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A REVIEW OF APPLICATION OF STEM CELL THERAPY IN THE MANAGEMENT OF CONGENITAL HEART DISEASE

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Abstract:

Research on stem cells has being rapidly growing with impressive break-throughs. Although merely a few of the laboratory researches have successfully transited to the clinical trial phase, the application of stem cells as a therapeutic option for some currently incapacitating diseases hold fascinating potentials. This review emphasis the various opportunities for application of stem cell in the treatment of fetal diseases. First, we provide a brief commentary on the common stem cell strategy employed in the treatment of congenital anomalies, thereafter we discuss how stem cell is being employed in the management of some fetal disorders.

Keywords: Stem cell, Fetal disease, cell therapy, in utero hematopoietic transplantation.

INTRODUCTION

In recent decades, intense attention and resources have been directed towards stem cell as a viable means of tissue and organ reparation owing to the proven vast regenerative and proliferation capacities of some unique cells(1). Advancements in molecular medicine and prenatal diagnostic sciences has made it possible for early detection and treatment of fetal illness which has significantly reduced the risk of potential death and disorders. Sophisticated fetal surgical techniques such as in utero stem cell transplantation have made remedial management of some congenital malformation near clinical fruition(2). Prenatal mediation which first debut in 1982 (3) have been advanced rapidly to treat structural abnormalities with both orthodox and marginally invasive procedures(4). Fortunately, the availability of high-tech imaging techniques such as ultrasound has made possible a number of ways to precisely convey targeted stem cells to fetus during early developmental stages, thereby rekindling research interests and enthusiasm in prenatal stem cell interventions. Intrauterine stem cell remedy may have found potential application in hematopoietic illnesses for example sickle cell anemia, thalassemia, and immune system deficiencies. Hitherto, bone marrow (BM) transplantation was the common treatment approach for these disorders but critical issues resulting from graft rejection, scarcity of human leukocyte antigen (HLA)-matched donors in addition to myelosuppression of host BM before transplantation significantly constrained it effectiveness(2, 5, 6). Intrauterine cell delivery is particularly advantageous in the sense that the rapid growing fetus have immature immune system which considerably tolerate donor cells thereby supporting propagation and differentiation of transplanted cell hence facilitating engraftment(1). Although many studies have reported the effectiveness of in utero hematopoietic cell transplantation (IUHCTx) in several experimental animal studies on the other hand human clinical trials has not achieved the desired successes(1, 7).

Stem cell are a group of homogenous cells that are capable of differentiating into certain specialized cell types that make up the various tissues and organs of the body. Generally, stem cells are broadly classified into adult stem cell and embryonic stem cell(7). Due to the high potential of

differentiation, both classes of stem cell are extensively suitable for regenerative medicine(8). Based on the source of derivation, there are different types of stem cells namely; embryonic stem cell (ESC), bone marrow (BM) or hematopoietic stem cell, umbilical cord blood-derived stem cell, very small embryonic-like stem cell(9), mesenchymal stem cell (MSC), induced pluripotent stem cell (iPSC), and tissue stem cell which includes intestinal enteric nervous system/epithelial stem cells(10). Embryonic stem cell (ESC) can be defined as are somatic cells capable of differentiating to become part of all cell types found in tissues and organs of the body such as heart, nervous, immune system etc therefore are called pluripotent(11). Embryonic stem cells are considered as the best option for use in clinical research, but have triggered a lot of ethical controversies. Biomedical researchers have resorted to autologous or autogenous stem cell transplantation for a number of obvious reasons; First to minimize graft rejection there bypassing the need for suppression of recipients' immune system. This is hinged on the fact that fetal tissues collected before 15 weeks of gestation have low competent T cells to trigger an immune response to bring about rejection(1, 12). A number of studies have documented procedures of collection, processing, preservation and banking of these fetal cells (12-14). Secondly it would have addressed substantially ethical and legal constraints associated with allogenic transplant added to its natural cumbersome procedures(1).

IN UTERO HEMATOPOIETIC CELL TRANSPLANTATION

IUHCTx concept was founded with the presumed merits that the naive and evolving fetal immune system and unique environment would allow for transplantation of allogeneic cells. Unlike adults, fetus have no bones till 12 weeks of conception as such hematopoietic processes cannot occurs in the BM(15). Hematopoiesis instead for the nascent life takes place outside the yolk sac and in the para-aortic splanchnopleura of the embryo. Although liver hematopoiesis has been reported to commence at about 5 weeks of gestation and runs actively until half-term when BM hematopoietic activities takes dominance(16, 17). The realization that hematopoietic stem cells (HSC) are in present high concentration in fetus circulation further brighten the prospect of IUHTx on the

assumption that transplanted HSCs could also be conveniently engrafted in the targeted organ. It therefore logically follows that one of the most probable justifications for carrying out in utero HSC transplantation is it high potential to attain donor-specific tolerance (18).

PRE-CLINICAL EXPERIENCE WITH IUHCTX

Study designs of IUHCTx with research animals as listed in Table 1 has shed more light on some of the prevailing challenges militating against successful stem cell engraftment aimed at treating of some inborn diseases. As early as 1953, donor specific tolerance was demonstrated in mice skin engraftment by Billingham *et al* with allogenous stem cell in what has been acknowledged as the debut of IUHTx(19). Researcher later realized that engraftment conducted in immunosuppressed mice yielded better results due to reduced or near absence of immune mediated graft rejection when compared to immuno-competent , wild mice which bared poor engraftment(20, 21). Further review of IUHTx methodologies with the aid of improved techniques shifted focus to ways of attaining higher donor-specific tolerance(22). Different mechanisms such as chimerism and anergy were experimented in mice in order to achieve induced T cells tolerance post IUHTx. Interestingly the results showed remarkable engraftment in the mice and point to the opinion that achieving tolerance involves the interplay of the balance of two critical factors namely; level of engraftment and presence of chimerism(23-25). Armed with this achievement in mice, researchers attempted to replicate and study the practicability of IUHTx in higher mammals specifically sheep, dog and pigs. Special attention was accorded the sheep model as a prototype for examining engraftment of human cells, primarily because there was no rejection of the xenogeneic cells, ease of tracking, and enhancing their levels via human granulocyte-colony stimulating factor(26, 27).

Furthermore, researchers have seized the suitable, natural and unperturbed environment offered by IUHCTx in sheep as a veritable avenue to comprehensively study the proliferation, differentiation and engraftment capacities of human stem cells especially ESCs and MSCs (28, 29). The dog and pig models also respectively revealed acceptance of xenogeneic transplants as observed in the sheep experiment. Peranteau *et al* reported that multi-lineage engraftment was achieved in

hematologically normal dogs, noting that IUHCTx in conjunction with postnatal enhancement effectively treated canine version of human leukocyte adhesion deficiency(18). Whereas the fetal pig with induced tolerance supported postnatal organ transplant as demonstrated with kidney allograft(30) thereby advancing tentative evidence aimed at adopting this approach in fetuses having congenital abnormalities which may require postnatal transplant. But on the contrary, there are also reports of insufficient engraftment in non-human primate models(31-33). While some distinctive challenges are expected of the human immune system, these higher mammal studies have successfully validated the practical viability of IUHTx and brighten the potential of achieving firm engraftment post IUHCTx in immune-competent host.

Table 1: Successful treatment of inherited disorders using animal models of IUHCTx, this image adapted from(2) with copyright permission.

Disease	Animal Model	Defective Gene
Anemia	Mouse	c-kit tyrosine kinase
Autosomal recessive Osteoporosis	Mouse	Tcirg1
Leukocyte adhesion deficiency	Dog	Leukocyte integrin CD18
Osteogenesis imperfect	Mouse	Col1a1
Severe combined immunodeficiency	Mouse	Scid
Sickle cell disease	Mouse	α-Globin
Thalassemia	***Mouse***	**β-Globin**

CLINICAL EXPERIENCE

Clinical trial of IUHCTx in humans has been rather disappointing as the outcome was not as successful as expected compared to the animal study with the exception of immunodecifient

cases. A pioneer case recorded successful transplant in a fetus suffering from bare lymphocyte syndrome(34). Subsequently, several studies reported effective handling of severe combined immunodeficiency (SCID) using IUHCTx strategies(35-38). IUHCTx Trails directed at managing non-SCID disorders such as metachromatic leukodystrophy, chronic granulomatous disease has been unsuccessful, thereby leading researchers to examine possible factors militating against it. One probable reason put forward was the failure to attain sufficient level of chimerism(39). However, efforts to further pinpoint the causal factors of poor engraftment in these cases remain challenging, primarily due to the wide range of variables involved. For instance, location of transplantations, different sources of donor cells, the gestational age at transplantation amongst others(33). Given these variations in the experiments it became practically impossible to link the failure to a particular element, hence animal models again became imperative to elucidate the barriers impeding engraftment after IUHCTx(2).

ROLE OF STEM CELL IN CONGENITAL HEART DISEASE

Congenital heart disease or defects (CHD) refers to structural malformation in the heart or blood vessels close to the heart of the developing fetus prior to birth(40, 41). With an established prevalence rate estimated as between 6 to 13 of every 1000 live birth, CHD is regarded as the commonest of congenital abnormalities in neonates accounting for about 31% of infant death from 1987 to 2005(42, 43). According to the American Heart Association, about 1 out of 150 adults are still living with CHD prompting the implementation of pulse oximetry as one of the necessary universal newborn screening within 24 hours of delivery before discharge from hospital(44). There are different forms of CHD basically classified according to the blood flow pattern as listed in table 2(43). These defects vary widely in their degrees of severity which ranges from simple to highly complex cases. Comparatively simple issues such as holes between the heart chambers are usually fixed via surgery, while complicated deformities like absence of chamber (s) or valve(s), leading to deficits in blood oxygen levels, defective circulation, heart failure (HF), and ultimately death(40). The principle of stem cell remedy involves targeted delivery of seeded cells to the

affected site to induce regeneration of the defective tissues which could lead to possibly recovery of its biological function(45). This targeted delivery into the heart can be achieved by various experimented methods namely; intracoronary which delivers into artery or heart, intra-myocardial, intravenous, and epicardial(46). Following from studies on special biomolecules (proteins and micro-RNAs) produced, a new paracrine strategy has evolved(47). In pursuit of a comprehensive solution for complex CHD, researchers have attempted diverse types of cells: from PSC to fetal and umbilical cord cells, to MSC, to hematopoietic stem cells. Research progression from preclinical to clinical trials have been somewhat cumbersome with CHD primarily due to its complex nature which makes it very difficult to induce in study animals(48, 49). Nonetheless, conditions of pressure or volume overload, a characteristic decimal in hypoplastic left heart syndrome (HLHS) or tetralogy of Fallot (ToF) patients was successfully induced in rats and sheep with the aid of pulmonary artery banding. In order to ascertain improvement of RV function, these animals heart were subjected to stem cell treatment sourced from the myoblast and umbilical cord(50). The design proved quite helpful in assessing the safety and efficacy of the cell therapy transplant for single ventricular laceration cases(49, 51). Also Reddy et al in another design used sutures to successfully manipulate the pulmonary valve leaflets of a murine rat to give rise to right ventricle (RV) volume overload(52). Furthermore, a trial of another ToF model in a swine neonate through intra-myocardial injection of human derived ESC though confirmed it viability and safety but at 3 months, a review confirmed RV remodeling in the test group which received cell therapy but no marked increment in RV function compared to the control animal (53).Luckily in 2015, Cantero *et al* successfully established the viability and safety of RV intra-myocardial transplant of autologous umbilical cord blood mononuclear cells in the heart of piglets(54). In the aspect of clinical trials for children with CHD, the focus has mainly been centered on postnatal management of pediatric dilated cardiomyopathy, whereas only very few stem cell based therapy studies has been conducted for children with severe CHD(55-57) .Rupp *et al*., in 2010 recounted a successful case of 11-month male baby with hypoplastic left heart syndrome whose RV ejection fraction (RVEF) doubled

to 44% from 22% barely 3 months after cell therapy administration via intracoronary injection of autologous BMCs (58). Likewise, a clinical trial which deployed autologous BMMNCs via intracoronary route for 9 kids at risk of terminal heart failure paid off in terms of its safety and feasibility and achieved stability of their conditions as early as 3 month post administration and over 4 years thereafter(59). The report of yet another infant (4 month of age) whose RVEF shot up to 50% from 30% in 2015 after being treated with autologous umbilical cord blood mononuclear stem cells was at least reinvigorating to researchers(57). It is even more encouraging to state that quite recently the result of a successfully completed pioneer longstanding phase I controlled clinical trial with a number of CHD affected children using autologous cardiac stem cells (CSCs), was published on a positive note [59]. The trail which enrolled 7 kinds (with hypoplastic left heart syndrome; age ranging from 5 months to 3years) receiving autologous CSCs after about 5 weeks of surgery. Comparing the RVEF determined percentages at 18th month review showed an average rise from 47% to 54% (7% gain) for CSCs treated group as against 47% to 49% (a marginal 2% gain) for the control group with no CSCs treatment(60). This trial thus giving an initial hint at the safety and feasibility of CSCs based approach as a viable option for CHD afflicted infants. On the basis of the findings from phase I trial, the Japan Okayama University has reported execution of the phase II pilot trail. The clinical trial which had 34 participants (with maximum age capped at 20 years) was designed to further verify the efficacy and safety of CSCs therapy for CHD infants targeting specifically HLHS, single right ventricle and single left ventricle disorder cases. These patients where carefully selected to include only those who were highly likely to resort to heart transplant as a result of either complicated preoperational state or failure to recover after first operation i.e post-operation. 14 HLHS patients underwent cell therapy treatment delivered intracoronary 4 weeks after surgery while the control group received normal procedural care post-surgery. Report of several weeks and upto 36-months of monitoring and evaluation supported by echocardiography showed no adverse effect, stable heart functions and substantially better RVEF output for the CSCs treated patients compared to control subjects(61). These successes though limited has paved the

way for other upcoming stem cell based CHD clinical trials such as the phase I project at Duke University with an expanded secondary aim of testing the suitability of intravenously administered autologous umbilical cord blood-derived stem cell in HLHS infants while the Maimi University proposed phase I trial hopes to utilize intramyocardial infused allogenic MSCs(62). The outcome of this trials will no doubt add to the slowly growing experimental database on cell therapy driven CHD management strategies but what remains uncertain is whether it will positively consolidate the gains recorded previously, address some current challenges and support the advancement of the pediatric CHD project.

Table 2: CHD Classification Based of Blood Flow this image adapted from (43) with copyright permission.

Acyanotic with Increased Blood Flow	**ACyanotic-Obstruction of Pulmonary Blood Flow**
Atrial septal defect (ASD)	Coarctation of the aorta
Ventricular septal defect (VSD)	Interrupted aortic arch (IAA)
Patent ductus arteriosus (PDA)	Aortic stenosis (AS)
Complete atrioventricular canal (CAVC)	**Cyanotic-Variable of Pulmonary Blood Flow**
Cyanotic-Obstruction of Pulmonary Blood Flow	Hypoplastic left heart syndrome (HLHS)
Pulmonary stenosis (PS)	Truncus arteriosus (TA)
Pulmonary atresia (PA)	Transposition of the great arteries (TGA)
Tetralogy of fallot (TOF)	Total anomalous pulmonary venous rectum (TAPVR)

PROSPECT OF TISSUE ENGINEERING REMEDY FOR CHD

In spite of the tremendous advancement in diagnosis, management and improved surgical techniques, CHD still remains a worrisome cause of infant mortality(63). This stems from the prevailing limitations inherent in the surgical approach which mainly revolves around availability and insufficient durability of graft prostheses(46). The commonest types of graft often used include; xenograft sourced from bovine and porcine(64) , human derived cryopreserved homograft, autografts obtained from patient's body but has been reported to develop dilatation and regurgitation issues(65) , and artificial or synthetic prosthetics (Gore-Tex mechanical valves) which are actually durable but obviously lack the capacity for growth(66). Although biomedical researchers succeeded in largely overcoming the immune related complications associated with most of these graft types using decellularization and immunosuppressive strategies, the resultant inadequate mechanical, electrical, and regenerative capacities in addition to its inability to sustain long term satisfactory performance has brought the option of tissue engineering into serious consideration(67, 68)(46). Tissue engineering in conjunction with stem cell has been projected as a viable means of finding a lasting remedy for CHD patients as it offers a 3- dimensional biodegradable scaffold which provided near natural support for seeded autologous stem cells to grow, proliferate, differentiate and remodel into the desired cardiac tissues(69, 70). Fortunately studies in this perspective have reached advanced levels with some biomedical researchers identifying some ideal properties of the 'would be' bioengineered tissue graft suitable for single ventricular septal defect patches, ROVT reconstruction, and pediatric defective valve replace to include; inability to trigger immunogenic reactions, ability to grow at a rate commensurate to the general somatic expansion, availability in variety of sizes, malleability, structural stability and resistance to calcification(71-74). Though a number of such scaffold have been fabricated and tested, an optimized one encompassing all the above qualities is yet a work in progress(72, 75). Interestingly, a lot of stringent preclinical and clinical studies have been executed to establish the safety and practicability of TE stem cell scaffold in both small and larger animal model. Studies have been conducted using BMMNCs, and endothelial cells (ECs) seeded onto a variety of TE

scaffold *in vivo* before implantation have been tested in SCID mice(76), rat(77), sheep(78, 79) / lamb(80, 81) and dog(82). Results of these animal experiments at several weeks and months post transplantation were all impressive and positive with no adverse reactions or complication such as stenosis, aneurysmal dilatation.

Given the impetus of the animal model studies, a few cases of controlled clinical trials have been conducted. Shin'oka et al., in 2001 attempted the first stem cell engineered scaffold on a 4 years old female CHD patient suffering from univentricular heart and pulmonary atresia. The scaffold was made of Poly-L-lactic acid (PLLA) and poly-caprolactone (PCL) with poly-glicolyc acid(PGA) reinforcement carefully designed to gradual degradation at 8 weeks had peripheral vein derived autologous stem cells embedded on it. Follow-up examination upto 7 months' post implantation revealed the implant was successfully integrated with on sign of occlusion or aneurysm of the conduit (83). In 2003 another positive clinical cases of CHD children treated with BMMNCs seeded on a TE biodegradable graft (designed to breakdown in 3 to 5 years) showed no incidence of stenosis(84). Similarly, a trial with 25 CHD children all less than 7 years of age who received a PLLA/PCL and PGA reinforced graft engineered with autologous bone marrow cells (BMCs) and billed to degrade in 2years was judged highly successful at 16th month monitoring with no complications of stenosis, thrombosis or occlusion and the conduit graft grew in its diameter(83). In a related clinical trial in 2010 involving 25 patients below the age of 7 with univentricular disordered CHD, a TE based autologous BMMNCs conduit transplant was carried out, 21 patients had excellent recovery with no complication while 4 experience graft occlusion corrected via a secondary procedure(85).

Although this initial result shows cardiovascular TE based cell therapy holds promising potential to achieving a long lasting recovery of CHD patients especially in children, may works needed to be done. A major challenge faced with this novel approached is the limited data on clinical trials conducted thus far. More studies with long term monitoring of participants are required in order to ascertain TE graft efficacy, performance and it capacity to sustain adult livelihood.

FUTURE DIRECTION FOR PEDIATRIC CHD HEART REPAIR RESEARCH

The experiences garnered from treatment and management of CHD cases in adult patients had helped our understanding of it complex nature in children. The characteristic delicate environment of pediatric CHD has impeded direct translation of treatment strategies deemed helpful in adults cardiac practice to these population of children and the major reason is the obvious pathobiological differences between adult and pediatric cardiac disorders. For instance, intracoronary delivery of stem cell gives uninterrupted access to the myocardium in adult's ischemic disease but in pediatrics open chest surgery is required to access it, hence delivery method cannot be the same(62). Also immunological sensitivity resulting from allogenic graft or cell products is feared to expose children to higher difficulty of securing donor match should a heart transplant be the last resort in future than in adults hence autologous stem cell based approaches might be a better option for pediatric centered researches(86). Human placenta-derived MSCs have been reported to possess some innate immuno-regulatory potentials. It capacity to display multi-lineage differentiation into a series of specialized cells as well as immunosuppression of T lymphocyte proliferation and function may come in handy as a potential source of allograft transplant(87). Although this discovery is in its preliminary stage, if further investigations prove beyond doubt the above attributes, then a perfect alternative may have been found to other sources of MSCs especially bone marrow in which the differentiation capacity has been reported to degenerate with age(88). The relative ease of availability of human placenta is another attractive advantage(89). Recent findings have proved that not all cell types are capable of inducing de novo cardiogenesis and re-muscularization of the diseased heart(90). Induced pluripotent stem cells have been found to possess this rear potential therefore future regenerative pediatric cardiac restoration interventions might need to realign its choice of cell type in order to achieve a heart with tissues born anew. More preclinical studies with animals and if successful, clinical trials would be needed in this direction to establish it safety and feasibility. Although the success recorded in the field of cardiovascular tissue engineering and stem cell based therapy are limited by virtue of the numerical data strength, a combination of

both approaches might finally give the answer to the CHD treatment conundrum. Such synergy is hypothetically seen to consolidate the gains of each method at the same time compensating for the inherent limitations thereof.

CONCLUSION

While it is now obvious that stem cell therapy alone cannot provide all that is required to comprehensively tackle the health challenges of CHD, the approach does offer opportunities which if successful, are likely to enhance lifelong outcome of infants. Researchers are still not clear on whether stem cell promotes differentiation of cells into cardiomyocytes or influence the release of cytokines and growth factors via paracrine mechanism to bring about cardiac repairs.

Since the uncertainty beclouding the precise mechanism by which stem cell ameliorate cardiac diseases persist, future study designs might consider a synergy between stem cell therapy and tissue engineering to overcome current challenges. Any successful hybrid approach should among other factors take into critical consideration stem cell types, source of cell, seeding technique, and time of intervention. As the gains of advancement in stem cell field of biology and tissue engineering is making its gradual and steady way into clinical practice a renewed hope in sight as it ripples effect would sooner than later impact on the expectant population of CHD patient especially infants and children thereby availing them a brighter chance to lead a normal life.

Conflict of interest: The authors declare that they have no conflict of interest.

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