Effects of Statins and Xuezhikang on the Expression of Secretory Phospholipase A2, Group IIA in Rat Vascular Smooth Muscle Cells

Qiang Xie, PhD and Dan Zhang, MD

Summary

Atherosclerosis is a multifactorial vascular disease characterized by formation of inflammatory lesions. Secretory phospholipase A2, group IIA (sPLA2-IIA) is involved in this process and plays a critical role. However, the exact role of sPLA2-IIA in cardiovascular inflammation is more complicated and remains unclear. Furthermore, both statins and Xuezhikang (XZK) are widely used in the prevention and treatment of cardiovascular disease risk because of their pleiotropic effects on the cardiovascular system. However, their effects on sPLA2-IIA are still controversial. We investigated the regulation of sPLA2-IIA by rat thoracic aorta smooth muscle cells (VSMCs) in culture. Cells were first incubated with IL-1β alone to induce expression of sPLA2-IIA and then treated with several concentrations of statins or XZK for different times in the absence or presence of IL-1β. We tested the expression of sPLA2-IIA, including sPLA2-IIA mRNA, protein, as well as activity. We found that statins or IL-1β increase the expression of sPLA2-IIA in VSMCs and the effect is based on a synergetic relationship between them. However, for the first time, we observed that XZK effectively reduces sPLA2-IIA expression in IL-1β-treated VSMCs. Our findings may shine a new light on the clinical use of XZK and statins in the prevention and treatment of atherosclerosis-related thrombosis. (Int Heart J 2017; 58: 115-124)

Key words: Lovastatin, Simvastatin, IL-1β

Statins are widely used in the prevention and treatment of cardiovascular disease risk because of their effective inhibition of 3-hydroxy-3-methylglutaryl coenzyme-(β) reductase and they decrease the pathogenic reaction of the cholesterol biosynthesis enzyme. Statins have many other roles in addition to lowering cholesterol. Importantly, statins are also involved in inhibition of the mevalonate pathway. Furthermore, a lot of effort has been expended to study the anti-inflammatory effects of statins. Among them, a reduction in C-reactive protein is thought to be directly related to the reduction of myocardial ischemia. Indeed, statins can increase the stability of atherosclerotic plaques by increasing the release of vascular endothelial nitric oxide, improving vascular smooth muscle cell adhesion/migration, and promoting a T helper 2 bias and suppressing the secretion of T helper 1 cytokines. Recently, more and more gene products have been shown to be regulated by statins, such as cyclooxygenase2, inducible nitric oxide (NO) synthase, tumor necrosis factor (TNF), and interleukin (IL)-6, which play a role in inflammation and atherosclerosis.

Interestingly, Xuezhikang, which is an extract of Chinese red yeast rice containing statin-like compounds, was shown to reduce the concentrations of serum triglycerides, total cholesterol, and low-density lipoprotein cholesterol in a clinical trial. XZK is also associated with potent anti-inflammatory and lipid-lowering effects, which help improve endothelial function. It is closely related with significantly enhanced vascular endothelial cell proliferation and adhesion ability, which are not significantly different to those of statins. Therefore, XZK can not only inhibit the actions of HMG-CoA reductase, fatty acids, and isoflavones, but also has lipid-lowering as well as anti-inflammatory actions.

Secretory phospholipase A2 of type IIA plays a key role in regulating the lipid production process by enlarging the neointimal inflammatory response of vascular smooth muscle. In the past two decades, a number of in vitro and in vivo studies have indicated that the expression of sPLA2-IIA was constitutively induced by pro-inflammatory stimuli in a wide variety of cells and tissues in various animal species, such as platelets, neutrophils, macrophages, and mast cells, as well as spleen, tonsil, intestine, and bone marrow. The sPLA2 IIA gene is highly expressed in VSMCs upon stimulation by cytokines, interleukin-1β (IL-1β), IL-6, and tumor necrosis factor alpha (TNF-α), as well as oxysterols, PGE2, high mobility group B1 (HMGB1), and serum amyloid A (SAA). The sPLA2 IIA is elevated in inflammatory states, such as sepsis, acute pancreatitis, and chronic rheumatoid arthritis. There is also evidence that the amount of SPLA2 in atherosclerotic tissue is correlated with the severity of the lesion.
Thus, statins modulate the expression of different cytokines, growth factors, and enzymes in inflammatory conditions, and this is strongly correlated to the expression of sPLA2-IIA. However, at the present time the specific biological function of sPLA2-IIA has not yet been fully clarified, which led us to explore the synthesis of sPLA2-IIA when giving statins and Xuezhikang in rat vascular smooth muscle cells in the presence of IL-1β. The results show that both IL-1β and statins increase the expression of sPLA2-IIA, and the effect has an obvious synergistic interaction between them. Xuezhikang alone, however, does not influence the expression of sPLA2-IIA, while in the presence of IL-1β, it can effectively inhibit the sPLA2-IIA expression.

**METHODS**

**Materials:** Lovastatin and simvastatin were purchased from Merck KGaA (Darmstadt, Germany). Xuezhikang (XZK) was kindly provided by the Beijing WBL Peking University Biotech Co. Ltd (WPU) (Beijing). Recombinant rat IL-1β was purchased from PEPROTECH (Rocky Hill, USA) and dissolved according to the manufacturer’s protocol. Statin was purchased from PEPROTECH (Rocky Hill, USA) was used to extract total RNA according to the supplier’s protocol. Enzyme activity is expressed as a function. This function represents the enzyme content of each well. The reaction plate was incubated as follows: 95°C for 30 s and 40 cycles of denature (95°C for 5 s) and anneal/extend (60°C for 31 s). Each run contained a control group and a blank group, and all of these run results were negative. Each sample cDNA was divided into the 3 same reaction tubes as parallel comparisons in every run. The reaction data were determined by using threshold cycles (Ct) values. The sPLA2-type IIA mRNA relative quantity expression was calculated using the ΔΔCt method, and these data were based on GAPDH as a reference of housekeeping genes.

**Quantitative determination of sPLA2-IIA by ELISA:** The sPLA2-IIA protein is mostly released into the medium. To determine the content of enzyme, a specific enzyme-linked immunosorbent assay (ELISA) kit was used according to the manufacturer’s protocol (RB). Data were analyzed in triplicate.

**Assay of sPLA2 activity:** sPLA2 activity was assayed in cell culture supernatants using a commercially available kit (Cayman Chemical, Ann Arbor, MI). Activity assay was performed according to the manufacturer’s protocol. Enzyme activity is expressed as a function. This function represents the enzyme activity in the volume of cell culture medium (μmol/minute/mL), because one unit of enzyme hydrolyzes one μmol of diheptanoyl Thio-PC per minute at 25°C. Experiments were performed in triplicate.

**Statistical analysis:** Data were analyzed by one-way ANOVA followed by Tukey’s multiple comparison test (V5.00, GraphPad Prism Software, Inc., San Diego, CA). Values of *P* < 0.05 were considered as significant. Each experiment was performed at least 3 times.

**RESULTS**

Up-regulation of sPLA2-IIA expression in VSMCs by IL-1β:
In the initial study, rat vascular smooth muscle cells (VSMCs) were tested for induction of sPLA2-IIA expression by IL-1β for different concentrations or time. In order to investigate the expression of sPLA2-IIA induced by the increasing dose of IL-1β, a series of experiments were conducted. These experiments were performed with VSMCs incubated for 24 hours with IL-1β (Figure 1A-C). As shown in Figure 1A-C, sPLA2-IIA...
mRNA, sPLA2-IIA protein, and sPLA2-IIA activity were modestly but significantly increased in a dose-dependent manner in VSMCs incubated for 24 hours with 5 ng to 20 ng/mL IL-1β. More than 20 ng/mL led to a decline in sPLA2-IIA expression instead. Therefore, all subsequent experiments were performed with 20 ng/mL IL-1β. After being exposed to 20 ng/mL IL-1β for 0 to 36 hours, VSMCs were harvested for the purpose of estimating sPLA2-IIA gene expression, and the culture supernatants were removed to investigate sPLA2-IIA protein and activity. There was also a time-dependent increase in sPLA2-IIA expression in VSMCs incubated with 20 ng/mL IL-1β for 0 to 24 hours, and after 24 hours of cultivation, the expression of sPLA2-IIA was reduced (Figure 1D-F). These results were similar to previously published data.28

With concentrations of more than 20 ng/mL or more than 24 hours of incubation time with 20 ng/mL IL-1β, we detected multiple cellular apoptosis phenomenon by fluorescence microscopy (data not shown). This may be associated with decreases in the sPLA2-IIA-mRNA/GAPDH-mRNA ratio, secreted sPLA2-IIA protein, and activity. Furthermore, IL-1β stimulated the increase of sPLA2-IIA released into the medium, both at the protein and activity levels, although only modestly. Under the same experimental conditions, the increase in transcription level was more significant. This different behav-

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**Figure 1.** Effects of IL-1β on the expression of sPLA2-IIA in vascular smooth muscle cells (VSMC), including gene, quantity, and activity of sPLA2-IIA expression. Before the measurements, VSMCs were treated with various concentrations of IL-1β for 24 hours (A-C) or 20 ng/mL IL-1β for different times (D-F). Dose- and time-dependent up-regulation of sPLA2-IIA expression in rat VSMC by IL-1β. A and D represent the results of real-time PCR analysis, which show that the relative increase in the ratio of sPLA2-type IIA mRNA/GAPDH mRNA in comparison to untreated cells in which the ratio was set at 1.0. (B, E) ELISA detected the content of sPLA2-IIA protein released into the culture medium. (C, F) sPLA2-IIA activity in culture medium. Results are the mean ± SD from 3 experiments. *P < 0.05, **P < 0.01, ***P < 0.001 versus untreated cells.
ior is consistent with previous research results. Pruzanski, et al reported that only when the binding sites of sPLA2-IIA on membranes are saturated in advance, will sPLA2-IIA be released into the medium.

Therefore, IL-1β activated sPLA2-IIA expression in rat VSMCs in a dose- and time-dependent manner, not only at the mRNA level, but also at the protein and activity levels. It is worth noting that all of these improvements were moderate, especially the protein and activity levels.

Effects of statins on sPLA2-IIA expression in VSMCs: In recent years, although the regulation effects of statins on many inflammatory cytokines have been confirmed, the specific regulatory role of statins in the process of sPLA2-IIA expression in inflammation remains controversial. In order to verify the exact effect of statins on sPLA2-IIA, VSMCs were respectively exposed to two kinds of statins for different times (D-F). A, D: sPLA2-IIA transcription was measured by reverse transcription/real-time PCR. GAPDH mRNA was used as an internal standard. The results are expressed as the relative increase in the ratio of sPLA2-type IIa mRNA/GAPDH mRNA in comparison to untreated cells in which the ratio was set at 1.0. B, E: sPLA2-IIA release into the supernatant was quantified by ELISA. C, F: sPLA2 activity was measured in the supernatant. All data are the mean ± SD of one experiment representative of 3 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 versus cells treated without lovastatin. #P < 0.05, ##P < 0.01, ###P < 0.001 versus cells treated without simvastatin. A-C: *P < 0.05, **P < 0.01, ***P < 0.001 versus cells treated with the same concentration but with lovastatin. D-E: *P < 0.05, **P < 0.01, ***P < 0.001 versus cells treated for the same time but with lovastatin.

Figure 2. Dose- and time-dependent up-regulation of sPLA2-IIA expression in rat VSMCs by lovastatin and simvastatin. VSMCs were incubated with several concentrations of lovastatin or simvastatin for 24 hours (A-C) or respectively exposed to 2 μmol/L of the two kinds of statins for different times (D-F). A, D: sPLA2-IIA transcription was measured by reverse transcription/real-time PCR. GAPDH mRNA was used as an internal standard. The results are expressed as the relative increase in the ratio of sPLA2-type IIa mRNA/GAPDH mRNA in comparison to untreated cells in which the ratio was set at 1.0. B, E: sPLA2-IIA release into the supernatant was quantified by ELISA. C, F: sPLA2 activity was measured in the supernatant. All data are the mean ± SD of one experiment representative of 3 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 versus cells treated without lovastatin. #P < 0.05, ##P < 0.01, ###P < 0.001 versus cells treated without simvastatin. A-C: *P < 0.05, **P < 0.01, ***P < 0.001 versus cells treated with the same concentration but with lovastatin. D-E: *P < 0.05, **P < 0.01, ***P < 0.001 versus cells treated for the same time but with lovastatin.
Figure 2 D-F shows a time-dependent induction of sPLA2-IIA expression following 2 \( \mu \)mol/L statin stimulation for 0 hours, 6 hours, 12 hours, 24 hours, or 36 hours. Statins (2 \( \mu \)mol/L) induced sPLA2-IIA expression which peaked at 36 hours (in the lovastatin group, there was a 12-fold increase in sPLA2-IIA mRNA over control, protein increased to 265 pg/mL, and sPLA2 activity up to 0.24 \( \mu \)mol/minute/mL. Interestingly, we found the increase in sPLA2-IIA expression was slightly more pronounced in the simvastatin group. However, both increases were moderate.

Effects of statins on sPLA2-IIA expression in IL-1\( \beta \)-treated VSMCs: Our previous experiments have verified that either IL-1\( \beta \) or statins enhanced sPLA2-IIA expression in a dose- and time-dependent manner, but both stimulations were at a low level. We wondered whether a combination IL-1\( \beta \) and statins might markedly improve the expression. To test this hypothesis, we divided the following experiments into two parts and chose lovastatin as a representative statin. In the first part, VSMCs were incubated with various concentrations of lovastatin for 12 hours, and then with 20 ng/mL IL-1\( \beta \) for 24 hours. D-F: VSMCs were treated with 2 \( \mu \)mol/L lovastatin for the indicated time in the absence or presence of IL-1\( \beta \). A, D: sPLA2-IIA transcription was measured by real-time PCR. GAPDH mRNA was used as an internal standard. The results are expressed as the relative increase in the ratio of sPLA2-type IIA mRNA/GAPDH mRNA in comparison to untreated cells in which the ratio was set at 1.0. B, E: sPLA2-IIA released into the supernatant was quantified by ELISA. C, F: sPLA2 activity was measured in the supernatant. A-C: *\( P < 0.05 \), **\( P < 0.01 \) versus untreated cells; *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \) versus cells treated with IL-1\( \beta \) alone. D-F: **\( P < 0.01 \), ***\( P < 0.001 \) versus untreated cells; ****\( P < 0.0001 \) versus cells treated for 24 hours with lovastatin and IL-1\( \beta \). All data are the mean + SD of one experiment representative of 3 independent experiments.
significantly enhanced sPLA2-IIA expression. The highest increase was a 300-fold improvement in sPLA2-IIA mRNA (Figure 3A), the release of 883 ng/mL sPLA2-IIA protein (Figure 3B), and 0.5 μmol/minute/mL activity (Figure 3C).

More interestingly, this marked up-regulation was also dose-dependent. The combination of IL-1β and 0.5 μmol/L lovastatin resulted in a 94-fold increase in sPLA2-IIA mRNA compared to that of control, ie, an 8-fold increase compared to that with IL-1β alone. In the second part, VSMCs were treated with 2 μmol/L lovastatin for the indicated time in the absence or presence of IL-1β. Similar to the stimulation with IL-1β or lovastatin alone, the combined effect could still stimulate sPLA2-IIA expression in a time-dependent manner, no matter what the sPLA2-IIA mRNA level, protein level, or activity level (Figure 3D-F). Furthermore, compared to IL-1β or lovastatin alone, incubation of IL-1β-treated cells with lovastatin consistently and dramatically increased sPLA2-IIA expression, especially in sPLA2-IIA mRNA (30-fold IL-1β alone and 40-fold lovastatin alone), the increase in protein or activity was not more than 10-fold.

These experiments demonstrated that the combination IL-1β and lovastatin dramatically improved the expression of sPLA2-IIA in VSMCs in a dose- and time-dependent manner, and that this induction occurred mainly at the transcriptional level. Therefore, we speculate that this effect is due to a synergetic mechanism between lovastatin and IL-1β in some pathways.

**XZK inhibited the expression of sPLA2-IIA in IL-1β-treated VSMCs:** XZK, which contains statin-like compounds, also has effective anti-inflammatory and lipid-lowering actions. Some research has indicated that there was no significant difference between XZK and statins in terms of improving the role of endothelial proliferation and adhesion ability. Thus, XZK and statins are closely linked to each other in many ways. In the case of the results above, we considered whether or not the expression of sPLA2-IIA in VSMCs could also be affected by XZK. To test our assumption, the following experiments were performed. First, VSMCs were exposed to various concentrations of XZK (based on previous experiments) without IL-1β. However, neither increased XZK to a high concentration of 500 μg/mL and extending the cultivation time to 48 hours showed little to no effect on the expression of sPLA2-IIA mRNA as demonstrated by real-time PCR (data not shown). Second, VSMCs were pre-incubated with different concentrations of XZK for 12 hours, and then the cells were refreshed with medium containing IL-1β (20 ng/mL) and the original concentrations of XZK for another 24 hours. Interestingly, XZK effectively improved the expression of sPLA2-IIA at the mRNA, protein and activity levels in a dose-dependent manner (Figure 4A-C). This effect was in contrast to statins. As shown in Figure 4A-C, XZK 50 μg/mL reduced IL-1β-induced sPLA2-IIA mRNA expression, until 100 μg/mL could effectively decrease the expression of sPLA2-IIA protein and activity. Therefore, we took 100 μg/mL as the effective concentration of XZK in the subsequent experiments. VSMCs were treated with 100 μg/mL XZK for 12 hours, 24 hours, 36 hours, or 48
hours. Twenty-four hours before harvesting, IL-1β (20 ng/mL) was added to the medium. Surprisingly, the results showed that this attenuation effect of XZK was also a time-dependent inhibition and the most significant decrease was seen at 36 hours (Figure 4D-E). In addition, in the presence of IL-1β (20 ng/mL) for 24 hours, more than 48 hours of cultivation with XZK (100 μg/mL) resulted in an increase in sPLA2-IIA expression. This may be related to a weakening of the inhibitory effect. Taken together, we concluded that XZK effectively down-regulates the expression of sPLA2-IIA in IL-1β-treated VSMCs in a dose- and time-dependent manner.

**DISCUSSION**

Secretory phospholipase A2 of type II A is involved in the development of atherosclerosis, and plays a critical role. sPLA2 II A is crucial for the process of regulating lipid production because it enlarges the neointimal inflammatory response of vascular smooth muscle. In the present study, we investigated the effects of statins and IL-1β on the expression of sPLA2 II A in VSMCs. Lovastatin, simvastatin, XZK, and IL-1β were tested by focusing our attention on the modulation of the expression of sPLA2 II A gene protein and activity in VSMCs. The above findings have demonstrated that either IL-1β or statins could increase the expression of sPLA2 II A in a dose- and time-dependent manner. Furthermore, a dramatic synergistic relationship between them was observed. XZK alone, however, did not influence the expression of sPLA2 II A. In the presence of IL-1β, XZK effectively down-regulated sPLA2 II A expression. Our results provide a new perspective for statins and inflammation.

As is known, VSMCs are the main constituent stromal cells of the vascular wall, and they play a variety of different structural and physiological functions in the process of atherosclerosis. After percutaneous coronary intervention (PCI), the proliferation and migration of VSMC participate in intimal hyperplasia and plaque progression, which are important to restenosis. Traditionally, smooth muscle cells are divided into quiescent contractile and proliferative synthetic phenotypes. In fact, there are several transitional states of middle subtype VSMCs within the same blood vessels. It has been shown that phenotypic modulation of VSMCs was mediated by the influence of transcription factors and co-factors, and also determined by transcription repression. There are multiple signaling pathways regulating this transcription process, including extracellular signal-regulated kinase (ERK), c-Jun-N-terminal kinase, p38 mitogen-activated protein kinases (p38MAPK), Akt, and Rho/Rho-kinase. In addition to their pleiotropic effects (anti-oxidation, anti-inflammation and anti-thrombosis), statins can also inhibit proliferation and migration of VSMC by regulating the activities of different pathways. In vitro and in animal models, the inhibition of Rho with statins was related to the reduction of calcium storage in VSMCs. It was also reported that statins prevented VSMC proliferation via inhibition of the Rho/Rho-kinase pathway. Furthermore, there is evidence that statins can suppress the change in phenotype and proliferation of VSMCs via inactivating the p38 MAPK pathway. Furthermore, in our experiment we found statins could moderately up-regulate the expression of sPLA2-IIA. Previous studies have found that statins may stimulate the expression of sPLA2-IIA synergistically by inhibiting RhoA and activating the 1/2ERK and MAPK signaling pathways. These are similar to the signaling pathways in which statins modulate the proliferation and migration of VSMCs. Therefore, we believe that the effects of statins on the proliferation and migration of VSMC and the expression of sPLA2-IIA in VSMCs were at least through the inhibition of the RhoA, 1/2ERK, and MAPK signal pathways. Atherosclerosis is a progressive and complex disease. To date, there is still controversy about the mechanism of statins on signal pathway inhibition in different situations. It has not yet been well documented as to whether sPLA2-IIA expression is involved in the inflammation process of atherosclerosis and in the phenotypic modulation of VSMC from contractile phenotype to synthetic phenotype during plaque formation. The underlying molecular mechanisms still need to be ascertained. Further research on these points is needed.

In the case of vascular inflammation, particularly during atherogenesis, VSMCs are activated by several proinflammatory cytokines, including IL-1β. It has been shown that IL-1β-induced sPLA2-IIA expression in VSMCs was regulated at the level of transcription by C/EBPβ and C/EBPδ. Recently, El Hadri, et al noted that PPARβ/δ ligand and AMPK signaling were also involved in this regulation. Our results indicate that IL-1β modulated sPLA2-IIA expression in VSMCs in a dose- and time-dependent manner, including sPLA2-IIA mRNA, protein, and activity. Therefore, on the basis of previous research, we were able to more comprehensively characterize the kinetic parameters of its induction. Hence our study could provide some information for future studies about the mechanisms behind the modulation of sPLA2-IIA expression induced by IL-1β.

As inhibitors of HMG-CoA reductase, in addition to their lipid-lowering effects, statins have anti-inflammatory effects on atherosclerosis: reduction in C-reactive protein, and modulation of cyclooxygenase 2, inducible NO synthase, TNF, and IL-6. However, very few reports have described a link between statins and sPLA2-IIA expression. Menschikowski, et al found that the expression of sPLA2-IIA in human aorta smooth muscle cells (HASMC) can be increased by statins, and this effect is based on both bJak2-, C/EBPδ- and NF-κB-dependent transcriptional regulation and the stabilization of sPLA2-IIA mRNA. Similarly, in rat VSMCs, for the first time, we found that statins alone can moderately up-regulate the expression of sPLA2-IIA, while this effect becomes dramatic when combining statins with IL-1β. This surprised us because numerous studies, both in vitro and in vivo, provided extensive evidence indicating that high expression of sPLA2-IIA leads to the occurrence of inflammation and atherosclerosis. Therefore, we need to consider how to understand our findings in a reasonable way since statins can indeed effectively slow down the progression of atherosclerosis. One possible explanation is that statins exert multiple protective effects on the cardiovascular system. Among them, the pro-atherogenic effect of sPLA2-IIA up-regulation is weak, even negligible, when compared to the other potent and main anti-atherogenic effects, such as lipid-lowering, a reduction in C-reactive protein, an increase in the release of vascular endothelial nitric oxide, or an improvement in vascular smooth muscle cell adhesion/migration. On the other hand, several lines of evidence have indicated that sPLA2-IIA is also involved in anti-inflam-
matory and anti-apoptotic progression. In a model in which sPLA2-IIA was over-expressed in macrophages, sPLA2-IIA exacerbated lesions but led to more stable lesions that were not prone to rupture. Likewise, sPLA2-IIA also plays an anti-atherogenic role via its inhibitory effect on thrombosis after plaque rupture. Recently, Wang, et al reported that autocrine sPLA2-IIA release could protect brain microvascular endothelial cells from LPS-induced injury. Furthermore, sPLA2-IIA retarded apoptosis in oligodendrocytes and protected the cells against oxysterol-triggered apoptosis, suggesting high expression of sPLA2-IIA in inflammation might trigger cell protective machinery to influence the final effect of inflammation. Others have reported on the anti-bacterial properties of sPLA2-IIA and its capability to enhance the clearance of oxidative modified lipoproteins during inflammation. Therefore, our data may provide new insight into statin treatment applied to sepsis and late cardiovascular disease.

Next, the reason why combination of statins and IL-1β dramatically stimulates sPLA2-IIA expression should be clearly explained. First, it is known that the NF-κB binding site is a crucial promoter in the sPLA2-IIA gene in rat VSMCs stimulated with IL-1β, suggesting those factors that can activate NF-κB may also at least partially induce sPLA2-IIA expression. There is ample evidence showing that two NF-κB binding elements (_214/223and _380/388 bp) are activated by statins in human monocytes, aortic smooth muscle cells, and human hepatocyte cells. Moreover, Menschikowski, et al demonstrated that statins may stimulate the expression of sPLA2-IIA synergistically with IFN-γ by the NF-κB signaling pathway. Therefore, similar to our results, we believe the NF-κB signaling pathway also responsible for the synergistic effects of IL-1β and statins, which lead to the dramatic expression of sPLA2-IIA in VMSCs. Secondly, in IL-1β-treated human corneal endothelial cells, RhoA was inhibited. Likewise, according to a previous study, human cells expressing unpreyalted RhoA produce increased levels of IL-1β mRNA, and in simvastatin-treated THP-1 cells, RhoA activity was significantly reduced. This suggests RhoA activity could be augmented by IL-1β and statins. These studies support our data. Taken together, we inferred that IL-1β and statins induce sPLA2-IIA expression in VSMCs by inhibiting the RhoA signaling pathway. In addition to the signaling pathways, we finally focused our attention on sPLA2-IIA mRNA stability. A previous study demonstrated that the half-life of sPLA2-IIA mRNA could be significantly prolonged in cells exposed to IFN-γ in the presence of a statin when compared to cells treated with IFN-γ or statin alone. Similarly, Laufs, et al reported that the treatment of human endothelial cells with simvastatin resulted in the increased stability of endothelial NO synthase mRNA. Therefore, we wonder whether the combination of IL-1β and statins increases sPLA2-IIA mRNA stability and further results in sPLA2-IIA expression. This needs to be verified in future experiments.

Xuezhikang is a traditional Chinese medicine with pleiotropic effects on the cardiovascular system, especially anti-inflammatory and lipid-lowering effects. Although its active agent is thought to be a 2-hydroxy-3-methylglutaral coenzyme, a reductase inhibitor commercially available as the pharmaceutical lovastatin, the exact functions of XZK and the differences between XZK and statins have not been fully elucidated. The data we obtained show that XZK can effectively inhibit sPLA2-IIA expression in IL-1β-treated VSMCs. Therefore, in contrast to statins, to the best of our knowledge, this is the first time that IL-1β-induced sPLA2-IIA activity has been shown to be inhibited by XZK mainly at a transcriptional level. Nevertheless, the question arises as to how the finding of sPLA2-IIA down-regulation in IL-1β-treated VSMCs is reconcilable with the fact that XZK contains a family of naturally occurring statins, such as lovastatin, but modulates sPLA2-IIA expression in a manner opposite to that of statins. We believe this difference can be explained as follows. First, it is known that XZK contains 0.8% lovastatin and 8% unsaturated fatty acids, as well as essential amino acids, ergosterol, carbohydrate, protein, isoflavones, alkaid, and trace elements. The XZK concentration of 100 μg/mL corresponded to approximately 2 μmol/L lovastatin in our experiments. In other words, they contain the same dose of statins, but regulation of the enzyme was different. We proposed this effect may be related to other useful substances (> 10 times statin, unsaturated fatty acids, essential amino acids, and ergosterol) in addition to the lovastatin. It is also well known that unsaturated fatty acids and essential amino acids have beneficial effects in the cardiovascular system, such as anti-inflammation and antioxidation. Secondly, it has been shown that the bioavailability of lovastatin contained in XZK was 169% compared with that of purified lovastatin. Similarly, in human studies, the AUC and Cmax values for lovastatin and its active metabolite, lovastatin acid, were significantly higher in volunteers receiving XZK capsules than in those receiving lovastatin tablets. In addition, shorter and less variable Tmax values were observed in volunteers taking XZK capsules. These findings also suggest that the oral bioavailability of lovastatin is significantly improved in XZK as a result of a higher dissolution rate and reduced crystallinity. Therefore, although 100 μg/mL XZK contains 2 μmol/L statins, the concentrations of statins actually absorbed are much higher than lovastatin. We speculated that high concentrations of statins (much higher than 2 μmol/L) might instead reduce sPLA2-IIA expression. On the other hand, previous research has demonstrated that IL-1β-induced sPLA2-IIA expression in VSMCs was up-regulated by C/EBPβ and C/EBPδ. Moreover, a study on the molecular mechanisms of XZK reported that XZK was more potent than lovastatin in suppressing plaque rupture. Therefore, in contrast to statins, to the best of our knowledge, this is the first time that IL-1β-induced sPLA2-IIA activity has been shown to be inhibited by XZK mainly at a transcriptional level. Nevertheless, the question arises as to how the finding of sPLA2-IIA down-regulation in IL-1β-treated VSMCs is reconcilable with the fact that XZK contains a family of naturally occurring statins, such as lovastatin, but modulates sPLA2-IIA expression in a manner opposite to that of statins. We believe this difference can be explained as follows. First, it is known that XZK contains 0.8% lovastatin and 8% unsaturated fatty acids, as well as essential amino acids, ergosterol, carbohydrate, protein, isoflavones, alkaid, and trace elements. The XZK concentration of 100 μg/mL corresponded to approximately 2 μmol/L lovastatin in our experiments. In other words, they contain the same dose of statins, but regulation of the enzyme was different. We proposed this effect may be related to other useful substances (> 10 times statin, unsaturated fatty acids, essential amino acids, and ergosterol) in addition to the lovastatin. It is also well known that unsaturated fatty acids and essential amino acids have beneficial effects in the cardiovascular system, such as anti-inflammation and antioxidation. Secondly, it has been shown that the bioavailability of lovastatin contained in XZK was 169% compared with that of purified lovastatin. Similarly, in human studies, the AUC and Cmax values for lovastatin and its active metabolite, lovastatin acid, were significantly higher in volunteers receiving XZK capsules than in those receiving lovastatin tablets. In addition, shorter and less variable Tmax values were observed in volunteers taking XZK capsules. These findings also suggest that the oral bioavailability of lovastatin is significantly improved in XZK as a result of a higher dissolution rate and reduced crystallinity. Therefore, although 100 μg/mL XZK contains 2 μmol/L statins, the concentrations of statins actually absorbed are much higher than lovastatin. We speculated that high concentrations of statins (much higher than 2 μmol/L) might instead reduce sPLA2-IIA expression. On the other hand, previous research has demonstrated that IL-1β-induced sPLA2-IIA expression in VSMCs was up-regulated by C/EBPβ and C/EBPδ. Moreover, a study on the molecular mechanisms of XZK reported that XZK was more potent than lovastatin in suppressing ERLK1/2 and NF-κB activation. The ERK cascade is one of the best characterized major MAPK and MAPK activation significantly inhibited cytokine-stimulated sPLA2 promoter. Thus, we surmised that XZK down-regulation of sPLA2-IIA expression may occur at least partially due to the suppression of ERLK1/2 and NF-κB activation, as well as MAPK activation. Future experiments should examine the exact molecular mechanism and its multiple effects.

In conclusion, the present study found that statins or IL-1β increase the expression of sPLA2-IIA in VSMCs and that the effect is based on a synergetic mechanism between them. However, for the first time, we observed that XZK effectively reduced sPLA2-IIA expression in IL-1β-treated VSMCs. These findings may provide a pharmacological basis for the clinical use of XZK and statins in the prevention and treatment of atherosclerosis-related thrombosis.
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