Review

Mechanism of action and delivery possibilities for TGFβ1 in the treatment of myocardial ischemia

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Abstract

Myocardial ischemia-reperfusion (IR) injury is associated with structural alterations involving both the necrotic and the non-necrotic myocardium. These changes are referred to as myocardial remodeling. In addition to the loss of critical cardiomyocyte mass through cell death, there are further structural alterations associated with scarring, as well as changes in a family of endogenous enzymes, the matrix metalloproteases (MMP), which cause loss of myocardial extracellular matrix (ECM) [Janssens S, Lijnen HR. What has been learned about cardiovascular effects of matrix metalloproteinases from mouse models. Cardiovasc Res 2006;69:585–594., Wainwright CL. Matrix metalloproteinases, oxidative stress and the acute response to acute myocardial ischaemia and reperfusion. Curr Opin Pharmacol 2004;4:132–138.]. The chemokine TGFβ1, which has wide-ranging effects upon cells and tissues, is showing promise as a useful drug/agent for the limitation of IR injury. Coupled with the identification of TGFβ1 as a therapeutic agent for IR treatment are investigations into its mode of delivery to the patient. Gene therapy utilizing delivery by viral vectors is just one of many possible ways to deliver TGFβ1 for IR treatment. In this review we discuss the mechanisms of action of TGFβ1 and how it might be delivered successfully to patients under risk of or who are actively undergoing acute IR injury.

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1. TGFβ1 and its receptors

TGFβ1, a member of the TGFβ family, is secreted only in its latent form from cells and must be activated to mediate its functions [3,4]. Members of the TGFβ family are typically produced as dimeric latent precursors, which are subsequently activated in the extracellular environment to release the receptor-binding ligands that share a common structural motif known as the cysteine knot. Several members of the TGFβ family play a role in vascular biology. TGFβ1 (the first member of the family to be discovered and the best-studied member to date) is present in high levels in the healthy blood vessel wall, whereas the closely related TGFβ2 and TGFβ3 isoforms are either absent or present only at low levels. TGFβ1 affects a variety of biological processes, such as proliferation and differentiation of cells, remodeling, angiogenesis, inflammation, cytokines, lipid metabolism, apoptosis, fibrosis and immunoregulation [5,6].

There are three distinct TGFβ1-binding receptors on cellular membranes. It has been postulated that TGFβ1 exerts its functions by directly binding to type 2 receptors, which transfers the signal to type 1 receptors. Type 3 receptor, a part of it also termed endoglin, modulates this binding signal, but it may not directly participate in signal transduction [7]. The TGFβ1 and type 2 receptor complex, then signals, through an intracellular serine/threonine kinase domain on the type 1 receptor, which phosphorylates downstream substrates, such as Smad proteins [8].

In recent studies in cultured coronary artery endothelial cells, angiotensin II (Ang II) was shown, via MAPK activation,
to enhance endoglin, as well as TGF-β1 type 1 and 2 receptor expression in a concentration and time-dependent fashion [9]. This upregulation of endoglin expression was blocked by the Ang II type I receptor blocker losartan. Notably, Ang II concurrently decreased TGF-β1 levels in the endothelial cells. Previous studies have shown upregulation of MMP expression by Ang II [10].

### 2. Alterations in gene expression during myocardial ischemia

A number of genes are altered during and soon after myocardial ischemia. These include genes for RAS, cytokines [such as TNF-α, interferon-γ and interleukins (IL-β, IL-6, IL-8)], mitogens, ROS, NOS, MMPs, TIMPs, and leukocyte adhesion molecules (such as, P-selectin, ICAM-1 and VCAM-1). A number of studies have shown alterations in a number of transcription factors, particularly the oxidative stress-responsive transcription factor NF-κB, which translocates from cytoplasm to the nucleus during ischemia-reperfusion. The nature of NF-κB proteins (p50, p52, p65, RelB or RelC) varies from cell to cell depending on extracellular signals [11]. Gene transcription of NF-κB decoy, which blocks the transcription factor, appears to block ischemia-reperfusion injury in its early stages [12]. On the other hand, activation of NF-κB may represent an early response for the transcription of target genes whose products may have a role in long-term cardioprotection [13]. NF-κB also induces transcription of iNOS, which may be temporarily depres left ventricular function, but preserve cardiac function over the long-term [14]. Several other related signaling pathways, such as activation of protein kinases [12,13] are also important during ischemia-reperfusion injury. There is evidence for upregulation of genes for a number of growth factors during myocardial ischemia.

Li et al. [15] showed that induction of myocardial stretch in an isolated perfused Langendorff preparation by inflation of an intraventricular balloon to an end-diastolic load of 35 mm Hg for 30 min resulted in a nearly 6-fold increase in VEGF mRNA level not only in the chamber subjected to stretch (left ventricle) but also in the unstretched right ventricle, thus raising the possibility of a soluble factor mediating stretch-induced increase of VEGF expression. This was further confirmed by demonstrating that coronary venous effluent collected from the stretched heart and used to perfuse isolated hearts in which no balloon was present was able to induce VEGF expression in these normal hearts. Importantly, inhibition of TGF-β1 activity using a neutralizing antibody, but not antagonists/inhibitors of endothelin and angiotensin II, eliminated stretch-induced increase in VEGF expression. Staurosporine, a protein kinase C inhibitor, also blocked stretch-induced increase of VEGF expression. Measurement of TGF-β1 concentration in the perfusate demonstrated increased amounts of the cytokine after myocardial stretch, and addition of TGF-β1 protein to the perfusion buffer resulted in increased VEGF expression in control hearts. These results suggest that stretch-induced increase of VEGF expression in the heart is mediated at least in part by TGF-β1.

### 3. MMPs and cardiac remodeling

Structural alterations in the heart begin early during myocardial IR injury and involve a family of endogenous enzymes, MMPs, which cause loss of myocardial extracellular matrix (ECM) [1,2]. Although MMPs differ in substrate specificity, cellular sources and inducibility, they share similar function with regard to the degradation of ECM components. MMPs are activated in the early stage of ischemia, and are involved in long-term myocardial remodeling [1,2]. In the early stages, MMPs are responsible for breakdown of basement membrane, increase vascular permeability, and enhance leukocyte migration outside the vascular lumen [16], as well as for myocardial rupture and apoptosis [17]. In the later stages, production of MMPs is thought to relate to ventricular dilatation, aneurysm formation, and heart failure [1,2].

A recent study suggests that expression and activity of neutrophil-derived MMPs may relate to angiogenesis and the effect of MMPs can be blocked by TIMPs, endogenous inhibitors of MMPs [18]. Other recent work shows that ox-LDL, which is found in abundance in the IR heart, upregulates the expression of MMPs without significant effect on TIMPs expression, thereby altering the balance between these mediators of cardiac remodeling [19]. Together, the interaction between RAS, TGF-β1 and MMPs/TIMPs provides a model for the pathophysiologic alterations in different stages of myocardial ischemia.

### 4. Tissue protection by TGF-β1

A number of growth factors, such as TGF-β1, VEGF, basic fibroblast growth factor (FGF), human growth hormone (HGH) and hepatocyte growth factor (HGF), have been shown to exert cardioprotective effect in several experimental and clinical studies [20–22]. Early in its existence, the protective cytokine idea was expanded beyond the smooth muscle component of the vessel wall. Gene knockout studies demonstrated that TGFβ exerted a dramatic antiinflammatory signal in constitutive fashion. Loss of TGFβ, resulted in perinatal death after unbridled leukocyte extra-vasation in almost every organ system [23,24]. Because accumulation of leukocytes is a characteristic signature of the change from normal structure to early IR lesion, the value of this cytokine on cardioprotection has been examined in several studies. Initial studies were performed by Lefer et al. [25,26], who attributed its cardioprotective effect to preservation of endothelium-derived relaxing factor during IR in the feline myocardium.

Over a period of years, a range of other potentially protective effects of TGFβ in cell culture were identified, including suppression of proinflammatory adhesion molecule expression by the vascular endothelium. Mallat and Tedgui...
have provided an excellent overview of the immune-modulatory role of TGFβ1, in which they highlight the central role of TGFβ in the regulation of T-cell biology and its implications for chronic inflammation that characterizes chronic ischemia.

Unfortunately, early studies by Lefer et al. [25] on the potentially cardioprotective effects of TGFβ1 did not get much-needed attention. Later while examining the effect of platelets on cardiac tissues exposed to IR, Yang et al. in our laboratory identified that platelet-derived TGFβ1 exerts potent cardioprotection during acute IR [28]. Several studies in our laboratory, in different settings, confirmed the cardioprotection by this cytokine [20,28,29].

Studies from other laboratories also suggest that short-term exposure to TGFβ1 can have major salutary effect against IR injury in tissues besides the heart, such as the brain, lung, and kidney [30–33]. Our research has shown an upregulation of TGFβ1Latent during IR, but a reduction in TGFβ1ACT levels, suggesting a decrease in the conversion of latent to active form of TGFβ1 [20]. The increase in TGFβ1Latent during IR could represent body’s attempt to modulate tissue injury by upregulating the local survival mechanism(s). TGFβ1 protects the hearts against injury by several well-accepted mechanisms. These include preservation of nitric oxide (NO) degradation, prevention of ROS generation, and reduction in TNFα release [25]. Chen et al. showed that the cardioprotection by recombinant TGFβ1 is associated with modulation of NO synthase (NOS) and protein kinase B (PKB) [29]. It was shown that ox-LDL-mediated adhesion molecule expression in cardiac myocytes was blocked by rTGFβ1 in vitro. Furthermore, TGFβ1-treated rats had a smaller infarct following IR and reduced oxidative stress as measured by NADHP p67phox expression. More recently, our group selectively upregulated TGFβ1 in the hearts of SD rats and found these rats to be protected from IR injury (unpublished data). The cardioprotection by TGFβ1 was found to be associated with an inhibition of mitogen-activated protein kinases (both p38 and p42/44MAPK). (unpublished data). These observations suggest that TGFβ1 exerts cardioprotection and this salutary effect is mediated by modulation of TNF-α, ROS, PKB, MAPK and NOS pathways.

Taken together, these studies allow a plausible molecular model for the role of TGFβ in the ischemic heart. High levels of TGFβ maintain a feedback loop in which type 2 receptor remains high and the heart retains its differentiated phenotype with lots of contractile proteins and relatively little ECM. If some external factor intervenes to reduce TGFβ activity sufficiently in a particular region, the heart responds by reducing type 2 receptor expression. This is the “first hit” of reduced TGFβ activity that promotes early cardiac tissue injury.

5. Support for the concept of TGFβ1-mediated cardioprotection

Genetic manipulation has become the standard method for determining the importance of gene products in disease processes. The gene of interest is knocked out, and the impact on disease progression monitored. Unfortunately, the TGFβ1 null mouse dies soon after birth from severe multifocal inflammation thereby preventing us from studying myocardial ischemia in the absence of TGFβ1. Two approaches have been taken to get around this problem: TGFβ1 null mice can be bred on a SCID background, allowing them to survive into adulthood [34] alternatively, mice heterozygous for the TGFβ1 deletion can be studied [35–37]. The TGFβ1+/− mice have lower levels of TGFβ1 in a number of tissues [36]. Adult TGFβ1+/− mice have reduced levels of smooth muscle specific-α-actin and smooth muscle-specific myosin heavy chain in the aortic wall. Interestingly, when these mice were fed a high-fat diet, they exhibited endothelial activation that was not observed in their wild-type littermate controls [25]. These observations demonstrate that reduced TGFβ1 production renders the tissues susceptible to endothelial activation.

More recently, several other groups have used different methodological approaches to draw similar conclusions. Both Mallat et al. [38] and Lutgens et al. [39] used in vivo neutralization approaches to show that depletion of TGFβ1 in the blood vessel wall of adult ApoE-deficient mice was sufficient to exacerbate lipid lesion development. The amount of lipid deposited was increased in both studies, as was the number of macrophages and other inflammatory cells accumulating in the developing lesion. Lutgens et al. [39] went on to demonstrate that neutralizing TGFβ1 decreased ECM deposition, confirming the role of this cytokine in regulating the balance between an unstable, proinflammatory lesion phenotype and a stable, matrix-rich phenotype. Taken together, these studies provide strong arguments that TGFβ1 may protect against unstable disease.

6. Potential role of TGFβ1 in long-term remodeling of heart

The role of the cytokine TGFβ1 in vascular biology is not uncontroversial. TGFβ1 is expressed in the artery wall, and its expression is increased in animal models of vascular disease and in some types of human arterial lesions. The major concern on the role of TGFβ1 comes from the knowledge on its pro-fibrotic effect. Nevertheless, reasonable arguments can be formulated that support both pro- and anti-fibrotic roles for TGFβ1. For example, it may act primarily as a mitogen and as an inducer of cell migration and ECM synthesis. These activities would contribute to collagen formation. It is also possible that TGFβ1 acts as a cytostatic and immunosuppressive agent, preventing fibroblast division and decreasing vascular wall inflammation. These activities would prevent or mitigate fibrous tissue formation. Further, some cytokines are considered oncogenes; TGFβ1 is one of those cytokines with a mild, but significant, oncogenic potential.

Gene delivery has been used as a strategy for uncovering the biological roles of VEGF, FGF, and other secreted
over-expressing TGFβ1 heart will enable researchers to exploit the power of animals be confirmed.

The acute phase may limit the early signal that would this it was postulated that limitation of ischemia injury in inclusion TGFβ1, TGFβ1ACCT, in the hearts by gene therapy approach limited IR injury and simultaneously, significantly reduced collagen signal [41]. From this it was postulated that limitation of ischemia injury in the acute phase may limit the early signal that would otherwise lead to cardiac fibrosis. However, this needs to be confirmed.

Extension of vascular gene transfer techniques to the rat heart will enable researchers to exploit the power of animals over-expressing TGFβ1 to answer specific questions about the roles of individual molecules in the development or regression of cardiac remodeling during chronic ischemia. This approach is extremely useful since the specific upregulation of TGFβ1 does not alter the phenotype of the animal and does not result in excessive mortality from altered gene expression which may happen with the altered total body TGFβ1 expression.

7. Potential methods of delivering TGFβ1 for treatment of ischemic injury

While cytokine delivery by intravenous injection is now the predominant mode of delivery, it is clear that intravenous injection has many disadvantages, and new delivery and treatment strategies are needed. There are now a wide variety of new options of chemokine delivery, some of which are now in use with chemokines other than TGFβ1. Yet other delivery techniques are only in the planning stage.

Here we discuss a variety of these treatment/delivery options, any one of which might be mated with TGFβ1 for improved efficacy. Perhaps the most exciting of these new options is TGFβ1 gene therapy, which has many potential advantages over intravenous cytokine injection. Of these, perhaps most important, is that fresh, active, continuous TGFβ1 secretion results from gene therapy. Yet such constitutive expression might not be the ideal approach for treating acute ischemic injury or for modulating remodeling following chronic ischemic. Regulated expression, during acute and chronic phases might be more desirable. The approach of introducing encapsulated particles which release TGFβ1 might also be a beneficial alternative to direct gene therapy. Furthermore, injecting altered-TGFβ1 might be advantageous if its half-life could be extended.

8. Delivery of TGFβ1 by direct injection

As mentioned earlier, the most widely used method for the administration of a cytokine, TGFβ1 or some other cytokine, has been through direct intravenous administration. Direct injection has specific advantages, and these include ease of administration, for example in the emergency room, during the acute phase. This would allow for immediate therapeutic effect. But most cytokines, including TGFβ1, are broken down rapidly by proteases or are otherwise quickly eliminated. Thus, the delivery must be in a high dose. The commercially available cytokines are also subject to degradation before administration into the patient, and thus have low biological activity. For example, the biological activity of freshly secreted granulocyte macrophage-colony stimulating factor (GM-CSF) is 100 to 1000 times more potent than the commercial preparation [42]. Second, patient compliance is an important issue particular if frequent doses are to be administered. This is particularly important for agents such as TGFβ1 which must be present over an extended period, perhaps likely weeks to months, to have an effect on cardiac remodeling.

9. Latent and active forms of TGFβ1 and their half-lives

A critical issue for all proteins which might have therapeutic benefit are their half-life in plasma. The issue of half-life of TGFβ1 is a complex one as this cytokine is expressed in a latent form which is then cleaved into an active form TGFβ1ACCT with much higher biological activity. Wakefield et al. have reported that the half-lives of these two forms are vastly different, with a >100 minute half-life in plasma for the latent form and a 2–3 minute half-life for the active form [43]. Availability of this dual form may allow for an additional cytokine activity.

In the context of half-life, there are some noteworthy observations. Beck et al. showed that the effects of active TGFβ1 remained evident for over a day with the administration of the active form [44]. McCaffrey et al found that certain extracellular components could protect active TGFβ1 from proteolytic degradation and extend its half-life three fold [45]. One unconfirmed report by Rollins et al indicated that surface-bound TGFβ1 was not degraded and remained biologically active for one week [46].

10. Prolonging the half-life of TGFβ1

One way of improving the efficacy of TGFβ1 administered intravenously is through modification to improve its stability or reduce its clearance. There are a variety of methods for doing this, including fusion of the cytokine with longer-lived proteins, such as antibodies, antibody components, or albumin [47–50]. For example, the fusion of albumin with interferon-alpha resulted in an increase in its half-life from 1.7 h [51] to 159 h [49]. Another well-
documented approach to increasing a cytokine’s half-life is by conjugation of high molecular weight polymers of polyethylene glycol (PEG) [52]. This modification significantly decreases the rate of clearance from plasma, and thus increases the cytokine’s half-life. The longer half-life of PEG-proteins appears linked directly to the extent of pegylation. PEG-interferon-alpha-2a (PEG-IFNα-2a) and PEG-granulocyte colony-stimulating factor (PEG-G-CSF) are in present use. Shechter et al. coupled moieties of 2-sulfo-9-fluorenylmethoxycarbonyl (FMS) to interferon-alpha and this increased its half-life almost 10 fold, from 4 to 35 h [53]. However, the modified cytokine did have altered biological activity. Another approach taken by Adams et al. was the fusion of the latency-associated peptide (LAP) of TGFβ1 to IFN-beta which improved its half-life to 55 h [54]. To our knowledge, none of these modifications have employed TGFβ1.

11. Genes which might substitute for TGFβ1

While TGFβ1 has been demonstrated to have certain therapeutic uses it is a secreted protein and has the potential for systemic effects. Some of these effects might be undesirable. For example TGFβ1 is known to have significant immune suppression characteristics. Thus another, possibly better, approach is to utilize the genes downstream from TGFβ1 in its signal transduction pathway, through which TGFβ1 acts. These downstream genes will code for intracellular proteins. Thus the use of such genes would result in therapeutic effects limited only to those cells which are transduced, or specifically treated. For TGFβ1ACt mediated protection of cells from ischemia-induced injury one downstream gene that may be relevant is Akt/PKB[29]. This gene has also been shown to be downregulated in myocardial ischemia and has also been shown to protect cardiac myocytes and hepatocytes exposed to ischemia [55].

12. Viral gene therapy for delivery of TGFβ1

As just discussed, a major alternative to intravenous therapy is to introduce TGFβ1 as a gene. The TGFβ1 gene therapy has theoretical benefits of a more permanent treatment. The TGFβ1 gene can be delivered in its native wild type latent form or as a pre-activated (TGFβ1ACT) form. The TGFβ1 gene can be expressed with tissue-specific or disease-specific transcriptional promoters allowing for favored expression. For example, for ischemia the TGFβ1 gene could be selectively expressed with a hypoxia-inducible promoter [56]. The TGFβ1 gene could be delivered by vectors showing specific cell or organ tropism, resulting in targeted deliveries [57,58]. Alternatively, the TGFβ1 gene could be introduced into cells in culture and then the resulting cells reintroduced into the patient [59].

The in vivo approach: Simply put, the in vivo approach is the direct injection of the TGFβ1 gene therapy vector virus into the animal or patient with resulting gene transduction soon after the time of injection. The major advantage of this approach is its simplicity. However, there are significant disadvantages as well. While the site of TGFβ1 vector injection can be varied, gene delivery by this approach is occurring under poorly defined conditions. Thus variable results can be expected. Yet due to its simplicity, the in vivo approach is the most likely form of gene therapy treatment at this time.

The ex vivo approach: The major alternative approach to in vivo introduction of TGFβ1 genes is the ex vivo approach of gene therapy. While this approach requires more extensive resources and time there are benefits to this type of treatment. The ex vivo approach requires the removal of desired target cells from the body. These cells could be grown in tissue culture and treated with TGFβ1 vector under optimal conditions, allowing for high success rate. As these cells are treated in the laboratory the cells could be further characterized at this time, allowing for a complete knowledge of the situation before reintroducing the genetically altered cells back into the animal or patient. The disadvantages of this approach would be additional cost and time, and the difficulty in determining the specific cell type that results in best treatment efficacy.

13. Use of virus vectors for TGFβ1 delivery

There are three major virus types in use today for gene therapy: retroviruses/lentiviruses, adenoviruses and aden-associated virus. Here we discuss them with regard to TGFβ1 gene delivery briefly.

Retrovirus vectors: The first viruses used to transfer genes into cells were the retroviruses. This was in fact discovered early in the 20th century when isolated fluids from tumors when transferred to other animals caused tumors in the recipient animals. Although not understood until the late 1970s, the fluid contained retroviruses which accidentally recombined with cellular proto-oncogenes, resulting in viral oncogenes (v-one). First man-made recombinant retroviral gene delivery studies using Moloney murine leukemia virus (MoMuLV) were published in 1983–1984 [60,61]. Since then MoMuLV retroviral vectors have been used for TGFβ1 gene delivery [62]. Yet research and clinical studies demonstrate that these viruses cause at least two serious side effects. First, they can promote malignancies [63,64]; second, they genetically alter germ line cells [65,66]. Recently, the development of Lentivirus vectors, a subcategory of retroviruses, is reinvigorating the field of retroviral gene transfer. The Lentiviruses include immuno deficiency virus (HIV), and this virus type may offer some advantages, such as higher transduction levels, over the traditional murine MoMuLV-based vectors [67]. Yet their safety still remains an issue.

Adenovirus vectors: Adenovirus vector delivery was first demonstrated in 1984. These vectors have been used to deliver TGFβ1 [68]. The attributes of adenovirus-based delivery are now known. First adenovirus transduces at high
levels early on but the transgene expression is transient. Second, adenovirus infection is almost always associated with significant inflammation [69]. While the inflammatory reaction is often transient, it is a significant enough problem to act as a confounder in many experiments. In fact the use of an adenoviral vector has led to the death of one patient [70]. Yet, adenovirus may still be a viable option with safeguards.

Adeno-associated virus (AAV) vectors: Since the mid-1960s, AAV has been known to latently infect cells [71]. Its first use as a gene delivery vehicle was demonstrated in 1984 [72,73]. Due to the elevated risks of retroviral and adenoviral vectors discussed above, the use of AAV vectors has dramatically increased over the years. AAV vectors show major differences with the other two virus vector types. AAV vectors often display only low initial expression, but the expression rises over time and ultimately is superior to the other two types over the long-term [74]. The AAV vector provirus can also be quite stable either as a chromosomally integrated element or as a episomal element. AAV vectors are also not known to cause significant inflammation or malignancies [75]. Finally, AAV has been shown to be effective in the delivery of TGFβ1 [76,77].

14. Delivery of TGFβ1 by encapsulated engineered cells

Coupled with the development of gene delivery technologies and ex vivo cell modification is the ability to reintroduce these cells back into the body [59]. However, engineered primary cells are mortal and are lost over time. This necessitates the genetic engineering of stem cells which is a field of study of its own. An alternative is to genetically alter immortalized cells which will have a long life span and produce TGFβ1 in a sustained fashion. Yet these cells must be controlled and protected. As they are essentially cancerous, their growth must be limited. Further, these cells are not “endogenous”, they will also be subject to attack and destruction by the immune system. An artificial capsule would prevent movement of these engineered cells yet allow for enough permeability to allow nutrients and oxygen to reach the engineered cells, as well as to allow the secreted cytokine to diffuse out of the capsule. Stability, low stimulation of inflammation, and lack of cell toxicity are also significant advantages. The field of genetically engineered cells is now well established and overlaps with gene therapy approach.

Encapsulation requires the ability to utilize appropriate material to seal the cells. The first reported use of a semi-permeable aqueous microencapsulation dates back to the 1960s, and the spectrum of materials being developed is expanding. Some have even entered clinical trials. One recent example is a photopolymerizable PEG polymer to encapsulate islet cells for the treatment of type 1 diabetes [78,79]. This approach has been developed to introduce islet cells in monkeys for better control of blood glucose levels. The transplanted cells continued to perform for up to 20 months post-introduction. Another interesting approach is to use photolithography techniques adapted from the semiconductor industry to encapsulate living cells. There are as yet no studies employing encapsulated engineered cells for TGFβ1 delivery.

15. Potential of nanotechnology to deliver TGFβ1

Nanotechnology is still in its infancy so it is too early to accurately gauge its importance in the delivery of TGFβ1 and other chemokines. However it is widely believed that nanodevices could be used to deliver cytokines to needed locations. There are many forms of carbon nanostructures that can be generated, from balls, to barrels, to tubes, with the most studied being the tube. There are multiple ways for molecules such as TGFβ1 to be associated with nanotubes. Such cytokines, as protein, can be loosely bound or covalently bound on the outside of the nanotube. This “scaffolding” delivery seems a less useful approach to us. It would seem desirable that TGFβ1, whether as a protein or a DNA gene, to be protected and concentrated inside the nanotube. Thus, possibly the most useful application of nanotubes may be as “delivery trucks” for TGFβ1 [80–84]. This would minimize TGFβ1 loss and protect the molecules from degrading enzymes.

The nanotube vectors, carrying TGFβ1 protein or gene (for prolonged expression), can be further externally modified by the attachment of specific antibodies or ligands [85]. Such modifications would allow the targeting of specific cell types, resulting in nanotube tropism, similar to that of many viruses. Another exciting possibility is combining the “best of” nanotubes with the “best of” viral vectors such as AAV. The DNA encapsidated within the nanotube could include the AAV terminal repeats at the end of the DNA to provide for the favored phenotype imparted by these elements [72,73]. These and other exciting possibilities suggest that nanotube technology has a useful future for the delivery of biologically active proteins/genes, such as TGFβ1, as has been accomplished with interferon-alpha delivery into cells via carbon nanotubes [86].

16. Summary

Here we have provided the background on the cardioprotective effects of TGFβ1, certainly in the acute phase of ischemia and possibly during chronic ischemia. The issues that need to be sorted out are the best methods to deliver this cytokine into the appropriate cells. In this context, we have defined the potential of various existing and novel technologies.

References


