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CLINICAL VIEWPOINT

A Possible Cell Therapy for Critical Limb Ischemia in Women by Using Endometrial Adult Stem Cells

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Abstract. Critical limb ischemia is manifested by pain at rest, nonhealing wounds and gangrene. Approximately 20–45% of patients require amputation. Autologous bone marrow therapy for lower limb ischemia appears to be a promising solution to the current lack of treatments in patients ineligible for endovascular/surgical interventions. However, this is limited by the need for anesthesia during the bone marrow extraction procedure, which is dangerous in the lower limb ischemia population since numerous co-morbidities exist. We hypothesized that endometrial stromal cells are more suitable candidates for lower limb ischemia cell therapy in women.

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Abbreviations used: CLI, chronic critical limb ischemia; VEGF, vascular endothelial growth factor.

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1. Introduction

Chronic critical limb ischemia (CLI) is the end result of the arterial occlusive disease, and the most common form is atherosclerosis. In addition to artherosclerosis in association with hypertension, hypercholesterolemia, cigarette smoking and diabetes [1], CLI is an advanced form of peripheral artery disease that is responsible for approximately 100,000 amputations each year in the U.S. Angiogenesis therapy has been described as a "biological bypass", and the idea is that through administration of agents capable of inducing collateralization, a more natural type of "bypass" can be achieved. Indeed it has been observed that ischemic muscles secrete angiogenic factors in response to hypoxia and that to some extent natural angiogenesis does occur in animal models of CLI and also in humans [2,3]. One of the angiogenic factors noted under many ischemic conditions, including cardiac ischemia, stroke, and CLI, is the vascular endothelial growth factor (VEGF). Angiogenesis is defined as the formation of new blood vessels from the existing vasculature [4,5]. Angiogenesis occurs regularly in the endometrium throughout the reproductive life of females as part of the cyclic rapid growth and regression of this tissue that occurs during the menstrual cycle. It is now well known that angiogenesis plays a key role in the reproductive processes such as embryo implantation and endometrial regeneration following menstruation, evidence is reviewed for the hypothesis that the endometrium of women have an increased capacity to cell proliferate, and angiogenesis [6]. Experimental studies have demonstrated that bone marrow contains adult stem cells that can induce neovascularization and improve angiogenesis in ischemia [7]. Autologous bone marrow therapy for CLI appears to be a promising solution to the current lack of treatments in patients ineligible for endovascular/surgical interventions. However, this is limited by the need for anesthesia during the bone marrow extraction procedure, which is dangerous in the CLI population [8]. Endometrial layers contain a population of stromal stem cells and they exhibit stem cell properties [9,10]. The recent study of CD146, a marker of colony-forming human endometrial stromal cells, provided support for the concept that human endometrium contains a population of candidate stromal stem/progenitor cells [11]. Since endometrial stromal cells produced a higher overall clonogenicity, they may be used in cell therapy in place of bone marrow stem cells in CLI in women.

2. Hypothesis

Human endometrium is a highly regenerative tissue. The source of endometrial stromal and vascular regeneration is a resident stromal stem/progenitor cell population [11]. CD146 as a marker of colony-forming human endometrial stromal cells supporting the concept that human endometrium contains a population of stromal stem/progenitor cells [11]. Autologous bone marrow therapy for CLI is limited by the need for anesthesia during the bone marrow extraction procedure, which is dangerous in the CLI population since numerous co-morbidities exist [8]. Thus, it is hypothesized that endometrial adult stem cells are potential candidates for CLI cell therapy as autologus in women.

3. Discussion

Critical limb ischemia (CLI), the most severe stage of peripheral arterial disease, affects 250,000 new patients annually in the U.S. with an estimated 40% requiring amputation within 12 months of a CLI episode, in addition to an annual mortality rate of more than 20% [12,13]. Distal bypass surgery prompts healing of lower extremity ulcers associated with CLI if resulting arterial patency supports skin perfusion pressure of at least 35 mmHg [14]. Surgical bypass of the occluded arterial segment improves and extends primary arterial patency, though there is insufficient evidence to support improved amputation rates or mortality compared to most other modalities [15]. Some studies will help to determine whether therapeutic angiogenesis with HGF is a viable option in the treatment of patients with CLI [7]. Angiogenesis, the development of new capillaries from pre-existing blood vessels, is a tightly controlled phenomenon and generally

does not occur physiologically except in the female reproductive system [16]. Angiogenesis is dependent on soluble factors released from cells [17]. Several peptide growth factors, including FGF-α, FGF-β, PD-ECGF and VEGF, stimulate vascular endothelial cell growth in vitro and angiogenesis in vivo [18].

Endometrium angiogenesis has been demonstrated during the early proliferative phase and secretory phase [19]. Recently, the induction of an angiogenic phenotype in human endometriotic cells has been demonstrated through establishing their ability to implant [20].

The ability of endometrial stromal cells to form new blood vessels suggests the possibility for their use in the treatment of certain disease conditions [21]. The 3-D culture allows the quantification of cell proliferation and angiogenesis from endometrial stromal cells [22]. Culture of human endometrial stem cells in the fibrin matrix resulted in the proliferation of endometrial stromal stem cells and angiogenesis [23]. Recent studies identified CD146 as a marker of colony-forming human endometrial stromal cells, providing support for the concept that human endometrium contains a population of candidate stromal stem/progenitor cells [11]. This was also supported by studies which demonstrated the existence of colony-forming [24] and side-population positive stromal cells in human endometrium [25]. The search to identify mesanchimal stem cells in human endometrium is in its infancy [26]. Oct-4, a transcription factor crucial for stem cell pluripotency, was detected in some of the individual stromal cells in human endometrium using immunohistochemistry [27], and stem cell markers, CD117 and CD34, were expressed throughout the menstrual cycle in human endometrial stroma [28]. A recent study investigated STRO-1, CD133, CD90 and CD146 as potential markers of human endometrial stromal cells [11]. Population of stromal stem cells residing in the layers of human endometrium would exhibit clonogenic activity in vitro [29]. Stromal or mesenchymal stem cells have been identified in bone marrow [30]. Bone marrow stromal stem cells give rise to cartilage, vessle, bone, and adipose tissue [31]. Therapeutic angiogenesis can be induced by the implantation of bone marrow mononuclear cells and stem cells play a key role in the therapeutic angiogenesis induced by bone marrow cell implantation [32]. Some degree of similarity exists between endometrial stem cells and bone marrow derived mesenchymal stem cells, for example, expression of CD90, CD105 and lack of CD45 and CD34 [33].

Our hypothesis is using endometrial stem cells in CLI cell therapy is better than bone marrow stem cells to cause following reasons. this is limited by the need for anesthesia during the bone marrow extraction procedure, which is dangerous in the CLI population [8]. Although endometrial stromal cells produced a higher overall clonogenicity of 1.25%, only 1 in 1400–5000 formed large colonies, similar to endometrial epithelial cells. In comparison with the clonogenic activity of stromal cells in bone marrow, this is quite substantial, as only 1 in 10 000 are clonogenic [34]. Bone marrow stem cells, are notoriously difficult to purify, since there are no stem cell-specific markers. Various markers or combinations of markers have been used to isolate bone marrow stem cells. However, none was able to isolate a pure bone marrow stem cell population. Hence, the precise phenotypic identity of bone marrow stem cells remains unknown [35]. One unique aspect of using endometrial stromal cells for this indication is the possibility of administering allogeneic, standardized cell populations [36]. Previous studies involving long term follow up of animals treated with endometrial stromal cells, as well as karyotypic normality of these cells after extended passage (68 doublings) have demonstrated the lack of tumorogenicity. Endometrial stromal cells represent a unique population of stem cells and they have different rate of proliferation (these cells proliferate approximately once every 19 hours, whereas stromal cells every 24–36 hours) [37].

4. Conclusion

Endometrial layers contain a population of stromal stem cells and they would exhibit stem-cell activity. The recent study of CD146, a marker of colony-forming human endometrial stromal cells, provides support for the concept that human endometrium contains a population of can-

didate stromal stem/progenitor cells. Since endometrial stromal cells produce a higher overall clonogenicity, they can be used in cell therapy in place of bone marrow stem cells in CLI in women as autologus.

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