Gene Therapy with Hepatocyte Growth Factor Improves Left Ventricular Systolic Function After Myocardial Infarction: A Systematic Review and Meta-analysis of Pig Models

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Abstract

Background: Hepatocyte growth factor (HGF) is an angiogenic cytokine which can promote angiogenesis and inhibit fibrosis. Previous studies have shown that HGF may have significant therapeutic effects on ischaemic diseases, such as ischaemic heart disease and peripheral arterial occlusive disease. Due to insufficient clinical study evidence, we conducted a quantitative meta-analysis of HGF therapy in pigs with myocardial infarction (MI) to provide more reliable evidence for the feasibility and effectiveness of HGF therapy for MI patients. We also analysed the efficacy and characteristics of different gene therapy vectors and drug delivery routes.

Methods: PubMed, EMBASE, and the China National Knowledge Infrastructure were searched for randomised studies that corresponded to our subject. The search terms included (hepatocyte growth factor OR HGF) AND (heart failure OR HF OR myocardial infarction OR MI OR AMI OR coronary heart disease OR CHD). The retrieved articles were screened strictly according to the inclusion criteria. The endpoints were the left ventricular ejection fraction (LVEF) and capillary density in the ischaemic regions in the model pigs.

Results: A total of nine studies were eventually included in this meta-analysis. Our analysis showed that LVEF (with mean difference [MD]:9.73, 95%CI :8.70, 10.76, $P < 0.00001$) and capillary density (MD:79.98, 95%CI :24.58,135.39, $P=0.005$) in the HGF group were significantly higher than those in the control group several weeks after HGF treatment. Further analysis showed that there was no statistically significant difference in the improvement of LVEF caused by intracoronary adenovirus₅-mediated HGF (Ad5-HGF) gene transfer and intramyocardial plasmid HGF injection (12.63 ± 3.2 vs 9.4 ± 1.09 , $P=0.06$), while intramyocardial injection of plasmid HGF had a stronger angiogenic capacity than intramyocardial administration of Ad5-HGF and HGF in hydrogel (117.34 ± 27.82 vs 26.45 ± 22.11 vs 11.50 ± 5.28 , $P=0.00001$).

Conclusions: HGF therapy can effectively promote angiogenesis and recovery of cardiac function and is a promising cardiac repair method. Intracoronary Ad5 vector transfer and intramyocardial injection of plasmid vectors can be used as effective means of gene therapy, and hydrogel as a vector also has potential applications.

Background

Myocardial infarction (MI), the most severe form of ischaemic heart disease (IHD), causes irreversible apoptosis and necrosis of myocardial cells [1]. The infarcted area is then replaced by fibrous scar tissue, leading to ventricular remodelling and heart failure (HF) [2, 3]. In the process of ventricular remodelling, the oxygen consumption of the hypertrophic ventricular wall increases, the number of capillaries decreases, and the myocardial ischaemia is further aggravated [4, 5]. Although early revascularisation can improve blood supply in ischaemic area to a certain extent, cardiac remodelling is still ongoing, resulting in HF approximately 50% of the time [6]. The only way to reverse this process is heart transplantation, which is limited by donor organ shortage, high cost, and the need for immunosuppressive medications [7]. Therefore, new therapeutic strategies should be explored for inhibiting cardiac remodelling and improving cardiac repair, and promoting angiogenesis is one promising approach. Among numerous angiogenic strategies, cytokine-based gene therapy has been extensively studied.

At present, a variety of vascular growth factors have been attempted for use in gene therapy after MI, among which vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) have attracted wide attention [8]. It has been reported that HGF could regulate the expression of cardiomyocyte-specific transcription factors and structural genes through its unique tyrosine kinase receptor, c-Met [9], and then perform functions such as promoting angiogenesis, regulating inflammation, inhibiting fibrosis, and even activating tissue regeneration [10]. HGF, and the activation of c-Met, could alleviate chronic myocardial injury in myocarditis, cardiomyopathy and other disease models [11]. In animal experiments, local HGF administration has been mainly conducted through intracoronary gene transfer using a catheter and intramyocardial injection via thoracotomy [12, 13]. At present, there have been relatively few clinical studies on the local application of HGF for MI treatment which, to some extent, hinders the prospects for application of HGF therapy. In contrast, HGF has been extensively studied in animal models. However, the efficacy of HGF has varied between studies, and researchers have used different carriers and routes of administration. Considering that pigs and humans are relatively similar species, we pooled the preclinical experimental data to provide more reliable evidence for the feasibility of clinical transformation of HGF therapy. In addition, the prospects of different treatment methods were analysed.

This article aims to analyse and summarise the applicability of HGF therapy after MI, as well as to compare and systematically review different approaches, including delivery routes and vehicles, and introduce new ones. Finally, more effective treatment options and future research directions will be discussed.

Methods

Search strategy

Two investigators retrieved literature through the PubMed database, the Excerpta Medica Database (Embase), and the China National Knowledge Information database. The search terms used were as follows: (hepatocyte growth factor OR HGF) AND (heart failure OR HF OR myocardial infarction OR MI OR AMI OR coronary heart disease OR CHD). We did not set a starting date for our search, but the end date was set at 28 March

2020. The publication language was not limited. The retrieved studies were carefully examined to exclude similar articles, and the studies related to the topic in the references of each article were manually retrieved to prevent omissions.

Study selection

Two investigators independently reviewed all of the retrieved studies, read the full text of each study, including titles and abstracts, and then extracted relevant data. Only randomised studies examining the effect of HGF therapy on cardiac function and angiogenesis in swine models with MI were included. The included studies had to be designed with experimental controls, and article types such as reviews and reports were excluded. Studies were included as long as either LVEF or capillary density was quantitatively assessed.

Quality assessment and data extraction

Two authors independently assessed the quality of the included studies according to five aspects: randomisation and control, adequate allocation, adequate method of randomisation, blinding of the operator, and blinding of the functional analysis. The following information was extracted from the full text: pig breed, gender, weight, number of pigs, intervention form, follow-up time, and any other relevant information. Any ambiguities were resolved by a more experienced third individual.

Data analysis and statistical methods

Our primary endpoints were the differences in mean LVEF (%) and capillary density (capillaries/mm²) at follow-up between the HGF group and the control group. We also measured the effects of different delivery methods and vehicles on cardiac function and angiogenesis. The mean difference (MD) and 95% confidence interval (CI) were used to calculate and assess evaluation data of the continuous results between HGF treatment and control groups. Statistical analysis was performed using Review Manager (version 5.3). Furthermore, I^2 values were used to assess the heterogeneity among the included articles. If $I^2 \leq 50\%$, the random-effect model would be used; if $I^2 > 50\%$, the fixed-effect model would be adopted. To test the robustness of the results, sensitivity analysis was performed by excluding the studies one by one. In addition, we performed subgroup analysis according to different conditions and used funnel plots to describe whether there was potential publication bias.

Results

3.1 Search results

Using the above query method, 1,224 articles were retrieved. We excluded studies that did not address our research topic or did not focus on pigs. Non-controlled studies were also dismissed. We finally included nine studies after excluding similar and identical articles by analysing the full text. The detailed selection process for included studies is represented in Figure 1.

3.2 Risk of bias assessment and study characteristics

All nine studies included in the meta-analysis met our selection criteria. The methodological quality of each study was evaluated, and the specific content of the quality assessment is shown in Table 1. In all included studies, MI pig models were established, and were randomly divided into an HGF treatment group and a control group, which conformed to the randomisation and control method. However, not all studies indicated whether blinded analysis of cardiac function and angiogenesis was used. After completing the study quality assessment, we extracted basic characteristics of each study, including pig breed, gender, weight, number of pigs, intervention form, follow-up time, and other relevant information (Table 2). Finally, 115 pigs with MI were analysed, 57 of which received HGF treatment and 58 that received control treatment.

3.3 Meta-analysis

Six studies [14-19] provided data on LVEF (%) and included 81 MI pigs, of which 40 were treated with HGF and 41 were used as controls. Since there was no significant heterogeneity ($I^2=2\%$, $P=0.40$) among studies, the data analysis was based on the fixed-effect model. The results of the analysis suggested that, compared with the control group, local application of HGF significantly increased LVEF (MD:9.73, 95% CI:8.70, 10.76, $P=0.00001$; Figure 2). In addition, of the six included studies, three were conducted with intracoronary adenovirus₅-mediated HGF (Ad5-HGF) transferred into the myocardium via the right coronary artery, and the other three were conducted with plasmid HGF intramyocardial injection. Therefore, we performed a subgroup analysis based on these two methods, and the results showed that there was no statistically significant difference in the improvement of LVEF between intracoronary injection of Ad5-HGF and intramuscular injection of plasmid HGF (MD: 12.63, 95% CI:9.43, 15.83 vs MD:9.40, 95% CI:8.31, 10.48, $P=0.06$; Figure 3).

The effects of HGF on angiogenesis in ischaemic areas of MI pigs were studied in five articles [12, 17, 18, 20, 21] involving 64 pigs, of which 32 were treated with HGF and 32 were used as controls. Angiogenesis was measured by capillary density (capillaries/mm²). Considering the significant heterogeneity between studies ($I^2=98\%$, $P<0.00001$), we used a random-effect model to analyse the difference in capillary density between the HGF group and the control group. The results showed that the capillary density in the ischaemic area of the HGF group was significantly higher than that of the control group (MD:79.98, 95% CI:24.58,135.39, $P=0.005$; Figure 4). In addition, an intramyocardial injection

delivery method was used in these five studies, but the HGF vehicles used were different. Among them, plasmid vectors were used in three studies, an Ad5 vector in one study, and a hydrogel vector in one study. Therefore, we performed a subgroup analysis to compare the therapeutic effects of different vectors under the same route of administration. The results showed that plasmid-mediated HGF therapy had a stronger angiogenic effect than Ad5 and hydrogel when intramyocardial administration was applied, with a statistically significant difference (117.34 ± 27.82 vs 26.45 ± 22.11 vs 11.50 ± 5.28 , $P=0.00001$; Figure 5).

In addition, sensitivity analysis, performed by excluding the studies one by one, demonstrated the same results, which indicated the meta-analysis was robust. However, the funnel plot indicated that there might be some publication bias, as the values were not completely and symmetrically distributed around the overall estimate (Figure 6).

Discussion

Chronic myocardial ischaemia and ventricular remodelling after MI are important causes of HF. In recent years, gene therapy based on angiogenic factors has become a potential method to promote angiogenesis, inhibit ventricular remodelling, and improve cardiac function [22, 23]. The cytokines that have been studied in preclinical and clinical research include VEGF, HGF, fibroblast growth factor, and insulin-like growth factor, and some studies have achieved encouraging results [24, 25]. Recently, Wang et al. [26] injected adenovirus-mediated VEGF165 into the peri-infarct myocardium in an MI rat heart, and their results suggested that VEGF165 gene therapy could improve cardiac function by inducing angiogenesis and inhibiting cardiomyocyte apoptosis.

HGF and its potential angiogenic, anti-apoptotic, and anti-fibrotic effects [27, 28] have been extensively studied in various models of ischaemic disease, such as MI [29] and peripheral artery occlusion disease [30]. A Phase III clinical trial of intramuscular injection of plasmid HGF for the treatment of severe limb ischaemia has been successful [31], suggesting that HGF may also be used for the treatment of IHD. The biological function of HGF is mediated by its unique tyrosine kinase receptor c-Met [32]. Activation of the c-Met receptor further activates many intracellular signalling pathways, including RAS-mitogen activated protein kinase, signal transducer and activator of transcription, phosphatidylinositol-3 kinase, protein kinase B, mammalian target of rapamycin, and β -catenin pathway [33, 34]. Our group has previously shown that HGF therapy could promote cardiac repair and improve cardiac function in MI rats through the above mechanisms [35].

Although HGF has been widely studied in some large animal MI models, the clinical trials related to HGF therapy have just started [36], which prompted us to conduct a meta-analysis on the preclinical data. Our study showed that the cardiac pump function of the HGF group was significantly better than that of the control group within 1-2 months after MI, with an increase in LVEF of about 9.73%. In addition to the improvement of LVEF, local application of HGF promoted angiogenesis and increased blood supply to the ischaemic area. Compared with the control group, the capillary density in the HGF treatment group was significantly increased (about 97.33 capillaries/mm² difference). Our study fully demonstrates that HGF treatment after MI can promote angiogenesis and improve the cardiac pump function. Neovascularization can improve the blood supply in ischaemic areas, provide preconditions for the tissue repair of the heart and, to some extent, reverse the fibrosis induced by hypoxia [12].

In terms of vectors and routes of delivery, plasmids, adenoviruses and injectable hydrogels have been widely accepted for gene therapy [37]. As mature and traditional vectors, plasmid- and adenovirus-mediated HGF gene transfer can significantly improve HGF expression in myocardial tissue. Wang et al. [15] transferred Ad5-HGF (4×10^9 pfu) into the myocardium via the right coronary artery four weeks after left anterior descending coronary artery (LAD) ligation in pigs, and detected the expression level of HGF by ELISA three weeks later. The results showed that the expression level of HGF in the experimental group increased to nearly 18 times that of the control group (109.3 ± 7.8 vs 6.2 ± 2.6), and the LVEF value increased by nearly 45% (43.9 ± 4.3 vs 30.4 ± 2.8). Similarly, Funatsu et al. [38] injected 125 microg of plasmid encoding human HGF (hHGF) into the peri-infarct regions of canines four weeks after LAD ligation, and the expression of hHGF was specifically detected by ELISA after four weeks (endogenous HGF could not be detected). The results showed that hHGF expression in the HGF group was 4.7 ± 1.7 ng/g, while no hHGF protein expression was found in the control group, and the number of capillaries in the ischaemic area of the HGF group also increased to 140% of that in the normal area. Therefore, both plasmids and adenoviruses as vectors can effectively improve the expression of HGF. In addition, studies have shown that local application of plasmid HGF or Ad5-HGF is reliable in terms of safety [39]. As illustrated by the above points, plasmids and adenoviruses are ideal vectors for gene therapy. Our analysis showed that intracoronary Ad5-HGF gene transfer and intramyocardial plasmid HGF injection had similar effects on cardiac pump function at conventional doses, and both treatments could bring significant improvement in LVEF (12.63 ± 3.2 and 9.4 ± 1.09 ; $P=0.06$). Injectable hydrogel is a promising synthetic biomaterial, with advantages such as mild gelation and cardiac-compatible properties [40, 41]. Recent studies have shown that the injection of hydrogel in ischaemic areas after MI could promote cardiac repair and improve cardiac function [42]. Hydrogels can also encapsulate therapeutic drugs and deliver them to target areas to achieve sustained therapeutic effects [43]. Steele et al. [44] encapsulated two cytokines, dimeric fragment of HGF and engineered stromal cell-derived factor 1 α , in hydrogels and delivered them to the peri-infarct regions, and found that the release time of the cytokines was significantly prolonged and the therapeutic effects were enhanced when the cytokines were encapsulated in hydrogels. These unique advantages of hydrogels potentially make them a more attractive method of exogenous administration.

All of the studies included in this meta-analysis adopted local administration. The traditional route of local administration is either intracoronary delivery of therapeutic drugs through a catheter or intramyocardial injection through thoracotomy, which can be used locally in coronary artery bypass grafting [45]. Our analysis showed that intramyocardial injection of plasmid HGF had a stronger angiogenic capacity than intramyocardial injection of Ad5-HGF and HGF in hydrogel ($P=0.00001$). Recently, Shi et al. [46] designed phase-transition microneedles coated with adeno-associated virus (AAV), which achieved uniform distribution of AAV delivery and were superior to direct muscular injection. This new delivery system may further optimise the efficacy of intramuscular injection. The advantage of local administration is that it effectively avoids the accumulation of drugs in other tissues and makes the concentration of drugs in the heart reach its peak. The disadvantage is that it limits the convenience and repeatability of its application. In contrast, systemic administration is more convenient, but it is necessary to find and apply heart-specific vectors to reduce the distribution of drugs in other tissues and organs.

This meta-analysis had certain strengths and limitations. First of all, this is the first meta-analysis pooling data from preclinical large animal studies, which provides strong evidence for the feasibility of HGF therapy in the treatment of MI. A second strength was that we analysed the therapeutic effects and characteristics of different vectors and delivery routes, and introduced a new intramuscular delivery system, providing a methodological basis for HGF gene therapy and other drug gene therapy. In terms of limitations, some studies did not provide relevant statistical data (three without LVEF data, four without capillary density data), which may partially affect the accuracy of the results. Secondly, most studies only used western blot to qualitatively analyse the expression difference of HGF between the experimental group and the control group without quantifying HGF expression. Therefore, we were unable to compare the differences in the expression of HGF between studies. In addition, there was some methodological heterogeneity in the included studies; however, they all observed the effects of HGF on cardiac function and angiogenesis in the chronic phase of MI, so we relaxed this aspect of our inclusion criteria and conducted some valid subgroup analyses to address the issue.

Conclusion

The safety of local HGF application has been preliminarily confirmed by previous Phase I clinical studies, which should be further verified by multicentre studies with more rigorous experimental design and longer follow-up times. Based on the current evidence, we demonstrated that HGF therapy after MI could promote angiogenesis and improve cardiac function, and that intramyocardial injection of plasmid vector and intracoronary Ad5 vector transfer are effective means to achieve these effects. Although differences exist between humans and pigs, our study provides reliable evidence for clinical transformation of HGF therapy. In addition, HGF can combine with other therapeutic factors to have a synergistic effect. For example, a stronger therapeutic effect may be achieved through combined application of HGF with bone marrow mesenchymal stem cells, VEGF, and other therapeutic factors, although this needs to be confirmed by further preclinical and clinical trials.

Declaration

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

Chong Du designed and wrote the manuscript. Xiao-Wen Chen and Ze-Mu Wang collected the data and participated in the design. Hao-Yu Meng, Ya-Fei Li and Tian-Wen Wei conducted the analysis and developed the Figs. Lian-Sheng Wang (corresponding author) supervised and performed manuscript editing. All authors approved the final version of the manuscript.

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None.

Abbreviations

IHD: Ischemic Heart Disease; HGF: hepatocyte growth factor; VEGF: Vascular endothelial growth factor; MI: myocardial infarction; LVEF: left ventricular ejection fraction; HF: heart failure; MD: mean difference; CI: confidence interval; Ad5-HGF: adenovirus5-mediated HGF; LAD: left anterior descending coronary artery; hHGF: human HGF; AAV: adeno-associated virus

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Tables

Table 1. Methodological quality of the included studies

Study	randomized controlled study	Adequate allocation	Method of randomization described	Operator blinded	Analyst blinded
Koudstaal et al. 2014 [12]	Y	Y	N	N	N
Carlsson et al. 2008 [14]	Y	Y	N	N	N
WANG et al. 2006 [15]	Y	Y	N	N	N
Yang et al. 2010 [16]	Y	Y	N	N	N
Saeed et al. 2008 [17]	Y	Y	N	N	N
Cho et al. 2008 [18]	Y	Y	N	N	N
Zhang et al. 2008 [19]	Y	N	N	N	N
Saeed et al. 2011 [20]	Y	Y	N	N	N
Chen et al. 2013 [21]	Y	Y	N	N	N

Note: The study conducted by Zhang et al. [19] was published on the China National Knowledge Information database. Y, yes; N, no

Table 2. Summary of characteristics of the included studies

Author, year	pig breed	Sex	Weight (kg)	n _t /n _c	intervention	dose	administration method	Timing of therapy	Control factors	follow-up
WANG et al. 2006	Suzhong swine	male	30±5	6/6	Ad5-HGF	4×10 ⁹ pfu	Intracoronary gene transfer	4 weeks post MI	null-Ad5	7 weeks
Saeed et al. 2008	not mentioned	Female/male	30±4	8/8	plasmid pCK-HGF-X7	2 mg	intramyocardial injections	3 hours post MI	saline	50 days±3
Carlsson et al. 2008	not mentioned	Female/male	30–40	8/8	plasmid pCK-HGF-X7	2 mg	intramyocardial injections	3 hours post MI	saline	50 days±3
Koudstaal et al. 2014	Dalland landrace pigs	female	~70	5/5	HGF in hydrogel	1 mg	intramyocardial injections	4 weeks post MI	hydrogel	8 weeks
Cho et al. 2008	Yorkshire swine	male	31.5±2.1	7/7	plasmid pCK-HGF-X7	1 mg	intramyocardial injection	4 weeks post MI	pCK-LacZ	8 weeks
Chen et al. 2013	mini-pigs	male	36±3.4	6/6	Ad5-HGF	1×10 ¹⁰ genome copies	Intramyocardial Delivery	4 weeks post MI	saline	8 weeks
Yang et al. 2010	not mentioned	male	23 ± 3	6/6	Ad5-HGF	5×10 ⁹ Pfu	Intracoronary gene transfer	4 weeks post MI	null-Ad5	7 weeks
Saeed et al. 2011	not mentioned	Female/male	30–32	6/6	plasmid pCK-HGF-X7	3.96×10 ¹¹ viral copies	intramyocardial injection	5 weeks post MI	pCK-LacZ	10 weeks
Zhang et al. 2008	mini-pigs	Female/male	26 ± 3	5/6	Ad5-HGF	5×10 ⁹ Pfu	Intracoronary gene transfer	4 weeks post MI	null-Ad5	7 weeks

n, number of animals (n_c, control; n_t, treated); HGF, Hepatocyte growth factor; Ad5-HGF, adenovirus-mediated HGF

Figures

Figure 2

Forest plot showing the impact of HGF therapy on LVEF. LVEF: left ventricular ejection fraction;

Figure 3

Subgroup analysis based on intracoronary Ad5-HGF gene transfer and intramyocardial plasmid HGF injection. Ad5-HGF: adenovirus5-mediated hepatocyte growth factor.

Figure 4

Forest plot showing the impact of HGF therapy on angiogenesis. Angiogenesis is measured by capillary density (capillaries/mm²).

Figure 5

Subgroup analysis based on different vectors under the same route of administration (intramyocardial injection). Ad5-HGF: adenovirus5-mediated hepatocyte growth factor.

Figure 6

Publication biases. A funnel plot illustrating publication biases for included studies comparing LVEF between the HGF group and the control group.

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