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The cardiac toxicity of PAMAM dendrimer drug delivery systems can be attenuated with the adjunct use of cardioprotective agents

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ABSTRACT

Polyamidoamine (PAMAM) dendrimer nanoparticles are efficient drug delivery vectors with potential clinical applications in nanomedicine. However, PAMAMs can compromise heart function, and strategies to mitigate cardiotoxicity would be beneficial. In this study, we investigated whether the adjunct use of three key cardioprotective agents could prevent the cardiac injury induced by a seventh-generation cationic PAMAM dendrimer (G7). Isolated rat hearts were subjected to ischemia and reperfusion (I/R) injury in the presence or absence of G7 or the cardioprotective agents Losartan, Epidermal Growth Factor (EGF), or S-nitroso-N-acetylpenicillamine (SNAP). I/R injury significantly compromised cardiac function, in terms of left ventricular hemodynamics, contractility, and vascular dynamics, which were markedly improved ($p<0.05$) by the administration of Losartan, EGF, or SNAP alone, confirming their cardioprotective effects. The administration of G7 significantly worsened cardiac function recovery following I/R ($p<0.05$). G7-induced impairments in cardiac and vascular dynamics were significantly improved by co-administration of Losartan, EGF, or SNAP. Treatment with G7 also significantly increased cardiac enzyme levels and infarct size, both of which were markedly reduced upon co-infusion of Losartan, EGF, or SNAP ($p<0.05$). Thus, G7 deteriorates the recovery of cardiac function in isolated hearts subjected to I/R injury, which can be rescued by co-administration of Losartan, EGF, or SNAP. These findings enhance our understanding of the nanotoxicology of PAMAM dendrimers in the mammalian heart and suggest that the adjunct use of cardioprotective agents is an effective strategy for mitigating the cardiotoxicity of these dendrimers and potentially other drug delivery systems.

Keywords: Ischemia/reperfusion, cardiac injury, cardiotoxicity, PAMAM dendrimer, nanoparticle, Losartan, EGF, Nitric oxide, SNAP.

INTRODUCTION

The hyperbranched, multivalent polyamidoamine (PAMAM) family of dendrimers are nano-sized nanoparticulate drug delivery systems with well-defined molecular architecture, and tunable surface chemistry that have potential applications in nanomedicine(1-3). However, in addition to improving cellular and tissue drug delivery, PAMAM dendrimers can also exhibit intrinsic toxicological and biological actions that, in some cases, can modulate key signaling pathways of vital importance for organ function (2, 4-13). The net consequences of PAMAM-cell interactions *in vivo* might be beneficial or detrimental depending on the physicochemical properties of the dendrimers, such as surface charge and molecular weight/size/generation, as well as the cell- or tissue-type affected (2, 4, 14). For example, we have previously reported on the beneficial vascular effects of PAMAM dendrimers in improving diabetes-induced vascular dysfunction (8). However, the direct effects of PAMAM dendrimers on the heart and cardiac hemodynamics as well as pharmacological strategies that might counter any adverse effects are not completely understood.

Higher-generation PAMAMs are known to accumulate in the heart, especially in ischemic regions within cardiac tissue (15). Consistent with this finding, we showed that PAMAMs (especially higher generations like cationic amino-terminal group G6 and G7) could compromise cardiac recovery from ischemia/reperfusion (I/R) injury with overall cardiotoxicity being markedly dependent on dendrimer generation and surface charge (16, 17). Cationic PAMAM dendrimers could also completely abrogate the beneficial/protective effects of postconditioning in rat hearts (17). Therefore, strategies that can mitigate the cardiotoxicity of these important classes of drug delivery systems are highly desirable and would be beneficial for their potential safety in the clinic. We recently reported that PAMAM-induced cardiotoxicity might be mitigated via co-administration of the heptapeptide, Angiotensin (1-7), a member of renin-angiotensin system (16). In the present study, we extend our previous findings to determine whether other cardioprotective agents with differing mechanisms of action like Losartan - an angiotensin II type 1 receptor ((AT1R) blocker, epidermal growth factor (EGF)- a growth factor ligand for EGF receptor (EGFR) tyrosine kinase, or S-nitroso-N-acetyl penicillamine (SNAP)- a nitric oxide (NO) donor, could also prevent PAMAM-induced cardiac dysfunction following I/R injury.

Losartan is a well-established cardioprotective drug used in the clinic for the treatment of various cardiovascular diseases (18). Losartan is a selective antagonist of type 1 angiotensin II receptors (AT1R) that are part of the detrimental arm of the renin-angiotensin system (RAS)

(e.g. see (19). It is known to be protective of the heart in I/R injury (20, 21) and especially the diabetic heart (22-24). However, the role of Losartan in protecting the heart from PAMAM dendrimer-induced injury is not known and is the subject of this study.

Epidermal growth factor (EGF) is a ligand for receptors from the EGFR/ErbB family of receptor tyrosine kinases that have important roles in the cardiovascular system and is a proven cardioprotective agent (7, 25). We previously reported that administration of EGF and subsequent activation of EGFR signaling was protective of the diabetic heart from I/R injury (26). Additionally, EGF was recently reported to also reduce infarct size and myocardial apoptosis due to I/R injury (27). Hence, with these cardioprotective characteristics, EGF appears to be a suitable candidate for the potential mitigation of PAMAM dendrimer-induced cardiac dysfunction.

Endothelial nitric oxide synthesis (eNOS) plays an essential role in the protection of the heart from I/R injury (28-30). Blockade of nitric oxide (NO) production was reported to result in a lack of cardioprotection from I/R injury (31) whereas NO induced during I/R protected the heart from I/R injury (32). Indeed, NO protects the myocardium from I/R by improving vascular blood flow (33). Furthermore, NO is a potent scavenger of reactive oxygen species (ROS) that are known to be detrimental to the heart during I/R (34). NO can be released *in vivo* by use of NO donors such as organic nitrates that are routinely used clinically for the treatment of ischemic heart disease (e.g. angina pectoris) (35, 36). Thus, in an attempt to study the role of NO in mitigating the cardiotoxicity of cationic G7 PAMAM dendrimer, in this study we used the NO donor, SNAP, that is known to be cardioprotective following I/R injury (37, 38). To the best of our knowledge, this investigation represents the first time that these diverse set of cardioprotective agents have been shown to protect the heart from the cardiotoxic effects of PAMAM dendrimers.

MATERIALS AND METHODS

Male Wistar rats weighing between 250 and 350 grams were sourced from the Animal Resources Center at Kuwait University, Kuwait, with study approval obtained from the Health Science Center, Kuwait University Animal Ethics Committee, in accordance with the EU Directive 2010/63/EU for animal experiments. The rats were housed in plastic cages with two rats per cage, maintained under controlled conditions of temperature (21-24°C), a 12-hour (7 a.m. - 7 p.m) light/dark cycle, and humidity (50%), with *ad libitum* availability of food and water. Heart isolation followed previously established protocols (39). The isolated hearts were promptly placed in ice-cold (4°C) Krebs-Hensleit solution (40). Cannulation and perfusion of

the heart were carried out as previously described (41). A 30 minute occlusion of the left anterior descending (LAD) coronary artery was performed to induce regional ischemia. Preload was maintained at 6 mmHg, and perfusion pressure (PP) was consistently maintained at 50 mmHg throughout the experimental procedures. PP was measured in a branch of the aortic cannula downstream of the flow probe using a Statham pressure transducer (P23 Db). Electronic control of constant PP was attained using the perfusion assembly ("Module PPCM type 671 (Hugo Sachs Elektronik-Harvard Apparatus GmbH, Germany")), providing precise adjustment of PP between 5 mmHg to 150 mmHg with an accuracy level of ±1 mmHg.

The cationic G7 PAMAM dendrimer nanoparticles (nominally of 8.1 nm diameter, MW of 116,493 and bearing 512 surface amino groups) were synthesised by Dendritech (USA) and acquired from the Sigma Chemical Company (St Louis, MO, USA). Unless stated otherwise, all other reagents used in thi study were acquired from Sigma Aldrich (St. Louis, Missouri, USA).

Study protocol

One set of hearts (n=8 per group) underwent 30 minutes of regional ischemia followed by reperfusion, as previously described by Mohammad and Babiker (38). Subsequently, all hearts were reperfused for an additional 30 minutes. Control hearts were subjected to ischemia/reperfusion (I/R) injury without any additional treatment. Another set of hearts subjected to ischemia received an infusion of 100nM cationic G7 PAMAM dendrimer (refer to Figure 1). Alternatively, infusions of 1µM Losartan, 10nM EGF, 1µM SNAP, with or without 100nM cationic G7 PAMAM dendrimer, were administered at the onset of reperfusion in the presence of ischemia. All treatments were initiated 5 minutes before reperfusion and continued for the initial 10 minutes of reperfusion (refer to Figure 1). Cardiovascular functions were determined as described previously (39, 42, 43). Left ventricular (LV) contractility, hemodynamics, and coronary vascular dynamics were assessed throughout the experiment. LV dynamics were ascertained by evaluating maximum developed pressure (DPmax), LV end-diastolic pressure (LVEDP) and LV contractility indices (+dP/dt and -dP/dt). Coronary vascular dynamics were assessed by coronary vascular resistance (CVR) and measuring coronary flow (CF). CF (ml/min) was monitored using an electromagnetic flow probe that was attached to the inflow of the aortic cannula, as previously described by Ismaeil et al. (44) and was digitally computated using software developed by Hugo-Sachs (Hugo-Sachs Electronik, Germany). CF values were manually verified via collection of coronary effluent as a function of time. CVR and hemodynamic data were sampled every 10 seconds through an established

data acquisition program (Hugo-Sachs' Isoheart software V 1.524-S). Following conclusion of each experiment, hearts were snap-frozen in liquid nitrogen and stored for further analysis at -80°C.

Measurements of infarct size and cardiac enzyme levels as indicators of cardiac injury

Infarct size was assessed using triphenyl tetrazolium chloride (TTC) staining, following established procedures (45). Images were captured with a Nikon camera. Red and pale unstained areas on each slice were assessed using Leica ImageJ software (Image J, Wayne Rasb and National Institute of Health, USA). The percentage (%) of infarct area was determined relative to the total left ventricular (LV) area. Cardiac injury was determined through the release of creatine kinase (CK) and lactate dehydrogenase (LDH) enzymes into the coronary effluent during reperfusion, as previously described (46).

Data analysis

The acquired data underwent analysis using two-way analysis of variance (ANOVA), followed by post hoc analysis with the least significant difference (LSD) method utilizing SPSS software. Comparisons were made between group means and their respective controls. Results were expressed as mean ± standard error of the mean, with statistical significance considered at $P<0.05$.

RESULTS

In the animals used for this study, the mean body weight of rats (295 ± 55 g) and the heart size (1.45 ± 0.32 g) at sacrifice did not significantly differ among the animal groups investigated. Regional cardiac ischemia for 30 mins resulted in a significant ($P<0.05$) deterioration in the LV hemodynamics, contractility, and coronary vascular dynamics compared to baseline data (expressed as % of baseline; see Figure 2).

To confirm their cardioprotective actions, we treated hearts subjected to I/R injury with Losartan, EGF, or SNAP (see Figure 1for protocol). Infusion of either of these three agents 5 min before reperfusion and continued for the first 10 min of reperfusion, significantly ($P<0.001$) improved all measured cardiac function parameters (Figure 2) as well as reduced infract size and cardiac enzyme levels for LDH and CK compared to I/R alone controls (Figure 4 and Table 1). For example, the LV function parameter DPmax was improved by

approximately 2-fold for all treatments from around 30% for I/R alone to over 60% of baseline when treated with either Losartan, EGF or SNAP (Figure 2A). Similarly, recovery of the LV contractility indices (+dP/dt and -dP/dt) upon treatments with cardioprotective agents were almost doubled compared to I/R alone (Figure 2D and 2E). In contrast, LVEDP, that is markedly raised following I/R injury to around 250% of baseline value, was improved to half that value upon treatment with any one of the cardioprotective agents (Figure 2B). In terms of the coronary vascular dynamics, treatment of hearts with either Losartan, EGF or SNAP significantly increased CF by about 50% and reduced CVR to approximately half the values for I/R alone (see Figure 2B and 2C) confirming the beneficial cardioprotective actions of all three agents in cardiac I/R injury.

Infusion of G7 PAMAM dendrimer to isolated hearts significantly exacerbated by 2 to 4-fold the LV hemodynamics, contractility, and coronary vascular dynamics parameters compared to hearts subjected to I/R alone (Figure 3). However, co-infusion of Losartan, EGF, or SNAP with G7 PAMAM dendrimer resulted in a significant improvement ($P<0.001$) in DPmax, LVDP, and LV contractility compared to infusion of G7 PAMAM dendrimer alone (Figure 3). Similarly, coronary vascular dynamics in terms of CF and CVR that were markedly impaired by G7 PAMAM dendrimer infusion following I/R injury were significantly improved by the co-infusion of Losartan, EGF, or SNAP ($P<0.001$) compared to G7 PAMAM treatment alone (Figure 3C and 3D). This recovery of cardiac function parameters was mirrored with data on infarct size and cardiac enzyme levels (Table 1). Indeed, adjunct treatment of the heart with Losartan, EGF, or SNAP significantly ($P<0.01$) rescued G7 PAMAM dendrimer-induced increase in infarct size and cardiac CK and LDH enzyme levels (Fig. 4 and Table 1).

DISCUSSION

The structurally defined and highly versatile, polyvalent PAMAM series of dendrimer nanoparticles are increasingly being considered for drug delivery and other potential clinical applications in nanomedicine (12). However, their full safety and toxicological profile especially in target organs like the mammalian heart are not fully understood. We previously showed that ex vivo and *in vivo* administration of cationic PAMAM dendrimers can compromise cardiac function recovery of mammalian hearts following I/R injury (16, 17). The extent of PAMAM dendrimer-induced cardiac dysfunction was dependent on the physicochemical properties of the nanoparticles such as their molecular size or weight (generation) and surface-group chemistry (16). Generally, higher generation/ molecular weight

PAMAMs (e.g. G6 and G7) with cationic surface chemistry (amino-group) exhibited the greatest cardiac toxicity (16). However, since higher generation PAMAMs also impart the best cellular uptake and delivery-enhancing properties for drugs such as nucleic acid-based gene silencing and gene-editing therapeutics (12), strategies to overcome their adverse cardiac effects will likely be required for their potential use in the clinic. Although several different strategies for decreasing the general cellular toxicity of PAMAMs are possible such as PEGylation or reducing surface cationic charge density (e.g. see (2) for a recent review), the direct effects of such modifications on heart function are not known. As an alternative novel approach, we previously reported that adjunct use of the cardioprotective agent, angiotensin (1-7), a heptapeptide member of RAS, could mitigate the cardiotoxicity of a cationic G7 PAMAM dendrimer (16). In this study, we extended these findings to investigate whether other known cardioprotective agents could rescue the adverse cardiac effects of a cationic G7 PAMAM dendrimer in the mammalian heart. Thus, the major findings of this study are that the adjunct administration of any of three different cardioprotective agents Losartan, EGF or SNAP, can significantly rescue the adverse cardiac effects of a cationic G7 PAMAM dendrimer in the isolated mammalian heart following I/R injury. Thus, the adjunct use of such general cardioprotective agents might be a useful new strategy in mitigating the cardiotoxicity of PAMAM dendrimers and, potentially, other drug delivery systems *in vivo*.

Cationic PAMAM dendrimers are known to biodistribute to the heart (along with other organs of the reticuloendothelial system) following systemic administration (12, 47) and furthermore, have been shown to preferentially accumulate within the ischemic regions of the myocardium (15). The latter implies that passive targeting of ischemic myocardial tissue is possible with PAMAM delivery systems, but it might also suggest that the ischemic heart may be more vulnerable to any adverse effects elicited by charged PAMAM dendrimers relative to healthy heart tissue. Although the precise mechanisms by which cationic PAMAM dendrimers compromise cardiac function are also not fully elucidated, we have previously proposed that they can either interfere with key recovery or salvage pathways activated in the heart in I/R injury and/or, due to their nanoparticulate nature, they may physically occlude coronary vasculature to reduce CF and increase CVR (16, 17). Although it is quite likely that both mechanisms are at play, given the fact PAMAMs exhibit vasculoprotective effects and are known to prevent vascular dysfunction and remodeling in an animal model of diabetes (8), it is quite likely that they have a greater effect on attenuating one or more cardiac survival (or salvage) signaling cascades that are normally elicited by the heart to aid its recovery from I/R

injury. We have previously identified that activation of EGFR signaling via PI3K/AKT pathway is critical for cardiac function recovery in I/R injury and thus represents a key salvage pathway in the ischemic heart. Additionally, we have shown that naked cationic PAMAM dendrimers are effective inhibitors of EGFR signaling cascades (5, 6, 8, 48). Thus, it is very tempting to speculate that cationic G7 PAMAM dendrimer-induced impairment in cardiac recovery of hearts following I/R injury occurs via blockade of the key survival pathway mediated via EGFR/P13K/AKT signaling. This would also be consistent with our finding from this study that cardioprotective EGF, a ligand for EGFR that is known to activate EGFR/P13K/AKT salvage pathway in ischemic hearts (22), was able to mostly abrogate the adverse cardiac effects of G7 PAMAM dendrimer. Indeed, we have already shown that cardiac EGFR signaling is attenuated following ischemia (22) leading to impaired recovery of hearts from I/R injury as seen in this study (Figure 2 and 3). The fact that G7 dendrimer treatment further exacerbated cardiac recovery in all measured cardiac function parameters (Figure 3) would be consistent with a further inhibition of EGFR signaling by G7 PAMAM dendrimer and subsequent attenuation of the EGFR-mediated salvage pathway necessary for cardiac function recovery. Rescue of ischemia and/or G7 dendrimer-mediated attenuation in cardiac EGFR signaling by exogenous EGF administration would thereby explain the improved recovery of hearts from I/R injury (see Figures 2 and 3), though this clearly needs further study. Indeed, EGF/EGFR signaling also can impact cardiovascular function in multiple other ways including through modulation of reactive oxygen species formation, NO production and regulation of myocardial apoptosis (for a recent review see (25). The latter may also explain the reduction in infarct size and cardiac enzyme levels observed in the present study upon infusion of EGF following ischemic injury (See Table 1 and Figure 4). Thus, the adjunct use of EGF appears to be a useful strategy for mitigation dendrimer-induced cardiotoxicty. This may be achieved through co-administration of EGF or via conjugation of EGF to PAMAM dendrimers as has been reported for targeting to the surface of cancer cells (49, 50).

Losartan is a well-established cardiovascular drug with an acceptable safety profile that has been on the market since 1995 and is clinically approved for use in the treatment of cardiovascular disorders including hypertension, diabetic nephropathy and stroke (51). Consistent with the confirmatory findings of our present study (see Figure 2), it has previously been shown, by us and others, that Losartan is also cardioprotective in I/R injury in most studies reported in the literature though not all (20-24, 40, 52, 53). Although its precise effects in I/R injury need clarification, in the present study, Losartan infusion markedly improved recovery

from I/R injury in all cardiac parameters studied (namely DPmax, LVEDP, CF, CVR and the contractility indices (+dP/dt and -dP/dt) implying it has broad cardioprotective properties that impact on both cardiac contractility and coronary hemodynamics. As a blocker of AT1R (termed ARB), Losartan opposes the detrimental effects of Angiotensin II (Ang II) on the heart that classically lead to increased formation of reactive oxygen species, vasoconstriction and apoptosis amongst others (e.g. see (19, 25, 52). The fact that we also observed a reduction in infarct size and cardiac enzymes with Losartan treatment (Figure 4 and Table 1) might be explained by its ability to reduce Ang II-induced myocardial apoptosis. Some of beneficial effects of Losartan might also be mediated in part by increasing endogenous Ang-(1-7). It has been shown that ACE inhibitors and ARBs increase Ang-(1-7) formation in the heart and Ang-(1-7) receptor antagonists attenuate their cardiovascular benefits (54-56). Ang-(1-7) is a coronary vasodilator and has anti-inflammatory, antioxidant and antithrombotic effects (54, 57-59). Ang-(1-7) has been shown to decrease I/R-induced calcium overload and reactive oxygen species production leading to cardioprotective effects (60). Interestingly, Ang-(1-7) potentiates bradykinin-induced vasodilation of porcine coronary arteries by acting as an ACE inhibitor (61). Chang et al showed that Ang-(1-7) protects cardiomyocytes from long term hypoxia-stimulated apoptosis. Indeed, Ang-(1-7) can mitigate G7 PAMAM-mediated cardiac dysfunction in I/R injury (16). Of note is that Losartan also ameliorated lung injury caused by a PAMAM dendrimer (62). Despite its already significant cardioprotective effects, Losartan's benefits can be significantly improved by co-administration of EGF (22). The reason for this is thought to be that EGF can rescue the partial Losartan-induced reduction in the otherwise beneficial cardiac EGFR signaling as a result of its ability to block Ang II-mediated transactivation of EGFR (22). Though not attempted in the current study, a combination of Losartan and EGF (or indeed other cardioprotective agents) might afford greater protection against G7 PAMAM induced cardiotoxicity- though this needs to be further researched and any potential drug-drug interactions taken into account.

Losartan and EGF are both known to increase NO production, most likely through increased eNOS activity, (22, 63, 64) which is an important regulator of microvascular flow and exhibits known cardioprotective actions in the heart following I/R injury (22- 26). Thus, the mechanisms by which Losartan and EGF exert their cardioprotection, might conceivably converge at the level of NO. Indeed, this notion is supported by our data showing similar magnitude of cardiac recovery in the different cardiac contractility and hemodynamic parameters assessed for each of these two agents and the NO donor, SNAP (see Figure 2 and

3). Although NO is a potent vasodilator with potential to attenuate cardiac I/R injury, it can also mediate tissue injury, but this appears to be concentration dependent (37). By using a previously defined 1μM dose of SNAP that is known to be cardioprotective and was shown to reduce infarct size in rodent hearts subjected to ischemia (37, 38), in the present study we found that infusion of this NO donor improved all cardiac parameters studied as well as reduced infarct size and cardiac enzyme levels due to I/R (Figures 2 and 4; Table 1) or as a result of further exacerbation of I/R injury with G7 PAMAM dendrimer (Figures 3-4; Table 1). These data confirm that in our hands, administration of exogenous NO at the right dose can be protective of the heart function and can reduce cardiac injury and myocardial infarction. Further, these results suggest that increasing NO levels with SNAP might be a useful strategy to reduce G7-mediated cardiotoxicity. Indeed, conjugates of a G4 PAMAM and SNAP have been reported (65, 66) with one study showing that by using a glutathione-initiated release of NO from these dendrimers, I/R injury could be reduced (65) that is supportive of our report of using free (unconjugated) SNAP as a strategy to rescue PAMAM-mediated toxicity. Additionally, the potential co-administration of other cardioprotective drugs as either free drugs, or associated with PAMAM delivery systems themselves (e.g. either entrapped within or covalently conjugated to the surface of nanoparticles) might provide similar cardioprotection to that observed in this study with free Losartan, EGF and SNAP. Indeed, a PAMAM dendrimer conjugated to an agonist of the A3 adenosine receptor, that activates key recovery pathways in hearts, improved cardiac function recovery and reduced infarct size following I/R injury (67, 68). Thus, by using appropriate dendrimer-drug combinations, or through simple adjunct administration of "free" drugs known to exert cardioprotective actions such as Losartan, EGF or SNAP, as reported here (see Figure 5 for summary), or even Ang-(1-7) shown by us previously (16), the cardiotoxicity of PAMAM dendrimers might be abrogated. Indeed, such cardiotoxicity-rescuing strategies might be necessary for improving the safety profile of PAMAM dendrimers for their potential use in the clinic.

As to whether this strategy will be effective in mitigating cardiotoxicity of other dendrimer drug delivery systems such as polypropylyiminine (PPI) and polyethyleneimine (PEI) [69-] is not known. Studies investigating the direct cardiotoxicity of these dendrimer-based delivery systems are sparce. However, we have previously shown that PPI dendrimers elicit multiple gene expression changes in cells (69) and are known to exhibit neurotoxicity (74) - changes that could potentially also modulate cardiac function. A common feature of the above-mentioned dendrimers is their polycationic nature that is also the most likely cause of cellular toxicity *in vitro* and *in vivo* (2). Although not studied, it is quite likely that PAMAM

nanoparticles and the related polycationic delivery systems will also exhibit toxicity via similar cellular mechanisms in the heart (2, 16-17). If confirmed, the use of diverse cardioprotective agents such as the clinically acceptable ARBs and NO donors as well as growth factors, as reported here for PAMAMs, might also be effective in mitigating the cardiotoxicity of these related delivery systems.

CONCLUSION

PAMAM-induced cardiac toxicity could be significantly attenuated by adjunct administration of the well-established cardioprotective drugs, Losartan, EGF, or SNAP (see Figure 5). Thus, co-administration of such pharmacological agents might constitute a novel strategy to rescue or prevent the cardiotoxicity elicited by PAMAM dendrimers as well as potentially other nanoparticle-based drug delivery systems.

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TABLES AND FIGURES

Table 1. Effects of I/R injury with or without treatments with Losartan, EGF, SNAP and/or cationic G7 PAMAM dendrimer on cardiac enzyme levels. LDH= lactate dehydrogenase; CK = Creatinine kinase.

Treatment	CK (IU/L)	P Value	LDH (IU/L)	P Value
I/R	37.52±2.85	-	30.27±1.23	-
+Losartan	30.13±2.42*	0.01	24.90±1.98*	0.01
+EGF	29.53±1.72*	0.01	25.12±1.78*	0.01
+SNAP	25.71±1.85*	0.001	22.21±1.73*	0.001
+G7	48.61±2.54$^{\$}$	0.01	39.92±1.7$^{\$}$	0.01
+G7 + Losartan	37.83±1.25**	0.02	29.87±2.16**	0.01
+G7 + EGF	37.31±1.12**	0.01	29.17±1.28**	0.02
+G7 + SNAP	35.51±1.27**	0.03	28.92±1.34**	0.01

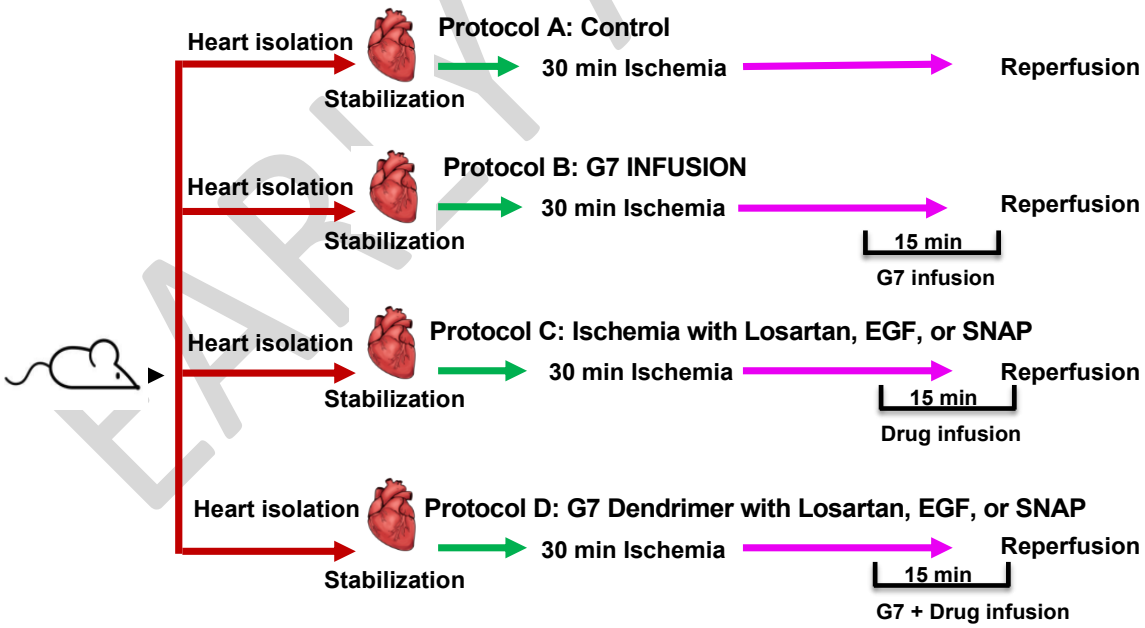

Figure 1. Diagrammatic representation of the study groups and protocols used in the study. Isolated rat hearts were divided into four main groups (n=8) subjected to the different experimental protocols labelled as protocols A, B, C, and D). Hearts underwent 30 min

ischemia followed by 30 min of reperfusion (I/R) (control) (Protocol A), I/R hearts treated with only cationic G7 PAMAM dendrimer starting 5 min prior to and then continued for first 10 min of reperfusion (Protocol B), or I/R hearts treated only with Losartan, EGF, or SNAP commencing 5 min prior to and then continued for first 10 min of reperfusion (Protocol C) and lastly, I/R heats treated with G7 PAMAM dendrimer in the presence of Losartan, EGF, or SNAP commencing 5 min prior to and then continued for first 10 min of reperfusion (Protocol D).

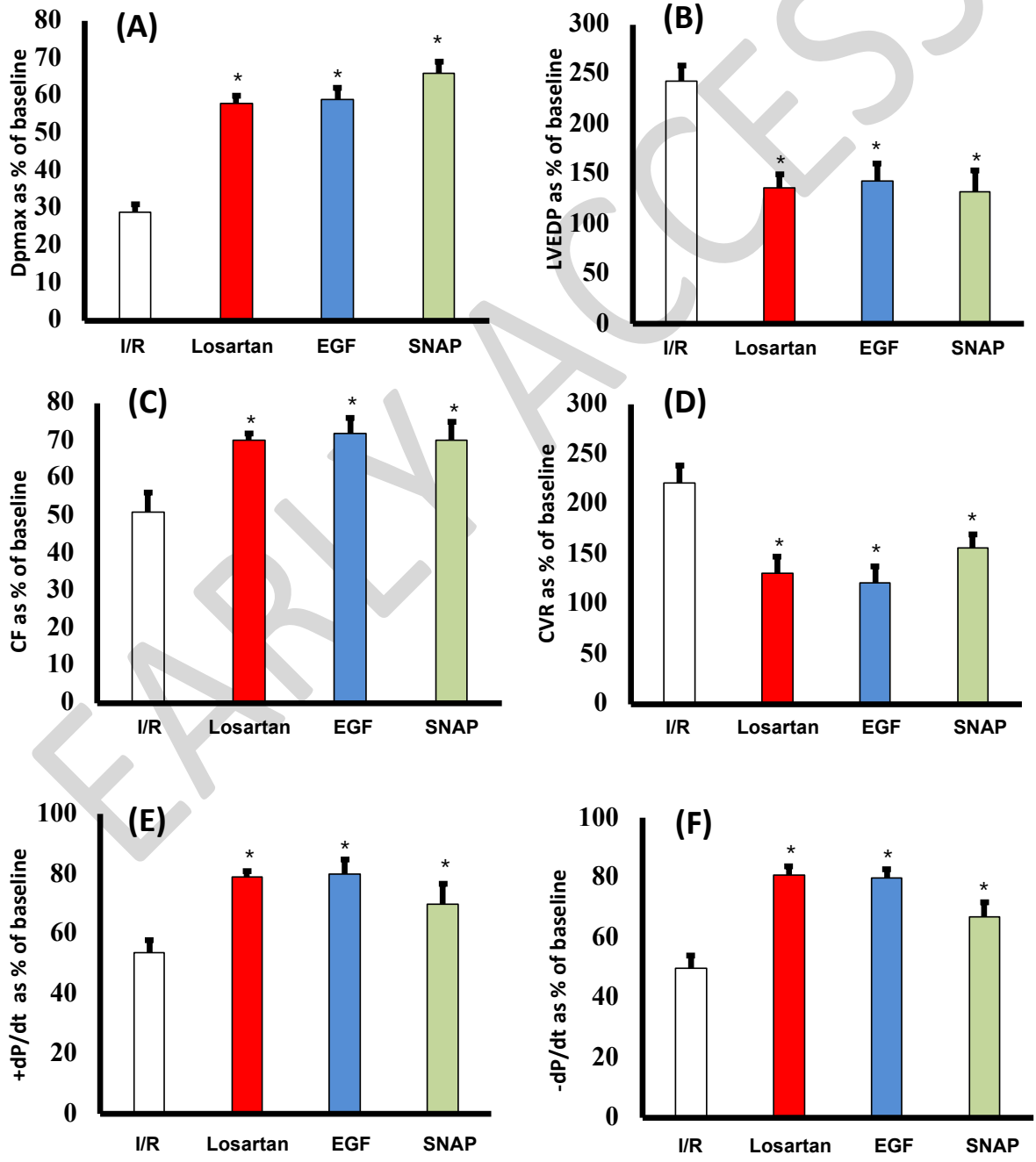

Figure 2. I/R injury compromises recovery of cardiac function that is improved by treatment with Losartan, EGF or SNAP. Post I/R recovery in the left ventricle function (DPmax (A) and LVEDP (B)), coronary vascular dynamics (CF (C) and CVR (D)) and contractility indices (+dP/dt (E) and −dP/dt (F)) without and with treatment with Losartan, EGF, or SNAP is shown. N=8. Mean +/- SEM. Asterix indicates significant difference ($p<0.05$) from I/R controls.

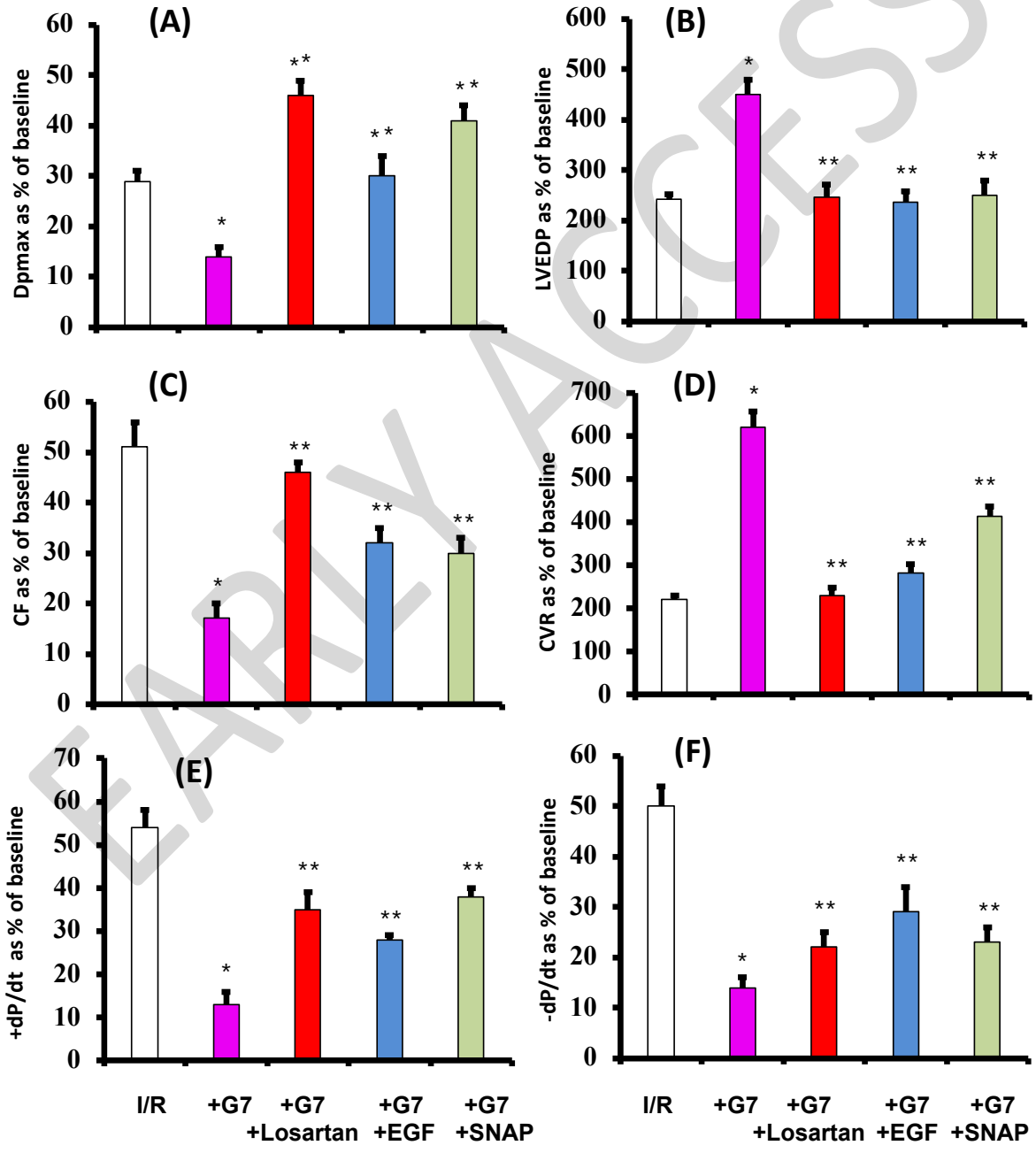

Figure 3. Cationic G7 PAMAM dendrimer exacerbates cardiac function recovery from I/R injury that is rescued by co-administration of either Losartan, EGF or SNAP. Post I/R recovery in the left ventricle function (DPmax (A) and LVEDP (B)), coronary vascular dynamics (CF (C) and CVR (D)) and contractility indices (+dP/dt (E) and −dP/dt (F)) without and with treatment with G7 PAMAM dendrimer (G7) or G7 together with Losartan, EGF, or SNAP is shown. N=8. Mean +/- SEM. Asterix indicates significant difference ($p<0.05$) from I/R controls. Asterix (*) indicates values significantly different from I/R controls whereas double Asterix (**) indicates values significantly different from G7 treatment only in I/R injury ($P < 0.05$).

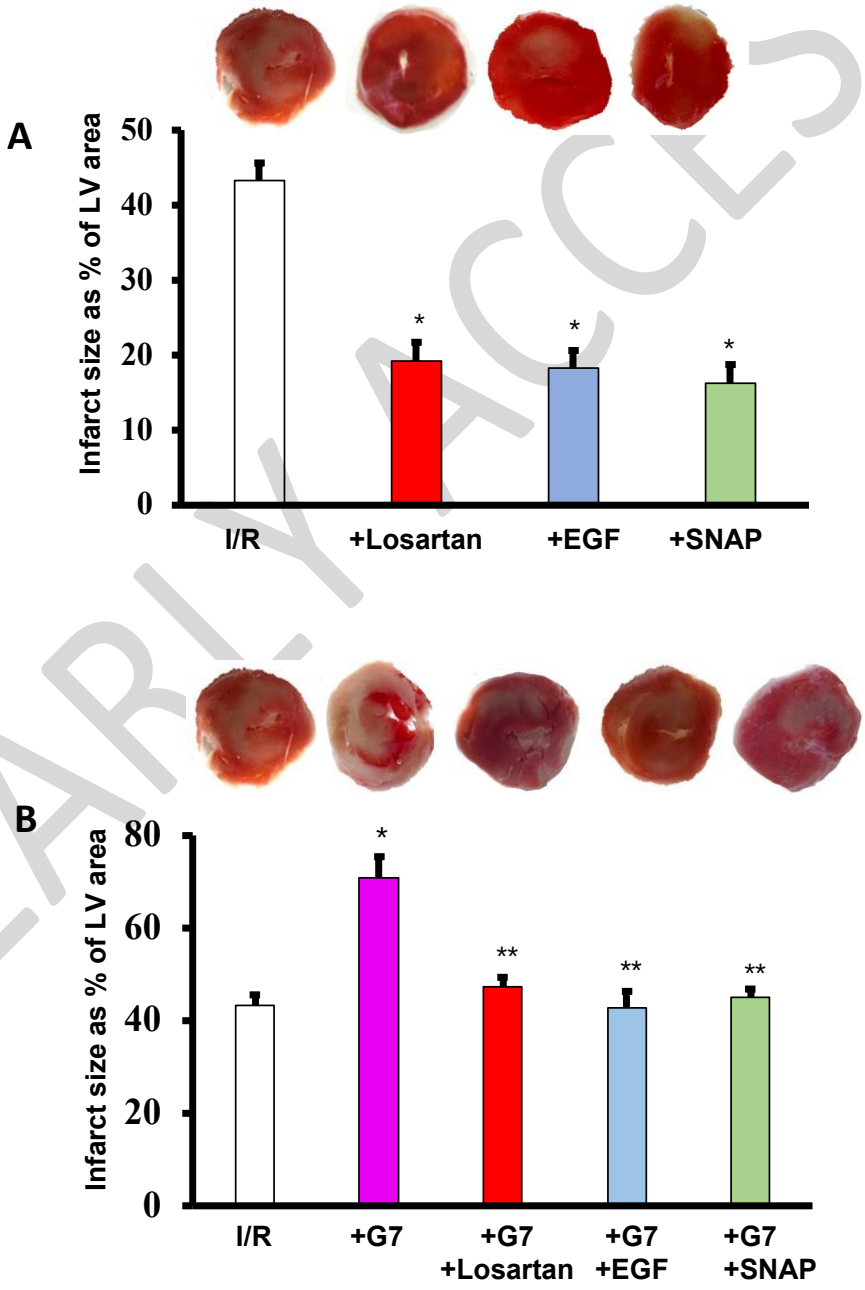

Figure 4. Histological assessment of ischemic injury via determination of infarct size (expressed as infarcted area as a percentage of left ventricle area). Panel A: Infarct size following I/R injury and upon treatment with Losartan, EGF, or SNAP 5min before and in first 10 min of reperfusion. Panel B: Infarct size following I/R injury, and I/R injury hearts treated with G7 PAMAM (+G7) dendrimer and/or co-infused with Losartan, EGF or SNAP 5min before and in first 10 min of reperfusion. In both figure A and B, the top panel shows representative 2,3,5-triphenyl-2H-tetrazolium chloride-stained heart slices for each indicated experimental group. Asterix (*) indicates values significantly different from I/R controls whereas double Asterix (**) indicates values significantly different from G7 treatment only in I/R injury ($P < 0.05$).

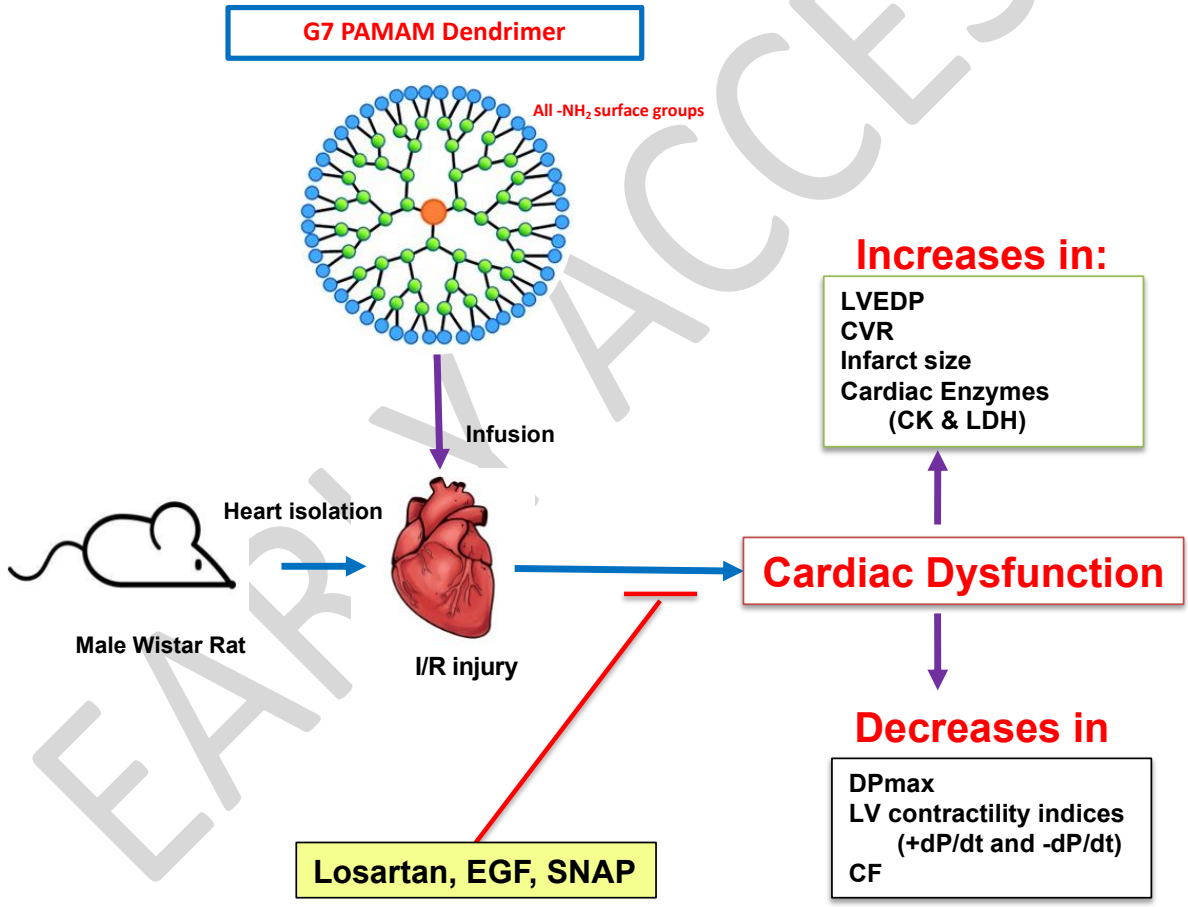

Figure 5. Cardiac effects of cationic G7 PAMAM dendrimer and the ability of cardioprotective agents to abrogate their cardiotoxicity. Cardiac administration of G7 PAMAM dendrimer bearing all amino (-NH2) surface groups impaired post-I/R recovery of hearts by increasing LVEDP, CVR, cardiac enzyme levels (LDH, CK), infarct size and decreasing DPmax, LV contractility indices ((+dP/dt and −dP/dt) and CF, all indicative of

cardiac dysfunction and myocardial injury. These adverse cardiac effects of G7 PAMAM dendrimer were markedly attenuated by adjunct administration of the cardioprotective agents, Losartan, EGF or SNAP. Thus, adjunct use of these cardioprotective agents may represent a viable pharmacological strategy to mitigate or abrogate the cardiotoxicity of PAMAM dendrimers as well as potentially other polymeric nanoparticulate drug delivery systems.