

Adipose-derived adult stem cells and new technique for intracavernosal injection for erectile dysfunction: novel approach to treat diabetes mellitus. Erectus Shot Technique

Carlos Mercado, MD*

Medical Director StemCellsMD, 1307 S. International Pkway, Suite 2091, Lake Mary, FL 32746, USA

Abstract

Erectile Dysfunction (ED) affects 10 percent of the population worldwide. In 1995 it was estimated that 152 million men were affected by ED. It is estimated that by 2025 352 million of population worldwide will be affected. Currently there is not available treatment that can restore or regenerate the tissues in the corpus cavernosum or arterial system that will cure ED a 20 percent failure rate with present therapies and high rate of drop out in patients with ED due to radical prostatectomy and post radiation. Testosterone replacement is the only independent risk factor that might help endothelial dysfunction and consequently ED. Adipose tissue is abundant and easy to collect from a miniliposuction. The procedure consists in collecting a lipoaspirate of 60 ml. No general Anesthesia is required. It is an outpatient procedure. Adipose tissue is processed using a non animal collagenase enzyme. Stromal vascular fraction (SVF) is isolated. Blood is obtained through a venipuncture. Platelet rich-plasma (PRP) is obtained. The SVF is suspended in PRP. The mixture is injected into the corpus cavernosum using our designed protocol for injection.

Four types of patients: DM, ED due to prostatectomy and radiation, Peyronie's disease, and ED organic cause/aging/vascular. Patients filled out the intensity score questionnaire before and after procedure. Both reported better erections in the morning, firmer, and better response to PDE5 inhibitors for which they were not responders prior to the treatment. This might be due to stem cells induced regeneration of the cavernous nerve and endothelial cells enhancing the nitric oxide production hence improved response to PDE-5 inhibitors.

Abbreviations: ED: erectile dysfunction; SC: stem cells; ADSC: adipose-derived stem cell; BMSC: bone marrow stem Cell; SVF: stromal vascular fraction; CC: corpus cavernosum; IC: intracavernous; refers IC injections; SMCs: smooth muscle cells; CSMCs: corpus smooth muscle cells; CN: cavernous nerve; NO: nitric oxide; NOS: nitric oxide synthase; eNOS: endothelial nitric oxide: refers to NO coming from endothelial cells; nNOS: neuronal nitric oxide: refers to NO coming from nerve endings like CN; TA: tunica albuginea; DM: diabetes mellitus; PD: peyronie's disease; CNI: cavernous nerve injury; MPG: major pelvic ganglion; RP: radical prostatectomy; PDE5 (I): phosphodiesterase type 5 (inhibitors); PVN: paraventricular nucleus; GTP: guanosine 5'-triphosphate.; cGMP: cyclic guanosine monophosphate; ATP: adenosine triphosphate; C AMP: adenosine monophosphate

Introduction

It appears that ED is becoming an epidemic [1-4]. Unfortunately the present standard care of treatment doesn't offer a therapy to reestablish function of the penile organ. The penile structure is a vascular organ and it is the area of the vascular system that contains the most endothelial cells when compared to other vascular tissues. ED has to be seen as a systemic disease that in many occasions merit a cardiac evaluation to rule out plaque in the coronary arterial system [5-7]. ED is defined as failure to initiate, failure to fill, failure to store necessary blood volume within the lacunar network [8]

The reason for the high prevalence of ED might be related to humans being now developing the devastating consequences of a poor life style such as smoking, hyperlipidemia, obesity, hypertension, sleep

apnea among some of the risk factors [8-10]. The worldwide prevalence of ED was estimated to be 152 million in 1995 and predicted to be 322 million by 2025. Almost the same size as the population in the United States

The other issue to address is the high failure rate of PDE5 (I) in patients such as DM and after RP and or after radiation to the pelvic area [10]. It appears that main reason for the high failure rate in these subset of patients of patients is due to minimal or lack NO production due to the damage to the CN creating apoptosis and eventually fibrotic tissue of the CC [10]. The other condition to review is PD (Peyronie's Disease) that causes penile deformity but also is associated with ED. The present urologic surgery for this condition entitles plaque removal from the TA (Tunica Albuginea) which eventually causes more scar tissue and then later on more deformity and ED problems. Currently, we are left with no real option treatments that can reestablish function and then improve the erection process in humans.

The main objectives of this review is to present our outpatient

Correspondence to: Carlos Mercado, MD, Medical Director StemCellsMD, 1307 S. International Pkway, Suite 2091, Lake Mary, FL 32746, USA, Tel: 407-771-0404; **E-mail:** carlosmercadomd@gmail.com

Key words: erectile dysfunction, adipose-derived adult stem cells, corpus cavernosum, nitric oxide, stromal vascular fraction (svf), platelet-rich plasma (prp), erectus shot technique

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protocol of IC injection technique using autologous adipose derived mesenchymal stem cells for ED and review the proposed mechanism of action and to propose the use the IC route as a way of systemic delivery without the need to go directly into the affected organ. The IC injection of a suspension of SVF in PRP appears promising in ED patients due to DM, prostatectomy and radiation for which at present there is not a cure furthermore they have a high rate of Failure to PDE5 inhibitors. The procedure is done as outpatient and requires no general anesthesia. The potential to treat diabetes as early as diagnosed might be a possibility since lower fasting glucose levels and improvement in HbA1C were noticed.

Erection process

The erection is a very complex process that requires the integrity of several organ systems in the body as explained below [10,11]. One is the integrity of the corpus cavernosum (CC) (Figure 1). The main components of the CC are: The endothelial cells, smooth muscles and the connection with the CN (cavernous nerves). The main ingredient appears to be NO (nitric oxide). The erection process requires equilibrium between sympathetic and parasympathetic system (Figure 2). An overdrive of the sympathetic system will make it difficult to have an erection.

Neurotransmitters in the brain are very important such as oxytocin, dopamine among others. Blood entering into the sinusoids (small venules in the CC) will cause the erection process mediated by neurotransmitters in the brain and predominance of parasympathetic system. The neurotransmitters in the brain elicit a positive effect on the PVN (paraventricular nucleus) such as oxytocin, dopamine, glutamic acid, and VGF peptides causing release on NO (nitric oxide) causing calcium influx then increasing NOs (nitric oxide synthetase) then increasing conversion of L- arginine to NO (nitric oxide). Oxytocin has a positive effect on extra-hypothalamic areas such as hippocampus, amygdala, spinal cord, ventral caudal tegmental areas (Figure 3). The end result is release of NO that is needed by androgens to produce an erection (Figure 3). Clinically speaking brain neurotransmitters play an important role in the erection process. For example, patients using opioids or cannabinoids has a negative effect on the production of NO, blocking the effects of NOs then causing minimal amount of NO. This explains why many patients on Pain medications such as opioids had problems with ED (Figure 3 and 4). So it is very important to understand the mechanisms of action in order to have an erection as follow [10-18]

NO (nitric oxide) released from the endothelial cells and CN (cavernous nerve) at the CC (corpus cavernosum) activate guanylyl cyclase enzyme converting GTP to cGMP [18,19]. When cGMP are sufficiently high, activation of c GMP dependent kinase occurs, leading

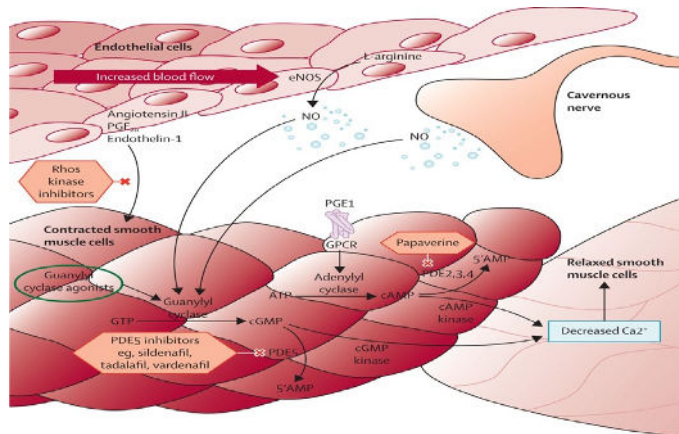


Figure 1. Erection Process. Interaction between Cavernous Nerve, Endothelial cells and Smooth muscle cells. NO (Nitric Oxide) is released by the CN and the Endothelial Cells. NO enters the smooth muscle cells. NO activates the enzyme Guanylyl Cyclase converting GTP to c GMP. PDE5 enzyme converts the cGMP to 5' AMP. The PDE5 inhibitors block the PDE5 enzyme maintaining sufficient levels of cGMP. This leads to decreased calcium causing relaxation of smooth muscle cells. In the relaxed state blood enters into the CC causing and maintaining an erection. The other enzyme is the Adenylyl cyclase that is non-dependence of NO. ATP is converted to c AMP by an Adenylyl Cyclase. cAMP is converted by a cAMP Kinase. The end result is decreased Calcium then allowing blood to enter into CC, causing and maintaining an erection that is not NO dependant.

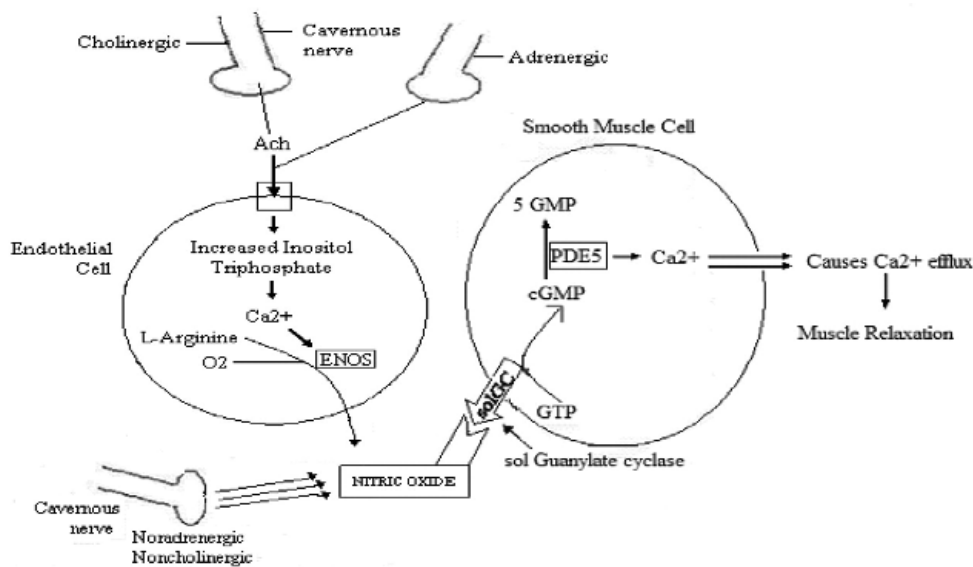


Figure 2. Erection Process in relationship with synergy between Adrenergic and Cholinergic System, Endothelial cells and Smooth Muscle Cells and Cavernous Nerve. The CN by the mediation of the Noradrenergic, Noncholinergic produces NO and the Cholinergic, Adrenergic produces Acetylcholine(Ach). Ach enters the endothelial cell, increasing Inositol Triphosphate and leads to Calcium to convert L- Arginine by ENOS (Endothelial Nitrous Oxide Synthase) leading to NO production. NO activates the conversion of GTP to cGMP . PDE5 Enzyme converts cGMP to 5 GMP. Calcium efflux causes muscle relaxation then allowing blood to enter the CC, then an erection.

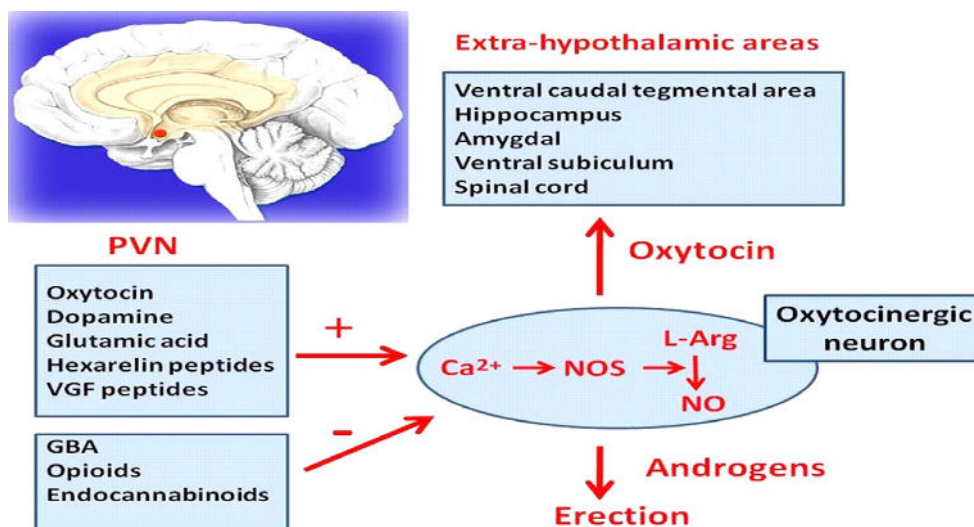


Figure 3. Erection process in relationship with Brain Neurotransmitters. Paraventricular Nucleus (PVN) has positive or negative Mediators in relationship with the production of NO. Oxytocin, Dopamine, Glutamic Acid, VGF has a positive effect. Contrary GBA, opioids, Endocannabinoids has a negative effect. Oxytocin has a positive effect in the Extra-hypothalamic areas such as Hippocampus, spinal cord. NO has a positive effect on androgens that mediate an erection.

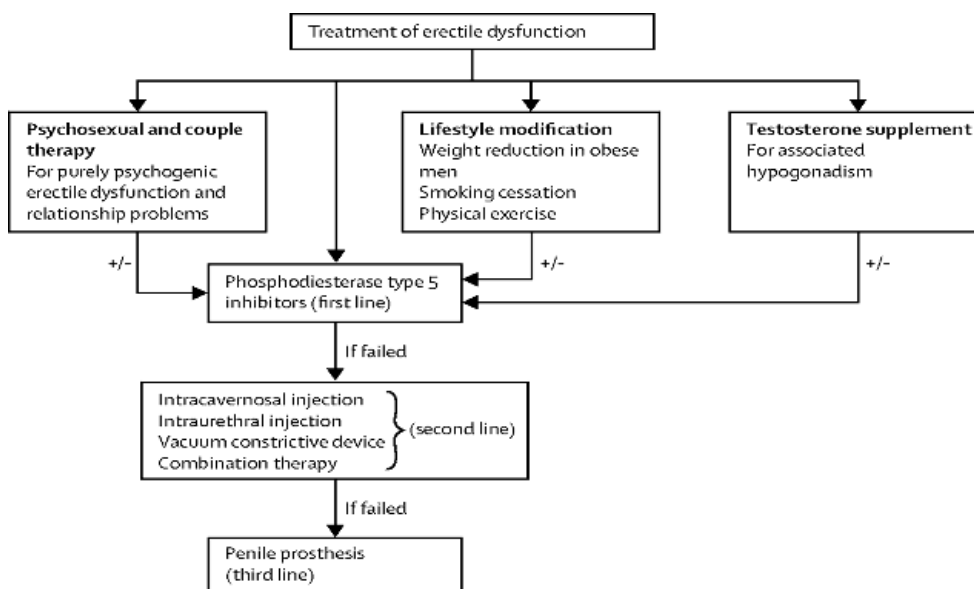


Figure 4. Treatment of erectile dysfunction. First line treatment includes Psychosexual and couple therapy, life style modification, testosterone supplementation for associated hypogonadism all in combination with Phosphodiesterase Type 5 inhibitors. If the above fails then use the second line options such as intracavernosal injection, intraurethral injection, vacuum constrictive device or combination therapy. If the above fails then the third line is a penile prosthesis.

to phosphorylation of several downstream target (ion channels), causing CSMCS to relax, and causing an erection (Figure 1).

PDE5 enzyme [19-25] converts cGMP into 5' AMP then lowering cGMP levels that are needed for the erection process. Medications such as sildenafil block the PDE5 enzyme then increasing and maintaining levels of cGMP necessary for the erection process. This is a key point that will be discussed later in relationship to high failure rate of PDE5 (I) in the setting of DM or after radical prostatectomy. In order for the PDE5 (I) to work, NO is needed, otherwise they might be ineffective [25].

When sexual stimulation stops, c GMP production is also halted. Then cGMP is hydrolyzed by PDE5 enzyme. Then cGMP is deactivated and converted to 5' AMP. Lowering the levels of cGMP will cause then

the smooth muscle cells of the CC to contract and the erection process to stop (Figure 1).

On the contrary there is another enzymatic process that is INDEPENDENT from NO [25,26]. It relates to the conversion of ATP to cAMP being converted by Adenylyl Cyclase enzyme (Figure 1). It involves another type of PDE enzyme: PDE 2, 3, 4 that converts cAMP to 5'AMP. C AMP by a CAMP kinase decreases calcium then relaxing the smooth muscle cells causing influx of blood and then an erection. For instance medications such as papaverine block the PDE 2, 3, 4 increasing levels of c AMP that leads to phosphorylation trough a c AMP Kinase and then causing relaxation of smooth muscles and then an erection. Currently many patients, who had DM or after RP that produce minimal or no NO, are on PDE 2, 3, 4 inhibitors, since the medication works without need of NO [25,26].

Hemodynamics of erection

In the flaccid state, the arteries, arterioles and sinusoids are CONTRACTED. The intersinusoidal and subtunical venous plexuses are wide open, with free flow through the emissary veins. The pO₂ is venous (35 mm Hg). In the erect state, the muscles of the sinusoidal wall and the arterioles relax, allowing maximal flow to the compliant sinusoidal spaces. Most of the venules are compressed because expanding sinusoids. Larger venules of sub tunical plexus are sandwiched because the distended sinusoids and the tunica Albuginea that effectively reduces the venous capacity to a minimum. The pO₂ here is arterial (>90 mm Hg) and ICP >100 mmHg [25,26].

Causes of ED

The risk factors for ED are similar to the one for cardiovascular disease. As mentioned before the PENIS is a vascular organ and it

Table 1. Number of published stem cells for erectile dysfunction studies.

Years	2004-2011	2014-2013	2004-2013
Number of Studies			
Total	15	20	35
Disease Model:			
Aging	2	1	3
CN injury	7	12	19
Hyperlipidemia	1	0	1
T1DM	2	6	8
T2DM	2	0	2
TA injury	0	2	2
SC type			
ADSC	5	13	18
BMMNC	1	0	1
BMSC	4	5	9
EPC ^b	1	0	1
ESC	1	0	1
SkMSC	2	1	3
Testis SC	0	1	1
UCBSC	1	0	1

Table 2. Published studies of stem cell therapy for cavernous nerve injury erectile dysfunction.

Publication year	First author	CN injury type	Stem cell type	Modification/supplementation*	Transplantation route	References
2004	Bochinski	Crush	Allogenic ESC	BDNF transduction	IC or intra-MPG	[98]
2006	Kim	Transection	Allogenic SkMSC	None	IC	[99]
2009	Fall	5-mm resection	Allogenic BMMNC	None	IC	[100]
2010	Albersen	Crush	Autologous ADSC	None	IC	[101]
2010	Kendirci	Crush	Allogenic BMSC	P75LNGFR selection	IC	[102]
2011	Lin	5-mm resection	AutologousADSC	None	Nerve graft	[103]
2011	Woo	Transection	Allogenic SkMSC	None	IC	[104]
2012	Fandel	Crush	Autologous ADSC	None	IC	[105]
2012	Kim	Crush	Allogenic BMSC	BDNF transduction	Intra-MPG	[106]
2012	Kovanecz	5-mm resection	Mouse SkMSC	Oral Sildenafil	IC	[107]
2012	Piao	Crush	Human ADSC	BDNF PLGA	CN scaffold	[108]
2012	Qiu	Radiation	Allogenic ADSC	None	IV	[109]
2012	Qiu	Crush	Autologous ADSC	None	IC	[110]
2013	Jeong	Crush	Human ADSC	BDNF PLGA	CN scaffold	[111]
2013	Kim	Crush	Human ADSC	NGF hydrogel	CN scaffold	[112]
2013	You	Not described	Human BMSC	None	IC + Periprostatic	[113]
2013	You	Stretch	Human ADSC	None	IC + Periprostatic	[114]
2013	Choi	Crush	Human testis SC	None	Periprostatic	[115]
2013	Ying	Crush	Autologous ADSC	None	IC	[116]

is the richest area of endothelial cells. Hypertension, dyslipidemia, diabetes causes the following effects as atherosclerosis and impaired endothelium-dependent relaxations. Atherosclerosis causes arterial stenosis and impaired endothelium-dependent relaxations causes impaired vasodilation. Then both of them cause ARTERIAL Insufficiency that leads to Reduced Penile inflow.

On the other hand atherosclerosis causes damage to the trabeculae by causing smooth muscle atrophy and fibrosis then leading to veno-occlusive dysfunction. The final result is EXCESSIVE penile outflow. By the same token, impaired endothelium-dependent relaxations causes veno-occlusive dysfunction due to impaired relaxation of the trabeculae causing excessive penile outflow. So the final result of this 2 conditions leads to reduced penile inflow and excessive penile outflow so no blood is constantly present causing ED.

Another risk factor for ED that is important is sleep apnea or lung disease that is overlooked many times. Chronic hypoxia appears to be an independent risk factor associated with ED. Hypoxia affects the tone of the blood vessels and induces production of vascular growth factors, inhibiting endothelial-mediated relaxation and causing vasoconstriction. Oxygen may also regulate NO synthesis in the corpus cavernosum (CC). Perhaps hyperbaric chamber treatment might be a proposal to add to the stemcells injection protocol (more data and studies will be needed). The aging process is another risk factor and it is caused by the oxidative stress then leading to ED.

Vascular endothelial dysfunction is the most likely pathophysiology linking ED and coronary artery disease which all share almost the same risk factors such as Hypertension, hyperlipidemia, smoking, obesity, metabolic syndrome. patients with ED have high rates of macrovascular atherosclerosis. Low Testosterone [26,27] is associated with ED as well as with cardiovascular morbidity and mortality. Testosterone regulates the expression of NO synthase and PDE5 and maintains the integrity of the vascular smooth muscle and endothelium. Medications [27] such as Statin used to treat hyperlipidemia has being implicated to ED. B-Blockers potentiating the Alpha 1- adrenergic system can cause ED. SSRI, Risperidone, Olanzapine have the highest likelihood if causing sexual dysfunction.

Present treatment options for ED

There are 4 treatment options presently that are used routinely (Table 4). However in many occasions patients just receive PDI5 Inhibitors as option one [27-31]. It is very important to address the cardiovascular risk factors and to be treated accordingly. First line of treatment includes the following (Table 4): Psychosexual and couple therapy; this is for purely psychogenic ED and relationship problems, lifestyle modification; weight reduction in obese men, smoking cessation, physical Exercise, testosterone supplementation for hypogonadism. Second line treatment if the above fails or it can be added it on at the same time are the PDI5 Inhibitors. Third Line if PDI5 Inhibitors failed is (Table 4), intracavernosal injection, intraurethral injection, vacuum constrictive device, and combination therapy. Fourth line if all above failed is a penile prosthesis which has a satisfaction rate of 98% (Table 4). All of the above treatment options

offer no restration to the penile tissue and then consequently no cure available for ED. Important to mention that a cardiac evaluation might be necessary since ED is considered a presenting symptom of coronary artery disease.

In a study of 327 patients by A. A. Carvalheira, N. M. Pereira, J. Maroco *et al.* [31], showed the following: only 148 (45.3%) of patients were taking the PDE5 inhibitors. 160 (48.9%) had discontinued the PDE5 inhibitors 19 (5.8%) never took the PDE5 inhibitors. The reason to discontinue the PDE5 Inhibitors was 38% said the medication was not effective. 15.7% were concerned about heart safety 13.7 were concerned about cost. The highest dropout rate was in DM, 78% drop out rate, RP, 66%, and 8.7% discontinue because of lack of spontaneity. All current treatments treat only symptoms but do not address the underlying causes. The current research is focus to find a therapy that can reverse the pathogenesis of ED and potentially restore the ability to

Table 3. Published studies of stem cell therapy for noncavernous nerve injury, nondiabetic ed.

Publication year	First author	Animal model	Stem cell type	Modification/supplementation*	Transplantation	Reference
2007	Bivalacqua	Aging rat	Allogenic BMSC	eNOS transduction	IC	[117]
2008	Nolazco	Aging rat	Mouse SkMSC	None	IC	[118]
2010	Abdel Aziz	Aging rat	Allogenic BMSC	None	IC	[119]
2010	Huang	Hyperlipidemia rat	Autologous ADSC	None	IC	[120]
2012	Ma	TA injury rat	Autologous ADSC	None	SIS graft	[121]
2013	Castiglione	TA injury rat	Human ADSC	None	Intraunical	[122]

Table 4. Published studies of stem cell therapy for diabetic erectile dysfunction.

Publication year	First Author	Patients/animal model	Stem cell type	Modification/supplementation*	Transplantation route	reference
2010	Bahk	T2DM patients	Allogenic UCBS	None	IC	[123]
2010	Garcia	ZDF rat	Autologous ADSC	None	IC	[124]
2011	Gou	STZ rat	Allogenic EPC ⁺	VEGF transfection	IC	[125]
2011	Qiu	STZ rat	Allogenic BMSC	None	IC	[126]
2012	Qiu	STZ rat	Allogenic BMSC	VEGF transfection	IC	[127]
2012	Sun	STZ rat	Allogenic BMSC	None	IC	[128]
2012	Nishimatsu	STZ rat	Allogenic ADSC	None	IC	[129]
2012	Ryu	STZ mouse	Syngeneic SVF	None	IC	[130]
2013	He	STZ rat	Allogenic BMSC	KCNMA1 transduction	IC	[131]
2013	Liu	STZ rat	Human ADSC	VEGF transduction	IC	[132]

Table 5. Erectile Intensity Score.

	Almost never or never	A few times (much less than half the time)	Sometimes (about half the time)	Most times (much more than half the time)	Almost always or always
How often were you able to get an erection during sexual activity?	1	2	3	4	5
When you had erections with sexual stimulation, how often were your erections hard enough for penetration (entering your partner)?	1	2	3	4	5
When you attempted intercourse, how often were you able to penetrate (enter) your partner?	1	2	3	4	5
During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?	1	2	3	4	5
	Extremely difficult	Very difficult	Difficult	Slightly difficult	Not difficult
During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?	1	2	3	4	5

ED Intensity Score

Note: The following questions should only be completed by individuals who have been sexually active and have attempted sexual Intercourse in the past 3 months. For sexually inactive individuals, the questionnaire may be answered for the last period of time (3 months or longer) during which the individual was sexually active. All questions are preceded by the phrase over the past 4 weeks.

Note: Score: Severe: 1-7. Moderate 8-11. Mild 17-21. No ED: 22-25

have and or maintain a natural penile erection. One of those options is stem cells that have the potential to regenerate and restore tissue.

How do stem cells travel after IC injection?

It appears after IC injection the mesenchymal stem cells remain in the CC for at least 4 weeks. In several studies (preclinical data) showed between 1 hour to up to 4 weeks [32-52]. In a study by genomics/stem cells in urology” use of nanoparticles to monitor human mesenchymal stem cells transplanted into penile cavernosum of rats with erectile dysfunction” [32]. This study was performed to examine the treatment of erectile dysfunction by use of superparamagnetic iron oxide nanoparticles-labeled human mesenchymal stem cells (SPION-MSCs) transplanted into the cavernous nerve injured cavernosa rats as monitored by molecular magnetic resonance imaging (MRI)

Transplanted MSPION-MSC existed for up to 4 weeks in the cavernous nerve injured cavernosa rats. Erectile dysfunction recovered as demonstrated by increased ICP (intracavernosal pressure) and Mean arterial pressure and could be monitored by MRI. In previous preclinical data there were problems with cell tracking due to lack of specific markers. This recent Korean study using nanotechnology and MRI proved that MSC's remain in to the CC for 4 weeks. Mesenchymal stem cells can be differentiated into osteogenic, chondrogenic, or adipogenic components [32]. Stem cells have the capacity to differentiate into endothelial cells leading to new blood vessel formation. Also they differentiate into smooth muscle cells then leading to regeneration of the CC. However since they travel very fast from the CC from 1 hour to up to 4 weeks the proposed mechanism of action seems to be a paracrine effect [32-52]. The stromal vascular fraction (SVF) is obtained from a lipos aspirate (60 ml of adipose tissue usually is required). The description of our protocol will be depicted below. SVF is composed of mesenchymal stem cells, endothelial cells, fibroblasts, growth factors, pericytes, and smooth muscle cells. The SVF pellet contains most of the time 250.000 cells, but only 2 % can adhere in the plastic dish, so only 2% become ADSC (adult stem cells).

RP/Pelvic radiation

Prostate cancer is the most common malignancy in men: Approximately 80% prostate cancer is mainly localized [53-67]. In the USA in 2010: 217.730 new cases were diagnosed and reported deaths of 32.050. One of the major and devastating complications of RP is ED approximately 60.8% and ED post radiation therapy is 93.90 percent rate of discontinuation of PDE5 inhibitors in patients with ED after RP (radical prostatectomy) is close to 66% and they are only successful in 43%.

Cavernous nerve runs alongside the prostate. RP even with the introduction of nerve-sparing RP 30 years ago, inadvertent injury to the CN's or just even the CN's intact causes subtle changes that are not seeing or obvious during the RP. There are CN's injury models either by “crushing” or “resection” of the CN's (Table 5B).

In patients after Radical Prostatectomy or Radiation of the prostate, stem cells after IC injection travel to the major pelvic ganglia of CV (cavernous nerve) and then from there start regenerating the fiber nerves that had gone wallerian degeneration and consequently trying to improve the release of NO (nitric oxide) to try to reestablish the connection between the CN (corpus nerve) and the corpora cavernosa. Unfortunately after RP (radical prostatectomy) the CN gets injured and consequently no release of NO (nitric oxide) in the CC (corpus cavernosum) leading to no engorgement of small sinusoids

located in the CC and then this leads to hypoxia of the CC then smooth muscle cells are replaced by collagen. The preclinical data using CN injury models showed reduced smooth muscle to collagen ratio. This eventually leads to atrophy of smooth muscle cells in the CC (corpus cavernosum). The lack of NO explains the reason why PDE5 inhibitors don't work properly.

Diabetes mellitus

In 2011 in the USA, 25.8 million (8.3% of the population) and 10.9 million were 65 year or older affected by DM [68-74]. In 1980 in the world there were 153 million affected by DM. In 2008 in the world were 347 million affected by DM. It is estimated that by the year 2030 it would be 552 million affected by diabetes mellitus.

In patients with diabetes mellitus

50 to 75% of patients with diabetes mellitus have ED. Important to mention that ED in patients with DM appears 10 to 15 years earlier [74-76]. The success rate of ED in patients with DM taking PDE5 Inhibitors is only 44 percent and diabetic patients has the highest drop out rate closing to 78 percent. It is here where the IC Adult stem cell injection might be beneficial and perhaps a novel treatment to treat not only ED but also DM at earlier age or even before their insidious beginning.

Mechanism of action of DM causing ED

The proposed mechanism of how diabetes mellitus causes ED has to do mainly with apoptosis [76-81]. Preclinical data have shown mitochondrial fragmentation as well as reduced contents of CN (corpus nerve) cell death nNOS- positive neurons in the MPG (major pelvic ganglia), CEC (cavernous endothelial cells) and CSMC (cavernous smooth muscle cells). One of the main functions of adult stem cells (MSC) is their antiapoptotic capacity and ability to regenerate tissue, angiogenesis, antifibrotic and stem cell differentiation.

Peyronie's disease- associated ED

PD affects 7.1% of the general population [82-93]. There is a PLAQUE formation on the TA (tunica albuginea) that might cause pain, penile deformity and shortening of the erect Penis. PD also is accompanied by ED. However most PD patients are able to obtain an erection. In a study by Lopez [84]: 76 out of 95 (80%) had associated ED and 36 % had abnormal arterial blood flow. 59 % of PD in Lopez study had veno-occlusive dysfunction which seems to be the main cause of ED. However the plaque was never examined to evaluate it as a cause of the ED. No study so far provides evidence that PD (TA plaque) is a DIRECT cause of ED. It appears that TA abnormalities can cause ED. IC Stem cells injection improves the penile deformity as well as the associated ED.

Studies

Interesting enough the first stem cell ED was published in 2004 (Table 1) [25,94-131]. Then up to November of 2013, there have been a total of 34 additional studies (Table 2). 20 studies were published after 2011 (Table 2). So the question is why the acceleration on performing studies on ED that obviously occurred after 2012. There are 35 Preclinical Studies and the US and the French clinical trial; 19 studies for CN injury ED (Table 1 and 2) and 10 diabetic ED studies (Table 1 and 4). Most of the studies Stem cells were obtained from adipose tissue (Table 1). Stem cells were injected IC, IV or Periprostatic. All 35 studies Improve Erectile Function in ED patients and Animal Models (Tables 2-4). Studies showed increment of ICP by 70% compared with non-ED

models in order to produce erection enough for penetration (Tables 1-4). Studies showed improvement in Smooth muscle, endothelium and Nerve of erectile tissue (Tables 2-4). Few studies documented SC engraftment or differentiation, demonstrating that the benefit of the stem cells seems to be mediated by a paracrine effect perhaps mediated by Fibroblast growth factor, CXCL5 cytokine which is a potent angiogenic and Neurotrophic factor.

In DM it showed lowering of glucose and Hb A1C: Preclinical data showed 2 phases: Early phase in which the SC promote B-cell function. LATE phase improves insulin resistance. Mediated by increase in GLUT 4 expression and elevation of phosphorylated insulin receptor substrate 1 (IRS-1) and AKT protein kinase B in insulin target tissues.

As mentioned previously the majority of SC exit within 1 day to up to 4 weeks (recent study done by nanotechnology and MRI showed at 4 weeks exiting) and time dependent decline. Preclinical data has difficulty showing SC in the CC even though improvement in erectile function and the SC appear to have preference to travel to bone marrow, supporting the role of being mesenchymal cells. In CN injured rat model the SC travel to MPG (major pelvic ganglia) mediated by upregulated SDF-1. Then they travel to the bone marrow supporting their origin and then they act as a reservoir for sustained regenerative and repair activities. In preclinical data the average amount was 1 million stem cells injected into the CC.

A very important point is that the IC (intracavernous) route is very similar to IV (Intravenous). Cavernous sinusoids is just bundle of venules similar to Intravenous. Blood flow in a flaccid state in the CC is SLOWER compared to systemic circulation, thus allowing superior retention into the CC. As mentioned previously the penile vasculature is the most endothelial-rich area in the body so even small amount of cells would be enough for grafting. Since the rationale of how stem cells work seems to be more a paracrine effect that engrafting then small amount of stem cells will be enough to be injected and to see improvements. The theory proposed is that from IC route SC can travel to Bone marrow since they are mesenchymal stem cells and had predilection to go to one of their site of origin which is the bone marrow and then from there perhaps modulate the immune system, prevent apoptosis, vasculogenesis, to other organs perhaps the heart, kidneys as example and then to continue the regeneration process. The IC stem cell injection perhaps might be an alternate route that is less technical difficult than to attempt to seed SC into the pancreas or heart as an example. This alternate route needs more study. One way would be to use nanotechnology markers and track them by MRI and see what other actions take in other organs. Perhaps once in the bone marrow they just direct or send signals to other cells and work in that way. Present approaches for stem cell therapy is to deliver the cells on the site of injury as example doing a Cardiac Catheterization and putting them in the vasculature or injecting into the myocardium, which is a high risk procedure compared to IC injection.

Preclinical and clinical data also showed reduced peak velocities in ED models with an average of 22 cm/sec in cavernosal arteries (Table 4). After IC SC injection peak velocities increased to more than 70 cm/sec which seems to be the peak velocity needed to obtain an erection.

Allogenic umbilical mesenchymal stem cells have been injected as well into the CC. No adverse effect. No allergic reactions. In the Korea Study in 2010 by JY Bahk and Lee 15 million Allogenic Umbilical cord blood were injected into the CC. It showed an Antidiabetic effect in the 7 Diabetic patients Type 2.

There are currently 2 clinical trials. The French Trial Phase I-II looking at the safety of IC bone marrow mononucleated cells in post RP ED patients. The USA trial: looking at the safety and treatment efficacy of IC injections of autologous ADSC in organic erectile dysfunction.

Technique of IC stem cell injection: erectus shot technique and protocol

There is no description in the literature depicting exactly the IC stem cell injection. Preclinical and clinical data describe it as just IC injection. I will describe the ERECTUS SHOT Technique that if done properly will have minimal risk for complications. The protocol for ED will be described as well.

Since the 2 main mechanism of action of Stem cells work are either by Cell differentiation/engraftment and or Paracrine action. Cell engraftment has been poorly documented sustaining that is more the Paracrine effect of the Stem cells. Based on that principle, SVF is placed in PRP (Platelet-Rich Plasma). PRP is rich in growth factors and it is a simple procedure.

Patients undergo a detailed targeted history looking at risk factors as mentioned previously such as cardiovascular risk factors such as Hypertension, Hyperlipidemia, obesity, Metabolic Syndrome, sleep apnea as among others.

Questionnaires before treatment are used: ED Intensity score (Table 5). The ED intensity score relates to ability to have an erection and able to do partner penetration and ability to maintain an erection. A physical exam is performed looking for carotid bruits, heart murmurs, gallops, abdominal bruits, pulses and vascular and perfusion. Also a Genitalia exam, penis evaluation such as size at flaccid state and deformities, penile plaques, testicular size and testicular nodules. Also Rectal and Prostate Examination.

Penile examination

Length

Normal length is variable from race to race, but there is an accepted average. Measurement are taken in the non-erect state by pulling on the glans forwards to stretch the penis and dipping the ruler into the pubic fat that surrounds the penis down to the bone, so that the measurement is taken from the bone to the glans, and not from the skin to the glans. This measures the actual length of the penis and not the apparent length. The stretched non-erect length should at least be 7 cm. In the erect state, length measured in the same way should be at least 11 cm. The length of the vagina is 10 cm on average, which makes 11 cm a normal length for the penis.

Width and diameter

There is no consensus about the normal diameter, but if measured with a taper, the perimeter should at least be 10 cm. It has to be noted that the vagina is surrounded by muscles that tighten it around the penis, regardless its diameter.

Straightness

The penis is mostly straight. However, many men have variable degrees of curvature affecting the penis. This poses cosmetic problems. This may also make intercourse difficult or painful to the female if curvature is more than 30 degrees, especially if it is downwards or to either side. By curvature we mean that the penis is straight at its base but has a bending point distally. A straight penis pointing upwards,

downwards or to a side without a bent is not considered curved.

Angle

The penis usually makes an angle of around 90 degrees relative to the surface of the abdomen. This may vary. The angle may be acute with the penis pointing upwards, or obtuse with the penis pointing downwards. This is acceptable as long as it does not make intercourse difficult or disturb the individual or his partner psychologically. A rigid penis pointing downwards is an erect penis. Erection does not necessarily mean that the penis should point forwards or upwards.

Urethral meatus

This is the urine outlet at the tip of the penis in the glans. Hypospadias is a disorder where the urethral meatus is not at the tip but is somewhere along the lower surface of the penis. This may require surgical correction.

A protocol has been designed exclusively as part of the erectus shot procedure and includes: CBC, CMP, TSH, HBA1C, insulin, lipids, testosterone total and free, estradiol, LH, prolactin, PSA, U/A. Medical clearance with EKG is performed as well. Informed consent for IC injection and liposuction and informed consent for risks/benefits/possible side effects are discussed with the patient. Cardiac status evaluation as required.

Methods

Patient undergoes a fat harvesting using water assisted liposuction. One of the main reasons this technology is used is because of the integrity of the fat and even ability to see attached blood vessels to the adipose tissue. Also a fast recovery for the patient when compared to traditional liposuction or manual syringe aspiration. Water-assisted liposuction (WAL) is a liposuction procedure that provides favorable results concerning patient comfort and aesthetic outcome.

WAL adipose tissue is a valid source of adMSC, yielding numbers of cells that lie within the range of cell numbers isolatable from adipose tissue harvested with other techniques. The adMSC isolated from WAL tissue are viable and possess mesenchymal differentiation potential. In this respect WAL tissue may be used for autologous fat grafting and is an appropriate source for adMSC isolation. The stromal vascular fraction of WAL tissue showed high viability and contained an average of 2.6×10^5 CD34-positive cells per milliliter of tissue. The yield of stromal vascular fraction (SVF) from WAL harvested fat is reasonably high, with an average of 6.1×10^5 cells per gram of tissue and a high fraction of CD34 positive cells. The MSCs isolated from WAL harvested fat are adherent to tissue culture plastic and proliferate in culture [132].

Also simple learning curve for the new physician learning liposuction. As well as the ability to infiltrate the tumescent fluid in a very rapid way and very homogeneous. Also the equipment is able to remove 30 percent of the tumescent fluid infiltrated it so minimizing the risk of lidocaine toxicity that can be lethal. Also it has the ability to place more tumescent fluid as necessary. The system is closed and fat goes to a special sealed container called the lipocollector that keeps the adipose tissue sterile.

The areas chosen for liposuction are clean with a warm skin cleanser (chlorhexidine) in a sterile fashion preparation. The physician wears sterile gloves and surgical gowns for body, head and mouth mask. 100 ml of adipose tissue is usually harvested. Important to mention is that during the fat harvesting procedure no general anesthesia is used.

Minimizing the risk for complications from liposuction. The patient remains alert and awake. No IV sedation is necessary.

Laboratory suite is adnexa to our surgical room suite. Once fat is harvested then is taken to the Lab in a sterile fashion in the closed lipocollector. Laminar Hood has been activated previously. The lipocollector with the Fat is placed into the laminar Hood. Then fat is processed as per our Protocol. An animal origin free (AOF) enzyme is used for the fat digestion process or using our non-enzymatic fat processing that is operator dependent. Our non-enzymatic process focuses on washing and centrifugation. The amount and availability and clinical response of the SVF are similar but use no enzyme. Optionally you may use a flow cytometry or cell counter. Once the SVF is obtained, pellet is separated. The pellet is mixture with 12 ml of PRP.

Erectus shot technique

Anatomy and rationale for technique IC Injection

It is important to understand the anatomy of the penis in order to do the proper IC injection. Avoiding complications such as urethral perforation, perforation of the dorsal artery, vein rupture causing hematomas, priapism and or nerve damage. There are 3 cylinders in the penis surrounded in 2 thin layers of muscle with fascia and a layer of skin. One of the three cylinders is the CORPUS SPONGIOSUM that covers the urethra. The other 2 cylinders are: Right corpus cavernosum and left corpus cavernosum.

Each CC is composed of erectile tissues in the form of microscopic spherical spaces called sinusoids that accommodate blood and expand when blood flow into them increases. Each sinusoid is surrounded by smooth muscle that controls its diameter. The sinusoids are enclosed in the outer wall of the CC that is firm, expandable layer of fibrous tissue named the Tunica Albuginea.

The neurovascular bundle is a collection of nerves, arteries and a vein that course along the penis on its upper surface, from the glans to the base of the penis. It includes the nerves that convey sensation from the glans: the dorsal nerve of the penis. As described below one of the areas to avoid injection is at 12 o'clock because of the dorsal location of the neurovascular bundle. At the base of the penis (towards the abdomen), the corpora cavernosa are attached to the bony pelvis by adhesions to between their deepest parts (the crura) and bone, and by a ligament that attaches the corpora cavernosa to the middle of the pelvis and to the anterior abdominal wall: the suspensory ligament.

Each corpus cavernosum has a feeding artery that fills it with blood, and a draining vein that lets blood out. The vein starts inside the corpus cavernosum between its wall (tunica albuginea) and the sinusoids, such that if the sinusoids expand, they may compress the vein and prevent blood from exiting the corpus cavernosum. The penis is covered by skin, underneath which is a thin layer of tissue named the Colle's fascia, beneath which is a thin muscle called Dartos Muscle or Buck's fascia.

Description: erectus shot IC injection

A triple anesthetic cream is placed on the penile area, left for 45 minutes. Patient is also instructed on the use of self-administered oxygen and nitrous oxide 50-50 concentration. It is a patient-controlled analgesic for In-Office use and takes effects in seconds, offering immediate relief of pain and anxiety. Important to mention that in this concentration of 50-50 oxygen/nitrous oxide it behaves as an analgesic and not as an anesthetic. Patients undergoing the Erectus shot procedure might anticipate pain which in terms creates anxiety.

The erectus technique includes proper marking of the penis in the standing position. The IC injection is performed in the supine position with head elevated at 45 degree angle. The penis is marked as follow: In the standing position in which the penis is in a flaccid state the physician then elevates and grabs the penile organ in a 90 degree angle, parallel to the abdomen.

A clock imaginary marking is performed. Use a marker pass 3 lines: one at 12 o'clock, the second one at 3 o'clock and the third one at 9 o'clock. Use a marker to identify the point of injections which will be at 2 and 10 o'clock. Usually 2 or 4 points of injections are done, depending on the size of the penis or sometimes the amount of PRP available from the patient. The 12 ml suspension of PRP and pellets is divided in 3 ml each in syringes of 3 ml. A 28^{3/4} needle is used for injection.

The needle will penetrate the following structure: skin, then a thin layer called the colle's fascia, then a thin muscle called dartos muscle or buck's fascia then the injector will feel the sensation I called "rupturing a thin membrane". Upon entering the corpus cavernosum the injector will experience a sensation of "rupturing a thin membrane". Once inside the CC there should be no resistance to the injection process and the mixture of PRP and SVF should flow easily.

At the end of the procedure a compression garment is placed on the area of the liposuction. Patients can have sexual intercourse the same day. As a measure of precaution patients are asked to void before discharging home to make sure there is no gross hematuria. The Erectus technique if performed correctly should no cause any echymoses, hematomas.

Proper training is required: The main elements are: Stem cells Fat digestion process, laboratory requirements, stem cell knowledge, blood processing protocols to obtain the PRP (Platelet Rich plasma) liposuction technique with proper patient selection and tumescent anesthesia, erectus IC injection technique. It is imperative that the physician providing this treatment is properly trained.

The erectus shot procedure and protocol has being designed to be done as an outpatient. No general anesthesia is required. Using the tools mentioned the procedure can be performed in an office setting following basic laboratory and liposuction guidelines. The linear curve to learn the procedure depends on many factors. Those factors might include prior liposuction training or some basic surgical skills. For a physician with no experience at all the linear curve goes exponentially and easy to achieve depending on their training. Adipose tissue from water assisted- liposuction.

Conclusions

Our technique it is a safe procedure obtained from lipoaspirate from liposuction. Our preferred technique is the WAL (water- assisted liposuction) due to higher counts after fat digestion and easy on the patient and excellent integrity of the adipose tissue. Preclinical data and clinical data showed recovery in erectile dysfunction. Proper training and stem cell protocol guidance are important in order to avoid complications. Complications are rare if proper procedure technique is followed. Laboratory guidelines and fat digestion process using either Animal Free origin enzyme or non-enzymatic allows for proper SVF with good viability and excellent counts of stem cells.

The mechanism of action of the IC stem cells appears to be related to a trophic mechanism. The engraftment appears to be poorly documented, even though improvement in ED is achieved. However the stem cells appear to remain the CC for at least 4 weeks. Then they

travel to the bone marrow as they are mesenchymal in origin.

The objective of this article is to bring preclinical data that can be applied to the outpatient clinical setting bringing a new protocol. The other main point of this review is to propose that perhaps using the IC injection might be an alternate route to deliver Mesenchymal cells to the circulation since it resembles the IV. The proposed idea is that perhaps using the IC injection the stem cells travel to the bone marrow and from there direct and or sent signals to other organs such as the pancreas, obviating the need to place stem cells into the pancreas directly which is a very high risk procedure for complications such as bleeding, pancreatitis, sepsis among others. The same might perhaps apply to other organs such as the heart, which currently studies deliver stem cells into the coronary circulation being flushed rapidly or injected into the myocardium, which again is a very high risk procedure for complications.

All preclinical and clinical data showed the IC stem cells injection to be safe and all showed improvement in IC peak systolic pressure. Also studies showed to be antidiabetic, lowering blood sugar and HbA1C. ED appears at least 10 to 15 years earliest before DM onset, so perhaps injecting those patients at earlier time might ameliorate not only the devastating effects of ED but also Diabetes Mellitus. More studies are needed to see the trafficking of stem cells from the CC to other organs. Perhaps using nanotechnology and MRI to track down the stem cells.

Patients with ED due to RP and or radiation, diabetes have a high failure rate to PDE5 (I) inhibitors and unfortunately this therapy offers no restorative function since they need nitric oxide production from the endothelial cells and cavernous nerve to be effective. Unfortunately DM and RP patients have a very low production of Nitric Oxide. Stem cells have the potential to reestablish function, in the cavernous nerve and endothelial function enhancing the nitric oxide production making the PD5 inhibitors to work better in the setting of DM and RP.

In conclusion, the use of intracavernosal autologous adipose derived stem cells injection appears to be a safe, and effective alternative option for patients suffering with ED related to DM, Post RP, and organic causes. More studies are underway to hopefully make this procedure available to the general population.

Conflicts of interest

None.

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