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Research Article

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Plasmid mediated kallistain gene expression via intramuscular electroporation for treating acute ulcerative colitis in mice

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ABSTRACT

Ulcerative colitis (UC) is a kind of the intestinal immune system disorder disease, the exact pathogenesis of UC remains undetermined. Current therapeutic strategies for treating UC mainly relied on nonspecific immune-suppressive therapies. Thus, new therapies are highlight to develop. We have investigated the effects of a kallistatin (Kal)-encoding plasmid in mice acute ulcerative colitis (AUC) by intramuscular electroporation. The model of mice AUC was induced by giving 4% dextran sulfate sodium (DSS) solutions as drinking water 6 days, which was evidenced by Disease activity index (DAI) estimation. Plasmid DNAs were electrotransfered into skeletal muscle, with the best electroporation conditions. Our results suggested that Kal was sufficiently expressed after injection of naked plasmid by intramuscular electroporation. In the Kal gene-transferred group, the colonic myeloperoxidase (MPO) activity, malondialdehyde level and TNF-α content were declined , the superoxide dismutase(SOD)activity was enhanced, and all had significant differences ($p < 0.05$) compared to those in non-Kal-treated group. The electrotransfed Kal plasmid can express transgene effectively in vivo and this might be a promising approach for treating UC.

Keywords: Kallistatin, electroporation, ulcerative colitis, gene therapy

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INTRODUCTION

Ulcerative colitis (UC), which is an idiopathic, chronic, non-specific intestinal inflammatory disorder resultant from a dysfunctional epithelial, innate and adaptive immune response to intestinal microorganisms in gastrointestinal tract [1, 2].UC is thought to be a combination of multifactorial, polygenic and environmental factors disease, and the prevalence of UC is continuously increasing worldwide [3].However, the exact pathogenesis of UC remains undetermined [4, 5]. Till now, there are no ideal therapies on UC, seeking effective therapeutic method on UC has important clinic significance.

Kallistain (Kal) is a member of the serine protease inhibitor (serpin) family, and play an important role in anti-inflammatory [6], antioxidant [7],anti-fibrosis [8],anti- tumor growth [9].Thus, Kal is a candidate anti-inflammatory drug for treatment of UC, tissue injury. However, the molecular mechanism by which Kal modulates inflammation has not been determined. Muscle electroporation has recently become a promising tool for efficient delivery of plasmids and transgene expression in vivo. This technology has been mainly applied to use of muscle as a bioreactor for production of therapeutic proteins [10]. In vivo electroporation has a variety of advantages over viral vectors as: any types of cells and tissues could become a target, handling is easy and quickly done within a matter of second, repeated administration of DNA is possible, no immunogenicity is expected, and there is no constraints on amounts and sizes of DNA to be used. Gene transfer efficiency of electroporation was found even superior to that of in vivo direct DNA injection methods.

Our previous studies have shown that electroporation associated muscle damage was minimal [11].In this study, we

investigated the therapeutic effect of pKal in DSS induced mice AUC by intramuscular electroporation.

EXPERIMENTAL SECTION

Materials: DNA Delivery Device was purchased from TERESA Healthcare Sci-Tech Company (Shanghai, China); Kallistatin and TNF-α ELISA kits were purchased from R&D Systems (Minneapolis, America). Myeloperoxidase (MPO), malondialdehyde (MDA) and superoxide dismutase（SOD）kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).DSS was purchased from MP Biomedicals (California, America), Salazosulfapridine(SASP)was purchased from Shanghai Xinyijiahua Pharmaceutical (Shanghai, China).

Plasmid DNA: The expression plasmid pEGFP under the control of the cytomegalovirus enhancer/chicken β-actin promoter, and the pKal under the control of the same promoter and driving a coding sequence for the human Kal, were constructed as reported previously [12] and kept in our laboratory. Plasmids were purified with the EndoFree plasmid Giga kit in accordance with the protocol. DNA was dissolved in Endofree TE buffer and kept frozen at a concentration of 1 mg/mL.

Induction of AUC: 6-8 wk old male BALB/c mice were obtained from the Slaccas Company (Shanghai, China). The model of mice AUC was induced by by oral administration of 4% DSS solutions in drinking water 6 days as Cooper *et al* described previously [13].All animal experiments were approved by Animal Ethics committee of Huaqiao University.

Experimental design: Thirty mice (Normal, pEGFP and pKal groups, each group containing ten mice) were used to verify the plasmid-mediated Kal expression by intramuscular electroporation. Then, fifty mice were divided into five groups: Normal, Model, pEGFP, pKal and SASP groups.

Group Normal (n = 10) was the control group and no procedures were carried out.
Group Model (n = 10) was subjected to DSS without any treatment.
Group pEGFP (n = 10) was treated with pEGFP by intramuscular electroporation before giving DSS solution.
Group pKal (n = 10) was treated with p Kal by intramuscular electroporation before giving DSS solution.
Group SASP (n = 10) was injected with SASP 320 mg/kg/day by intragastric administration from modeling. SASP group was set as positive control.

In vivo electroporation: The mice were anesthetized by intraperitoneal injection of pentobarbital sodium at a dose of 30 mg/kg body weight. The plasmid DNA was injected into mice skeletal muscle using the TERESA DNA delivery device, with the best electroporation condition of 60 V voltage, 10 ms pulse width, 12 pulse times, the longitudinal electric field direction, plasmid concentration of 1.0 mg/ml, dose volume of 100 μl [14].

Disease activity index (DAI) estimation: The DAI was estimated and recorded daily according to a classic scoring system by Cooper et al [13] in the process of modelling: body weight loss (0, none; 1, 1–5%; 2, 5–10%;3,10–20%; 4,> 20%), stool consistency (0, normal; 2, loose stool; 4, diarrhoea) and stool blood (0, negative; 2, foecal occult blood test positive; 4, gross bleeding). The scoring parameters were added, resulting in a total DAI ranging from 0 to 12. The DAI values can verify whether the modeling is success.

Histopathologic examinations: The length of colons were measured to observe the hispathological changes .After measuring the length and weight of the entire colon, it was cut longitudinally and fixed in 10% formaldehyde, embedded in paraffin, cut into 5 μm sections, stained with hematoxylin and eosin (H&E) for general morphology analysis.

Measurement of colonic MPO, SOD and MDA: The colons also homogenated for measurement of MPO, SOD activity and MDA levels. The MPO activity was determined as described by Ceyran et al [15], and MPO activity was expressed as U/g tissue. The SOD contents were measured according to the inhibition of nitroblue tetrazolium (NBT) reduction with xanthine-xanthine oxidase used as a superoxide generator. The calculated SOD activity was expressed as U/mg protein. The concentration of MDA was quantified by thiobarbituric acid method [16]. MDA results were expressed as nmol/mg protein in the homogenate.

Enzyme-linked immunosorbent assay (ELISA): The expression level of Kal protein in the serum was measured every two days using Kal- ELISA kit; After mice were sacrificed, the colons were homogenated and assayed for TNF-α concentration by ELISA using an ELISA kit.All ELISA experiments were performed according with manufacturer's instructions.

Statistical analysis: The results were presented as mean ± SD. The data were subjected to Student's t test, $p<0.05$ was considered statistically significant.

RESULTS

Transgene expression: After the plasmids were electrotransfed into mice, the expression of Kal protein in the serum of mice was verified every two days, pKal can express transgene effectively in vivo,and reach peak at day 8 (Fig.1).

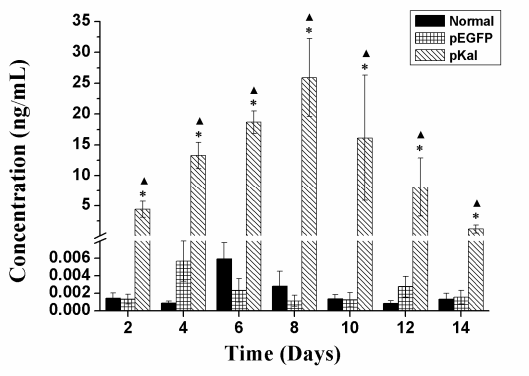

Fig.1: The expression of Kal in vivo by intramuscular electroporation. BALB/c mice were given pEGFP, pKal, respectively
*The values shown are mean ±SD, n =10.（*p<0.05 vs Normal group, ▲p<0.05 vs pEGFP group）*

Histopathologic changes: The DSS can led to obvious colitic signs in the mice, such as diarrhoea, weight loss and bloody stools, with the DAI values increased. Intramuscular electrotransfed with pKal significantly attenuated the DSS-induced colitic signs and the DAI values (Fig.2A).

As shown in Fig.2B, the decreased colon length caused by DSS was abrogated by treating with pKal, and the changes were statistically significant. The pathological section showed that DSS administration resulted in extensive colonic lesions, including vanished crypts, submucosal oedema, severe ulceration, destruction of epithelial integrity in the Model and pEGFP groups, Consistent with the DAI scores, SASP and pKal treatment exhibited an evidently attenuated morphologic damage in DSS-induced AUC mice (Fig.2C).

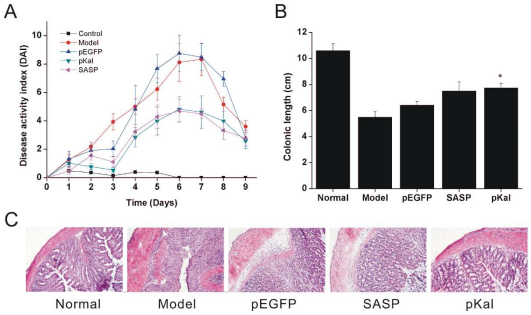

Fig.2: Histopathologic assessment of colon at day 9 in Normal, Model,pEGFP, pKal,SASP groups.(A) Changes in the DAI values.The mice treated with pKal or SASP significant lower DAI compared to Model or those treated with pEGFP.(B) The measurement of colonic length in each group at day 9.(*p<0.05 *vs* Model group).(C) Histology (H&E staining; magnification ×100) of colonic samples taken at day 9

MPO, MDA and SOD assay in colonic tissue:MPO activity was measured to indicate the severity of colonic inflammation, MDA is an indicator of lipid peroxidation in vivo. The DSS oral administration significantly descended colonic MPO (Fig.3A) and MDA (Fig.3C) activity in the pKal group when compared with pEGFP or Model group ($p<0.05$).SOD is a metalloenzyme that catalyze the conversion of the superoxide anion into hydrogen peroxide and dioxygen. Compared to pEGFP or Model group, colonic SOD increased significantly (Fig.3B, $p<0.05$). Thus, pKal treatment decreased MPO activity and MDA levels and significantly increased SOD activity in the colonic tissue.

TNF-α in colonic tissue: To examine the effect of pKal on the colonic expression of proinflammatory cytokines induced by the administration of DSS, TNF-α present in the homogenates of colonic tissue were quantified using ELISA. The increased levels of TNF-α in colonic tissue of mice was significantly suppressed in pKal and SASP

group compared with pEGFP or Model group (Fig.3D, p<0.05).This indicated that the pKal-mediated suppression of DSS-induced colitis appeared to be derived from the down-regulation of TNF-α.

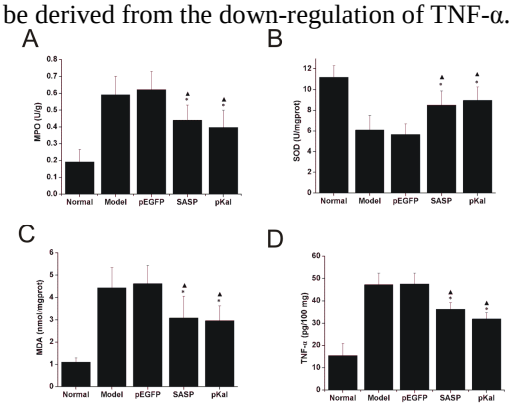

Fig. 3: Measurement of MPO,SOD,MDA and TNF-α.(A)Measurement of colonic MPO.(B) Measurement of colonic SOD C)Measurement of colonic MDA content.(D)Measurement of colonic TNF-α. (*p<0.05 *vs* Normal group, ▲p＜0.05 *vs* pEGFP group)

DISCUSSION

As the exact etiology of UC is still unclear, DSS-induced colitis was used to study many important aspects of the pathogenesis in UC. In the present study, we demonstrated that electroporation mediated Kal plasmid could express transgene efficiently in vivo and significantly attenuates DSS-induced AUC by down-regulating the expression of TNF-α.

Our results identify the therapeutic role of Kal in AUC, thereby this approach will facilitate the further clinical application for treatment of inflammatory disease.

Acknowledgments
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