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Immunological safety assessment of intravenous injection of rat embryonic proteome extract for in vivo regenerative therapies

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ABSTRACT

The branch of regenerative medicine has been advancing in recent years, thanks to milestone discoveries in stem cell biology and tissue engineering; now driven paradigm shifts in the embryonic molecular therapy of traumatic injuries, organ failure, cancer, degenerative, and ischemic diseases. Mother's womb is the manufacturing unit of all tissues and organs, and thus embryo is a plethora of tissue differentiation, organ generation, and growth factors. Embryonic molecules of the embryo mediates significant in vivo replenishment of lost and/or dysfunctional tissue and organs. Simultaneously, it is advancing by overcoming major limitations like tumorigenecity, heterogeneity, and immunogenicity. Present study was focused on immune response through intravenous injection of 14th, 16th and 19th day embryonic proteome extract.

Methods: Isolation of embryonic proteome for 14th, 16th, and 19th embryonic days of 3 SD rats were injected for the assessment of serum CRP and IL-6

Results: EPE injected rats were compared with control. Both CRP and IL-6 has shown no significant difference between the control and treated and were found within normal limits ($p < 0.05$). In conclusion, present study has confirmed that embryonic proteome extract are safe without immunological rejections and thus can be used for the in vivo regenerative therapies.

Keywords: Embryonic Proteome Extract (EPE),tumorigenecity, heterogeneity, immunogenicity, intravenous injection, Sprague Dawley Rats (SD)

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INTRODUCTION

In recent years, there has been a relentless advancement in regenerative medicine, ensuring reliable treatment for diseases such as age-associated pathologies, injuries, cancer, cardiovascular diseases and other chronic organ failures [1-3]. Among all these diseases, cancer is one of the leading causes of illness and mortality around the globe [4]. Cancer cells may lack the distinct molecular systems that embryos have for immune evasion, cell differentiation, migration, invasion, gene expression, protein profiles, and other processes. Studies done in the past compared the characteristics of cancer and embryos and looked into how they might be used therapeutically. Over the past 20 years, there has been a significant increase in scientific knowledge and literature exploring the parallels between cancer and embryos [5]. Simultaneously, as shown in Figure 1, the embryonic molecular cascade provides a reliable therapeutic tool for a wide range of diseases [6].

During the early phases of embryonic development, many similarities and disparities are shown between embryogenesis and carcinogenesis. Both embryonic and cancer cells have infinite proliferation potency, with the disparity that embryonic cells proliferate under stringent regulatory factors whereas tumour cells lack such factors. By comparing organized cell cycle of embryo and disorganised cell cycle of cancer cells may serve as benchmarks during the discovery of new cancer drugs, we can control cancerous cell growth [7].

On the other hand, immunogenicity, tumorigenicity, and heterogeneity are three disadvantages of stem cell therapy. The regenerative field will reach a great milestone if we can get beyond these challenges and regenerate every organ in an organism. To that end, the current study was designed to overcome these constraints. Immunogenicity refers to immune cells activates by producing antibodies in response to any protein entry, transplanted cells tissues and organs, so that any foreign object should be eliminated, the same mechanism working in elimination of transplanted tissues organs and stem cells [8]. Tumorigenicity

refers to uncontrolled cell division. Because embryonic cells have an infinite proliferation capacity when injected directly, these cells may develop into tumours or teratomas, which is a major disadvantage of embryonic stem cells [9]. Heterogeneity in which a single pluripotent cell generate multiple cell types and also with HLA typing variation with paternal or maternal origin.

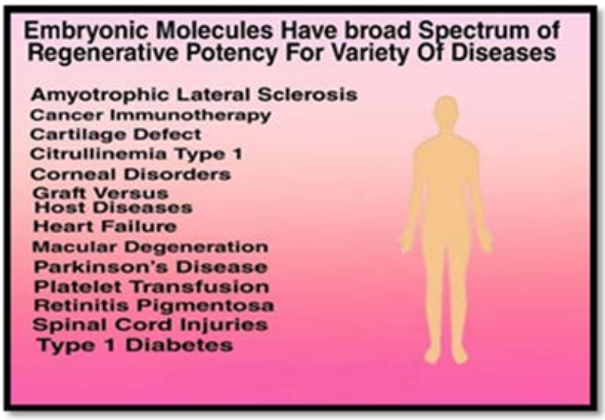

Fig. 1. Embryonic proteins and their utility in the treatment of a variety of diseases

Heterogeneity is a phenomenon in which all tissues and organs will develop from a single cell and thus final fate of a cell is not unique and cannot be manipulated [10]. While this study is going to ratify three major advantages apart from regenerative potency, in the usage embryonic proteins: non-tumorgenicity, non-heterogeneity and non-immunogenicity. First two limitations are tumorigenicity and heterogeneity are totally cellular characteristics, so total embryonic cell structure can be decellurized by homogenization, so that these two barriers can be resolved [11, 12]. Now the immunogenicity of embryonic proteins has to be studied to confirm that embryonic proteins are non-immunogenic.

Many studies have proven the in vitro organogenesis, which is nothing but a regenerative function. In vivo regenerative therapy, safety evaluation for EPE is required, as embryo is a universal source of regenerative and rejuvenate molecules. The total embryonic proteome is included in this study, so that future safety studies is not to be repeated as our confirmed the total embryonic proteome safety and future in vivo regenerative therapies can be started directly. Rat tissue and organogenesis occur at different days during gestation. The early organogenesis period of rat embryos is between 9.5 and 11.5 days of gestation [13]. The late organogenesis period is between the 14th and 19th embryonic days, where various important growth factors can be isolated [14].

MATERIALS AND METHODS

In the present Immunological potency has been determined, by isolating SD rat's Embryonic Total Proteins (ETP) of the 14th, 16th, and 19th days were injected intravenously. EPE and its self-antigen recognition was determined through serum immunological markers CRP and IL6.

SD Rats segregation

A single pair of Sprague Dawley rats was housed on a 12:12 h light cycle, with free access to food and water. According to the recommendations of the animal ethics commission, every animal was kept in an isothermic habitat. Six pregnant rats underwent examination based on visible vaginal plug formation and the cytology of vaginal smear microscopy in order to ensure biological replication [15].

In order to create the first filial generation of six female and five male rats, this initial set of one female and one male was let to mate. Again, these five female and five male rats was for them to mate and produce their second filial generation. Five females are allowed for mating in the second filial generation. Three pregnant rats were allocated for scarification of isolated embryos on the 14th, 16th, and 19th days for EPE preparation. Proteins in this latte organogenesis can be used for safety studies and regenerative purposes. The remaining two rats were meant for the full gestational period, and these two rats were given birth to 20 offspring. All these rats were used in further experimental studies, including immunogenicity and represented in Figure 2.

Embryonic protein extract preparation and quantification

On 14, 16, and 19 days of the embryonic stage, thirty embryos were separated from three pregnant rats. Proteolytic enzyme activity was inhibited by removing an embryo every day and cleansing it six times with 10x PBS buffer, and subsequently, ultrasonicator homegenization was performed with 0.85 mg/ml of EDTA. Extracted proteins were used for in vivo injections, and the extraction procedure was carried out in a cold-chain environment at 4 degrees Celsius. Non-homogenised embryos are stored at -80°C, and protein quantification was done using a nanodrop spectrophotometer [16].

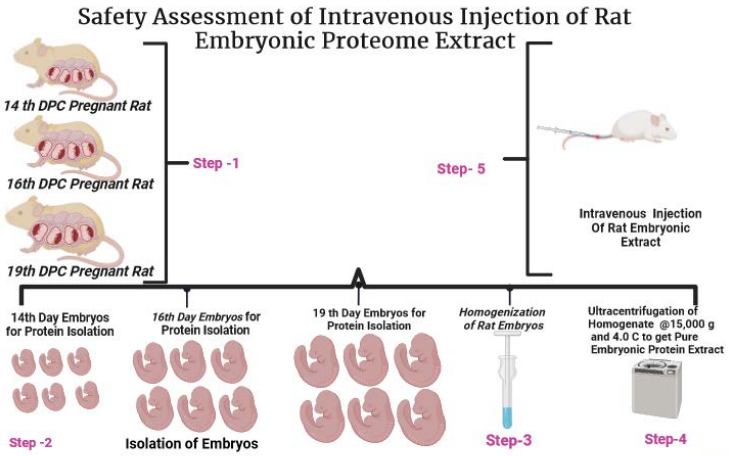

Fig. 2. Schematic flow of the work, 1) Pregnant Rats 2) Isolated embryos from pregnant rats, 3) Homogenization of embryos, 4) Ultracentrifugation of embryos, 5) Intravenous EPE Injection Rat

Injection of embryonic extract through intravenous route

Several in vitro investigations have validated the biological roles of several embryonic components. Confirming in vivo regeneration function and safety need more research [17-20]. In order to assess the safety in vivo, we extracted embryonic protein and injected them intravenously in the current work, which is cantered on safety confirmation [18]. 12-13 week old adult rats were split into two groups, with six rats each assigned to the control and treatment groups for the injection of EPE. Rats in the treatment group received an injection of 120µL of EPE extract, which was made from the 14th, 16th, and 19th days of embryogenesis [19]. Concentration of proteins EPE are 0.724 mg/ml, 1.548 mg/ml, and 2.532 mgml, respectively. After intravenous injection CRP, IL6, levels were estimated by ELISA method (Elabscience kits).

RESULTS

Pro-inflammatory markers like CRP and IL6 were quantified both in control and EPE treated groups after 48 hours. It is evident that IV EPE injected 14th, 16th and 19th SD rats has shown the serum levels of CRP as 156.48, 157.5 and 164.07 with an average SEM values of 3.22 which are in the normal range. With an aver-age of 18% to 19% of CRP variation has been shown on compari-son with the control (198.5 ± 11.5).

Similarly, IL-6 levels IV EPE injected rats are 17.1 ± 0.38, 16.78 ± 0.41, and 16.67 ± 0.28 respectively with an average SEM of 0.36. Approximately, an average of 15% of variations were observed between the treated and control groups (19.5). Considering kit normal ranges, the variation falls within the normal range which is statistically not significant. Both CRP and IL-6 data were graphi-cally displayed in Figure 3 and Table 1.

Tab. 1. Serum CRP and IL-6 levels in IV EPE treated and control SD rats. Data were represented in mean ± SEM (n=12)

S.No	Parameters	Control (n=6)	14th Day (48h)	16th Day (48h)	19th Day (48h)
1	CRP pg/mL	198.5 ± 11.5	156.48 ± 3.58	157.5 ± 2.68	164.07 ± 3.4
2	IL-6 pg/mL	19.5 ± 0.8	17.1 ± 0.38	16.78 ± 0.41	16.67 ± 0.28

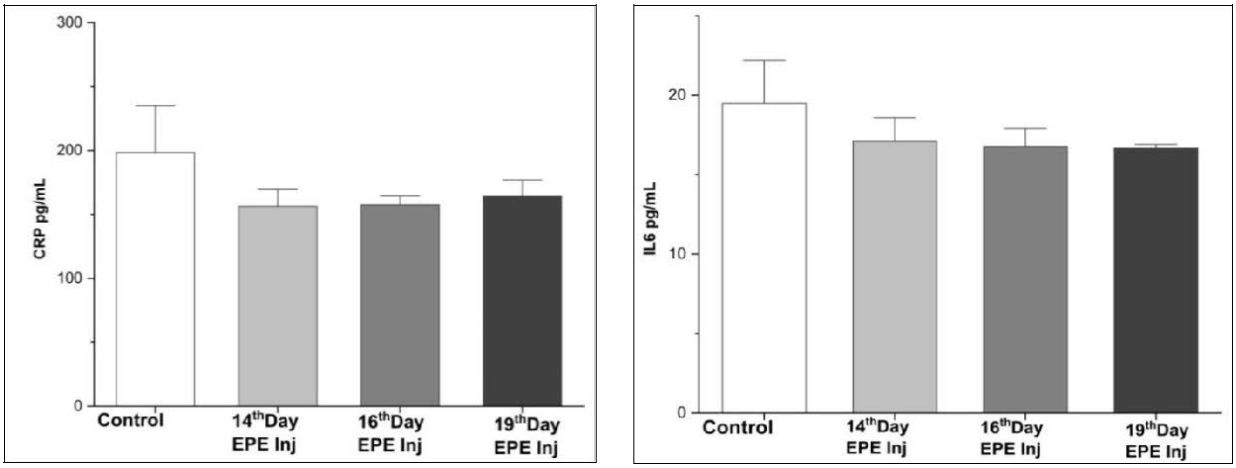

Fig. 3. CRP and IL-6 levels in IV EPE-injected, 48-hour-incubated SD rats. Data were represented as bar graphs with a mean, and the variations as SEM by performing a t-test (two-sample assumption of unequal variances). P value was considered to determine its significance ($p<0.05$) for all parameters

Inflammatory Markers

Protein concentration in the EPE

Before intravenous injection of EPE protein concentration is quantified and their concentrations are as follows 14th, 16th, and 19th embryonic day's are 5.21 mg/mL, 12.19 mg/mL, and 24.1 mg/mL, respectively.

DISCUSSION

Earlier research studies on in vitro tissue differentiation and organ differentiation are live evidences for biological function of embryonic proteins. Many in vitro studies have confirmed the regenerative function of embryonic proteins; induced pluripotent stem cells are generated by using the Yamanaka factor [19]. Another important in vitro study confirms the early nephrogenesis with epigenetic factors Cbx1, Cbx3, and Cbx5, in which ureteric bud formation, renal vesicles elongate to form comma- and S-shaped bodies [20]. The structure of which the Amniotic fluid stem cell vesicle derived fraction has significant reno-protection capacity [21]. In a recent study Yamanaka factors genes are injected through recombinant adenoviral vector in to the eyes aged mouse retinal ganglion and allowed to express Yamanaka factor proteins, which had reversed the vison to young stage. These Yamanaka factors are exclusively embryonic origin and at same time in this study they did not eliciting immunogenicity [22]. As Yamanaka factors are all these studies have paved the way for embryonic molecules' application in the regeneration of organs.

Now regenerative medicine is stepping towards molecular regenerative therapy in which embryonic proteome is considered as best source safety is required for direct clinical usage. So in this study we confirmed the safety of embryonic proteins by testing immunological, markers after 48 hours of EPE injection. Immunological parameters are crucial in safety trails studies of vaccine as well as therapeutic protein injection studies [23]. Intravenous injection of embryonic protein extract is a part of new therapeutic approach, so our immunological parameters in this study can provide critical information about the acceptance, efficacy, and safety. C-reactive Protein (CRP), an acute-phase protein secreted by the liver, is widely used in clinical settings as an inflammatory biomarker. Elevated levels of CRP are associated with the prognosis of various diseases and inflammatory conditions elicited by foreign protein invasions. In recent years, accumulating evidence has shown that CRP correlates with various new vaccines and drug trials. In the present study, despite injecting a load of EPE, elevated levels of CRP have not been found, indicating that the injected proteins are considered self-antigens and exhibit the property of not eliciting the immune response [24, 25].

Interleukin-6 (IL-6) is a pleiotropic cytokine that alters haematopoiesis, metabolism, and organ development in addition to controlling the immunological and inflammatory response. Few studies has stated that acute and intermittent production of interleukin 6 (IL-6) in response to infections, molecular, graft transplantations [26]. There exists a convincing role for IL-6 in innate immune responses and adaptive immunity, including those associated with cellular and antibody-mediated rejection after solid organ transplantation, vaccine and molecular protein therapy trials. The present study has confirmed that IV-injected EPE proteins are not considered foreign antigens and thus might be showing IL-6 levels in the normal range. Based on the results, we can infer that quantified pro-inflammatory markers CRP and IL-6 after IV EPE-injected rats are found to be in the normal range and are non-immunogenic [27]. Thus, this pilot study has confirmed that embryonic proteins are safe, can be used for in vivo therapies, and may be crucial in all vaccine and therapeutic studies.

CONCLUSION

In conclusion, embryonic proteomes are immunologically safe and might be used in the field of regenerative therapy to regenerate different organs, including cancer-associated organ resurrections. Embryos may not be a future source for regenerative molecules, as the total genome is present in all somatic cells. Desired proteins can be produced on a large scale by isolating specific genes.

CONFLICT OF INTEREST

Authors declared that they have no conflicts of interest.

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