together, it is speculated that pitavastatin may attenuate the stress-induced PAI-1 expression through the inhibition of TNF-α-induced NF-κB activation and its anti-oxidative effect. Although there have been some reports on the inhibitory effect of atorvastatin on PAI-1 expression in vitro or ex vivo [11], this agent may have less anti-oxidant potential and less ability to block NF-κB activation than pitavastatin [12], resulting in the lack of suppressive effect on the stress-induced PAI-1 expression. Finally, the finding in this study suggests that pitavastatin contributes, in part, to the prevention of thrombotic cardiovascular diseases associated with physicopsychological stress although further studies are required to elucidate its mechanism.

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