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Short Communication

Effect of umbilical cord mesenchymal stem cells on hypoxia-inducible factor-1 alpha (HIF-1α) production in arteriovenous fistula (AVF) animal model: A preliminary study

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Abstract

Hypoxia-inducible factor-1 alpha (HIF-1α) is a transcription factor that plays a crucial role in cellular responses to hypoxia, such as in the development of intimal hyperplasia, a common complication in arteriovenous fistula (AVF) creation. While the application of umbilical cord mesenchymal stem cells (UC-MSCs) has shown promise in various regenerative medicine applications, including tissue repair and angiogenesis, the effect of UC-MSCs on HIF-1α level in the AVF has not been tested. Therefore, the aim of this study was to evaluate the effect of UC-MSCs administration on HIF-1α levels in the AVF animal model. An experimental study was conducted on 28 local male rabbits (*Lepus domestica*) using a post-test-only design. The rabbits were divided randomly into four groups: normal rabbit group (negative control), placebo-treated AVF rabbit group (positive control), AVF rabbits treated with *in-situ* UC-MSCs injection (one dose, 10^6 UC-MSCs/kg body weight), and AVF rabbits treated with intravenous UC-MSCs (one dose, 10^6 UC-MSCs/kg body weight (BW). HIF-1α level was measured using ELISA method after 28 days post-treatment. All data were analyzed using the one-way analysis of variance (ANOVA) and continued with the Duncan's post-hoc test. The data indicated that the levels of HIF-1α were different among all four groups ($p<0.001$). The post-hoc analysis revealed that the HIF-1α levels in both UC-MSC treated groups were significantly lower compared to untreated AVF rabbits ($p<0.05$). This study suggests that UC-MSCs could be a promising therapy to prevent and reduce intimal hyperplasia in AVF.

Keywords: Arteriovenous fistula, intimal hyperplasia, HIF-1α, UC-MSC, rabbit model

Introduction

Hypoxia-inducible factor-1 alpha (HIF-1α) is a transcription factor that plays a crucial role in angiogenesis [1] and cellular responses to hypoxia [2]. During hypoxic condition, HIF-1α is stabilized and enters the nucleus to bind to other cofactors and activates the hypoxia response element of certain gene promoters [2]. This activation stimulates various pathways that help the cells adapt to low oxygen environments. One such pathway involves in the development of intimal hyperplasia (IH), a condition characterized by the thickening of the inner lining of blood vessels,

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which is a common complication in arteriovenous fistula (AVF) creation [3]. In the case of AVF creation, the local oxygen diffusion from the arterial lumen may be insufficient due to intimal thickening and inflammation, resulting in a hypoxic environment that leads to the overexpression of HIF-1α [1].

The increased expression of HIF-1α has been observed during AVF maturation, in particular in the venous endothelium [4], which allows HIF-1α to serve as a valuable biomarker for preventing IH after AVF creation [5]. HIF-1α has also been implicated in regulating genes involved in the forming of IH [6]. By closely monitoring the expression of HIF-1α levels in patients undergoing AVF creation, clinicians could identify individuals at higher risk for IH development and initiate targeted interventions [8].

One potential approach for preventing IH is the use of umbilical cord mesenchymal stem cells (UC-MSCs). UC-MSCs have shown promise in various regenerative medicine applications, including tissue repair and angiogenesis [7]. The potential of UC-MSCs could enable early preventive measures and interventions to reduce the incidence and severity of IH. However, the effect of UC-MSCs on HIF-1α level in the AVF has not been studied. The aim of this study was to evaluate the effect of UC-MSCs on HIF-1α levels in the rabbit AVF models.

Methods

Study design and animal model

An animal experiment using a post-test-only design was conducted at the Experimental Animal Hospital, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia. The local male rabbits (*Lepus domestica*) were used. Animals were 8–12 weeks, weighed 2–2.5 kg and had no visible anatomical abnormalities, signs of previous infection, and other diseases. Disabled rabbits males, drop out before 2 weeks post AVF period, sick or died since treatment and behavior changed during the study (limp and not agile) were excluded.

Sample size and study groups

The sample size was calculated using the sample formula from Federer. The minimum number of samples was rounded up to six subjects. The estimated number of animals that dropped out was 10%; therefore, the minimum number of research subjects for each group was seven samples. The total samples to be examined in this study was 28 animals divided randomly into four different groups: normal rabbit group without AVF (negative control), placebo-treated AVF rabbit group (positive control), AVF rabbits treated with *in-situ* UC-MSCs group, and AVF rabbits treated with intravenous UC-MSCs group (**Figure 1**).

AVF model procedure

In both positive control and treatment groups, AVF was created to induce IH. To ensure proper anesthesia, the animals were injected with 50 mg/kg body weight (BW) intramuscular ketamine and 5 mg/kg BW intramuscular xylazine. The incision areas were shaved to improve visibility during surgery and disinfected with povidone-iodine. A vertical incision was made in the neck to access the right carotid communis artery. A dose of 100 IU/kg BW intravenous heparinization was given before the right carotid communis artery was anastomosed end-to-side to the right internal jugular vein. The anastomosis was completed by suturing with a 7-0 polypropylene. The left internal jugular vein was left untouched.

Intervention

The animals within the untreated AVF group (positive control) were given an intravenous placebo solution of 2.5 ml of normal saline. The treated AVF groups were treated with UC-MSCs injections either *in-situ* or intravenously. *In-situ* administration of the UC-MSCs was directly delivered to the AVF anastomosis site at the adventitia layer of the vessel, while intravenous administration to the draining vein of the internal jugular vein. The UC-MSCs were administered one time only at a dosage of 10^6 UC-MSCs/kg BW. The UC-MSCs were obtained from Prodia Stem Cell (Prodia, Jakarta, Indonesia).

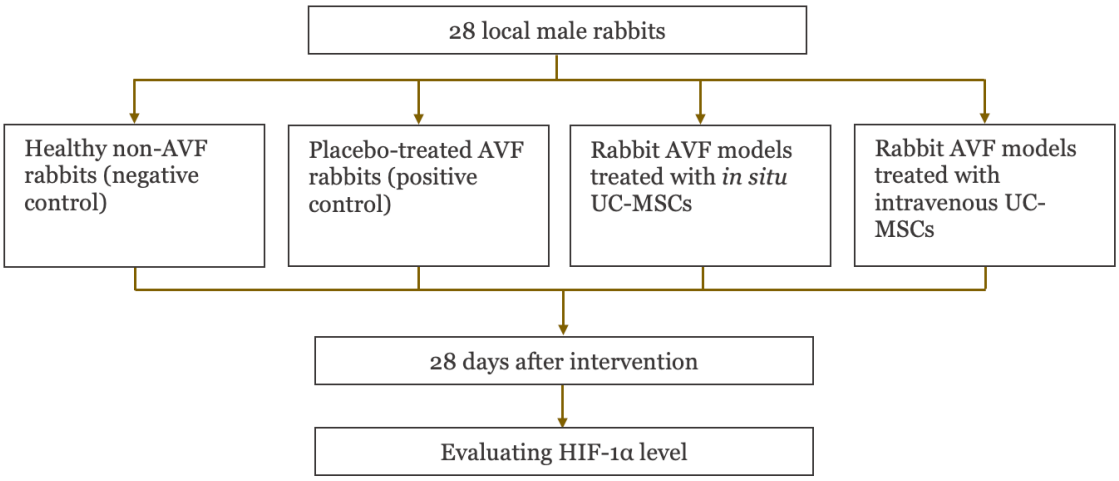

Figure 1. Schematic of study groups and the treatment received.

End point

The HIF-1α levels were determined after 28 days of intervention using ELISA sandwich methods using Rabbit Hypoxia Inducible Factor 1 Alpha (HIF-1 α) (Bioenzy, Jakarta, Indonesia). The levels of HIF-1α were measured from AVF anastomose tissues. The procedure for measuring HIF-1α levels was conducted following the manufacture protocol. The absorbance value and level of HIF-1α were measured using an ELISA reader at 450 nm wavelength (Biolegend, San Diego, USA). The levels of HIF-1 α were presented for each mg of tissue.

Statistical analysis

HIF-1α level data obtained were tested for normality using the Shapiro-Wilk test and it were distributed normally. To determine differences in HIF-1α concentrations between groups, one-way analysis of variance (ANOVA) was used and continued with Duncan's post-hoc test. Statistical tests were performed using SPSS 25 (SPSS Inc., Chicago, IL, USA).

Results

The highest mean of HIF-1α level were found in untreated AVF rabbit group (1.31±0.45 ng/mg), followed by the AVF rabbits treated with intravenous UC-MSCs (0.33±0.12 ng/mg), the *in-situ* UC-MSCs (0.21±0.08 ng/mg), and the negative control group (0.09±0.02 ng/mg) (**Table 1**). The statistical analysis showed that was significant differences of HIF-1α levels among groups (p<0.001).

Table 1. Comparation of hypoxia-inducible factor-1 alpha (HIF-1α) in healthy, arteriovenous fistula (AVF), and UC-MSCs treated AVF rabbits (n=28)

Group	Mean±SD (ng/mg tissue)	*p*-value
Negative control	0.09±0.02	<0.001
Positive control	1.31±0.45	
AVF with *in-situ* UC-MSCs injection	0.21±0.08	
AVF with intravenous UC-MSCs injection	0.33±0.12	

The results of the further post-hoc analyses found that the levels of HIF-1α in rabbits with AVF received UC-MSC treatment, either *in-situ* or intravenously, was not significantly different from healthy rabbits (p>0.05) (**Figure 2**). The HIF-1α levels were also not statistically different between *in-situ* and intravenous UC-MSC treatment groups (**Figure 2**). Our post-hoc analysis found that the HIF-1α levels in rabbits with AVF that received UC-MSC treatment either *in-situ* or intravenously were significantly lower compared to AVF animals that received normal saline (both comparisons had p<0.05) (**Figure 2**).

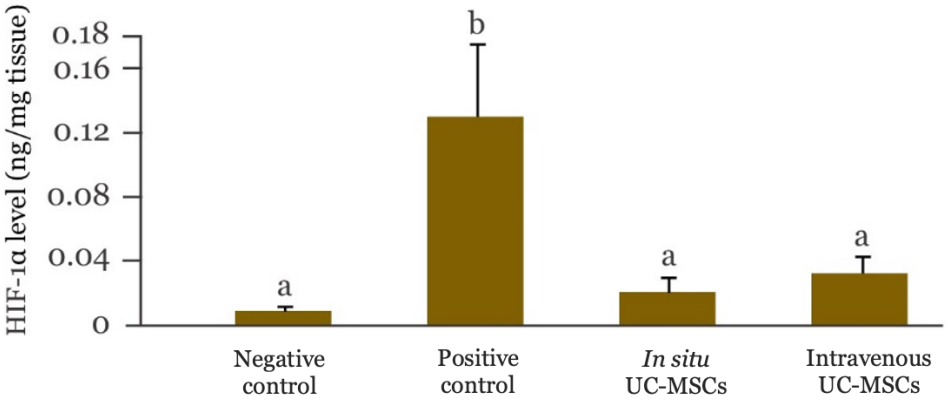

Figure 2. Post-hoc analyses comparing hypoxia-inducible factor-1 alpha (HIF-1α) levels between two groups. A different letter (a and b) above the histogram indicates significant different (p<0.05). The data indicates that HIF-1α levels in AVF animals treated with *in-situ* or intravenous UC-MSCs are significantly lower compared to AVF rabbits treated with normal saline (positive control).

Discussion

Our data suggested that the administration of MSCs can regulate HIF-1α levels and potentially prevent the development of IH in AVFs. HIF-1α is a transcription factor that regulates the adaptive response to hypoxia in cells [9]. During hypoxia, HIF-1α is stabilized and translocated to the nucleus, which regulates the expression of genes involved in angiogenesis, cell survival, and inflammation [10]. The stability of HIF-1α under hypoxic conditions enhances the regenerative potential of adipose-derived stem cells by increasing the secretion of angiogenic factors, such as vascular endothelial growth factor, which may contribute to the reduced levels of HIF-1α in AVF-related hypoxia [11,12]. Particularly in the context of AVF-related hypoxia, HIF-1α has been found to play a crucial role in vascular remodelling and angiogenesis [9].

HIF-1α could be used as a biomarker for preventing IH in AVF by monitoring its expression levels. Increased expression of HIF-1α occurs early during the maturation of AVF and this expression is localized to the venous endothelium [4]. The venous neointimal hyperplasia, a specific type of IH that causes stenosis in arteriovenous grafts and late AVF, has a well-described pathogenesis involving both upstream and downstream events. Initial insults lead to endothelial injury, triggering a cascade of mediators that regulate oxidative stress, endothelial dysfunction and inflammation [13]. The response to this injury results in the migration of smooth muscle cells from the media to the intima, eventually forming neointimal hyperplasia [14]. The activation of HIF-1α can promote angiogenesis and inhibit IH in AVF by promoting the formation of new blood vessels and inhibiting the excessive growth of smooth muscle cells in the vessel wall [2].

Moreover, the stabilization of HIF-1α can improve the functions of MSCs, including cell adhesion, migration, and proliferation [15]. UC-MSCs administration has shown potential in the prevention of IH formation in arterial prosthetic grafts [16]. The mechanism through which UC-MSCs exert their anti-IH effects is multi-faceted. As these stem cells have the ability to differentiate into various cell types, such as smooth muscle cells, endothelial cells, and fibroblasts [17,18], they can promote tissue repair and inhibit the development of IH when introduced into the site of AVF creation [19]. One possible mechanism is that the transplanted stem cells release factors or cytokines that promote angiogenesis and vasculogenesis, thereby enhancing the formation of new blood vessels in the AVF. These newly formed blood vessels may support the delivery of oxygen and nutrients to the surrounding tissues, reducing hypoxia and preventing IH formation [20]. Another possible mechanism is that UC-MSCs have immunomodulatory properties [21] which are important factors in IH development by modulating the immune response and can suppress inflammation in the vessel wall [22]. This anti-inflammatory effect may prevent the activation of smooth muscle cells and the subsequent proliferation of these cells, which is a key process in IH development [23].

In the context of AVF creation, the combination of UC-MSCs and HIF-1α can provide a synergistic effect in preventing IH [24]. UC-MSCs may be useful in reducing HIF-1α levels in

AVF-related hypoxia by modulating HIF-1α expression and activity through hypoxic preconditioning, which can enhance their angiogenic properties and contribute to the reduction of HIF-1α levels [25]. Hypoxic preconditioning of UC-MSCs has also been shown to upregulate the HIF-1α/FASN/mTORC1 axis, which promotes proliferation and migration of human MSCs derived from umbilical cord blood [26]. Another study found that human UC-MSCs produced exosomes promote angiogenesis through HIF-1α which is beneficial to fracture healing [27]. This synergistic effect is based on the ability of UC-MSCs to secrete angiogenic cytokines and promote the proliferation of hematopoietic stem cells, which contributes to the formation of new blood vessels [28]. Additionally, UC-MSCs express adhesion molecules that interact with hematopoietic cells, facilitating their implantation and homing to the site of AVF [10,28].

By measuring and monitoring HIF-1α expression, clinicians could potentially identify patients who are at a higher risk of developing IH. This could allow for early intervention and the implementation of preventative measures to minimize the incidence and severity of IH. Additionally, understanding the role of HIF-1α in angiogenesis and its link to IH formation can provide insight into potential therapeutic strategies. Since this study was limited to HIF-1α in rabbit AVF model, further studies examining other biomarkers are necessary to develop beneficial and reliable UC-MSCs therapies for inhibiting IH in AVF creation.

Conclusion

Our studies have shown that the administration of UC-MSCs may prevent IH in AVF by controlling HIF-1α to the normal level. HIF-1α can be used as a biomarker to develop better ways in preventing IH and promoting angiogenesis in AVF. However, more study is needed in humans to understand how UC-MSC could control HIF-1α level and to optimize its use in preventing IH.

Ethics approval

Ethical clearance approval was received from the Research Ethics Committee Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia, No.190/KEPH/XII/2022.

Competing interests

The authors declare that there is no conflict of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author.

How to cite

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