Research and Reviews: Drug Delivery

Drug Delivery and Nanoformulations for the Cardiovascular System

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ABSTRACT

Therapeutic delivery to the cardiovascular system may play an important role in the successful treatment of a variety of disease state, including atherosclerosis, ischemic-reperfusion injury and other types of microvascular diseases including hypertension. In this review we evaluate the different options available for the development of suitable delivery systems that include the delivery of small organic compounds [adenosin A_{2A} receptor agonist (CGS 21680), CYP-epoxygenases inhibitor (N-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide, trans-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy] benzoic acid), soluble epoxide hydrolase inhibitor (N-methylsulfonyl-12,12-dibromododec-11-enamide), PPARγ agonist (rosiglitazone) and PPARγ antagonist (T0070907)], nanoparticles, peptides, and siRNA to the cardiovascular system. Effective formulations of nanoproducts have significant potential to overcome physiological barriers and improve therapeutic outcomes in patients. As per the literature covering targeted delivery to the cardiovascular system, we found that this area is still at infancy stage, as compare to the more mature fields of tumor cancer or brain delivery (e.g. blood-brain barrier permeability) with fewer publications focused on the targeted drug delivery technologies. Additionally, we show how pharmacology needs to be well understood when considering the cardiovascular system. Therefore, we discussed in this review various receptors agonists, antagonists, activators and inhibitors which will have effects on cardiovascular system.

INTRODUCTION

The cardiovascular system plays a major role in health and disease in the body, and any deregulation in the cardiovascular system can lead to cardiovascular diseases, including atherosclerosis, myocardial infarction and microvascular disease [1,2]. One of the major risk factors for cardiovascular disease is high blood pressure or essential hypertension (HTN). According to the CDC in 2011, around 75 million adults in America has high blood pressure, which is one in every three adults. Despite the fact that HTN is easy to diagnose, it can be maintained through a healthy diet, regular exercise, medication, unfortunately, the serious condition can develop in untreated hypertensive patients. Also, hypertension alters blood vessels structures and functions, lead to organ damage including kidneys, brain, and eyes [3,4]. Numerous antihypertensive drugs are used to control hypertension including beta-blocking agents, angiotensin converting enzyme inhibitors (ACEI), angiotensin II receptor antagonists, diuretics, calcium antagonist and alpha-receptor blocking agents. The optimal results for blood pressure control are obtained by combinations of two or more of antihypertensive agents from various categories were mostly recommended [5]. Several factors are involved in the blood pressure regulation, including adenosine receptors, nitric oxide synthase, cyclooxygenase, CYP-epoxygenases, soluble epoxy hydrolase, ω-hydroxylases and their derived metabolites, etc.

Deregulation in the cardiovascular system can lead to cardiovascular diseases. Before targeting any system, it is important to understand the physiology and pharmacology; otherwise compensatory systems may overshadow the effects of the target. For instance, Adenosine is a purine nucleoside, involved in different physiological and metabolic activities [6,7]. The adenosine has its physiological effects in most tissues and organs [8-12]. Thus, it plays an important role in vascular regulation by the interaction with four subtypes receptors: A_1, A_{2A}, A_{2B}, and A_3 adenosine receptor (ARs) [13]. In vascular tissue, the vasodilation effect is mainly induced by both A_{2A} AR and A_{2B} AR [14-19], whereas the vasoconstriction effect is through A_1 AR and A_3 AR [20,21]. As mentioned earlier, A_{2A}AR is involved in vascular relaxation is through an endothelium-dependent mechanism [7,17,19,22-25]. Another study demonstrated the involvement of CYP-epoxygenases in vascular relaxation [26]. They concluded that the A_{2A}AR activation is associated with an elevation of CYP-epoxygenases, which converts arachidonic acid (AA) to epoxyeicosatrienoic acids (EETs) that result in vascular relaxation [17]. Moreover, the data also suggested the involvement of ATP-sensitive K^+ channels in A_{2A} AR-mediated vascular relaxation through CYP-epoxygenases [27]. In contrast, the absence of A_{2A} AR in mouse aorta contracted though 20-hydroxyeicosatetraenoic acids (20-HETE) via PKC-α/p-ERK pathway [26,27].

The relationship between adenosine receptors activation and the role of soluble epoxide hydroxylase (sEH) was explored using soluble epoxide hydroxylase knockout ($sEH^{-/-}$) and their respective wild-type ($sEH^{+/+}$) mice. In $sEH^{-/-}$, the adenosine-induced relaxation involved an upregulation of A_{2A}AR, CYP-epoxygenases, and PPARγ, accompanied with downregulation of A_1 AR and PPARα [28]. The cytochrome P450 (CYP450) family is divided into two subfamilies (enzymes), epoxygenases and ω-hydroxylases that involve in maintaining vascular tone [29-31]. The main function of CYP-epoxygenases is to metabolize AA into EETs (vasodilator), whereas the ω-hydroxylases metabolizes AA to 20-HETEs (vasoconstrictor). Further metabolism of EETs through sEH generates less active metabolites Dihydroxyeicosatrienoic acids (DHETs), which attenuate vasodilator effects of EETs. Also, due to sEH activity, many polyunsaturated fatty acids (PUFA) are formed, they are also called oxylipins or oxylipids. Our lab has investigated the role of oxylipins in coronary reactive hyperemia (CRH) using isolated mouse heart model against various drugs, like A_{2A}AR agonist (CGS 21680), CYP-epoxygenases inhibitor [N-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide (MS-PPOH], sEH inhibitor [trans-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid (t-AUCB)], ω-hydroxylases inhibitor [N-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS)], PPARγ agonist (rosiglitazone) and PPARγ antagonist (T0070907). CRH is a protective mechanism against myocardial injury [32,33]. It was concluded that inhibition of sEH enhances CRH (protective) against ischemia, whereas inhibition of CYP-epoxygenases reduces CRH (not protective) against ischemia. Activation of PPARγ and blocking of ω-hydroxylases activity also enhances CRH [32,33].

Our main goal in this review is to discuss the current state of the drug delivery technologies or therapeutic agents to the cardiovascular system particularly related to nanomedicine and delivery to the vascular endothelial cells in the cardiovascular system. We expand here on the reviews done previously, not only evaluating small molecule delivery [34,35] but also including in a discussion of the newer methods and therapeutic agents including siRNA, DNA, peptides, proteins, small molecules [CGS 21680, N-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide, trans-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid, N-methylsulfonyl-12,12-dibromododec-11-enamide, rosiglitazone and T0070907] and antibodies.

METHODOLOGY

Delivery of Small Molecules

Traditionally small molecules are used to treat cardiovascular system diseases. Examples of commonly used drugs include atorvastatin, metoprolol, valsartan and ezetimibe. These drugs are mostly available in oral drug delivery systems and are used in the chronic management of the disease. Due to the market share of over several billions of dollars in the treatment of cardiovascular disease, there is a keen interest in developing novel drugs as well as for the delivery of these compounds **(Table 1)** [36]. Small molecule drug delivery, therefore, is an area of interest which as seen several technologies emerge over the past few decades [34]. Compounds fail in clinical trials mainly due to poor pharmacokinetic behavior, which has led a recent paradigm shift in drug discovery has resulted in the inclusion of pharmacokinetic properties of drugs, including absorption, distribution, metabolism, elimination and toxicology (ADME/Tox) to the early stages of drug discovery [37,38]. This new inclusion of ADME properties has led to a designed inclusion of the drug-like properties earlier in the discovery pipeline [39].

Table 1. Examples of different systems used in the cardiovascular delivery of therapeutics.

Polymer system	Drug/Therapeutic	Disease State	Citation
Liposomes	Telmisartan	Hypertension	[59]
PLGA	Pitavastatin	Atherosclerosis	[8,58]
	Pioglitazone	Atherosclerosis	[48]
PEG		Myocardial Infarction	[40]
Liposome	siRNA	Atherosclerosis	[37,42]
Exosomes	siRNA	Inflammation	[71]
PEG	Peptides	Atherosclerosis	[30]

For some compounds, simple medicinal chemistry approaches have not necessarily been able to overcome the challenges faced when dosed in a preclinical model or human disease. In this case, formulations technologies have been employed to deliver the compound to the target site optimally [37,40,41]. Several physicochemical properties of compounds which are impacting adequate drug delivery such as solubility can be overcome by using these nanoformulation approaches [34,42-44]. An additional feature of drug delivery of small molecules using nanomedicine in contrast to the classical achieving fair distribution at the drug target is that it also allows for the possible shielding of a compound to prevent the toxic effect on target organs [34]. An example, nanoformulation strategy for the cardiovascular system about augmenting toxicity, is the recent publication by the group of Liu et al. [45], where they reduced organ toxicity of platinum-containing drugs used in the treatment of cancer. Here they used a hyaluronic acid polymer nanoparticle as well as Intralipid 20% to reduce the toxicity significantly, in organs such as liver, spleen and kidney [46]. Intralipid is currently in human clinical trials to assess its effect on reperfusion, Cardiac Reperfusion With Intralipid® at Reperfusion (CREW-I) NCT02807727. Liposomes specifically have been used in therapeutic delivery more often, and the FDA has developed sets of specifications to address the use of it [47].

Several types of nanomedicine have been formulated in the past few years and can be utilized for specific projects [43]. The choice of which carrier formulation to use is dependent on several factors such as the drug inherent chemical properties (e.g. solubility (logS, logP, and logD7.4), molecular weight as well as the therapeutic goal. For instance, if the compound is simply to treat the peripheral organ systems, then simple protection against metabolism may be the only formulation target. In other cases, the nanoformulation may help in the distribution of compound to various target organs. A classic example is the delivery of drugs to the brain. The microvascular unit in the brain, the blood-brain barrier (BBB) is selectively permeable to organic compounds due to the presence of tight junctions. Due to this restriction by the tight junctions, only transcellular or transporter-mediated uptake can occur into the brain. **Figure 1** shows the differences between the more leaky peripheral vascular system and the restrictive BBB. Interestingly, a recent study found that the A_{2A} AR may play a role in the opening of the BBB. When mice were infused with adenosine, the BBB was opened due to the simulation of the A_{2A} AR. This could be used as a stealth technology or Trojan horse to allow for the delivery of compounds to the brain such as chemotherapeutic agents to treat brain cancers which are excluded from the CNS due to the BBB [47,48].

The types of formulations used for small organic compounds include liposomes, nanoparticles, nanocapsules, nanotubes, polymeric conjugates, and micelles **(Figure 2)** [43]. Each of these types of nanoformulation have been used for different disease states, of which cancer and central nervous system (CNS) disease [49] arguable are the most represented, with other areas such as orthopedics and cardiovascular delivery and emerging as novel delivery rich areas [35,50]. With small molecules, the general strategy for nanoformulations is the encapsulation of a drug inside a polymer carrier system. The principle of these formulations is that the lipophilic compounds associate with the lipophilic parts of the polymer, which then self-assembles and forms a barrier between the aqueous environment and the compound **(Figure 3)** [51]. Other methods include the conjugation of a compound to the polymer or by forming a complex with the system such a glutathione or folate [52,53]. For many of these systems, an additional component is the additional of a targeting system which could either include as a complex with antibodies or drugs [CGS 21680, N-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide, trans-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid, N-methylsulfonyl-12,12-dibromododec-11-enamide, rosiglitazone and T0070907] coated in the nanoparticle.

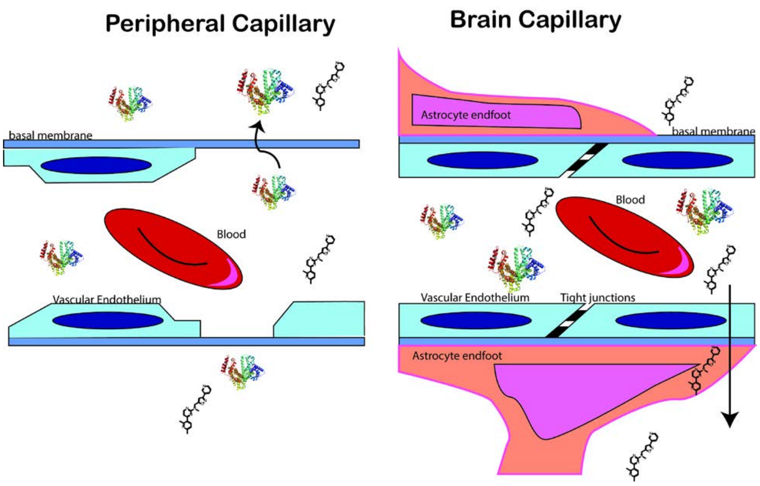

Figure 1. Physiological differences exist between the peripheral vasculature which is leaky versus the selectively permeably blood-brain barrier vasculature. Tight junctions in the BBB restricts paracellular transport [1].

Drug Delivery Systems

PLGA-based nanoparticles

Polymers have been used and developed for use in nanoformulations. These nanoformulations largely consist of nanoparticles of a size range generally of <300 nanometers in diameter [54]. These include poly lactic-co-glycolic acid (PLGA) which is a polymer of poly lactic acid (PLA) and poly glycolic acid (PGA) which is an FDA-approved biomaterial [54]. The group of Katsuki et al. successfully used PLGA nanoparticle loaded with pitavastatin to inhibit the rupture of atherosclerotic plaques by regulating monocyte recruitment to the vascular plaques [55]. PLGA nanoparticles were also used for the delivery of the anti-diabetic drug pioglitazone, an agonist of peroxisome proliferator-activated receptor-γ (PPARγ) [56]. In a study done by Nakashiro et al. the thiazolidinedione (TZD) pioglitazone-encapsulated nanoparticles were able to inhibit the activation of macrophages in hyperlipidemic ApoE -/- mice and prevent the atherosclerotic plaques in the mice [57]. PLGA nanoparticles which encapsulate the statin pitavastatin were able to deliver the drug to the vascular endothelium and show effective therapeutic neovascularization [58]. The work with pitavastatin PLGA nanoparticles was further studied to determine the utility of delivery of compounds to the heart after a myocardial infarction to prevent the ischemic tissue damage seen in these patients. These nanoparticles were successful in reducing ischemic-reperfusion (I/R) injury in the heart, by way of activation of the AKT/PI3K kinase signaling pathway. Additionally, these nanoparticles were able to reduce the inflammation which leads to the secondary tissue damage seen in MI [59]. These pitavastatin nanoparticles could be useful in the treatment of organ ischemia in several diseases states [60,61] and represent an excellent example of cardiovascular drug delivery using nanoformulations.

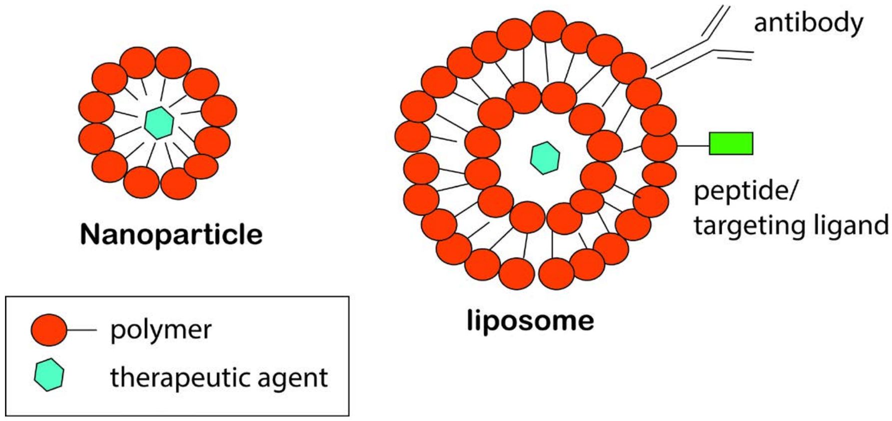

Figure 2. Nanoformulations can be used to deliver therapeutics agents including small organic compounds, peptides, proteins and siRNA. These can be targeted to a specific area by antibody conjugation or use of a ligand/peptide. The best-case scenario for drug delivery is the presence of a tissue-specific target which minimal expression in the other types of tissue in the body.

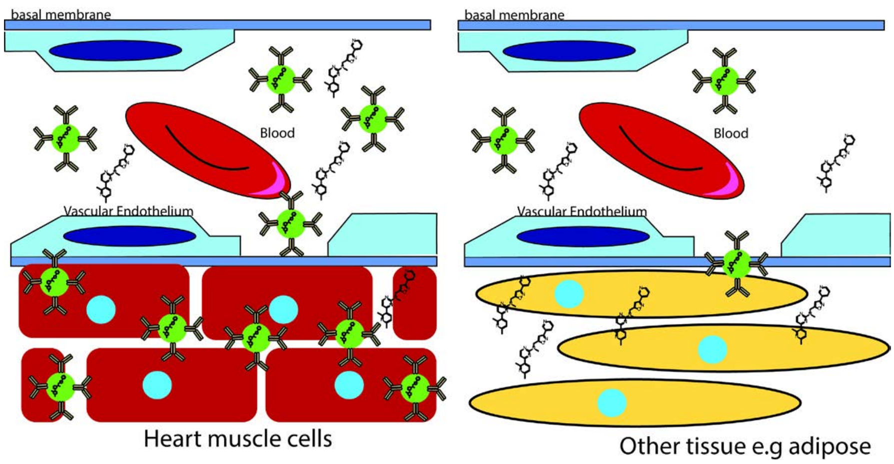

Figure 3. Targeting the cardiovascular system with nanoparticles. The principle behind this method is that the therapeutic drug may not be reaching the target tissue in sufficient concentration to allow for pharmacological action, for instance, a drug may not be reaching the myocardial tissue in sufficient concentration as compared to other tissues. In the case of nanoparticles, the drug will concentrate at the target tissue in sufficient concentration to have an effect. The use of targeting moieties such as antibodies can significantly help with that aspect.

PEG-based nanoparticles

Polyethylene glycol (PEG) has been extensively used in the literature due to the broad utility in drug delivery formulations as well as the ability of PEG to be used to prevent/slow down the clearance of the nanoparticles from the blood stream into the reticuloendothelial system (RES). An example of PEG-based nanoparticle delivery to the vascular endothelium is showed in **Figure 4.** PEG has also been linked to peptides and antibodies to increase the retention time in the body. The successful use of PEG to increase retention time in the body has led to the use of PEG in many formulations, as well as the advantage that formulations containing PEG have been approved for use in humans [62]. Modifications to the PEG motif have been published, for instance the use of PLGA-PEG diblock and triblock polymers, which then can be used for different delivery options as well as controlled delivery systems. Recently the group of Lundy et al. showed that there was a size-dependent effect with the use of PEG-modified polystyrene nanoparticles following an MI and the reperfusion injury in the heart. They concluded that the optimal size for this nanoparticle was able to target the ischemic tissue after an MI, which was between 20 and 200 nm. One caveat, which was pointed out in this study, is that a suspension of nanoparticles (labeled with fluorescent FITC) distribute to the organ tissue in relatively low concentrations. Additionally, the majority of the nanoparticles seem to be taken up by the RES [63]. Similarly, the group of Paulis et al. showed that liposomes with a size of 100 nm were able to slowly move out of the vasculature (extravasation) as well as showed slower to go to the tissue and release the cargo over a period of time. Thereby they arguing that this type of delivery be suitable for treatment of MI [64,65]. The group of Takahama et al. used liposomal adenosine to increase the cardioprotection in a rat model of ischemia/reperfusion (IR) injury [66].

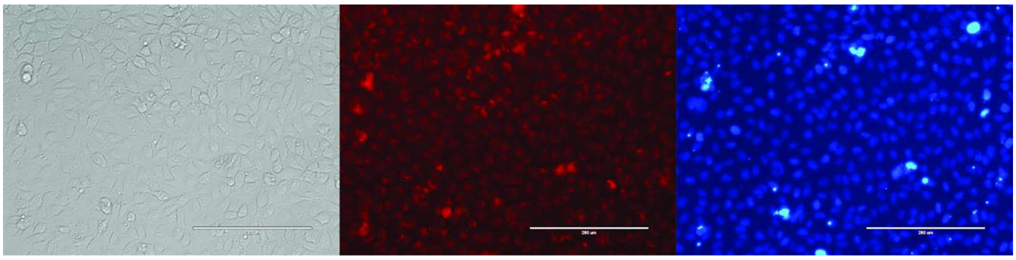

Figure 4. Uptake of fluorescently labeled rhodamine nanoparticles using PLGA-PEG polymers. These data show the uptake of sEH inhibitor (t-AUCB) in human endothelial cell line (EA.Hy926) with a counterstain of blue (DAPI) showing the cellular nucleus.

Liposomal delivery systems

Liposomes are vesicle based systems commonly used in drug delivery system. They form when lipids and surfactants, are suspended into an aqueous environment and self-assembled into spherical liposomes. For an excellent review see the work published by the group of Rao et al. [35]. The lipophilic interior of the liposome naturally allows for the inclusion of compounds such as drugs which normally share this lipophilic nature. A major reason for the formulation into liposome for small molecules has traditionally revolved around the improvement of oral bioavailability. A good example of this formulation strategy was recently published by the group of Patel et al. who describe the increase of the delivery of the antihypertensive agents which target the angiotensin II receptor as antagonists, telmisartan and irbesartan [67,68]. Both these drugs are poorly water-soluble, and the use of castor oil in combination with surfactants Tween 20 and Carbitol as co-solvent formed the necessary self-emulsifying drug delivery system (SEDDS) [35] which improved the oral uptake of the compounds more than 7.5 fold [67, 68].

Delivery of Biologicals

Traditionally, drugs which are used in the treatment of the cardiovascular disease were limited to small organic molecules. Inherent challenges come with the use of drugs to treat different disease states, such as the chemical nature of the compound which may not lend itself to sufficient distribution or in other cases the pathological condition of the tissue in a disease state. Currently, there is a paradigm shift in the therapeutics field for the treatment of cardiovascular diseases, which now includes biological antibodies, proteins, peptides, siRNA and DNA.

RNA-based delivery

Silencing RNA (siRNA) has been successfully delivered to animals using nanoformulation, with the advantage that this therapeutic system can be utilized for precision medicine. In a study done by Leuschner et al. [69], siRNA was formulated in liposome using cholesterol, C12-200 lipid, disteroylphophatidyl choline, and PEG-DMG which forms a spontaneous micellular liposome. The siRNA liposome was able to knock down the expression of CCR2 in monocytes in atherosclerotic-prone animals [70,71].

Another example is the use of exosomes, which have also been used for the delivery of therapeutic siRNA [69]. Exosomes have recently been in the spotlight due to the role they play in cellular communication. Cells use exosomes to transfer several cytosolic components from one cell to another including RNA and microRNA [72]. The group of Shtam et al. was able to use exosomes to deliver siRNA and knockdown RAD51 [71]. Similarly, exosomes which obtained from human iPSCs were able to deliver siRNA to pulmonary microvascular endothelial cells reducing inflammation [69]. Other types of polymer have been successfully used to deliver siRNA to the cardiovascular system, for example, Want et al used a polyethyleneimine-based system to deliver siRNA to the heart [69].

Therapeutic proteins and peptides

Therapeutic peptides have also been important approaches to the treatment of cardiovascular disease. The nature of peptides has made them more challenging to delivery in the body, due to being susceptible to enzyme degradation in the blood, as well as significantly reduced permeability via the vascular endothelial cells and diminished distribution into tissues [73]. One classical method to increase the residence time in the blood is to attach PEG linkers to the peptide [73,74]. Another approach is to develop a cyclic analogy of the linear peptide, which is less prone to metabolic degradation in the blood stream. For example, the group of Gebhard et al. cyclized the peptide HYD1 to form MTI-101 which had superior activity in animals models [75]. In some cases, the peptide may be useful as targeting tool to target the nanoparticles to a specific tissue organ.

The group of Dvir et al. used a peptide sequence to the angiotensin II type I receptor (AT1) for the delivery of PEGylated liposome to the cardiac cells of an infarcted heart. This targeting nanoparticle can be used to deliver a variety of payloads including cytokines, growth factors or other types of therapeutic compounds [76]. The therapeutic potential of proteins has also been shown in the cardiovascular system. Apolipoprotein A-I was used to treat and stabilize atherosclerotic plaques in apolipoprotein E knockout mice [77]. To overcome some of the natural challenges of peptides for drug delivery, development of non-peptide mimetics is a viable option. For instance, the non-peptide Ang-1(1-7) mimetic AVE 0991 was shown to have significant anti-atherosclerotic activity in ApoE-/- mice [78]. Similarly, the group of Kamaly et al developed an anti-inflammatory peptide nanoparticle Ac2-26 which can reduce chronic inflammation in diseases such as atherosclerosis [79].

CONCLUSION

The cardiovascular system presents itself with several therapeutic targets ranging from ischemic/reperfusion injury, myocardial infarction to atherosclerosis. Several novel technologies have been developed for both targeted and prolonged delivery of novel therapeutics which includes compounds and biological, and the field of specifically targeted drug delivery to the cardiovascular system has large potential with main advantages. These new delivery methods open up a host of possibilities of obtaining the necessary tissue specificity and reduced system exposure that will allow us to use new pharmacological agents [CGS 21680, N-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide, trans-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid, N-methylsulfonyl-12,12-dibromododec-11-enamide, rosiglitazone and T0070907] for better treatment of patients in future.

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