The Protective Effect of Daming Capsule on Heart Function in Streptozocin-Induced Diabetic Rats with Hyperlipidemia

Jing Ai, a,b Xinxin Yan, a,b Limei Zhao, a Yuan Lu, a Feng Liang, a Benzhi Cai, a Guoyu Li, a Yanjie Lu, a,b and Baofeng Yang a,b

Department of Pharmacology, Harbin Medical University; and State-Provincial Key Laboratories of Biomedicine-Pharmaceutics of China, Harbin Medical University; 194 Xuefu Road, Nangang District, Harbin 150081, China.

Received March 4, 2008; accepted January 9, 2009; published online May 19, 2009

Impaired heart function is the main reason for increased mortality of diabetes mellitus. Development of drugs with cardioprotective effects against diabetic myocardialopathy would benefit patients with diabetes. In this study, we tested the cardioprotective effects of Daming capsule (DMC), a traditional Chinese formula, on heart function in streptozocin (STZ)-induced diabetic rats with high fat-diet (HFD). DMC 100 mg/kg/d markedly decreased fasting blood glucose (FBG) and total cholesterol (TC), but did not affect triglycerides (TG) in diabetic rats at 30 d. The decreased heart rate (HR) and prolonged QT and PR interval induced by diabetes mellitus were significantly reversed by DMC (p<0.05). The mechanism may involve that DMC attenuated L-type calcium channel α1 subunit increasing and Kv4.2 decreasing at both mRNA and protein level in diabetic rats. Additionally, DMC could obviously ameliorate the impaired heart function of diabetic rats by decreasing elevated left ventricular end-diastolic pressure (LVEDP) and increasing the attenuated maximum change velocity of left ventricular pressure in the isovolumic contraction or relaxation period (±dp/dt max). Transmission electron microscopy (TEM) results showed that myocardium injury was attenuated by DMC (100 mg/kg/d) in STZ-induced diabetic rats with HFD. In conclusion, DMC could recover the prolonged QT interval and PR interval and elevated diastolic and systolic function of diabetic heart. This protective effect may partially be mediated through affecting the mRNA and protein expression of Kv4.2 and α1, as well as preventing cardiomyocyte morphological remodeling.

Key words Daming capsule; diabetes mellitus; heart function; α1; Kv4.2

Abnormal heart function is a common syndrome in patients with diabetes mellitus (DM) and is becoming a major cause of mortality.1,2) Drugs with protective effects on heart function and prevention of myocardial remodeling might decrease mortality associated with DM. Hypoglycemic agents used for the treatment of diabetic patients, such as thiazolidinediones (TDs), usually act primarily by decreasing insulin resistance and regulating lipid metabolism. Although some clinic trials have investigated the relationship between the TDs and the risk of congestive heart failure (CHF), conflicting results have led to limited use of TDs in diabetic patients with coexisting cardiovascular disease.3–8) Recently, some studies showed that statin therapy could slow the progression of diabetic nephropathy and potentially exert other renoprotective effects,9) and one meta-analysis of >18000 diabetic patients across 14 trials supported that all diabetic patients at “sufficiently high risk” for vascular events could benefit from statins.10,11) Nevertheless, long-term treatment with statins could easily induce rhabdomyolysis. Angiotensin II type 1 receptor blocker (ARB) could effectively attenuate myocardial remodeling and preserve diastolic function in diabetic heart.12) The cardioprotective effects of ARB in diabetic patients were a result of its anti-hypertension action. However, DM itself can cause a specific form of cardiomyopathic state independent of vascular complications, such as hypertension.13) Therefore it is very important to develop drugs for preserving the impaired heart function induced by DM.

Traditional Chinese medicine formulas have prominent advantages because of their stable curative effects with low toxicity. The principle of traditional Chinese medicine is integrity. Traditional Chinese formulas treat diseases from different aspects at the same time, by aiming to arouse the whole body’s potential to recover health. Daming capsule (DMC) was designed and carefully organized in accordance with the rule of the traditional Chinese medical compatibility, jun, chen, zuo, shi (monarch, minister, adjuvant and messenger) after selecting proper herbs and relevant dosage, and mainly comprises Rheum Palmatum, Cassia obtusifolia L., Salvia miltiorrhiza, and Panax ginseng C.A. The idea was that these herbs used together would synergize the desirable effects from different mechanisms and decrease side effects. In the formula, Rheum Palmatum is considered as monarch drug because of its actions on promoting digestion and removing food retention as well as promoting blood circulation to dissipate blood stasis (Pharmacopoeia of the People’s Republic of China, 2000). Worldwide studies have shown that Emodin (a mainly effective element of Rheum Palmatum) has hypolipidemic and anti-cancer action.14,15) However its cardiac effects have sparsely been reported. Cassia obtusifolia L. also has hypolipidemic,16,17) hypotensive,18) antihypertotoxic,19) and antibacterial effects.20) In this formula, Cassia obtusifolia L. could enhance the pharmacological actions and compensate for the adverse effects of Rheum Palmatum due to its benefiting yin effects (Pharmacopoeia of the People’s Republic of China, 2000). Therefore Cassia obtusifolia L. is named as minister drug in the present formula. Salvia miltiorrhiza is also designed as a minister drug because of its similar function with Cassia obtusifolia L. (Pharmacopoeia of the People’s Republic of China, 2000). In studies of modern medicine, Salvia miltiorrhiza has cardioprotective effects, but research and clinical application mostly focus on myocardial ischemia.21–24) Panax ginseng C.A. is a kind of immunomodulatory drug25,26) used in physically weak patients.
with various diseases, including in elderly persons in China. In this formula, *Panax ginseng* C.A. is used as adjuvant drug to elevate the decreased physical state induced by DM. Hyperlipidemia is closely associated with DM and statins therapy could reduce cardiovascular events.\(^{27,28}\) Our previous study showed that DMC is effective in lipidemic modulation and the DMC is currently in phase III trial as a hypolipidemic drug. The present study was designed to investigate whether DMC could preserve impaired heart function in rats with DM.

**MATERIALS AND METHODS**

**Chemicals and Herbal Materials** *Rheum Palmatum* was harvested from Gansu Province after 3 years of cultivation; *Cassia obtusifolia* L. and *Panax ginseng* C.A. were from Sichuan Province with 1 year cultivation; *Salvia miltiorrhiza* was from Jilin Province aged 8 years. All of these plant materials were collected during September and November. Then, the plants were ground into powder and capsulated. Plant materials were collected during September and November. DM was induced in the rats by intraperitoneal (i.p.) injection of 40 mg/kg/d streptozocin (STZ) (Sigma Chemical Co., Ltd., China), QTC, which is used to rectify the influence of heart rate (HR) on QT interval, is equal to QT interval (ms) divided by the square root of RR interval.

**Quality Control by Quantitative Analysis of Total Anthraquinones** High-performance liquid chromatography (HPLC) analysis of the total anthraquinones in DMC was performed by marker compound Chrysophanol. Chromatographic separation was carried out with a ZORBAX C18 column (250 mm×4.6 mm, 5 μm) with a mobile phase composed of methanol–1% phosphate acid solution (85:15), detection wavelength at 254 nm, and flow rate at 1.0 ml/min. The content of Chrysophanol was more than 1.5 mg in each capsule (300 mg) estimated from standard calibration curve using authentic standard and the qualified standard.

**Animals** Male Wistar rats (the Animal Center of the 2nd Affiliated Hospital of Harbin Medical University, China) were used in this study. Rats were housed in a temperature (23 ± 1 °C) and humidity (55 ±5%)-controlled room with 12 h dark/light cycle and allowed food and water unlimited according to the policy of Good Laboratory Practice (GLP). Use of animals was in accordance with the regulations of the ethic committees of Harbin Medical University.

**Establishment of Diabetic Model** According to our previous studies,\(^{29,30}\) Wistar rats (180—220 g) were intragastrically administered with fat emulsion (10 ml/d) for 15 d. Fat emulsion was prepared with 20 g lard, 1 g threestat, 5 g cholesterol, 1 g sodium glutamate, 5 g sucrose, 5 g saccharu, 20 ml tween-80, and 30 ml propylene glycol, distilled water was then added to 100 ml. Fat emulsion was kept in a refrigerator at 4 °C. DM was induced in the rats by intraperitoneal (i.p.) injection of 40 mg/kg/d streptozocin (STZ) (Sigma Chemicals) in a 0.1 M citrate buffer solution (pH 4.2) for 2 d. Blood samples were collected and fasting blood glucose (FBG) level was measured at 72 h after the last injection of STZ by Grace glucometer (Grace Medical, Inc. America) to ensure induction of DM (glycemia ≥16.7 mm). Forty-eight rats with DM were randomly divided into 4 groups (12 rats/group), as follows: diabetic model (DM); high-dose DMC (200 mg/kg/d); middle-dose DMC (100 mg/kg/d); low-dose DMC (50 mg/kg/d). Six rats in each group were used for RT-PCR analysis; the remaining 6 rats were prepared for TEM test. DMC was intragastrically administered for 30 d. At the same time, all 4 groups were intragastrically administered with fat emulsion (10 ml/d). Ten healthy Wistar rats (200—250 g) were used as the control group (Ctr), they were treated with the same volume of 0.9% NaCl when other rats were treated with STZ and drug.

**Biochemical Estimations** Blood Samples were collected from tail vein to test FBG after fasting for 12 h. Blood was collected from heart, then separated and analyzed for total cholesterol (TC) and triglycerides (TG) using appropriate kits (Nanjing Jiancheng Bioengineering Institute, China).

**In Vivo Electrocardiogram (ECG) Studies** Rats were anesthetized with i.p. sodium pentobarbital (40 mg/kg). Pin electrodes were pricked into rats’ limbs for the recording of Lead II ECG. QT and RR interval were measured by BL-420 Data Acquisition & Analysis System (Chengdu Tme Technology Co., Ltd., China). The influence of heart rate (HR) on QT interval, is equal to QT interval (ms) divided by the square root of RR interval.

**Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis** Total RNA was extracted from heart tissue. Potassium channel Kv4.2 and L-type calcium channel α1C subunit were detected by RT-PCR. Primers used for amplification were as follows: Kv4.2, forward primer: 5'-CCGGCAGGGGAAGCTTCACT-3'; reverse primer: 5'-CCTGCGTAGACCCACATGGA-3'. α1C, forward primer: 5'-TCCTACGCCCCAAAACACAGG-3'; reverse primer: 5'-GAGATGGGGACAGCTAAC-3'. β-Actin, forward primer: 5'-AGGCCCTCTGTACAGCTACTT-3'; reverse primer: 5'-TGCCCAAGGAATCTTATACCC-3'. PCR was performed for 30 cycles. Each cycle consisted of denaturation at 94 °C for 30 s, annealing at 52.5 °C for 30 s, and extension at 72 °C for 30 s. For α1C, autoextension at 72 °C for 7 min was included after completion of the last cycle. The PCR products were size-fractionated by 2.0% agarose gel electrophoresis. The density and area of each band were analyzed by ChemiImager™ 4000 digital system (Alpha Innotech Corp., U.S.A.).

**Western Blot** Membrane protein samples were extracted from rat hearts for immunoblotting analysis of ion channel proteins, with procedures essentially the same as described in detail elsewhere.\(^{31}\) The protein content was determined with Sunrise-Basic Tecan (Austria) using bovine serum albumin as the standard.

Membrane protein sample was fractionated by SDS-PAGE (10% polyacrylamide gels) and transferred to PVDF membrane (Bio-Rad, Hercules, CA, U.S.A.). The sample was incubated 3 h and overnight at 4 °C with the primary antibodies of Kv4.2 (the pore-forming α-subunit of L<sub>a</sub>) and Ca<sub>a1.2</sub> (for the α<sub>1C</sub> subunit of I<sub>CaL</sub>) in 1 : 200. Affinity purified polyclonal primary antibodies against C-terminus of human Ca<sub>a1.2</sub> and Kv4.2 was both raised in goat. Inhibitory peptide for each antibody was used to test the antibody specificity. Next day, the membrane was washed in PBS-T 3 times (10 min each) and incubated for 2.5 h with HRP-conjugated donkey anti-goat Ig (1 : 1000) in the blocking buffer. Both primary and secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Bound antibodies were detected by Odyssey infrared imaging system (LI-COR, Inc. America).
Lincoln, NB, U.S.A.). β-Actin was used as internal control for equal input of protein samples, using anti-β-actin anti-
body purchased from Santa Cruz (CA, U.S.A.). Coomassie
staining was also performed to verify the sample quantity.
Western blot bands were quantified using Quantity One soft-
ware by measuring the band intensity (Area×OD) for each
group and normalizing to β-actin. The final results are ex-
pressed as fold changes by normalizing the data to the con-
trol values.

**In Vivo Heart Function Studies** After ECG was
recorded, catheter was inserted into the left ventricle via the
right common carotid artery to record cardiac electrophysi-
ologic parameters. HR, left ventricular end-diastolic pressure
(LVEDP), and maximum change velocity of left ventricular
pressure in the isovolumic contraction or relaxation period
(±dp/dt\text{max}) were measured by BL-420 Data Acquisition &
Analysis System.

**Transmission Electron Microscopy (TEM)** Analysis
Specimens were prepared by routine methods for TEM
administration. 1 Specimens were prepared by routine methods for TEM
analysis, 1 Specimens were prepared by routine methods for TEM
procedures.

**Statistical Analysis** Data were analyzed by one-way ANOVA. A significant difference was determined when
*p* < 0.05. Comparison between two groups was done using
Student’s *t*-test.

**RESULTS**

**Biochemical Estimations** After rats were treated with
STZ as well as high-fat emulsion for 45 d, FBG, TG, and TC
were significantly increased as shown in Table 1. DMC effect-
ively decreased FBG and TC after 30-d treatment. However,
the effects were not dose-dependent, because FBG and TC in
DMC (50 mg/kg/d) group were not significantly changed
compared with the diabetic group and those in DMC
(200 mg/kg/d) group were similar with those in the DMC
(100 mg/kg/d) group. No significant change of TG was ob-
served in all groups.

**In Vivo Cardiac ECG Studies** As shown in Table 2 and
Fig. 1, HR was significantly reduced in STZ-induced diabetic
rats with HFD compared with control group (Ctr) (*p* < 0.01).
The decrease in HR in diabetic rats was reversed by DMC
administration. Furthermore, the prolonged QT interval and
PR interval in diabetic rats were partially recovered by DMC
treatment (*p* < 0.01).

**RT-PCR and Western Blot Analysis** The mRNA level of
g_{\text{Ca}1.2} slightly increased in diabetic rat heart. However, it
was decreased by approximately 16% in the DMC groups
compared with the DM group (*p* < 0.05) (Fig. 2A). The level of
Kv4.2 decreased in the DM group, but increased by 19.2%
in the DMC group compared with diabetic rats (Fig. 2B).

**In Vivo Heart Function Studies** The decreased
+dp/dt\text{max} in diabetic rats induced by STZ with HFD was
elevated by 30 d DMC treatment (384.56 ± 59.08 kPa/S for
Ctr rats, 251.38 ± 37.23 kPa/S for STZ induced rats, and
294.41 ± 40.68 kPa/S for DMC rats) (Fig. 3A). So as to re-
moves the effects of pre-load and after-load of heart on the
+dp/dt\text{max}, we further calculated the Log value of +dp/dt\text{max}
max. The results implied the same trend as with +dp/dt\text{max} (Fig.
3B). Remarkably, LVEDP was notably increased in diabetic
rats (0.49 ± 0.04) compared with control rats (−0.5 ± 0.06,
*p* < 0.05, *n* = 6). LVEDP in rats with DMC treatment was
decreased (−0.35 ± 0.12) compared with diabetic rats (Fig.
3D). −dp/dt\text{max} is another index to evaluate diastolic func-

### Table 1. The Effects of DMC on FBG, TG and TC in Diabetic Rats (*n* = 8)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/d)</th>
<th>FBG (mmol/l)</th>
<th>TG (mmol/l)</th>
<th>TC (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctr</td>
<td>6.01 ± 1.42</td>
<td>1.1 ± 0.25</td>
<td>2.71 ± 0.46</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>24.91 ± 6.04**</td>
<td>2.9 ± 0.31*</td>
<td>3.42 ± 0.33*</td>
<td></td>
</tr>
<tr>
<td>DMC 200</td>
<td>13.95 ± 3.76*</td>
<td>2.6 ± 0.52</td>
<td>2.83 ± 0.22*</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>10.22 ± 3.34*</td>
<td>2.6 ± 0.48</td>
<td>2.81 ± 0.43*</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>22.82 ± 7.12</td>
<td>2.8 ± 0.36</td>
<td>3.26 ± 0.39</td>
<td></td>
</tr>
</tbody>
</table>

* *p* < 0.05 vs. Ctr, **p* < 0.01 vs. Ctr, *s* *p* < 0.05 vs. DM.

### Table 2. The Effects of DMC on ECG of STZ-Induced Rats (*n* = 6)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/d)</th>
<th>HR (min⁻¹)</th>
<th>QT interval (ms)</th>
<th>QTc</th>
<th>PR interval (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctr</td>
<td>367 ± 33</td>
<td>71.2 ± 8.5</td>
<td>5.85 ± 0.53</td>
<td>51.1 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>288 ± 32**</td>
<td>103.6 ± 10.2**</td>
<td>7.15 ± 0.65**</td>
<td>60.2 ± 8.2**</td>
<td></td>
</tr>
<tr>
<td>DMC 100</td>
<td>343 ± 20##</td>
<td>86.7 ± 10.4**##</td>
<td>6.32 ± 0.55#</td>
<td>52.7 ± 2.7##</td>
<td></td>
</tr>
</tbody>
</table>

* *p* < 0.01 vs. Ctr, *s* *p* < 0.01 vs. DM, ## *p* < 0.05 vs. DM.
tion. In the experiment, diastolic function was markedly impaired in diabetic rats (−225±83.54 kPa/S) compared with Ctr rats (−343.46±83.54 kPa/S). DMC (100 mg/kg/d) could effectively recover this parameter to −266±29.01 kPa/S (Fig. 3C).

DISCUSSION

We successfully established an animal model of DM with hyperlipemia using STZ treated Wistar rats with HFD. Our results showed that DMC could not only decrease elevated TC level but also effectively decrease FBG in diabetic rats, although FBG level was still higher than in control animals, which was in accordance with the results of clinical trial. However, the hypoglycemic action of DMC was not dose dependent. The hypoglycemic action of DMC at a dosage of 100 mg/kg/d was higher than that with 50 mg/kg/d, but similar with 200 mg/kg/d. This result may be due to the characteristics of herbal medicines. Low-dosage DMC (50 mg/kg/d) may contain insufficient Rheum Palmatu and therefore we could not observe enough drug action. However, the toxicity of Rheum Palmatu at highest dose of DMC (200 mg/kg/d) was increased, and not counter-balanced by accompanying elevated minister drugs, e.g. Cassia obtusifolia L. and Salvia miltiorrhiza. Hence DMC 200 mg/kg/d could not exert its hypoglycemic action due to decreased defense function of the body induced by Rheum Palmatu. Our result suggests that the optimal dose of DMC is 100 mg/kg/d. Therefore we selected the dosage of 100 mg/kg/d to study the effects of DMC on cardiac function of diabetic rats. We did not select any marketed drug as control drug because there is no acknowledged and specific drug indicated for diabetic myocardopathy.

Abnormal prolongation of QT interval is considered the most prominent electrical remodeling in diabetic heart. In our experiment, DMC significantly reduced prolonged QT interval in diabetic rats. Furthermore, PR interval, an index of AV nodal conduction, was also prolonged in the heart of DM rats and shortened by DMC treatment. These results imply that the decreased heart rate in diabetic rats is not only

Fig. 2. Alterations of mRNA and Protein Expression Levels of α1c and Kv4.2

(A) α1c mRNA level assessed by RT-PCR analysis with whole tissues from rat heart. (B) Kv4.2 mRNA level assessed by RT-PCR analysis with whole tissues from rat heart. (C) Ca1.2 (for the α1c subunit of I_{CaL}) protein expression level assessed by Western blotting analysis with membrane samples (200 μg) extracted from rat heart. Top: Western blot bands with anti-Ca1.2 and anti-β-actin antibodies. Bottom: densitometric analysis of bands corresponding to Ca1.2. Data were normalized to β-actin and expressed as fold changes over control. (D) Kv4.2 (the pore-forming α-subunit of Ito) protein expression level assessed by Western blotting analysis with membrane samples (100 μg) extracted from rat heart. Top: Western blot bands with anti-Kv4.2 and anti-β-actin antibodies. Bottom: densitometric analysis of bands corresponding to Kv4.2. Data were normalized to β-actin and expressed as fold changes over control. Values are mean±S.E. (n=6). *p<0.05 vs. Ctr; #p<0.05 vs. DM.

Fig. 3. Quantitative Analysis of Cardiac EP Parameters of Normal Rats and STZ-Induced Diabetic Rats with and without DMC Treatment

(A) Maximum change velocity of left ventricular pressure in the isovolumic contraction period (±dp/dt_{max}); (B) Log(±dp/dt_{max}); (C) maximum change velocity of left ventricular pressure in the isovolumic relaxation period (−dp/dt_{max}); (D) left ventricular end-diastolic pressure (LVEDP). *p<0.05 vs. Ctr; #p<0.05 vs. DM. n=6.

Fig. 4. Transmission Electron Microscopy Micrographs Exhibiting Ultrastructural Changes in Left Ventricular Myocardium

Regular structure of left ventricular myocardium from Ctr; DM showed ultrastructural changes of left ventricular myocardium of STZ-induced rats; DMC showed the effect of DMC on ultrastructure of ventricular myocardium.
derived by prolonged QT interval but also due to the prolonged PR interval. DMC could ameliorate decreased heart rate in diabetic rats by recovering abnormally prolonged QT interval and PR interval.

Calcium and potassium currents, especially transient outward K⁺ current (Iₒ), are two very important elements for QT interval in rats. We therefore carried out RT-PCR analysis to explore whether the recovered QT intervals by DMC treatment discovered in our experiments were associated with changed inward L-type calcium current and outward Ito currents. Kv4.2 is a protein of Iₒ, and α₁c is of L-type calcium channel. In this study, we found that mRNA expression of Kv4.2 was decreased, and α₁c increased in diabetic rat heart following high fat administration. DMC attenuated the expression of α₁c increasing and Kv4.2 decreasing in STZ-induced diabetic rats. As we know, the function of ion channels is actually conducted by its protein. The results of Western blotting analysis showed that DMC could also attenuate protein expression of Ca,1.2 increasing and Kv4.2 decreasing in STZ-induced diabetic rats, suggesting that an effect of DMC on both protein and gene expression of L-type calcium channel and transient outward K⁺ channel is possibly responsible for its effects on ECG.

Impaired heart function of diabetic patients, especially diabetic dysfunction, independent of vascular and other diseases even in patients with excellent glucose control by insulin suggests primary myocardial defect in DM.34,35) Our data showed that both LVEDP and \( \pm dp/dt_{\text{max}} \) of diabetic rats following HFD decreased compared with control group. DMC treatment retrieved cardiac dysfunction in diabetic rats. The results indicate that there are other mechanisms involved in the cardioprotective effect of DMC on diabetic rats.

Diastolic and systolic function of the heart is mainly determined by the morphology and structure of cardiomyocytes. Our study suggests that myocardial ultrastructures were severely damaged in diabetic rats. However, DMC partially prevented the impairment. In conclusion, the protective effects of DMC on impaired heart function in diabetic rats could be due to two effects. First, the effect of DMC on expression of Kv4.2 and α₁c subunit may recover prolonged QT interval and PR interval induced by diabetes; second, DMC elevated diastolic and systolic function of the heart by improving morphological remodeling of cardiomyocytes in DM rats.

In the present study, we could not identify which of these protective effects of DMC in heart are driven by what ingredient in the formula. In future, we will investigate the effects of individual ingredients in the formula on the heart. We also aim to uncover whether DMC could exert protective action on the impaired heart function by improving remodeled vascular function.

Acknowledgments This study was supported by the key Project of Natural Science Foundation of Heilongjiang Province (No. ZJY0703-02), Natural Science Foundation of China (No. 30870862), National Basic Research Program of China (973 program, No. 2007CB512006), New Century Excellent Talents in Universities, Chinese Ministry of Education and Special Fund for Innovation Talent by Harbin Science and Technology (2008RFLXS009).

REFERENCES