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Effects of antisense IRAK-2 oligonucleotides on PGI_2 release induced by IL-1 and TNF

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KEY WORDS interleukin-1 receptors; protein kinases; prostaglandins F; antisense oligonucleotides; interleukin-1; tumor necrosis factor; vascular endothelium

ABSTRACT

AIM: To explore the effects of antisense interleukin-1 receptor associated kinase-2 oligonucleotide (IRAK-2 ODN) on the prostacyclin (PGI_2) synthesis in human umbilical vein endothelial cells (HUVEC) induced by interleukin-1 (IL-1) and tumor necrosis factor (TNF). **METHODS**: The HUVEC were transfected with antisense IRAK-2 ODN and stimulated with IL-1 and TNF. The levels of PGI_2 release were analyzed by competitive ELISA. **RESULTS**: Pre-transfection with antisense IRAK-2 ODN could remarkably decrease the levels of PGI_2 synthesis induced by IL-1 in a time- and concentration-dependent manner, whereas it could not attenuate the one stimulated by TNF. **CONCLUSION**: The responses of antisense IRAK-2 ODN to IL-1 and TNF-stimulated PGI_2 release are different. IRAK-2 plays a key role in the IL-1 signaling events leading to PGI_2 release.

INTRODUCTION

IL-1 and TNF are cytokines released from a variety of cells in response to several chronic inflammatory diseases[1]. Most of the biological effects of IL-1 and TNF, such as stimulation of prostacyclin synthesis[1], are mediated by nuclear factor kappa B (NF-κB)[2].

Recently, interleukin-1 receptor associated kinase-2 (IRAK-2) was identified as early signal transducer for IL-1. IRAK-2 has been shown to be associated with IL-1 receptor, and overexpression of IRAK-2 activates NF-κB in a TRAF6 [a member of the TNF receptor associated factor (TRAF) family]-dependent manner[3]. Thus inhibition of IRAK-2 may block NF-κB activation and ultimately inhibit PGI_2 synthesis, thus inhibiting the inflammation. Since the antisense oligonuleotide provides an attractive protocol to block the transcription and translation of specific genes, in this study, we explored the effects of antisense IRAK-2 ODN on the PGI_2 synthesis induced by IL-1 and TNF.

MATERIALS AND METHODS

Materials IL-1β was purchased from Tianxiangren Co, Beijing. TNF was purchased from Dainippon Pharmaceutical Co, LTD, USA. 6-Keto-prostaglandin $F_{1\alpha}$ immunoassay kit was purchased from R&D System. DMEM and lipofectin were purchased from Gibco BRL.

Cell culture[4] Endothelial cells were isolated from human umbilical cords. After rinsing with PBS, the veins were incubated with 0.25 % trypsin at 37 ℃ for 10 min. Primary cultures were seeded at a density of 5×10^4 cells per well into 96-well plates. The culture medium used was DMEM containing 20 % fetal bovine serum purchased from Institude of Sijiqing Bioengineering Material, Hangzhou, China. The cells were cultured to confluency at 37 ℃ in a humidified atmosphere of 5 % CO_2 + 95 % O_2 and with a medium change on the first day after seeding and every 2 days thereafter.

Design of oligonucleotides sequence The sequences of ODN against IRAK-2 were: (1 to 18 of human IRAK-2 sequences).

Antisense: 5′-GTAGATGTAGCAGGCCAT-3′;

Sense: 5′-ATGGCCTGCTACATCTAC-3′

Oligonucleotides were synthesized by Sangon Co LTD, Shanghai, and modified with phosphorothioate at their 5′ end.

Lipofectin-encapsulated ODN Preparation Lipofectin 4 μL was diluted in 96 μL serum-free media for 45 min. Indicated dose of ODN was diluted in serum-free media to 100 μL. Then lipofectin was mixed with ODN for 15 min and kept at room temperature.

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Competitive ELISA for detecting 6-keto-$PGF_{1\alpha}$ The method[4] was described by kit directions.

Statistics Data were expressed as $\bar{x} \pm s$ and compared with t test.

RESULTS

The effect of antisense IRAK-2 ODN on the release of 6-keto-$PGF_{1\alpha}$ without IL-1 pre-treatment The cells were incubated with lipofectin-capsulated antisense ODN (3 μg) or washed with medium for 8 h followed by IL-1 (10 mg/L) stimulation. Twenty-four hours later, the 6-keto-$PGF_{1\alpha}$ was measured in the supernatant. The release of 6-keto-$PGF_{1\alpha}$ in both groups of cells stimulated with IL-1 were greatly increased compared with control, but between the two groups of cells stimulated with IL-1 there was no statistical difference. This indicates that without IL-1 pretreatment, the antisense IRAK-2 ODN cannot affect the IL-1-induced PGI_2 synthesis (Tab 1).

Tab 1. The effects of antisense IRAK-2 oligonucleotides on IL-1-induced 6-keto-$PGF_{1\alpha}$ synthesis without IL-1-pretreatment. $n = 4$ experiments. $\bar{x} \pm s$. $^cP < 0.01$ vs control.

Group	6-keto-$PGF_{1\alpha}$ production ($pg/10^5$ cells)
Control	18 ± 6
IL-1	467 ± 69^c
IL-1 + Antisense ODN	518 ± 6^c

The effect of IRAK-2 ODN on the release of 6-keto-$PGF_{1\alpha}$ with IL-1 pre-treatment After IL-1 pretreatment for 30 min, the cells were washed or incubated with sense or antisense ODN for 8 h followed by IL-1 stimulation for 24 h. Then the release of 6-keto-$PGF_{1\alpha}$ was measured. The antisense IRAK-2 ODN greatly decreased the level of 6-keto-$PGF_{1\alpha}$ production induced by IL-1 compared with control, whereas the sense IRAK-2 ODN had no effect (Tab 2).

Concentration-response for inhibitory effects of antisense ODN on IL-1-stimulated 6-keto-$PGF_{1\alpha}$ production After IL-1 pretreatment for 30 min, the cells were incubated with antisense ODN at different concentrations for 8 h followed by IL-1 stimulation for 24 h. Then the levels of 6-keto-$PGF_{1\alpha}$ were measured. The difference in the levels between the antisense IRAK-2 ODN concentration groups and control was significant. And at the concentration of 30 $mg \cdot L^{-1}$, the inhibitory effect reached a maximum (Fig 1).

Tab 2. The inhibitory effect of antisense ODN on IL-1-stimlated 6-keto-$PGF_{1\alpha}$ synthesis with IL-1 pretreatment. $n = 4$ experiments. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ vs control.

Group	6-keto-$PGF_{1\alpha}$ production ($pg/10^5$ cells)
Control	196 ± 38
Sense ODN + IL-1	173 ± 30^a
Antisense ODN + IL-1	42 ± 6^c

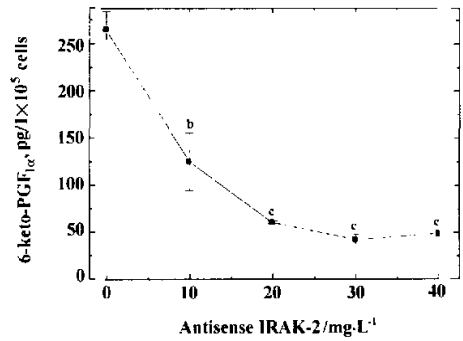

Fig 1. Concentration-response for inhibition of IL-1-induced PGI_2 release by antisense IRAK-2 ODN. $^bP < 0.05$, $^cP < 0.01$ compared with no ODN.

Time-course for inhibitory effects of antisense ODN on IL-1-stimulated 6-keto-$PGF_{1\alpha}$ production After IL-1 pretreatment for 30 min, the cells were incubated with antisense ODN at the concentration of 30 $mg \cdot L^{-1}$ for indicated time followed by IL-1 stimulation for 24 h. Then the 6-keto-$PGF_{1\alpha}$ release was measured. As indicated in Tab 3, between 5 h and 24 h,

Tab 3. Time course for inhibition of IL-1-induced PGI_2 release by antisense IRAK-2 ODN. $n = 4$ experiments. $x \pm s$. $^cP < 0.01$ compared with no ODN.

Group	6-keto-$PGF_{1\alpha}$ production ($pg/10^5$ cells)
IL-1	257 ± 49
IL-1 + Antisense ODN 5 h	183 ± 30^c
IL-1 + Antisense ODN 8 h	78 ± 16^c
IL-1 + Antisense ODN 24 h	89 ± 56^c

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the 6-keto-$PGF_{1\alpha}$ release was stably inhibited in cells transfected with antisense IRAK-2 ODN, which did not occur in control cells.

The effect of antisense ODN on TNF-stimulated 6-keto-$PGF_{1\alpha}$ production After TNF pretreatment for 30 min, the cells were washed or incubated with lipofectin containing antisense IRAK-2 ODN for 8 h followed by TNF stimulation for 24 h. TNF could induce the release of PGI_2, which was not affected by antisense IRAK-2 ODN (Tab 4).

Tab 4. The effects of antisense IRAK-2 ODN on TNF-induced PGI_2 release. $n = 4$ **experiments.** $\bar{x} \pm s$. $^cP < 0.01$ *vs* **control.**

Group	6-keto-$PGF_{1\alpha}$ production ($pg/10^5$ cells)
Basal	46 ± 69
TNF	429 ± 34^c
TNF + Antisense ODN	382 ± 61^c

DISCUSSION

In this study, we found that antisense IRAK-2 oligonucleotide produced different effects on IL-1- and TNF-stimulated PGI_2 release. The antisense ODN inhibited the IL-1-stimulated PGI_2 release, but not the TNF-stimulated release.

The inhibition of IL-1-stimulated PGI_2 release by antisense ODN was time- and concentration-dependent. The maximum inhibition occurred at 8 h thereafter, when the cells were treated with antisense ODN at the concentration of 30 $mg \cdot L^{-1}$. The concentration of antisense ODN required for blocking IL-1-stimulated PGI_2 release was about 10 $mg \cdot L^{-1}$. The inhibitory effects of antisense ODN on IL-1-stimulated PGI_2 release occurred only under the condition of IL-1 pretreatment. The reason for it is that IRAK-2 is constitutively expressed in the cytoplasm and can be degraded after activation, and new proteins are then synthesized to maintain its sensitivity to IL-1[5]. Although both IL-1 and TNF induce 6-keto-$PGF_{1\alpha}$ release, TNF-stimulated 6-keto-$PGF_{1\alpha}$ release could not be blocked by antisense ODN (even at high concentrations), suggesting that IRAK-2 is not involved in the TNF-stimulated 6-keto-$PGF_{1\alpha}$ production.

Taken together, IRAK-2 plays an important role in IL-1 signaling events. Whereas TNF-stimulated 6-keto-$PGF_{1\alpha}$ release is not related to IRAK-2. The antisense IRAK-2 oligonucleotide may be of potential use in blocking IL-1-induced inflammation.

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白细胞介素-1 受体相关激酶-2 反义寡核苷酸对 IL-1 和 TNF 诱导 PGI_2 合成的影响

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关键词 白细胞介素-1 受体；蛋白激酶类；前列腺素 F 类；反义寡核苷酸类；白细胞介素-1；肿瘤坏死因子；血管内皮

目的：研究白细胞介素-1 受体相关激酶-2（IRAK-2）反义寡核苷酸对白细胞介素-1（IL-1）和肿瘤坏死因子（TNF）诱导前列环素（PGI_2）合成的不同影响. **方法**：IRAK-2 反义寡核苷酸（IRAK-2 ODN）转染脐静脉内皮细胞以阻断 IRAK-2 的表达，以 IL-1 及 TNF 刺激细胞后，用竞争 ELISA 方法检测 PGI_2 的合成. **结果**：IRAK-2 ODN 可显著降低 IL-1 诱导的 PGI_2 合成，此效应强度存在时间和浓度依赖性，但 IRAK-2 ODN 对 TNF 诱导的 PGI_2 合成无影响. **结论**：IRAK-2 ODN 对 IL-1 和 TNF 诱导 PGI_2 合成有不同影响. IRAK-2 在 IL-1 诱导的 PGI_2 合成中起重要作用.

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