OMTM, Volume *9*

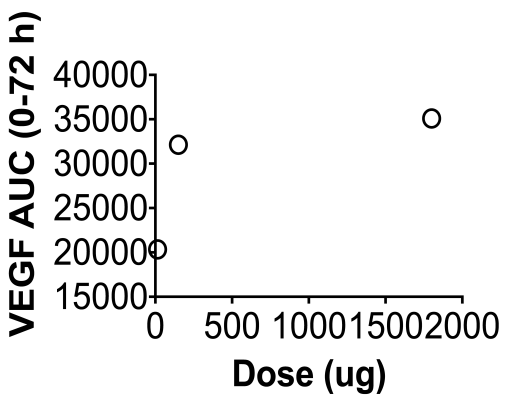
Supplemental Information

Biocompatible, Purified *VEGF-A* mRNA Improves Cardiac Function after Intracardiac Injection 1 Week Post-myocardial Infarction in Swine

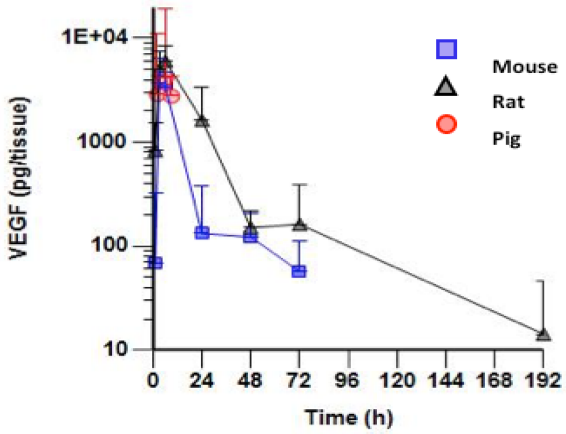
Leif Carlsson, Jonathan C. Clarke, Christopher Yen, Francine Gregoire, Tamsin Albery, Martin Billger, Ann-Charlotte Egnell, Li-Ming Gan, Karin Jennbacken, Edvin Johansson, Gunilla Linhardt, Sofia Martinsson, Muhammad Waqas Sadiq, Nevin Witman, Qing-Dong Wang, Chien-Hsi Chen, Yu-Ping Wang, Susan Lin, Barry Ticho, Patrick C.H. Hsieh, Kenneth R. Chien, and Regina Fritsche-Danielson

Supplemental Materials

A.



B.



C.

	T_{max} (h)	C_{max} (ng)	AUC_{0-72} (ng·h)	$t_{½}$ (h)
Mouse	3-6	3.86±0.60	40.8±6.9	25±40
Rat	3-6	6.47±0.90	125±20	39±21
Pig	6	4.15±15.2	NC	NC

Figure S1. Pharmacokinetics of VEGF protein from purified cs-*VEGF* mRNA. (A) Increasing doses of cs-*VEGF* mRNA were injected into a normal, non-ischemic rat heart and tissue levels were tested. With a ten-fold increase in dose, there was only a 1.6 fold increase in the area under the curve. (n=3 per time point and dose) (B) Pharmacokinetic profiles of VEGF protein in cardiac tissue in mouse, rat, and pig following single intra-cardiac injections of cs-*VEGF* mRNA (100 ug). (C) Pharmacokinetic parameters of VEGF protein in mice, rate, and pigs following single intra-cardiac injections of cs-*VEGF* mRNA (100ug). All data are mean +/- SD. Mouse and rat n=3, pig n=2.

Table S1. Plasma concentrations of IL-6 and IL-8 following intradermal and intravenous administration of *VEGF* mRNA in the Cynomolgus monkey

Dose (mg/kg)	Administration route	Analyte	Plasma concentration (pg/mL)			
			Week -2	Week -1	Day 1 6 h	Day 1 24 h
0	ID	IL-6	BLQ	BLQ	BLQ	BLQ
		IL-8	15.6 (11.8)	12.6 (6.0)	18.1 (16.7)	21.5 (5.6)
0.5	ID	IL-6	BLQ	BLQ	BLQ	BLQ
		IL-8	49.8 (48.1)	55.2 (66.7)	102 (89.2)	33.2 (35.3)
1	ID	IL-6	BLQ	BLQ	BLQ	BLQ
		IL-8	27.4 (34.9)	21.5 (15.2)	40.9 (22.7)	41.7 (14.8)
3	ID	IL-6	BLQ	BLQ	BLQ	BLQ
		IL-8	30.0 (18.6)	40.9 (29.9)	45.3 (27.2)	34.0 (16.4)
3	IV	IL-6	BLQ	BLQ	BLQ	BLQ
		IL-8	28.8 (11.3)	36.5 (37.2)	58.8 (28.1)	45.9 (39.0)

ID, intradermal, IV; intravenous, BLQ; below limit of quantification, NC; not calculated, NS; no sampling. Lower limits of quantification were 8.40 pg/mL (IL-6) and 2.88 pg/mL (IL-8), respectively. Results shown are means (SD), n=4 males/group.

Table S2. Plasma concentrations of IL-6 and KC following intradermal and intravenous administration of *VEGF* mRNA in the rat

Dose (mg/kg)	Administration route	Analyte	Plasma concentration (pg/mL)		
			Day 1 3 h	Day 1 6 h	Day 1 24 h
0	ID	IL-6	NS	BLQ	BLQ
		KC	NS	BLQ	BLQ
0.5	ID	IL-6	NS	BLQ	BLQ
		KC	NS	BLQ	BLQ
1	ID	IL-6	NS	BLQ	BLQ
		KC	NS	BLQ	BLQ
3	ID	IL-6	NS	BLQ	BLQ
		KC	NS	BLQ	BLQ
3	IV	IL-6	BLQ	NS	BLQ
		KC	106 (40.0)	NS	BLQ

ID, intradermal, IV; intravenous, BLQ; below limit of quantification, NC; not calculated, NS; no sampling. Lower limits of quantification were 4.37 pg/mL (IL-6) and 10.4 pg/mL (KC), respectively. Results shown are means (SD), n=10 males/group.

Table S3. Overview of cardiac therapy for preventing/repairing left ventricular dysfunction and timing of *VEGF* mRNA delivery after a myocardial infarction

Acute	Sub acute	Chronic
Anti-coagulation	Beta-blockers	+/- Beta-blockers
Anti-platelet	ACEI/ARB	+/-ACEI/ARB
Revascularization	VEGF mRNA	
Statins		
Beta-blockers		
ACEI/ARB		
VEGF mRNA		
If EF<40%:	If EF <40%:	IF EF <40%:
MRA	ARNI	+/- Revascularization
	Vasodilators*	Beta-blockers
	MRA	ACEI/ARB
		ARNI
		Vasodilators*
		Resynchronization
		MRA

ACEI; Angiotensin Converting Enzyme Inhibitors, ARB; Angiotensin Receptor Blocker, MRA; Mineralocorticoid Receptor Antagonists, ARNI;Angiotensin Receptor Blocker-Neprilysin Inhibitor. EF; Ejection Fraction. *Vasodilatory therapy for African-Americans.

Table S4. Complete codon optimized sequence of *VEGF* mRNA transcript used for production

Name	Sequence
VEGF mRNA Transcript	N7mGpppGmGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGAACUUU CUGCUGUCUUGGGUGCAUUGGAGCCUUGCCUUGCUGCUCUACCUCCACCAUGCCAAGUGGUCCCA GGCUGCACCCAUGGCAGAAGGAGGAGGGCAGAAUCAUCACGAAGUGGUGAAGUUCAUGGAUGUCU AUCAGCGCAGCUACUGCCAUCCAAUCGAGACCCUGGUGGACAUCUUCCAGGAGUACCCUGAUGAG AUCGAGUACAUCUUCAAGCCAUCCUGUGUGCCCCUGAUGCGAUGCGGGGGCUGCUGCAAUGACGA GGGCCUGGAGUGUGUGCCCACUGAGGAGUCCAACAUCACCAUGCAGAUUAUGCGGAUCAAACCUC ACCAAGGCCAGCACAUAGGAGAGAUGAGCUUCCUACAGCACAACAAAUGUGAAUGCAGACCAAAG AAAGAUAGAGCAAGACAAGAAAAUCCCUGUGGGCCUUGCUCAGAGCGGAGAAAGCAUUUGUUUG UACAAGAUCCGCAGACGUGUAAAUGUUCCUGCAAAAACACAGACUCGCGUUGCAAGGCGAGGCAG CUUGAGUUAAACGAACGUACUUGCAGAUGUGACAAGCCGAGGCGGUGAUAAUAGGCUGGAGCCUC GGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCC CGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAUCUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCCAGCCCCUCCU CCCCUUCCUGCACCCGUACCCCCGUGGUCUUUGAAUAAAGUCUGA
Open Reading Frame	ATGAACTTTCTGCTGTCTTGGGTGCATTGGAGCCTTGCCTTGCTGCTCTACCTCCACCATGCCAAGT GGTCCCAGGCTGCACCCATGGCAGAAGGAGGAGGGCAGAATCATCACGAAGTGGTGAAGTTCATGG ATGTCTATCAGCGCAGCTACTGCCATCCAATCGAGACCCTGGTGGACATCTTCCAGGAGTACCCTG ATGAGATCGAGTACATCTTCAAGCCATCCTGTGTGCCCCTGATGCGATGCGGGGGCTGCTGCAATG ACGAGGGCCTGGAGTGTGTGCCCACTGAGGAGTCCAACATCACCATGCAGATTATGCGGATCAAAC CTCACCAAGGCCAGCACATAGGAGAGATGAGCTTCCTACAGCACAACAAATGTGAATGCAGACCAA AGAAAGATAGAGCAAGACAAGAAAATCCCTGTGGGCCTTGCTCAGAGCGGAGAAAGCATTTGTTT GTACAAGATCCGCAGACGTGTAAATGTTCCTGCAAAAACACAGACTCGCGTTGCAAGGCGAGGCAG CTTGAGTTAAACGAACGTACTTGCAGATGTGACAAGCCGAGGCGG

Supplemental methods

Assessment of activation of the innate immune system in the Cynomolgus monkey and the rat

These studies were performed in accordance with the OECD principles of Good Laboratory Practice and in compliance with legislation (Animals (Scientific Procedures) Act 1986 and European Directive 2010/63/EU on the protection of animals used for scientific purposes). Three groups of Cynomolgus monkeys, each consisting of 4 males, were given VEGF mRNA intradermally, once on Day 1. The dose levels were 0.5, 1.0 or 3.0 mg/animal. An additional group of 4 males were given *VEGF* mRNA intravenously, once on Day 1 at a dose level of 3.0 mg/animal. A further group of 4 males were dosed with the citrate/saline vehicle (10 mmol/L sodium citrate, 130 mmol/L sodium chloride, pH 6.5) once intradermally on Day 1. Blood samples (using K_2EDTA as anticoagulant) for plasma analysis of IL-6 and IL-8 were taken from the femoral vein

at the following time points: twice pre study (week 2 and week 1) then 3 hours (intravenous dosing group only), 6 hours and 24 hours after dosing on day 1, respectively. The plasma concentrations of IL-6 and IL-8 were determined by a qualified method using Luminex technology. Calibration standards were prepared in Universal Assay Buffer and calibration standards were freshly prepared for each batch of samples. Analyses were performed in batches containing study samples, calibration standards at 7 concentration levels and blank samples. Lower limits of quantification for IL-6 and IL-8 were 8.40 and 2.88 pg/mL, respectively.

Three groups of Han Wistar rats, each consisting of 10 males, were given VEGF mRNA intradermally, once on Day 1. The dose levels were 0.5, 1.0 or 3.0 mg/animal. An additional group of 10 males were given *VEGF* mRNA intravenously, once on Day 1 at a dose level of 3.0 mg/animal. A further group of 10 males were dosed with the citrate/saline vehicle, as above. Blood samples (using K2EDTA as anticoagulant) for plasma analysis of IL-6 and keratinocyte-derived chemokine (KC, rodent functional counterpart to human IL-8) were taken from the lateral tail vein at the following time points: 3 hours (iv group), 6 hours (intradermal and vehicle groups) and 24 hours (all groups) after dosing. The plasma concentrations of IL-6 and KC were determined as described above. Lower limits of quantification for IL-6 and KC were 4.37 and 10.4 pg/mL, respectively.