

Original paper

# The superior role of coagulation factor FX over FVII in adenoviral-mediated innate immune induction of the hepatocyte: an *in vitro* experiment

Saeed Firoozi Ghahestani<sup>1</sup>, Alireza Shiri<sup>1</sup>, Afagh Moattari<sup>1</sup>, Jamal Sarvari<sup>1,2</sup>, Ali Moahammad Tamaddon<sup>3,4</sup>, Seyed Younes Hosseini<sup>1</sup>

<sup>1</sup>Department of Bacteriology and Virology, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>2</sup>Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>3</sup>Pharmaceutics Department, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>4</sup>Center for Nanotechnology in Drug Delivery, Shiraz University of Medical Sciences, Shiraz, Iran

## Abstract

**Aim of the study:** To better understanding the contribution of coagulation factors to the extent of adenovirus-mediated innate toxicity on the hepatocyte.

**Material and methods:** Adenovirus-36 (AD) and adenovector type 5-GFP (Ad5-GFP) were propagated and titered; then, they were loaded with coagulation factors VII or X. The complex of adenovirus with coagulation factor VII and X were for size and charge parameters. After adding AD-VII and AD-X complexes, the expression levels of innate inflammatory genes including protein kinase R (PKR), interleukin (IL)-1 $\beta$ , IL-8 and IL-18 were measured by Real-time PCR on a hepatocellular carcinoma cell line, HepG2.

**Results:** The loading of coagulation factors VII and X on Ad5-GFP enhanced the transduction rate up to 50% and 60% ( $p < 0.05$ ), respectively, compared to the adenovector alone (30%) ( $p < 0.05$ ). The formation of the coagulation factor-virus complex leads to multimodal size distribution with an increase in average hydrodynamic size and absolute zeta potential. The qPCR results showed that PKR expression increased significantly after treatment with all adenoviruses. These findings also showed that AD had a significant ( $p = 0.0152$ ) inflammatory impact on Hep-G2. However, AD which was loaded with FX (AD-X) exhibited the most inflammatory effect ( $p = 0.0164$ ). Significantly, the expression of IL-1 $\beta$  ( $p = 0.0041$ ), IL-8 ( $p = 0.0107$ ) and IL-18 ( $p = 0.0193$ ) were also enhanced following FX loading. On the other hand, the AD-VII complex showed the least effect of innate immune induction when compared to the negative control ( $p < 0.05$ ).

**Conclusions:** The loading of coagulation factors, particularly FX, could enhance the transduction efficiency of Ad5-GFP. Furthermore, adenovirus loaded with FX exhibited more innate toxicity on the hepatocytes, while it was not the case for FVII.

**Key words:** innate immunity, adenovirus, gene therapy, hepatocyte, coagulation factor.

## Address for correspondence

Dr. Seyed Younes Hosseini, Department of Bacteriology and Virology, Shiraz University of Medical Sciences, Shiraz, Iran, e-mail: [hoseiniy@sums.ac.ir](mailto:hoseiniy@sums.ac.ir)

## Introduction

Different types of viruses have been designed to deliver genes to cells or destroy a spectrum of the neoplastic cells [1]. Adenovirus is an effective oncolytic virus and delivery vector with appealing characteristics which made it more popular for cancer gene ther-

apy [2]. A major obstacle for efficient viral-based gene therapy and virus replication in the target tissue is the heavy induction of innate immunity, especially when employed systemically [1, 3]. Apart from the toxic inflammation and cytokine storm-related shock, adenovirus-mediated innate immunity is capable of mounting the adaptive immunity which could memorize the events

later on. As a result, this immunological memory destroys virus-producing cells and limits re-administration of the viruses [4].

The understanding of innate immune induction mechanisms by adenoviruses (Ads) is a field of interest to control their natural toxicity. Following intravascular administration, Ads have been shown to form complexes with a variety of blood components, more importantly, coagulation factors [5]. These factors are harnessed by viral particles to target them into permissive host cells [6].

Pioneer studies showed that coagulation factors interacted with adenoviruses hexon protein to enhance the entry and transduction process when assessed *in vitro* [6, 7]. Coagulation factor X was initially determined to be a critical factor of tropism which facilitates the hepatocyte infection by adenovirus [7]. Of note, despite the high-affinity interaction of coagulation factor VII to hexon, supported evidence that its cooperation in the entry process on the hepatocyte is lacking. It is shown that in the case of human adenovirus type 5 HAd5, coagulation factor X is sufficient to mediate virus transduction on hepatocyte when evaluated *in vivo* [8]. However, any data regarding the importance of FVII in this process need to be investigated. In addition, it is demonstrated that a complex of adenovirus with coagulation factors induces different patterns of innate immunity when investigated *in vivo* and *in vitro* [9]. Several studies have been performed to describe the role of FX in innate immunity induction when it is loaded on adenovirus. Doronin *et al.* showed that binding of FX to the adenovirus which has accessed the blood circulation permits the sentinel immune cells to sense the FX-adenovirus complex through triggering the TLR4 signal pathway, and subsequently developed a stronger innate immunity [10]. In contrast, Xu *et al.* suggested that the decoration of adenovirus with FX molecules is an effective strategy to protect viral particles from antibodies and complement detection [11]. In a recent study, Duffy *et al.* showed that in the human sera, adenovirus induced complement activation less when FX was available for virus interaction [12].

In spite of different studies, questions about the role of coagulation factors in innate immune induction of adenovirus were mostly remained unanswered. It is unclear how much adenovirus-mediated immunity may be influenced by coagulation factors as they come together in the human body. Hence, comparing the contribution of coagulation factors such as FX and FVII in this process has not been considered, yet. The delineation of the extent of adenovirus immune induction by coagulation factors, especially in hepatocytes, could be beneficial in finding ways of inflammation control during gene therapy with these viruses. Herein, the immune induction

potency of FVII or FX coated adenovirus was evaluated in the hepatocyte cell model.

## Material and methods

### Cell culture

Ad-293 (human embryonic kidney cell) and HepG<sub>2</sub> cells, a human hepatocellular carcinoma cell line (HB-8065, ATCC), were purchased from Iranian Biological Resource Center (Tehran, Iran). The cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) complemented with 10% fetal bovine serum (FBS), with 2 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin. The cells were distributed in 6-well plates in  $0.3 \times 10^6$  cells/well and treatment was performed when their confluence reached 80%. They were incubated under humidified standard conditions, at 37°C and 5% CO<sub>2</sub>. Ad-293 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented as above.

### Virus propagation and titration

Human adenovirus 36 (ATCC VR1610) was a kind gift from Dr. Ali Teimouri (Hamadan University of Medical Sciences). It was selected based on the similar attachment and entry process of common oncolytic adenoviruses which is CAR-dependent. A recombinant adenovector type 5 expressing green fluorescent protein (GFP) (Ad<sub>5</sub>-GFP) was also prepared in our laboratory, formerly [13]. In order to amplify these viruses, Ad-293 cells were seeded in T<sub>175</sub> culture flasks; then, the rounded cells containing viruses were harvested, washed with phosphate buffer (PBS), and freeze-thawed to release the viruses. The concentrated viruses were then prepared from supernatant of destroyed cells following 2 rounds of 4000 rpm centrifuge, passing through 0.4 μm size filter and Amicon Ultra-15 Centrifugal Filter Devices (EMD Millipore Corporation, Billerica, MA, USA). Then, the prepared viruses were stored with 10% (V/V) glycerol at -70°C until further experiments.

In order to determine the viral titer, we employed 50% tissue infective dose assay (TCID<sub>50</sub>). The endpoint was taken to be the highest dilution of the virus that produced a cytopathic effect (CPE) in 50% of the inoculated cells [14]. TCID<sub>50</sub> titer was converted to the number of infectious units per volume, and then divided by the number of cells to be infected in order to obtain the suitable MOI (multiplicity of infection) of viruses.

In addition, to estimate the suitable effective and non-cytotoxic MOI of the virus, the trypan blue exclusion test was performed. To do this, virus of different MOI ranging from 0.01 to 10 MOI was added into

the HepG<sub>2</sub> cells and incubated for 24 hours at 37°C. Afterward, the cells were harvested, then stained with trypan blue and evaluated under light microscopy. The percentage of live cells was considered in selecting the suitable dose of inoculation.

### Zeta potential and particle size distribution

Particle size and zeta potential of the adenovirus-coagulation factor complexes were measured after 1 hour at 37°C of incubation, using a NANO-flex – 180° DLS and ZETA-check (Microtrac, Meerbusch, Germany). The viscosity and refractive index of pure water at 25°C were used for data analysis. The intensity-average hydrodynamic size (Z-average) was determined at the suitable concentration. Zeta potential measurements were performed in phosphate-buffered saline (pH = 7.4) using the calibrated streaming potential method.

### Virus-coagulation factor complexes preparation and Hep-G<sub>2</sub> infection

Recombinant human blood coagulation factors zymogens VII and X were purchased from Hematologic Technologies, Inc. (Essex Junction, VT, USA). They were reconstituted in PBS and then stored at –80°C until further processes. Adenovirus was diluted in serum-free DMEM to reach 0.5 MOI, and then the coagulation factors were added at physiological concentration. They were then incubated for 1 hour at 37°C until the virus/factor complex formed. About 10 µg/ml and 0.6 µg/ml of FX and VII proteins corresponding to their physiological concentration in the blood (1 U/ml) were included in each tube, respectively [6]. The Hep-G<sub>2</sub> cells were seeded into 6-well plates one day prior to infection, while they were divided into 4 cell groups receiving Ad<sub>36</sub> (AD), Ad<sub>36</sub>-FX complex (AD-X), Ad<sub>36</sub>-FVII complex (AD-VII), or medium (Cell), respectively. For infection, the cells were washed twice with PBS then virus/coagulation factor complexes in a serum-free DMEM were added per well and incubated for 6 more hours at 37°C.

### Transduction efficiency

In order to evaluate the virus transduction efficiency on Hep-G<sub>2</sub> cells, different MOIs of recombinant Ad<sub>5</sub>-GFP were tested alone or in complex with coagulation factors (Ad<sub>5</sub>-GFP, Ad<sub>5</sub>-GFP-FVII and Ad<sub>5</sub>-GFP-FX). MOI = 1 was selected for further evaluation. In the 48-well plate, different complexes of Ad<sub>5</sub>-GFP vector were inoculated at MOI = 1 following 1-2 hours absorbance. Afterwards, the infected cells were fed with 2%

complete medium, and then they were left in CO<sub>2</sub> incubation atmosphere for 24 hours [8]. The transduced cells were finally estimated by fluorescent microscopy through evaluating the green fluorescence from cells, indicating GFP expression as well as adenovector presence. To assess the percentage of positive cells, GFP signals were simply counted on each field whilst light and fluorescent images were overlaid, then the mean of repeated tests was considered as the estimated percentage. Also the detailed quantification of GFP positive cells was performed by flow cytometric analysis using a BD FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). The data were analyzed with FlowJo software.

### Real-time PCR analysis

Total cellular RNA was isolated from normal HepG<sub>2</sub> as a control and treated HepG<sub>2</sub> (treated with AD, AD-VII, and AD-X) with an RNA extraction kit (CinnaGen Inc., Tehran, Iran). The quantity and quality of the obtained RNA were checked by measuring the ratio of optical density of 260/280 nm, using a Nanodrop spectrophotometer (Nanodrop; Thermo Fisher Scientific, Wilmington, DE, USA), and then it was stored at –80°C until cDNA synthesis. The cDNA was then synthesized using 1,000 ng of total RNA in a first-strand cDNA synthesis reaction using the PrimeScript RT reagent Kit (Perfect Real Time) (TaKaRa Bio, Siga, Japan). Quantitative polymerase chain reaction (qPCR) was performed using the ABI Biosystems 7500 and the RealQ Plus 2× Master Mix Green (Ampliqon A/S, Odense, Denmark). In each reaction, 100 nM of each primer was added to target the specific sequence. Specific primers targeting IL-1β, IL-8, IL-18, and protein kinase R (PKR) were designed, as shown in Table 1. Primer pairs were designed using Allel ID software (Applied Biosystems). The PGK housekeeping gene was also used as the internal control of qPCR normalization. The qPCR conditions were set

Table 1. Primers used in the study

Gene names	Sequences (5'-3')	Size (bp)
IL-1β	Forward: TGTGAAAGACACTATGCAAGCTC Reverse: GTGACACGATGAGAACAAAGTTG	83
IL-8	Forward: GAGGGCCAAGACGAAGACATC Reverse: CAGATCACGTCATCGACAAC	94
IL-18	Forward: TACTTCCACAGGTCCACAAC Reverse: GTTTGAGGGGATGGATAAAC	166
PKR	Reverse: CGACCACAAGGATCAGCATGA Forward: TGCTGACCATCTTGACTGTG	110
PGK	Forward: TAAAGCCGAGCCAGCCAAAA Reverse: CTCCTACCATGGAGCTGTGG	116

bp – base pair, IL – interleukin, PKR – protein kinase R, PGK – phosphoglycerate kinase

for 15 min at 95°C followed by 40 cycles of 15 s at 95°C, 30 s at 54-58°C and final extension of 30 s at 72°C. The amplification signals of different samples were normalized to the PGK cycle threshold (Ct), and then the  $2^{-\Delta\Delta CT}$  method was applied for comparing the mRNA expression levels of groups against the control, normal HepG<sub>2</sub>, which was represented as fold change in data analysis.

### Statistical analysis

All experiments were performed a minimum of three times. Data were expressed as the mean  $\pm$  standard error of the mean and analyzed using one-way ANOVA or the Kruskal-Wallis test using GraphPad Prism 7.03 (GraphPad Software, Inc., La Jolla, CA, USA). Comparisons of the groups for determinations of statistical differences were done using Student's *t*-test.  $P < 0.05$  was considered to indicate a statistically significant difference between the group means.

## Results

### Cytotoxic effects of adenovirus on HepG<sub>2</sub>

In order to investigate the cytotoxic effect of adenovirus on HepG<sub>2</sub>, adenovirus of different MOIs was added into the cell culture. The light microscopy and cell staining showed that at MOI  $> 1$  a portion of cells started to detach and acquire a necrotic shape ( $> 50\%$ ),

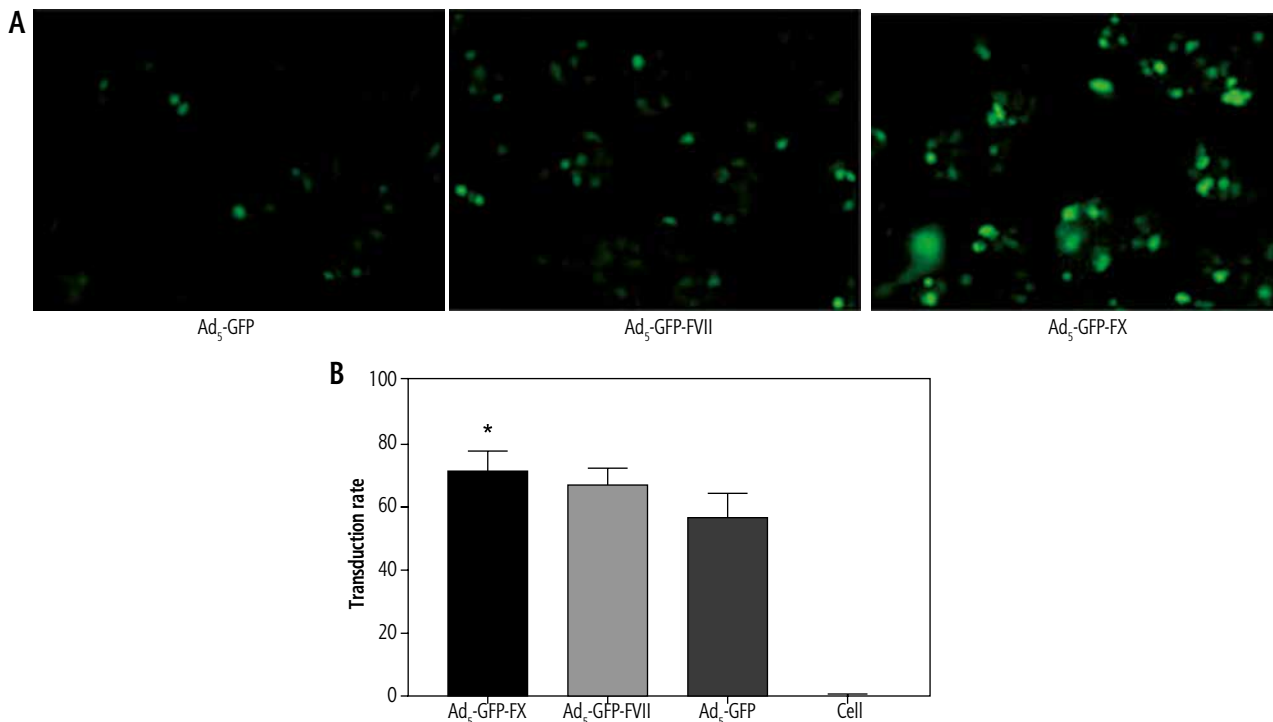
which indicated significant cytotoxicity of adenovirus in those doses. This finding also revealed that MOI = 0.5-1 as well as the incubation time of 6-12 hours could be effective as well as non-toxic for further immune response evaluation.

### Transduction assays of Ad<sub>5</sub>-GFP in Hep-G<sub>2</sub>

To assess whether loading coagulation factor VII and X on adenovector increase its transduction efficiency, fluorescent microscopy and flow cytometry were employed. Interestingly, GFP fluorescent numeration showed that different adenovector complexes could reach the nucleus, efficiently. Moreover, microscopic evaluation showed that loading with coagulation factors enhanced the transduction rate of Ad<sub>5</sub>-GFP as Ad<sub>5</sub>-GFP-FX (60%) and Ad<sub>5</sub>-GFP-FVII (50%) exhibited an increase in transduction potency ( $p < 0.05$ ) in comparison to Ad-GFP (35%) (Fig. 1A). In addition, flow cytometry results were consistent with fluorescent microscopy and indicated the superiority of FX over VII for boosting the transduction efficiency of Ad<sub>5</sub>-GFP in Hep-G<sub>2</sub> ( $p < 0.05$ ), as shown in Fig. 1B.

### Particle size and Zeta Potential

Volume-weighted distribution of AD after incubation with FVII and FX changes from unimodal to mul-



**Fig. 1.** Evaluation of vector transduction efficiency in Hep-G<sub>2</sub> cells. **A)** Fluorescent microscopy of Ad<sub>5</sub>-GFP, Ad<sub>5</sub>-GFP-FVII, and Ad<sub>5</sub>-GFP-FX expression on Hep-G<sub>2</sub> cells, **B)** Flow cytometric analysis of Ad<sub>5</sub>-GFP, Ad<sub>5</sub>-GFP-FVII, and Ad<sub>5</sub>-GFP-FX expression on Hep-G<sub>2</sub> cells; the results has described in the text

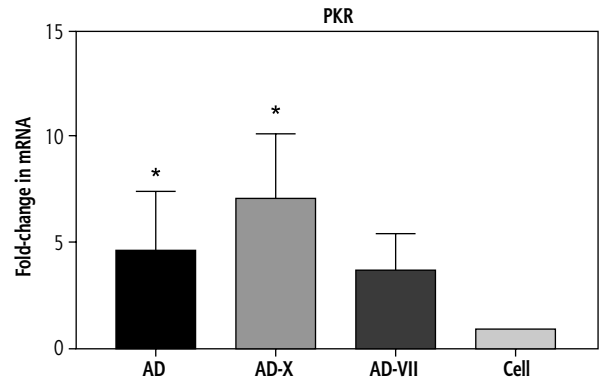
timodal pattern. Similarly, multiple peaks are observed in light intensity-weighted distribution with an increase in average hydrodynamic size following coagulation factor loading on AD. Furthermore, zeta potential of the viral particle ( $-27$  mV) undergoes subsequent changes by coagulation factors (data not shown). These findings demonstrate that loading of coagulation factors on the virus leads to an increase in the mean size and absolute zeta potential to different extents.

### Pattern of innate immune gene, PKR expression

In order to evaluate the innate immune induction, PKR was measured by qPCR. The results showed that all types of viruses were able to induce PKR gene expression. These findings demonstrated that the adenovirus-FX complex (AD-X) exhibited the most significant increase in PKR expression level ( $p = 0.0164$ ), followed by a similar level for AD ( $p = 0.0152$ ) and adenovirus-FVII (AD-VII) (4.6-, 7.1-, and 3.7-fold, respectively), when compared to the control cell (Fig. 2).

### Pattern of IL-1 $\beta$ , IL-8 and IL-18 gene expression

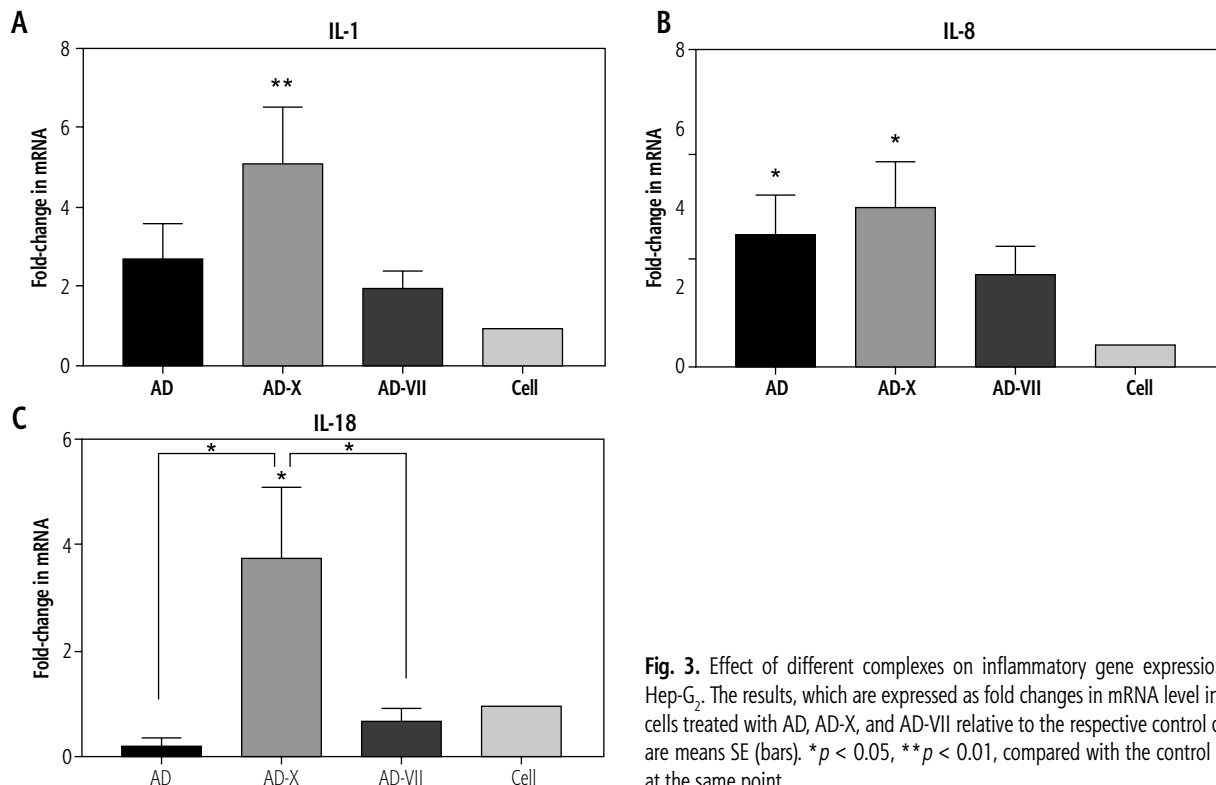
The pattern of IL-1 $\beta$  expression revealed that following treatment with AD, AD-X, and AD-VII, its level increased up to 2.7-, 5.1- and 2.0-fold compared with



**Fig. 2.** The effect of different treatments on PKR gene expression of Hep-G<sub>2</sub>. The results, which are represented as fold changes in mRNA level in the cells treated with AD, AD-X, and AD-VII relative to the control cells, are means  $\pm$  SE (bars). \* $p < 0.05$  compared with the control cells at the same point. AD – adenovirus 36, AD-X – adenovirus 36-FX, AD-VII – adenovirus 36-FVII

normal cells, respectively. These findings demonstrated that the AD-X virus complex exhibited the most significant increase in the IL-1 $\beta$  level ( $p = 0.0041$ ), followed by AD and AD-VII, when compared to the control cell. However, there was no statistically significant difference between adenovirus groups (Fig. 3A).

For IL-8, it was shown that Hep-G<sub>2</sub> infection by AD, AD-X, and AD-VII groups induced an increased expression up to 6.3-, 7.5-, and 4.3-fold when compared to the normal group, respectively. The AD-X virus complex showed a significant rise in the IL-8 level ( $p = 0.0107$ ),



**Fig. 3.** Effect of different complexes on inflammatory gene expression of Hep-G<sub>2</sub>. The results, which are expressed as fold changes in mRNA level in the cells treated with AD, AD-X, and AD-VII relative to the respective control cells, are means  $\pm$  SE (bars). \* $p < 0.05$ , \*\* $p < 0.01$ , compared with the control cells at the same point

followed by a similar level for AD ( $p = 0.0370$ ) and AD-VII ( $p > 0.05$ ). However, there was no statistically significant difference between these groups (Fig. 3B).

In contrast, IL-18 has no significant fold difference in AD and AD-VII treatment groups relative to the normal group, but instead rose up to 3.7-fold in the AD-X infection group ( $p = 0.0193$ ). In this case, AD-X showed a significant increase even when compared with other adenovirus groups including AD ( $p = 0.0360$ ) and AD-VII complex ( $p = 0.0122$ ) (Fig. 3C).

## Discussion

The systemic administration of adenovirus induces innate toxicity following viral components' sensing by different cells [4, 15]. Recent investigations suggested that coagulation factors, particularly FX, cause the virus to be targeted in the liver and infect the hepatocytes through a process that relies on the hexon interaction [6, 16]. Regarding the importance of innate toxicity, understanding the mechanisms and responsible molecules would be beneficial in finding new strategies to decrease inflammation and to reduce the burden of systemic administration [17]. In previous studies, the role of coagulation factors, especially FX, in increasing the efficiency of transduction in the epithelial and macrophage cells, as well as their effect on innate immune induction, has been suggested *in vivo* and *in vitro* [18-21]. Despite a study on the effect of FX [20], there are limited data about the role of other coagulation factors in the innate immunity process of the most important cells during systemic injection – the hepatocytes [18]. Moreover, a comparison between FVII and FX (important coagulation factors which interact with adenovirus) has not been performed yet to determine the extent of innate immune induction in the hepatocytes [6]. Herein, the immune-inflammatory response of adenovirus type 36 (AD) was compared with Ad<sub>36</sub> coated with FX (AD-X) and FVII (AD-VII) complexes on Hep-G2 cell line.

To determine the transduction rate between these groups, first we investigated the transduction efficiency of Ad<sub>5</sub>-GFP loading with FVII or FX. The GFP signal detection under fluorescent microscopy and flow cytometry showed that FX and FVII could increase the rate of entry [8]. The more detailed data of flow cytometry also showed that the FX can enhance the virus transduction rate up to 72%. A comparison of the role of FX and FVII in transduction efficiency of adenovector was reported for the first time.

It has been shown that FX simply binds to the Ad hexon via charge-dependent interaction through hy-

pervariable regions of the hexon [5]. In our study, Zetasizer evaluation demonstrated that simple mixing of the Ad<sub>36</sub> and blood coagulation factors (both FVII and FX) increased the average particle size as well as the negative zeta potential charge, demonstrating the true loading of coagulation factor on particles.

The immune gene expression results indicated that the FX enhanced the innate immunity and inflammation caused by the Ad<sub>36</sub> contrary to the FVII factor. Regarding the qPCR, the expression pattern of the PKR gene as an innate immunity marker revealed the significant role of FX. However, sole Ad<sub>36</sub> and AD-VII complex induced PKR expression in a similar pattern. It is necessary to investigate whether this result is a consequence of the virus entry into the hepatocyte or due to viral interaction with an undefined molecular sensor in the host. Similar to this study, Dronin *et al.* in 2012 showed that adenovirus coated with FX induced stronger immune inflammation when compared with the virus alone, in the mice macrophage [10]. In spite of this, another study on human macrophage and dendritic cells (DCs) showed that Ad<sub>5</sub>-FX was not capable of activating TLR/NF- $\kappa$ B-related innate immunity in the mononuclear phagocytes or induced proinflammatory cytokine [20]. In addition, adenovirus complexes with FX and FVII were observed to attach and enter into the hepatocyte with the help of heparan sulfate proteoglycan (HSP), so it seems that the entrance pathway does not influence the inflammatory response [22-25].

The expression patterns of IL-1 $\beta$ , IL-8, and IL-18 were also in accordance with the PKR result as all adenovirus treatments showed a significant degree of inflammation. Meanwhile, FX-coated virus exhibited the most significant inflammatory effect on the Hep-G<sub>2</sub> cells. In previous studies, it has been shown that adenovirus is largely capable of inducing inflammatory immunity in many cells, especially the hepatocytes [26-29]. Infection of cells by human adenoviruses/vectors induces the expression of pro-inflammatory molecules following the sensing of the capsid, dsDNA and other viral components [29]. Activation of different TLRs (Toll-like receptors) and DNA sensors that trigger downstream signaling pathways activates the NF- $\kappa$ B and IRFs (IFN-regulatory factors) and ultimately switches on the innate immune toxicity [29]. The ability of the blood coagulation factors, particularly FX, to induce or reduce the innate and inflammatory immunity has been considered by others; however, the exact role needs more investigation [2, 10, 11]. It has been shown that FX may protect the adenovirus from the complement system attachment and ameliorate the innate immunity when becoming complex with FX, while Dornin *et al.* demon-

strated an enhancing effect for FX in this purpose [10, 11]. They suggested that when FX binds to adenovirus, its structure might transform to a pathogen-associated molecular pattern (PAMP) to stimulate the innate immunity via the TLR/NF- $\kappa$ B pathway. Recently, the interaction of FX and FVII with surface glycoproteins of *Herpesviruses* has been demonstrated [30]. However, comparative reports about the innate toxicity of FVII versus FX molecules are limited.

In summary, irrespective of a similar rate of virus transduction enhancement, the results of this study for the first time suggested that coagulation factor X (FX) but not FVII has an inductive role in the adenovirus-mediated innate toxicity of hepatocytes. However, this study had some limitations including the lack of exact determination of the virus entrance steps by confocal microscopy or antagonists, absence of ELISA assay for cytokine measurement, and lack of any data about protein interactions with cell surface molecules. Another study suggests that an interaction between Ad-FX may result in a better attachment to TLR4, which will consequently enhance the systemic inflammatory response [20]. Hence, there are many factors which could be involved in this difference, but among them a closer interaction with cellular sensors seems to be more likely.

## Conclusions

In conclusion, loading FX molecules on adenovirus enhanced the innate inflammatory property of the virus when evaluated in Hep-G<sub>2</sub> cells, whereas coating with FVII alleviated this immunity. However, this finding needs more detailed investigation.

## Acknowledgments

We would like to thank Dr. Nasrin Shokrpour from the RCC department of Shiraz University of Medical Sciences for English editing of the manuscript. The manuscript was derived from the MSc thesis of Saeed Firoozi Ghahestani in the field of Virology. We are also thankful to Ali Arabi Monfared for all his assistance in the Central Research Laboratory, School of Medicine.

## Disclosure

The authors declare no conflict of interest.

## References

- Bommareddy PK, Patel A, Hossain S, et al. Talimogene laherparepvec (T-VEC) and other oncolytic viruses for the treatment of melanoma. *Am J Clin Dermatol* 2017; 18: 1-15.
- Duffy MR, Parker AL, Bradshaw AC, et al. Manipulation of adenovirus interactions with host factors for gene therapy applications. *Nanomedicine* 2012; 7: 271-288.
- Atasheva S, Yao J, Shayakhmetov DM. Innate immunity to adenovirus: Lessons from mice. *FEBS Lett* 2019; 593: 3461-3483.
- Suzuki M, Rooney CM. Adenovirus immunity: X marks the spot. *Mol Ther* 2012; 20: 2197-2198.
- Lyons M, Onion D, Green NK, et al. Adenovirus type 5 interactions with human blood cells may compromise systemic delivery. *Mol Ther* 2006; 14: 118-128.
- Irons EE, Flatt JW, Doronin K, et al. Coagulation factor binding orientation and dimerization may influence infectivity of adenovirus-coagulation factor complexes. *J Virol* 2013; 87: 9610-9619.
- Lopez-Gordo E, Denby L, Nicklin SA, et al. The importance of coagulation factors binding to adenovirus: Historical perspectives and implications for gene delivery. *Expert Opin Drug Deliv* 2014; 11: 1795-1813.
- Krutzke L, Prill JM, Engler T, et al. Substitution of blood coagulation factor X-binding to ad5 by position-specific pegylation: Preventing vector clearance and preserving infectivity. *J Control Release* 2016; 235: 379-392.
- Baker AH, Nicklin SA, Shayakhmetov DM. FX and host defense evasion tactics by adenovirus. *Mol Ther* 2013; 21: 1109-1111.
- Doronin K, Flatt JW, Di Paolo NC, et al. Coagulation factor X activates innate immunity to human species C adenovirus. *Science* 2012; 338: 795-798.
- Xu Z, Qiu Q, Tian J, et al. Coagulation factor X shields adenovirus type 5 from attack by natural antibodies and complement. *Nat Med* 2013; 19: 452-457.
- Duffy MR, Dospoly A, Turner G, et al. The relevance of coagulation factor X protection of adenoviruses in human sera. *Gene Ther* 2016; 23: 592-596.
- Hosseini SY, Sabahi F, Moazzeni SM, et al. Construction and preparation of three recombinant adenoviruses expressing truncated NS3 and core genes of hepatitis C virus for vaccine purposes. *Hepat Mon* 2012; 12: 6130.
- Petiot E, Proust A, Traversier A, et al. Influenza viruses production: Evaluation of a novel avian cell line duckcelt(r)-t17. *Vaccine* 2018; 36: 3101-3111.
- Appledorn DM, Kiang A, McBride A, et al. Wild-type adenoviruses from groups a-f evoke unique innate immune responses, of which had3 and sad23 are partially complement dependent. *Gene Ther* 2008; 15: 885-901.
- Gimenez-Alejandre M, Cascallo M, Bayo-Puxan N, et al. Coagulation factors determine tumor transduction in vivo. *Hum Gene Ther* 2008; 19: 1415-1419.
- Jonsson F and Kreppel F. Barriers to systemic application of virus-based vectors in gene therapy: Lessons from adenovirus type 5. *Virus Genes* 2017; 53: 692-699.
- Parker AL, Waddington SN, Nicol CG, et al. Multiple vitamin K-dependent coagulation zymogens promote adenovirus-mediated gene delivery to hepatocytes. *Blood* 2006; 108: 2554-2561.
- Lee BH, Kushwah R, Wu J, et al. Adenoviral vectors stimulate innate immune responses in macrophages through cross-talk with epithelial cells. *Immunol Lett* 2010; 134: 93-102.
- Eichholz K, Mennechet FJ, Kremer EJ. Human coagulation factor X-adenovirus type 5 complexes poorly stimulate an innate immune response in human mononuclear phagocytes. *J Virol* 2015; 89: 2884-2891.
- Findlay JS, Cook GP, Blair GE. Blood coagulation factor x exerts differential effects on adenovirus entry into human lymphocytes. *Viruses* 2018; 10: 20.

22. Waddington SN, Parker AL, Havenga M, et al. Targeting of adenovirus serotype 5 (Ad5) and 5/47 pseudotyped vectors in vivo: Fundamental involvement of coagulation factors and redundancy of CAR binding by Ad5. *J Virol* 2007; 81: 9568-9571.
23. Kalyuzhniy O, Di Paolo NC, Silvestry M, et al. Adenovirus serotype 5 hexon is critical for virus infection of hepatocytes in vivo. *Proc Natl Acad Sci U S A* 2008; 105: 5483-5488.
24. Zaiss AK, Lawrence R, Elashoff D, et al. Differential effects of murine and human factor X on adenovirus transduction via cell-surface heparan sulfate. *J Biol Chem* 2011; 286: 24535-24543.
25. Alba R, Bradshaw AC, Parker AL, et al. Identification of coagulation factor (F)X binding sites on the adenovirus serotype 5 hexon: Effect of mutagenesis on FX interactions and gene transfer. *Blood* 2009; 114: 965-971.
26. Muruve DA, Barnes MJ, Stillman IE, et al. Adenoviral gene therapy leads to rapid induction of multiple chemokines and acute neutrophil-dependent hepatic injury in vivo. *Hum Gene Ther* 1999; 10: 965-976.
27. Bowen GP, Borgland SL, Lam M, et al. Adenovirus vector-induced inflammation: Capsid-dependent induction of the C-C chemokine RANTES requires NF- $\kappa$ B. *Hum Gene Ther* 2002; 13: 367-379.
28. Kiang A, Hartman ZC, Everett RS, et al. Multiple innate inflammatory responses induced after systemic adenovirus vector delivery depend on a functional complement system. *Mol Ther* 2006; 14: 588-598.
29. Hartman ZC, Black EP, Amalfitano A. Adenoviral infection induces a multi-faceted innate cellular immune response that is mediated by the toll-like receptor pathway in a549 cells. *Virology* 2007; 358: 357-372.
30. Livingston JR, Sutherland MR, Friedman HM, et al. Herpes simplex virus type 1-encoded glycoprotein C contributes to direct coagulation factor X-virus binding. *Biochem J* 2006; 393: 529-535.