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Research Paper

Effect of Guanxin No.2 decoction on gene expression in different areas of the myocardial infarcted heart of rats using microarray technology

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Abstract

Objectives We have used microarray technology to detect the effect of Guanxin No.2 decoction on gene expression in different areas of the myocardial infarcted heart of rats.
Methods Male Sprague-Dawley rats (180–200 g) were randomly divided into three groups: sham-operated; coronary artery ligation; and coronary artery ligation plus administration of Guanxin No.2 decoction (10.0 g raw materials/kg per day by gavage). The experiment was carried out on day seven after ligation.
Key findings We found that the gene expression using microarray technology showed many differences in the border infarcted left ventricular area compared with the remote noninfarcted left ventricular area after administration of Guanxin No.2 decoction.
Conclusions Guanxin No.2 decoction has a long history in treating ischaemic cardiomyopathy in China, but the molecular mechanism has been unclear. In this study we found that some important genes may have contributed to the cardioprotective effect of Guanxin No.2 decoction.
Keywords collagen deposition; fibrinogen; Guanxin No.2; myocardial infarction; oxidative stress

Introduction

Microarray technology was founded in the 1990s, but it has been quickly developed and applied in many fields, especially in transcriptome analysis, because of its ability to detect thousands of millions of targets simultaneously. It has also been used to detect the gene expression in myocardial infarction (MI) samples.[1–8] However, there have not been any reports on the therapeutic effects of Chinese traditional medicine (CTM), which has been used to treat myocardial infarction, and the difference between the border area and the remote area of the infarct is still unknown.

Guanxin No.2 decoction (GXII) is a typical traditional Chinese medicine prescription. It was developed in the 1970s and consists of five herbs: *Salvia miltiorrhiza* Lamiaceae, *Ligusticum Chuanxiong* Hort, *Paeonia lactiflora* Pall, *Carthamus tinctorius* L and *Dalbergia odorifera* T. Chen. It has a long history of frequent use in treating cardiovascular disease in China, Japan and other Asiatic countries. In previous studies, it was found to promote blood circulation, removing blood stasis and scavenging the channels or blood vessels, so it has been applied in clinical practice to treat cardiovascular disease, such as acute heart attack, cardiac infarction and congestive heart failure.[9–12] Although it has been extensively applied clinically, the mechanism of action of GXII has remained unclear. In this study, we have used microarray technology to detect any difference in gene expression between the border infarcted left ventricular (LV) area and the remote noninfarcted left ventricular area of myocardial infarcted rats after administrating GXII, with the aim of finding some important target genes of GXII treatment.

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Materials and Methods

Preparation of phytochemical profile of Guanxin No.2 decoction

The GXII was prepared by traditional production methods. *Salvia miltiorrhiza* Lamiaceae, *Ligusticum Chuanxiong* Hort, *Paeonia lactiflora* Pall, *Carthamus tinctorius* L and *Dalbergia*

odorifera T.Chen were mixed in the dry weight ratio of 6 : 3 : 3 : 3 : 2. To keep the consistency of the herbal chemical ingredients, all of the herbal components were originally obtained from the standard native sources as stated above with GAP grade and authenticated by academician X. S. Yao. The voucher specimens were deposited in the Institute of Chinese Herb Medicine, China Academy of Traditional Chinese Medicine, Beijing, China. The drugs were extracted with standard methods according to the Chinese Pharmacopoeia.[13] GXII was made through boiling with distilled water at 100°C for 30 min twice and the drug solution was vacuum cool-dried to produce the drug powder, which was dissolved in distilled water to a final concentration of 1.8 g/ml (equivalent to dry weight of raw materials).

A high-performance liquid chromatography-diode array detection (HPLC-DAD) method has been developed for the analysis and the quality control of GXII decoction. The chromatographic separation was obtained on a Kromasil C_{18} (4.6 mm × 250 mm, 5 μm) column in Agilent 1100 (Agilent Technology, Beijing, China). The mobile phase was a gradient of CH_3CN–H_2O buffer. The effluent was monitored on a DAD detector. The results were compared at different wavelengths and the HPLC chromatograph was established based on the data at 254 nm. A typical chromatograph of GXII samples analysed by HPLC had been published previously.[14] In the chromatograph, seven peaks were marked. By using the HPLC-ultraviolet electrospray ionization (UVESI)-time-of-flight (TOF)-mass spectrometry (MS) system, they were identified according to compound molecular weight data. Peaks numbered 1–7 represented danshensu, protocatechuic acid, protocatechualdehyde I, hydroxysafflor yellow A, paeoniflorin, ferulic acid and salvianolic acid B, respectively (see Figure 1).

Animals and experimental protocol

The animal experiments were performed in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals of Zhejiang Province, and approved by the local ethics committee. Male Sprague-Dawley rats (180–200 g initially; Laboratory Animal Center of Zhejiang University) were housed individually at controlled temperature and humidity with a 12-h-light/dark cycle. Rats had free access to a standard diet and drinking water. The myocardial infarction model was carried out by ligation of the left anterior descending coronary artery. Briefly, the rats were anaesthetized with urethane (1.5 g/kg, i.p.) and ventilated by a volume-regulated respirator. The thorax was opened, the heart was exteriorized, and the proximal left anterior descending coronary artery was ligated 2–3 mm from its origin between the pulmonary artery conus and the left atrium with a 4-0 prolene suture. The heart was returned to its normal position, and the thorax was closed. Sham-operated rats underwent identical surgical procedures as described above except that the suture was not tightened around the coronary artery. The surviving rats were divided into three groups: sham-operated group, coronary artery ligation (CAL) group; CAL+GXII group (10.0 g raw materials/kg/per day by gavage) beginning within 0.5 h before surgery. Vehicle was administered to the sham-operated and CAL groups once daily for seven days. The experiment was carried out on the seventh day after ligation.

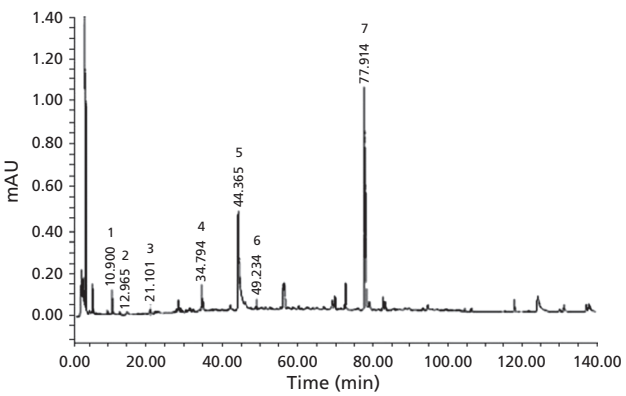

Figure 1 A typical chromatograph of the Guanxin No.2 decoction analysed by HPLC. 1, Danshensu; 2, protocatechuic acid; 3, protocatechualdehyde I; 4, hydroxysafflor yellow A; 5, paeoniflorin; 6, ferulic acid; 7, salvianolic acid B.

Preparation of reference RNA

Total RNA was extracted from heart, spleen, liver and kidney of three normal male Sprague-Dawley rats using Trizol reagent (Invitrogen, Shanghai, China). The RNA sample from each tissue was purified using phenol–chloroform extraction. RNA quality and quantity were evaluated by electrophoresis and UV absorbance at 260 (A260) and 280 nm (A280). The RNA yield of each sample was determined spectrophotometrically, assuming one optical density at 260 nm (OD_{260}) unit = 40 mg/l. The examined RNA of heart, spleen, liver and kidney was mixed with the ratio of 2 : 2 : 3 : 3.

Microarray design and construction

A total of 1285 genes were selected for microarray detection. These genes were selected from the literature in which they were reported as candidate genes, and from commercial cardiovascular microarrays and some related databases. Furthermore, another part of this gene set was a number of heart specific genes which were detected by Pearson's chi-square test (χ^2) from the UniGene database.[15,16]

A set of positive controls was added, including Actb (actin, beta), Ldha (lactate dehydrogenase A), Tuba1 (tubulin, alpha 1), Gapdh (glyceraldehyde-3-phosphate dehydrogenase), Rps5 (ribosomal protein S5), Rps12 (ribosomal protein S12), Rpl32 (ribosomal protein L32), Aldoa (aldolase A), A2m1 (alpha-2-macroglobulin). The negative control was the spotting solution, i.e. 3× saline–sodium citrate buffer (SSC).

Gene-specific oligonucleotides were designed and synthesized by Integrated DNA Technologies, Inc. (Skokie, IL, US), with an average length of 61 mers (range from 53 to 73 mers). Each oligonucleotide was printed three times on the poly-L-lysine treated glass slides by SpotArray 72 microarray printing systems (PerkinElmer Life Sciences, Boston, MA, US) according to the manufacturer's instructions, and the positive and negative controls were arranged in the origin and the last row of each block on the array.

RNA isolation, probe preparation and microarray hybridization

Seven days after ligation, the left ventricle of each rat heart was washed and separated into two parts, namely the border region of myocardial infarction and the remote noninfarcted left ventricular area (NMI). The tissues were quickly frozen in liquid nitrogen and stored at −80°C until RNA extraction. Total RNA was extracted from each tissue by RNA simple Total RNA Kit (Tiangen Biotech (Beijing, China) Co., Ltd) according to the manufacturer's instructions. The RNA yield of each sample was determined spectrophotometrically, assuming 1 OD_{260} unit = 40 mg/l. The quality of total RNA extracted from each sample was monitored by A260 : A280 ratio and 1.0% agarose formaldehyde gel electrophoresis. All samples had A260 : A280 ratios of approximately two and exhibited discrete 28S and 18S bands.

Due to a relatively high frequency of error, triplicate biological replicate samples of each group were obtained, and RNA obtained from each sample along with reference RNA was hybridized with two microarrays using dye-swap methods. This was also an indirect experimental design i.e. each sample was compared with reference RNA on a single array.

Cy5/Cy3-labelled cDNA was produced by reverse transcription of total RNA samples (25 μg each), using an established direct labelling protocol with minor modification.[17] The Cy5-labelled sample along with the Cy3-labelled cDNA were hybridized together to each array.[18] Hybridizations were performed overnight (approximately 18 h) at 42°C, and after hybridization the slides were washed.[18]

Measurement of spot intensity and normalization

Microarrays were scanned using a GenePix 4100A confocal laser microscope. GenePix pro 6.0 was used to quantify hybridization signals. Before channel normalization, microarray outputs were filtered to remove spots of poor signal quality by excluding those data points with a mean intensity less than two standard deviations above background in both channels.[19]

Background correction, normalization and statistical analysis were carried out in the R computing environment (2.11, Raqua on Windows) using the linear models for microarray data package (Limma).[20] Within Limma, normexp background correction method with an offset of 50 was used to remove the effects of nonspecific binding or spatial heterogeneity across the array, and printtip LOESS normalization was carried out for each microarray.[21–23] For between array normalization, the vsn method was executed.[24,25]

Validation of gene expression

Reverse transcription polymerase chain reaction (RT-PCR) for Ucp2 (uncoupling protein 2), Col1a1 (procollagen type I, alpha 1), Fgb (fibrinogen B beta polypeptide), Fxyd1 (FXYD domain-containing ion transport regulator 1), and Spp1 (secreted phosphoprotein 1) were carried out for validation. The reagents were from Promega Corporation, USA. Oligonucleotides for the primers were all synthesized by Invitrogen (Shanghai, China). Total RNA was extracted from 100 mg frozen noninfarcted and infarcted left ventricular area samples by using 1 ml Trizol, respectively. The total RNA was reverse transcripted into cDNA by AMV reverse transcriptase (Promega Corporation, Madison, WI, US). To ensure a fixed amount of initial mRNA in parallel with β-actin, amplification was performed using the following sequences: sense: 5′-GGT ATG GGT CAG AAG GAC TCC-3′, antisense: 5′-TGA TCT TCA TGG TGC TGC TAG GAG CC-3′, predenaturation at 94°C for 5 min, denaturation at 94°C for 40 s, annealing at 60°C for 60 s, extension at 72°C for 1 min, 30 cycles and final extension at 72°C for 10 min. The primers used for Ucp2, Col1a1, Fgb, Fxyd1, and Spp1 are listed in Table 1. PCR was performed according to the conditions listed in Table 1 by an authorized thermal cycler (Eppendorf, Hamburg, Germany).

The PCR amplified cDNA were separated by agarose gel electrophoresis, stained with ethidium bromide and analysed by densitometry. The β-actin was used as the loading control. The integrated optical density (*IOD*) of the band was measured and the following formula was used to establish the differences in the expression level of the genes:

$$\text{Relative expression level} = \frac{IOD_{target\ gene}}{IOD_{\beta\text{-}actin}} \times 100\%$$

Statistical methods

Due to a relatively high frequency of error, triplicate biological replicate samples of each group were obtained. RNA obtained from each sample was hybridized with two

Table 1 Primers and amplification conditions for reverse transcription polymerase chain reaction validation

Genes	Primers	Amplification conditions
fgb	Forward: 5′ AGCCTGACACCTCCAGCAAACC 3′ Reverse: 5′ GCCATCGTTGTCCCTGTCGTAG 3′	94°C, 4 min; (94°C, 40 s; 60°C, 60 s; 72°C, 60 s;) 32 cycles; 72°C, 10 min
Col1a1	Forward: 5′ ACTTCCCTACCCAGCACCTT 3′ Reverse: 5′ CCACCCCTTCACAGAGATGT 3′	94°C, 4 min; (94°C, 40 s; 55°C, 60 s; 72°C, 60 s;) 31 cycles; 72°C, 10 min
Ucp2	Forward: 5′ TTCTACAAGGGGTTCATGCC 3′ Reverse: 5′ GTTAGCAGGACTGAGGGTGC 3′	94°C, 4 min; (94°C, 40 s; 55°C, 60 s; 72°C, 60 s;) 30 cycles; 72°C, 10 min
Fxyd1	Forward: 5′ GCACCTCTCCACCACATCTT 3′ Reverse: 5′ CCCAGTTCTCTGCTGTTGGT 3′	94°C, 4 min; (94°C, 40 s; 56°C, 60 s; 72°C, 60 s;) 31 cycles; 72°C, 10 min
Spp1	Forward: 5′ GAGGAGAAGGCGCATTACAG 3′ Reverse: 5′ GCAACTGGGATGACCTTGAT 3′	94°C, 4 min; (94°C, 40 s; 56°C, 60 s; 72°C, 60 s;) 31 cycles; 72°C, 10 min

microarrays using dye-swap. Each microarray slide contained triplicate sets of gene fragments. Therefore, 18 data points were obtained for each gene. The three replicate spots per gene in each array were used to maximize the robustness of differential expression measurement of each gene via the 'lmFit' function within Limma. This step uses a pooled correlation estimation to generate a more robust estimation of the gene expression across replicate spots, compared with a straight average of replicate spots.[26] For statistical analysis and assessing differential expression, an empirical Bayes (eBayes) method was executed to moderate the standard errors of the estimated log-fold changes.[27] When the fold changed to more than two, and the *P* value was less then 0.05, then the gene was considered as being significantly differentially expressed.

Results

Components of Guanxin No.2. decoction

GXII contains five plants: Dan-Shen; Chuan-Xiong; Jiang-Xiang; safflower; and peony. The decoction consisted of seven chemicals: danshensu; protocatechuic acid; protocatechualdehyde I; hydroxysafflor yellow A; paeoniflorin; ferulic acid; and salvianolic acid B. Figure 1 shows a typical chromatograph of GXII analysed by HPLC.

General patterns of gene expression in border infarcted left ventricular area

After seven days of ligation, 182 significantly changed genes had been detected in the border infarcted left ventricular area of the CAL group, and 123 (approximately 67.6%) of them were upregulated. The most increased gene by the ligation was procollagen, type 1, alpha 1 (Col1a1) which was one-hundred-times upregulated. After administration of GXII, 41 genes were changed with statistical significance compared with the CAL group, and 34 (approximately 82.9%) genes were increased. That is, in the border infarcted left ventricular area, more than two-thirds of the changed genes were upregulated (Figure 2). The most activated gene after GXII intervention was glutathione peroxidase 1 (Gpx1) compared with the CAL group, but it did not show statistical significance in the CAL group. That means it was upregulated solely after GXII intervention. There were 17 genes which showed this pattern, and only one gene was repressed by GXII. Furthermore there were 10 genes which showed an opposite change in the CAL and GXII groups. Nine of them were recovered to the normal level; i.e. the expression level in the GXII-treated group was similar to the sham group. Five genes were upregulated to a higher level after GXII intervention in the border infarcted left ventricular area.

General patterns of gene expression in the remote noninfarcted left ventricular area

In the remote noninfarcted left ventricular area, 275 significantly changed genes were obtained, and 178 (approximately 64.7%) of them were downregulated. After treatment with GXII, 50 genes appeared to be changed significantly compared with the CAL group, and 28 (approximately 56%) genes were repressed. So in contrast to the border infarcted left ventricular area, in the remote noninfarcted left ventricular area downregulated genes formed the larger proportion. Among the 50 significantly changed genes, 11 of them were changed only by GXII, and 26 genes were changed oppositely by GXII compared with the CAL group, whilst six genes were upregulated to a higher level after GXII intervention. Meanwhile, administration of GXII for seven days resulted in 13 genes being recovered to the normal level.

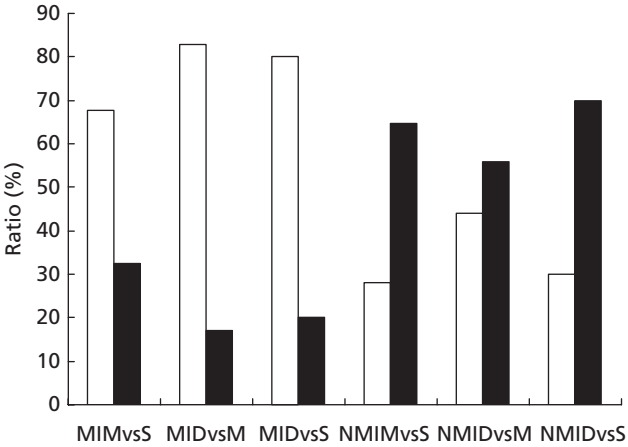

Figure 2 The ratio of up- and downregulated genes comparing different groups. MIMvsS, the coronary artery ligation (CAL) group compared with the sham group in the border infarcted left ventricular area; MIDvsM, the Guanxin No.2 decoction (GXII) group vs the CAL group in the border infarcted left ventricular area; MIDvsS, the GXII group vs the sham group in the border infarcted left ventricular area. The corresponding area away from myocardial infarction (NMI) was denoted by the remote noninfarcted left ventricular area. Upregulation □; downregulation ■.

Genes changed by the administration of GXII in the border infarcted left ventricular area and the remote noninfarcted left ventricular area

We detected only five genes which showed significant changes in the border infarcted left ventricular area as well as the remote noninfarcted left ventricular area (Table 2). They were: Ucp2 (mitochondrial, a proton carrier), Col1a1, Fgb, Fxyd1 and Col16a1 (procollagen type XVI, alpha 1). Ucp2 was activated solely by GXII in the border infarcted and remote noninfarcted left ventricular areas. Gene Col1a1 was extremely increased in the CAL group, but after treatment with GXII this gene was significantly repressed in the border infarcted and remote noninfarcted left ventricular areas. However, in the border infarcted left ventricular area the Fgb gene was increased in the CAL and the GXII groups; in the remote noninfarcted left ventricular area, it was significantly depressed in both groups. The Fxyd1 gene, which was repressed by the ligation in the remote noninfarcted left ventricular area, was activated after GXII intervention in the myocardial infarcted border and remote noninfarcted left ventricular areas. In the CAL group however, the Fxyd1 gene did not show significant changes in the border infarcted left ventricular area. Compared with the Fxyd1 gene, the Col16a1 gene showed the same pattern in the border infarcted left ventricular area, i.e. the gene's changes were solely dependent on the decoction, but in the remote

Table 2 Genes which showed statistically significant changes in the border infarcted and remote noninfarcted left ventricular areas

Accession number	Gene symbol	Area	Fold change		
			M vs S	D vs M	D vs S
NM_019354	Ucp2	MI	NA	2.755	3.785
		NMI	NA	1.372	1.359
XM_213440	Col1a1	MI	8	−3.018	4.982
		NMI	5.32	−3.169	2.151
NM_020071	Fgb	MI	2.325	3.925	6.25
		NMI	−3.447	−3.049	−6.497
NM_031648	Fxyd1	MI	NA	1.346	2.116
		NMI	−4.07	1.444	−2.626
XM_345584	Col16a1	MI	NA	2.277	1.624
		NMI	2.573	−1.174	1.399

NA, no statistical significance; MI, myocardial infarction, represented by the border infarcted left ventricular area; NMI, represented by the noninfarcted left ventricular area, namely the remote infarcted area; M vs S, the coronary artery ligation (CAL) group compared with the sham group; D vs M, the CAL+GXII (Guanxin No.2 decoction) group vs the CAL group; D vs S, the CAL+GXII group vs the sham group.

Table 4 The fold changes of the selected gene expression by reverse transcription polymerase chain reaction

Gene	Area	Fold change		
		M vs S	D vs M	D vs S
Fgb	MI	2.32	2.69	6.25
	NMI	−3.45	−1.92	−6.67
Col1a1	MI	8.00	−1.59	5.00
	NMI	5.32	−2.5	2.15
Ucp2	MI	1.03	3.67	3.78
	NMI	N/A	N/A	1.35
Fxyd1	MI	−1.30	2.75	2.12
	NMI	−4.00	1.53	−2.63
Spp1	MI	7.19	−1.69	4.25
	NMI	−3.13	−1.67	−5.26

MI, the border infarcted left ventricular area; NMI, the noninfarcted left ventricular area, namely the remote infarcted area; M vs S represents the coronary artery ligation (CAL) group compared with the sham group; D vs M, the CAL+GXII (Guanxin No.2 decoction) group vs the CAL group; D vs S the CAL+GXII group vs the sham group; N/A, data not available with our methods.

noninfarcted left ventricular area, the Col16a1 gene was activated in the CAL group and depressed in the GXII group.

Validation by RT-PCR

We have validated the expression of Spp1, Ucp2, Fgb, Fxyd1, and Col1a1 by RT-PCR (Tables 3 and 4). The overall changes of the above genes were verified by the concordant results of our microarray experiment.

Discussion

Myocardial infarction is the acute condition of necrosis of the myocardium that occurs as a result of an imbalance between coronary blood supply and myocardial demand. After the death of cardiocytes, macrophages, monocytes and neutrophils migrate into the infarcted area, initiating the inflammatory response. Infarcted expansion then begins to occur because of the activation of matrix metalloproteinases (MMPs), which degrade the extracellular matrix and result in myocyte slippage. This weakens the collagen scaffold, which then results in wall thinning and ventricular dilation. After the initial inflammatory phase, there is an increase in fibrin, cross-linked with collagen deposition, which protects the infarcted heart from deformation and rupture.[28] During the injury, a large part of the reactive oxygen species has been generated. Some of the less reactive of these species (such as superoxide) can be converted by oxidoreduction reactions with transition metals or other redox cycling compounds (including quinones) into more aggressive radical species that can cause extensive cellular damage.[29] The major portion of long-term effects is inflicted by damage on DNA.[30]

In this study, we have used an oligonucleotide microarray, which we constructed in-house, to detect the gene expressions in the border infarcted and remote noninfarcted left ventricular areas, and after ligation and administration of GXII. It was discovered that most of the genes were upregulated in the

Table 3 The integrated optical density (*IOD*) value of the validation of selected gene expression by reverse transcription polymerase chain reaction

Gene	Area	*IOD*		
		Sham	CAL	CAL+GXII
Fgb	MI	2.93(1.08–6.83)	6.81(3.68–9.35)	18.31(12.31–22.50)
	NMI	1.44(0.86–4.93)	0.42(0.13–1.22)	0.22(0.10–0.72)
Col1a1	MI	0.21(0.10–0.97)	1.68(0.85–4.31)	1.05(0.54–3.49)
	NMI	2.56(1.03–5.86)	13.62(10.74–16.92)	5.51(1.99–10.82)
Ucp2	MI	0.65(0.16–1.83)	0.67(0.16–1.83)	2.46(1.02–6.35)
	NMI	0.54(0.15–1.63)	N/A	0.73(0.16–1.83)
Fxyd1	MI	1.09(0.60–3.72)	0.84(0.33–3.65)	2.31(1.02–6.13)
	NMI	0.69(0.10–1.18)	0.17(0.10–0.73)	0.26(0.16–0.98)
Spp1	MI	1.26(0.69–3.92)	9.06(5.23–12.88)	5.35(1.87–10.56)
	NMI	0.79(0.22–2.98)	0.25(0.13–1.08)	0.15(0.10–0.96)

MI, myocardial infarction, represented by the border infarcted left ventricular area; NMI, represented by the noninfarcted left ventricular area, namely the remote infarcted area; sham, sham-operated group; CAL, the coronary artery ligated group; CAL+GXII; coronary artery ligation plus Guanxin No.2 decoction group; N/A, data not available with our methods.

border infarcted left ventricular area, but a larger part of the genes were repressed in the remote noninfarcted left ventricular area. We found that more genes were regulated in the remote noninfarcted left ventricular area (i.e. 50, compared with 41 in the border infarcted left ventricular area), but that GXII treatment regulated a smaller number in this area (just 11, compared with 17 in the border infarcted area). In the border infarcted left ventricular area, 41.5% of the genes were changed solely by GXII, and 24.4% of the genes were changed to a normal level. However, in the remote noninfarcted left ventricular area, only 22% of the genes were regulated solely by GXII, and 52% of the genes seemed to become normal. Although a large part of the genes became normal in the noninfarcted area, the effect was smaller compared with the effect of GXII in the border infarcted left ventricular area (Figure 3). That is, GXII seemed to have an increased effect in the border infarcted left ventricular area although more genes were regulated after GXII intervention in the remote noninfarcted left ventricular area.

Five genes were regulated after GXII intervention both in the border infarcted and remote noninfarcted left ventricular areas. Mitochondrial Ucp2 is a member of the larger family of mitochondrial anion carrier proteins (MACP). Ucp2 separates

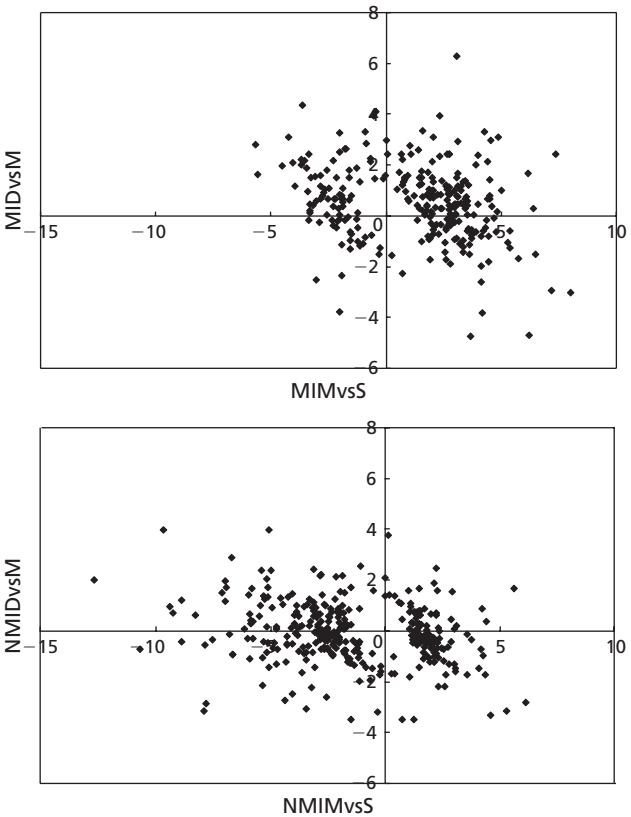

Figure 3 Dot plot of the gene expression in the border infarcted and remote noninfarcted left ventricular areas. The values are log 2 based transfer of the fold change. MIMvsS, the coronary artery ligation (CAL) group compared with the sham group in the border infarcted area; MIDvsM, Guanxin No.2 decoction (GXII) group vs the CAL group in the myocardial infarcted area. The corresponding area away from the myocardial infarction (NMI) was denoted by the remote noninfarcted left ventricular area.

oxidative phosphorylation from ATP synthesis with energy dissipated as heat, also referred to as the mitochondrial proton leak. It facilitates the transfer of anions from the inner to the outer mitochondrial membrane and the return transfer of protons from the outer to the inner mitochondrial membrane. It also reduces the mitochondrial membrane potential in mammalian cells. Ucp2 contains the three homologous protein domains of MACPs. It is thought to play an important role in response to superoxide and mitochondrial transport.[31,32] As we know, overproduction of oxygen free radicals can induce damage of heart tissue. It has been reported that GXII and its constituents in antagonizing and repairing myocardial damage scavenge the abnormal increased active oxygen free radicals in tissue.[11,33] In our study it was inferred that GXII scavenged the oxygen free radicals probably by activating the expression of Ucp2 gene, and the strength was much larger in the border infarcted left ventricular area.

Col1a1 gene encodes the pro-alpha1 chain of type I collagen, which is the major component of the cardiac extracellular matrix. Collagen genes are expressed throughout adult life in the heart, and these proteins are continuously synthesized and degraded.[34] When there is a shift in the balance between collagen synthesis and degradation, collagen accumulation occurs. After myocardial infarction, the appearance of myofibroblasts at the site of repair is accompanied by elevated type I collagen mRNA expression and collagen accumulation, indicating that active collagen synthesis is the responsibility of these phenotypically transformed fibroblast-like cells. The expression of the collagen at the infarct site remains elevated over four weeks. It has been reported that increased type I collagen mRNA expression at the site of myocardial infarction was seen in the rat heart 90 days after the infarction.[35] To a lesser extent than seen at the site of injury, but still evident, is the rise in procollagen mRNA and collagen volume that occurs remote from the infarction.[36] Thus the Col1a1 gene plays a very important role in myocardial infarction. In this study, this gene was extremely activated by the ligation, but was repressed by GXII. This meant GXII inhibited the collagen accumulation and reduced ventricular remodelling probably by repressing the expression of the Col1a1 gene.

The protein encoded by Fgb gene is the beta component of fibrinogen. Following vascular injury, fibrinogen is cleaved by thrombin to form fibrin, which is the most abundant component of blood clots. It plays a role in wound healing.[37] Interestingly, in our study this gene was activated in the border infarcted left ventricular area, and became more active after GXII administration. However, in the remote noninfarcted left ventricular area, this gene was repressed, and GXII could strengthen its repression. We inferred that the synthesis of fibrin was inhibited in the remote noninfarcted left ventricular area, but accelerated in the border infarcted left ventricular area. GXII could strengthen the inhibition in the border infarcted left ventricular area and the acceleration in the remote noninfarcted left ventricular area.

When compared with the gene expression in the infarcted and noninfarcted left ventricular areas, significantly changed genes were found in the GXII-treated group, even in the sham group. This indicated that gene expression was a dimensional process even in a single tissue such as the left ventricle. In the GXII-treated group more genes had been changed in the

infarcted area. It thus seemed that GXII might have a greater effect on the infarcted left ventricular area.

Conclusions

In this study, the genechip results suggested that gene expression of the left ventricle was a dimensional process, due to its cellular heterogeneity. The use of microarray technology allowed us to observe the many differences in gene expression shown between the border infarcted left ventricular area and the remote noninfarcted left ventricular area after GXII administration. Some important genes may have contributed to the cardioprotective effect of GXII. The changes in protein expression require further investigation.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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