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Original Article

Adenovector Mediated Inflammation in Mice Liver Boosted by Hydrodynamic Injection

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Abstract

Background and Aims: Adenovector gene transfer induces inflammatory response that finally leads to vector removal from delivered site. The effect of hydrodynamic pre-injection on Adenovector mediated liver inflammation remained elusive.

Materials and Methods: Different mice groups were pre-treated by hydrodynamic saline, dexamethasone or nothing prior to Adeno-Luciferase administration. Their samples were collected in 18 hours later and serum IL-1β level as a marker of inflammation was quantified. Also, liver histological assessment was performed to score the inflammatory grade.

Results and Conclusion: In spite of dexamethasone group, both hydrodynamic/Ad-Luc and Ad-Luc receiving groups induced the expression of IL-1β cytokine ($p<0.05$). The pathological views also indicated more inflammation and necrosis inside the different zones of liver tissue in these two adenovector injected groups. These results emphasized that Adenovector served as a suitable inflammatory model and hydrodynamic injection enhances this kind of inflammation.

Keywords: Hydrodynamic injection; Adenoviral vector; Inflammation

Introduction

Adenovirus is the most widely used vector for gene therapy in all over the world. However, main drawback for it's therapeutic applications is the induction of innate and inflammatory responses (1). All parts of the virus and related products such as non-coding small RNAs, capsid, genomic DNA and fiber protein activate different extra and intracellular sensing pathways that results in viral interferon and inflammatory cytokines production (2, 3). Consequently, the vectors will eliminate from the host and transgene expression reduces as well.

Hydrodynamic injection (HI) as a new physical method of gene delivery into human organs and tissues is proposed recently (4-7). It has been considered to improve the specificity of gene transfer to particular tissue in human being and thus would enhance the safety (7). Nevertheless, it was frequently employed to deliver genes into mice liver from past (8-10). Hydrodynamic injection leads to several times more transgene expression in liver than other non-viral delivery methods (11). The electron microscopy indicated that following saline hydrodynamic injection, delivery of different size molecules was improved into liver. Also, it has been demonstrated that HI protocol enhances the inflammation and leads to some

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liver cells death (11). However, it has been shown that with an unknown mechanism this method can cause the resumption of gene expression by previously injected plasmid vectors (11).

Theoretically, HI mediated targeting of adenovector to specific organ seems appropriate as an option. Nonetheless, the biological effect of HI injection on the adenovector mediated inflammation in liver has been studied rarely. Recently, HI injection of helper-dependent adenovector showed an increase in hepatic but not splenic transduction compared to systemic IV injection and induced less inflammation (12). Liver has been noted as a major site of adenovirus expression after systemic administration (13). So, it can be a suitable place to assess the biological effect of HI injection on the maintenance of adenovector and relevant inflammation.

Herein, to investigate the liver inflammation by Adenovector and its consequence when followed by HI injection, recombinant Ad-luciferase injected 24 hours after HI injection and then the extent of liver inflammation were evaluated. This study will be helpful to understand the inflammatory process during gene transfer by adenovector, hydrodynamic injection or their combination.

Methods

Materials

Recombinant Adenovector expressing luciferase, Ad-luc was gift from Dr. Gloria González Aseguinolaza (CIMA Research Center, Spain). The virus was extracted by two cesium chloride gradients and virus particle count was performed by simple spectrophotometry of viral DNA genome. Inbred BALB/c female mouse with 6 weeks age were purchased from the Animal facility House of Shiraz University of Medical Sciences. For IL-1β measurement ELISA kits was purchased from eBioscience company (USA).

Groups of test and study design

During animal experiments, approved ethical protocols of animal care by Shiraz University of Medical Sciences were taken into consideration profoundly. To evaluate the effect of hydrodynamic injection on adenovirus vectors inflammtion, matched mice were randomly divided into 3 groups. First group of mice received dexamethasone 5mg/kg 3 hours before and 24 hours after Ad-luc injection intraperitoneal, as control group. Second test group received HI injection 24 hours before Ad-luc injection and third group received just Ad-luc by IV administration. For HI injection, 2 mL of saline buffer which was equivalent to 10% of the animal's body weight introduced via tail vein with 1ml/ 4 second speed. Around 10^{10} Ad-luc particles were also diluted in 200 µl saline buffer and then injected systemically next day.

IL-1β expression Evaluation

For evaluating the IL-1β serum level in treated mice, ELISA assay was performed. After anesthesia via ether inhalation, blood was collected from heart and the sera was prepared by centrifugation at 3500 rpm then stored at -80°C until ELISA test. Mouse IL-1β platinum ELISA kits (EBioscience Company) was employed for this purpose. The ELISA method was performed according to company protocols and in final step, the plate was read at 450 nm wavelength by ELISA reader.

Liver histopathology

To investigate the level of inflammation, the liver samples were fixed in 10% formalin and then sections were stained by traditional Hematoxillin-Eosin (H&E) method. The tissue slides evaluated thoroughly, while comparing with negative controls. Mononuclear cell recruitments and parenchymal/periportal inflammation beside necrosis were considered during slide observation under light microscopy. The degree of liver inflammation was ranked by modified knodell method.

Statistical Methods:

In our analysis, for parametric tests mean+/-SD of each group was compared together by one-way Annova method then for checking pair's analysis tukey posttest employed. Also for histological test analysis kruskal-wallis non-parametric method was used.

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Results

HI procedure enhanced the level of serum IL-1β

The results of the ELISA test showed that HI injection prior to Ad-luc administration (Hydro/Ad-luc) leads to highest expression level of pro-inflammatory IL-1β cytokine comparing to other groups (Fig. 1). The expression level in this group was roughly twice as much as group received Ad-luc alone (Ad-Luc) and also was significantly higher than those group received dexamethasone before virus injection, Dexa/Ad-Luc group ($p \leq 0.001$). These findings emphasized the inflammatory role of HI injection before Ad-luc administration since serum IL-1β is considered as a pro-inflammatory cytokine. Furthermore, the results were in consistent with vector persistent test results.

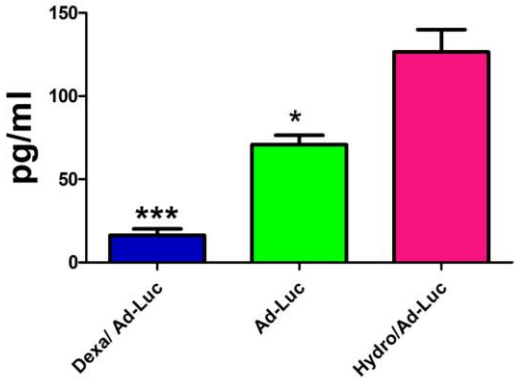

Fig. 1. The results of the expression level for IL-1β by ELISA. The bars are representing the mean±SD of ELISA results from at least 3 mice per group. The level of IL-1β in mice receiving HI procedure (HI/Ad-Luc) showed 2 times elevation compared to the group which received Ad-luc alone (Ad-Luc) and was significantly higher than those group pre-treated with dexamethasone before virus injection, Dexa/Ad-Luc group ($p \leq 0.001$).

Liver histopathology results

The primary results of liver histology indicated inflammation following Adenovector injection. These finding also showed that liver inflammation was accelerated by HI administration. In sum, histology finding displayed significant inflammation in the two groups receiving Ad-luc and HI injection (HI/Ad-luc) compared to dexamethasone received group ($p<0.01$) as shown in figure 2A. Tissue inflammation scored by modified Knodel method, resulted in average of inflammation grade in Hydro/Ad-Luc, Ad-Luc and Dexa/Ad-Luc groups as 4.5, 2.5 and less than 1, respectively. More detailed histology largely confirm that HI/Ad-luc and Ad-luc treatment caused significant infiltration of mononuclear cells in parenchymal and draining vessels as depicted in figure 2B and 2C. Mild necrosis of HI/Ad-luc group assessed by liver histopathology, indicated the more damage of mentioned group comparing other group. Dexa/Ad-luc group showed fine inflammation and no sign of necrosis under light microscopy.

Discussion

All generations of Adenovector are able to induce host innate immunity strongly by wide range of molecular pathways that ultimately eliminate vector harboring cells (2, 3). It supposed that Adenovector injection served as a reliable viral-induced inflammatory model in mice. Hydrodynamic injection (HI) is at the beginning of human use so, further studies are demanding (14). Herein, we tried to investigate the role of HI protocol on Adenovector inflammation in liver tissue.

At the beginning Liu and colleagues offered a novel plasmid transfer method into liver in vivo by rapid administration of high volume of diluent (15). HI due to forced permeability of surrounding endothelial cells aided in transfer of wide range of molecules albeit extensive inflammation and destruction of liver tissue is unavoidable (16, 17). However, previous studies reported the contradictory results of increasing or decreasing the inflammation degree by hydrodynamic tail injection repeatedly (17). Naomi et al. investigated the roles of pro-inflammatory cytokines and ROS elements in the reactivation of silenced transgene expression produced by a hydrodynamic injection of saline (11). Hydrodynamic injection significantly enhances the adenoviruses transduction efficiency in mouse liver due to the enhanced permeability of liver endothelium (18, 19). Recently, HI

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injection of helper-dependent Adenovector showed the increment of hepatic but reduced splenic transduction which consequently alleviates the inflammation comparing to systemic IV injection (12). On the other hand, while HI procedure in mice resulted in high ALT level and extensive hepatocyte necrosis, inflammatory cytokines and liver damage reduced following lower volume of HI saline treatment (17). It was shown that compared to conventional IV method this method of administration will cause further maintenance of Adeonovector and less immune inflammation (4). By our knowledge, the biological effects of pretreatment by HI injection on Adenovector mediated inflammation have not been studied yet.

Therefore, three experimental mice groups were selected to receive dexamethasone, HI procedure and nothing before administration of Adneovector. The results indicated the least inflammation for dexamethasone received group. Analysis of IL-1β inflammatory cytokine significantly showed that the expression level in Ad-luc alone group was significantly lower than group pre-dosed by hydrodynamic injection ($p<0.05$). This finding emphasized the induced inflammation by HI treatment as IL-1β level elevated near 2 times when compared with Ad-luc group. This data also revealed the suppressive role of dexamethasone injection on controlling the Adenovector inflammation as it was previously described by others (20).

Direct histological studies largely confirm also this finding. This data showed that both vector and HI treatment induce detectable inflammation even in the presence of dexamethasone. This result demonstrated that HI pre-dose induce the highest degree of inflammation (score 4.5) when compared to 2 other groups. Lymphocytic-mononuclear infiltration in this group was evenly distributed in liver parenchyma and preportal area beside mild necrosis. The results also revealed fine necrosis in Ad-luc group as sign of liver damage. In Ad-luc treated group, inflammation and small degrees of necrosis were scattered in liver tissue in comparison to evenly distributed inflammation of HI/Ad-luc. Conclusively,

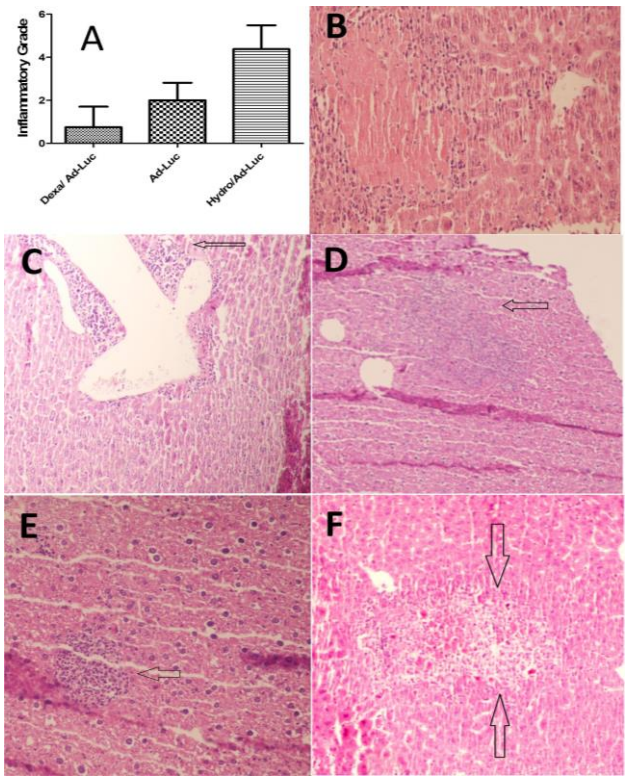

Fig. 2. The results of liver histopathology showed the signs of inflammatory responses (H&E×250). By Knodell modified method, the inflammation score of Hydro/Ad-luc group was shown to be significantly higher than other groups (A). Sections of liver showed intact architecture and nearly normal view for Dexa/Ad-luc treated group, but finely impaired architecture demonstrated in HI treated group. In Hydro/Ad-luc group distributed infiltration of mononuclear cells in parenchymal (B) and periportal (C) and scattered necrosis (D) were observed. For Ad-luc treated group less infiltration of mononuclear cells in parenchymal (E) and fine necrosis (F) was detected as shown by empty arrows.

Adenovector induced mild inflammation in mice liver and pre-dosing of liver with HI procedure boosted Adenovector mediated inflammtion in gene therapy approaches and seems to be contraindicative for human application.

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References

1. Crettaz J, Berraondo P, Mauleon I, Ochoa-Callejero L, Shankar V, Barajas M, et al. Intrahepatic injection of adenovirus reduces inflammation and increases gene transfer and therapeutic effect in mice. Hepatology (Baltimore, Md). 2006;44(3):623-32.
2. Fejer G, Freudenberg M, Greber UF, Gyory I. Adenovirus-triggered innate signalling pathways. European journal of microbiology & immunology. 2011;1(4):279-88.
3. Thaci B, Ulasov IV, Wainwright DA, Lesniak MS. The challenge for gene therapy: innate immune response to adenoviruses. Oncotarget. 2011;2(3):113-21.
4. Brunetti-Pierri N, Ng T, Iannitti DA, Palmer DJ, Beaudet AL, Finegold MJ, et al. Improved hepatic transduction, reduced systemic vector dissemination, and long-term transgene expression by delivering helper-dependent adenoviral vectors into the surgically isolated liver of nonhuman primates. Human gene therapy. 2006;17(4):391-404.
5. Suda T, Liu D. Hydrodynamic gene delivery: its principles and applications. Molecular therapy : the journal of the American Society of Gene Therapy. 2007;15(12):2063-9.
6. Herrero MJ, Sabater L, Guenechea G, Sendra L, Montilla AI, Abargues R, et al. DNA delivery to 'ex vivo' human liver segments. Gene therapy. 2012;19(5):504-12.
7. Khorsandi SE, Bachellier P, Weber JC, Greget M, Jaeck D, Zacharoulis D, et al. Minimally invasive and selective hydrodynamic gene therapy of liver segments in the pig and human. Cancer gene therapy. 2008;15(4):225-30.
8. Fukunaga S, Kanda G, Tanase J, Harashima H, Ohyama T, Kamiya H. A designed curved DNA sequence remarkably enhances transgene expression from plasmid DNA in mouse liver. Gene therapy. 2012;19(8):828-35.
9. Rodriguez-Madoz JR, Zabala M, Alfaro M, Prieto J, Kramer MG, Smerdou C. Short-term intratumoral interleukin-12 expressed from an alphaviral vector is sufficient to induce an efficient antitumoral response against spontaneous hepatocellular carcinomas. Human gene therapy. 2014;25(2):132-43.
10. Yan S, Fu Q, Zhou Y, Wang J, Liu Y, Duan X, et al. High levels of gene expression in the hepatocytes of adult mice, neonatal mice and tree shrews via retro-orbital sinus hydrodynamic injections of naked plasmid DNA. Journal of controlled release : official journal of the Controlled Release Society. 2012;161(3):763-71.
11. Takiguchi N, Takahashi Y, Nishikawa M, Matsui Y, Fukuhara Y, Oushiki D, et al. Positive correlation between the generation of reactive oxygen species and activation/reactivation of transgene expression after hydrodynamic injections into mice. Pharmaceutical research. 2011;28(4):702-11.
12. Brunetti-Pierri N, Stapleton GE, Palmer DJ, Zuo Y, Mane VP, Finegold MJ, et al. Pseudo-hydrodynamic delivery of helper-dependent adenoviral vectors into non-human primates for liver-directed gene therapy. Molecular therapy : the journal of the American Society of Gene Therapy. 2007;15(4):732-40.
13. Shayakhmetov DM, Li ZY, Ni S, Lieber A. Analysis of adenovirus sequestration in the liver, transduction of hepatic cells, and innate toxicity after injection of fiber-modified vectors. Journal of virology. 2004;78(10):5368-81.
14. Bonamassa B, Hai L, Liu D. Hydrodynamic Gene Delivery and Its Applications in Pharmaceutical Research. Pharmaceutical research. 2011;28(4):694-701.
15. Liu F, Song Y, Liu D. Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. Gene therapy. 1999;6(7):1258-66.
16. Herweijer H, Wolff JA. Gene therapy progress and prospects: hydrodynamic gene delivery. Gene therapy. 2007;14(2):99-107.
17. Racz Z, Godo M, Revesz C, Hamar P. Immune activation and target organ damage are consequences of hydrodynamic treatment but not delivery of naked siRNAs in mice. Nucleic acid therapeutics. 2011;21(3):215-24.
18. Brunetti-Pierri N, Palmer DJ, Mane V, Finegold M, Beaudet AL, Ng P. Increased hepatic transduction with reduced systemic dissemination and proinflammatory cytokines following hydrodynamic injection of helper-dependent adenoviral vectors. Molecular therapy : the journal of the American Society of Gene Therapy. 2005;12(1):99-106.
19. Suda T, Gao X, Stolz DB, Liu D. Structural impact of hydrodynamic injection on mouse liver. Gene therapy. 2007;14(2):129-37.
20. Seregin SS, Appledorn DM. McBride AJ. Transient pretreatment with glucocorticoid ablates innate toxicity of systemically delivered adenoviral vectors without reducing efficacy. Molecular Therapy. 2009;4:685-94.

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