Tohoku J. Exp. Med., 2007, **213**, 139-148

Colchicine, a Microtubule Depolymerizing Agent, Inhibits Myocardial Apoptosis in Rats

Kenya Saji,[1] Yoshihiro Fukumoto,[1] Jun Suzuki,[1] Shigefumi Fukui,[1] Jun Nawata[1] and Hiroaki Shimokawa[1,2]

[1]Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan
[2]The Technology Agency, CREST, Tokyo, Japan

Saji, K., Fukumoto, Y., Suzuki, J., Fukui, S., Nawata, J. and Shimokawa, H. *Colchicine, a Microtubule Depolymerizing Agent, Inhibits Myocardial Apoptosis in Rats*. Tohoku J. Exp. Med., 2007, **213** (2), 139-148 —— Heart failure is the most common cardiovascular disease with high mortality and morbidity. Both enhanced microtubule polymerization and cardiomyocyte apoptosis are involved in the pathogenesis of heart failure. However, the link between the two mechanisms remains to be elucidated. In this study, we thus address this important issue in cultured cardiomyocytes from Wistar rats in vitro and in angiotensin II (ATII)-infused rats in vivo. Confocal microscopy examination showed that in cultured rat cardiomyocytes, micrographic density of microtubules was increased by paclitaxel, a microtubule-polymerizing agent, and decreased by colchicine, a microtubule-depolymerizing agent, but not affected by ATII, isoproterenol, or tumor necrosis factor-α alone. Immunoblotting analysis showed that Bax/Bcl-2 ratio, which is associated with the activation of caspase-3, was significantly increased in ATII-stimulated cultured cardiomyocytes in vitro and in ATII-infused rats in vivo, both of which were inhibited by co-treatment with colchicine. Caspase-3 and TUNEL assay to detect apoptosis in vitro demonstrated that paclitaxel or ATII alone significantly enhanced and their combination further accelerated cardiomyocyte apoptosis, which was again significantly inhibited by colchicine. Caspase-3 and TUNEL assay in vivo also demonstrated that ATII infusion significantly increased myocardial apoptosis and that co-treatment with colchicine significantly suppressed the apoptosis. In conclusion, these results indicate that a microtubule-depolymerizing agent could be a potential therapeutic strategy for treatment of heart failure. ———— apoptosis; myocytes; microtubule; polymerization; colchicine

Heart failure is the most common cardiovascular disease with high mortality and morbidity, in which myocardial apoptosis is substantially involved in ventricular dysfunction and remodeling (Redfield et al. 2003). Indeed, in the onset and progression of heart failure, myocardial apoptosis is one of the major determinant factors (Gonzalez et al. 2003; Tada et al. 2006).

Accumulating evidence has indicated that two major pathways are involved in the induction of apoptosis: mitochondrial apoptotic pathway and the death receptor pathway (Ashkenazi and

Received July 11, 2007; revision accepted for publication August 20, 2007.
Correspondence: Yoshihiro Fukumoto, M.D., Ph.D., Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan.
e-mail: fukumoto@cardio.med.tohoku.ac.jp

Dixit 1998; Bishopric et al. 2001). The former pathway is activated by several vasoactive agents, such as angiotensin II and isoproterenol, while the latter by inflammatory cytokines, such as tumor necrosis factor (TNF)-α (Kajstura et al. 1997). These agents of angiotensin II, isoproterenol, and TNF-α, play an important role in the progression of heart failure (Tabet et al. 2002). In the mitochondrial apoptotic pathway, increased Bax/Bcl-2 ratio is associated with the activation of caspase-3 (Haunstetter and Izumo 1998). Indeed, caspases are activated by a variety of apoptotic stimuli, and the apoptotic process is largely suppressed by caspase inhibitors, suggesting that caspases play a central role for apoptosis (Nicholson and Thornberry 1997). By contrast, the death receptor pathway mainly stimulates caspase-8 with an interaction with the mitochondrial apoptotic pathway (Chen and Tu 2002; de Jonge et al. 2003).

Microtubules are one of the major components of cardiomyocyte cytoskeleton and linked with various pathological conditions, such as myocardial hypertrophy and ischemia, and ventricular failure (Sato et al. 1993; Ishibashi et al. 1996; Hein et al. 2000). Enhanced microtubules polymerization is associated with the development and progression of myocardial hypertrophy (Ishibashi et al. 1996). Moreover, contractile dysfunction of hypertrophied cardiomyocytes is in parallel with the increase in microtubule polymerization, while microtubule depolymerization restores contractile function (Ishibashi et al. 1996; Tsutsui et al. 1999; Koide et al. 2000). However, the pathogenic link between microtubule polymerization and myocardial apoptosis remains largely unknown. In the present study, we thus tested our hypothesis that a microtubule-depolymerizing agent inhibits myocardial apoptosis in rats.

Materials and Methods

The present study conformed to the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and was approved by the ethical committee on Animal Experiments of Tohoku University School of Medicine.

In vitro study

Cardiomyocytes were isolated from male Wistar rats (6-8 weeks old, 180~250 g in body weight, Japan Charles River Laboratories, Yokohama), as previously described (Li et al. 2002). After the animals were anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneal [IP]), the heart was excised and perfused with 15-30 mg/kg depyrogenated collagenase B (Roche, Basel, Switzerland). Cardiomyocytes were plated on dishes precoated with laminin and stored at 37°C in 5% CO_2 in a Dulbeccos modified Eagle's media.

Cardiomyocytes were incubated for 16 or 24 hrs in the presence or absence of apoptosis-inducing factors, such as angiotensin II (10 μmol/L, Sigma Chemicals, St. Louis, MO, USA), isoproterenol (100 μmol/L, Sigma Chemicals), or TNF-α (10 ng/mL, Sigma Chemicals) (n = 6~9), with or without co-treatment with a microtubule-polymerizing agent, paclitaxel (taxol, 10 μmol/L, Bristol-Myers Squibb Co., Princeton, NJ, USA), or a depolymerizing agent, colchicine (10 μmol/L, Sigma Chemicals) (n = 6~9), as previously described (Tsutsui et al. 1999; Sharma and Singh 2000; Ishibashi et al. 2003).

After 16 hrs incubation, we evaluated Bax/Bcl-2 expression by immunoblotting, and after 24 hrs incubation, we evaluated microtubule polymerization by confocal microscopy and apoptosis by caspase-3 activity and Terminal deoxy-nucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay.

Confocal microscopy for immunolabeling of microtubules

Microtubules were labeled with monoclonal antibody to β-tubulin (1 : 50; abcam) and fluorescein isothiocyanate (FITC)-conjugated donkey anti-mouse IgG (1 : 50; Jackson Immuno Research, West Grove, PA, USA) (Howarth et al. 1999). Immunofluorescent images were obtained using a confocal microscope (C1, Nikon, Tokyo). Analysis was performed using Axio Vision 4.5 for Windows software (Carl Zeiss, Jena, Germany) by selecting an area of the cell and measuring mean fluorescence (Howarth et al. 1999).

Immunoblotting for Bax/Bcl-2

Cultured cardiomyocytes were prepared with lysis buffer containing the protease inhibitors including 2 mM phenylmethylsulphonyl fluoride (PMSF), 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 mM Na_3VO_4, and centrifuged at 14,000 rpm for 10 min at 4°C. The supernatants were collected and 50 μg of protein were electrophoresed

on a 15% SDS-polyacrylamid gel (PAGE). Gels were transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA, USA) and exposed to rabbit polyclonal anti-human Bax (N20; Santa Cruz Biotechnology) at a concentration of 1 : 200, or anti-rat/mouse Bcl-2 (554087; BD Biosciences) at a concentration of 1 : 1500. Immunodetection was accomplished using rabbit anti-mouse secondary antibody (1 : 5000 dilution, DAKO, Carpinteria, CA, USA) and an enhanced ECL or ECL plus reagents (Amersham Biosciences, Upplala, Sweden). Due to technical reasons, we used the separate blots obtained from different gels, but performed on the same day. The intensities of the bands were quantified using the Scion Image for Windows software (Scion Corporation, Frederick, MD, USA) and normalized to those in the control group.

Caspase-3 activity

Caspase-3 activity was determined using a caspase-3 colorimetric activity assay kit (CHEMICON international, Inc., Temecula, CA, USA) by means of detection of chromophore p-nitroaniline cleaved from labeled substrate Ac-DEVD-p-nitroaniline (Tada et al. 2006). Briefly, after 24 hrs of incubation, cardiomyocytes were dissolved in the lysis buffer provided with the kit. The lysates were centrifuged at 10,000 g for 5 min at 4°C, and the supernatants protein were used for caspase-3 assay. Aliquots of the protein lysates were then reacted with an equal volume of 3 mg/ml Ac-DEVD-p-nitoroaniline for 2 hrs at 37°C. Caspase-3 activity was assessed in microtiter plate reader at 405 nm and was calculated using the calibration curve obtained with a known concentration of p-nitroaniline (p-NA) (Tada et al. 2006).

TUNEL assay

Cardiomyocytes were fixed with 10% formalin phosphate buffered saline for TUNEL assays with over 2,000 cells scored for each group. Apoptosis was measured by TUNEL staining of heart slices using CardioTACS in situ apoptosis detection kit (R&D Systems, Minneapolis, MN, USA) (Fukumoto et al. 2001; Tada et al. 2006).

In vivo study

We made the following 4 groups of male Wistar rats (n = 6 each); (1) control rats, (2) control rats treated with colchicine (1 mg/kg, intraperioneal injection, for 2 days), (3) angiotensin II (1 mg/kg/day, SC, for 16 or 24 hrs)-infused rats, and (4) angiotensin II-infused rats treated with colchicine. Bax/Bcl-2 expression was evaluated after 16 hrs by immunoblotting, while the extent of myocardial apoptosis was evaluated after 24 hrs by caspase-3 analysis and TUNEL assay.

Animal preparation

Cardiomyocyte Bax/Bcl-2 expression or apoptosis in vivo was examined by infusion of angiotensin II for 16 or 24 hrs, with or without administration of colchicine (1 mg/kg) dissolved in 0.05% dimethyl sulfoxide (DIMSO) intraperitoneally for two days before angiotensin II infusion. Human synthetic angiotensin II (Sigma Chemicals) was dissolved in 0.01N acetic acid in 0.15 mol/L saline. Male Wistar rats were anesthetized with ketamine (50 mg/kg, IM) and xylazine (10 mg/kg, IM) to implant Alzet miniosmotic pumps (model 2002, Alza Corp., Mountain View, CA, USA) behind the neck subcutaneously under sterile conditions (De Angelis et al. 2002; Suzuki et al. 2003). The pumps were filled with saline or saline with angiotensin II to infuse solution at 0.5 μl/hr, with delivery of angiotensin II at the dose of 1 mg/kg body weight. After 16 or 24 hrs, the rats were anesthetized with pentobarbital (50 mg/kg, IP), the hearts were excised and rinsed in saline. For immunoblotting for Bax/Bcl-2 expression and caspase-3 assay, the hearts were stored at −80°C and homogenized with lysis buffer containing protease inhibitor cocktail (1 : 100) (Sigma, St. Louis, MO, USA). The homogenates were centrifuged at 15,000 rpm for 20 min at 4°C, and the supernatants were collected and immunoblotting for Bax/Bcl-2 or caspase-3 assay was performed, as described above. For TUNEL staining, the hearts were fixed in 10% buffered formalin solution for 48 hrs, embedded in paraffin, and sliced with 2 μm cross-sections mounted onto glass slides, and then TUNEL staining was performed, as described above. In order to depolymerize myocardial microtubules, colchicine (1 mg/kg) was intraperitoneally administered to rats, a day before and on the day of angiotensin II infusion.

Statistical analysis

Results are expressed as the mean ± S.D. Statistical analysis was performed by ANOVA followed by Fischer's post-hoc test for multiple comparisons with Stat View (SAS Institute, Cary, NC, USA). P values less than 0.05 were considered to be statistically significant.

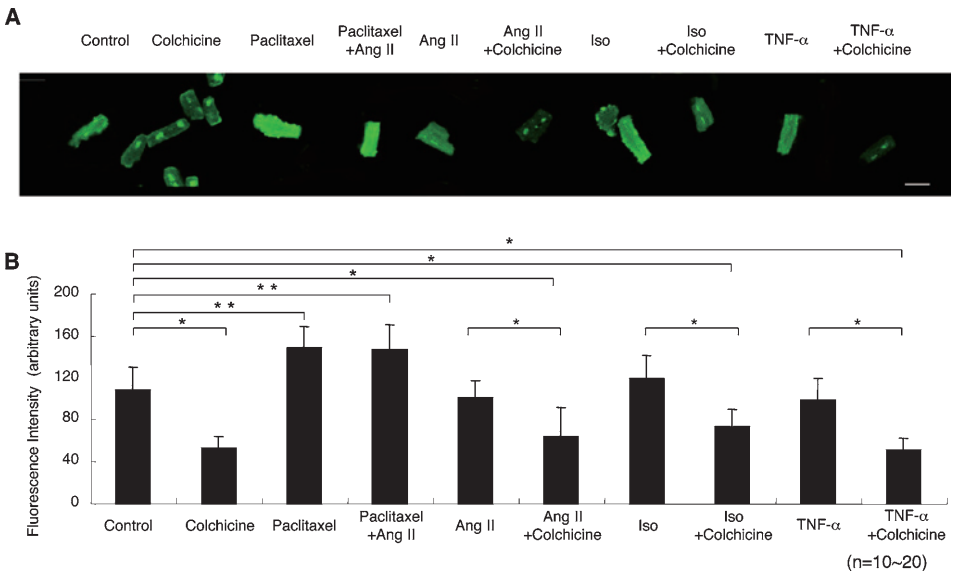

Fig. 1. Immunofluorescence micrography and density of microtubules in cultured rat cardiomyocytes.
A: Representative β-tubulin immunostaining in cultured rat cardiomyocytes. Scale bar, 100 μm.
B: Quantitative analysis of fluorescence intensities of β-tubulin.
AngII, angiotensin II; Iso, isoproterenol; TNF-α, tumor necrosis factor-α. Results are expressed as means ± S.D. $^{*}p < 0.05$, $^{**}p < 0.01$.

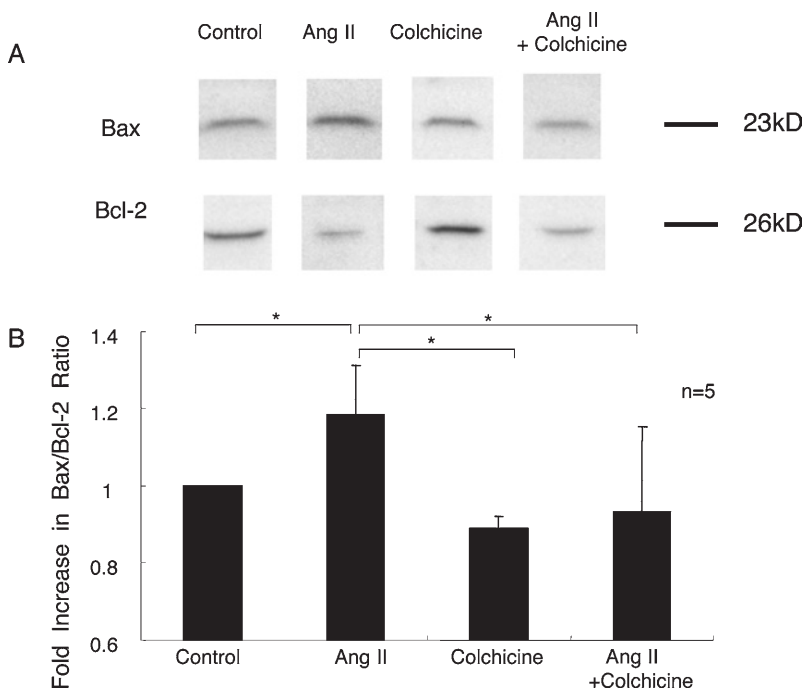

Fig. 2. Bax/Bcl-2 ratio in cultured rat cardiomyocytes.
A: Representative blottings for Bax and Bcl-2. B: Quantitative analysis of the Bax/Bcl ratio in the 4 different groups. AngII, Angiotensin II. Results are expressed as means ± S.D. $^{*}p < 0.05$.

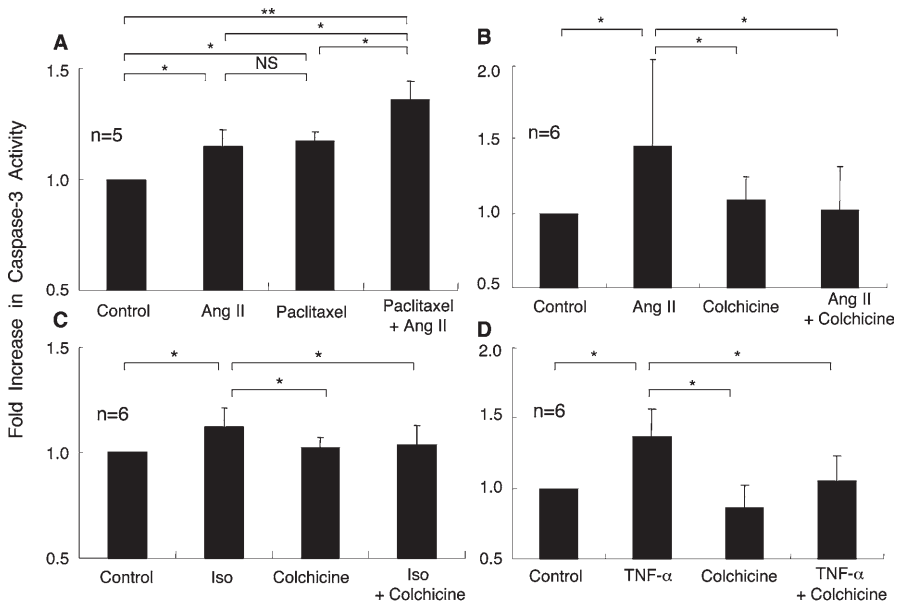

Fig. 3. Caspase-3 activities in cultured rat cardiomyocytes.
A: Effects of paclitaxel alone, angiotensin II (AngII) alone, and their combination. B: Effects of angiotensin II (AngII) alone, colchicine alone, and their combination. C: Effects isoproterenol (Iso) alone, colchicine alone, and their combination. D: Effects of TNF-α alone, colchicine alone, and their combination. Results are expressed as means ± S.D.
*$p < 0.05$, **$p < 0.01$.

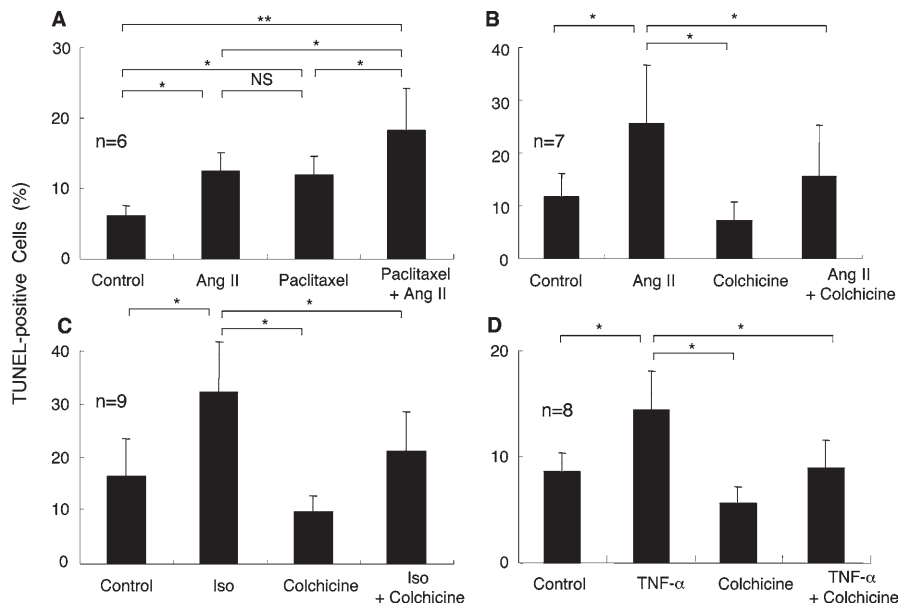

Fig. 4. Terminal deoxy-nucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assays in cultured rat cardiomyocytes.
A: Effects of paclitaxel alone, angiotensin II (AngII) alone, and their combination. B: Effects of angiotensin II (AngII) alone, colchicine alone, and their combination. C: Effects isoproterenol (Iso) alone, colchicine alone, and their combination. D: Effects of TNF-α alone, colchicine alone, and their combination. Results are expressed as means ± S.D.
*$p < 0.05$, **$p < 0.01$.

Results

In vitro study

The density of cardiomyocyte β-tubulin, as a marker of microtubule network, was significantly decreased by colchicine and increased by paclitaxel as compared with controls (Fig. 1). Although angiotensin II, isoproterenol, or TNF-α alone did not significantly affect the β-tubulin density, co-treatment with colchicine significantly inhibited the density (Fig. 1). To evaluate the relationship between microtubule density and apoptotic pathway, we first examined Bax/Bcl-2 ratio by immunoblotting. Angiotensin II significantly increased Bax/Bcl-2 ratio, which was inhibited by simultaneous treatment with colchicine (Fig. 2). Next, we evaluated the extent of apoptosis of cultured cardiomyocytes in terms of caspase-3 activity and TUNEL staining. Paclitaxel or angiotensin II alone significantly enhanced cardiomyocyte apoptosis and their combination further accelerated the apoptosis (Figs. 3A and 4A). Angiotensin II, isoproterenol, and TNF-α significantly increased caspase-3 activity (Figs. 3B-D) and the number of TUNEL-positive cardiomyocytes (Figs. 4B-D), both of which were also significantly inhibited by co-treatment with colchicine.

In vivo study

Characteristics of the animals, including body weight, systolic blood pressure, heart weight, heart/body weight ratio, and heart rate, were all comparable among the 4 groups (data not shown). Among the 4 groups, myocardial Bax/Bcl-2 ratio was significantly increased in angiotensin II-infused rats, which was prevented with co-treatment with colchicine (Fig. 5). Angiotensin II-infusion significantly increased caspase-3 activity (Fig. 6) and the number of TUNEL-positive cardiomyocytes (Fig. 7), both of which were significantly inhibited by co-treatment with colchicine (Figs. 6 and 7).

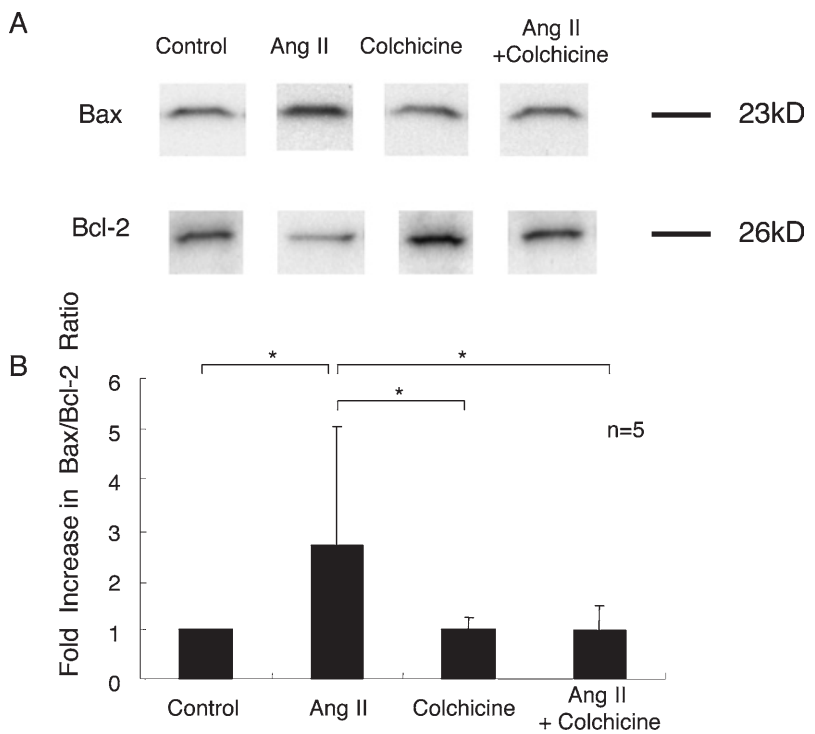

Fig. 5. Cardiac Bax/Bcl-2 expression ratio in rats in vivo.
A: Representative blottings for Bax and Bcl-2. B: Quantitative analysis for cardiac Bax/Bcl-2 expression ratio. AngII, angiotensin II. Results are expressed as means ± S.D. $*p < 0.05$.

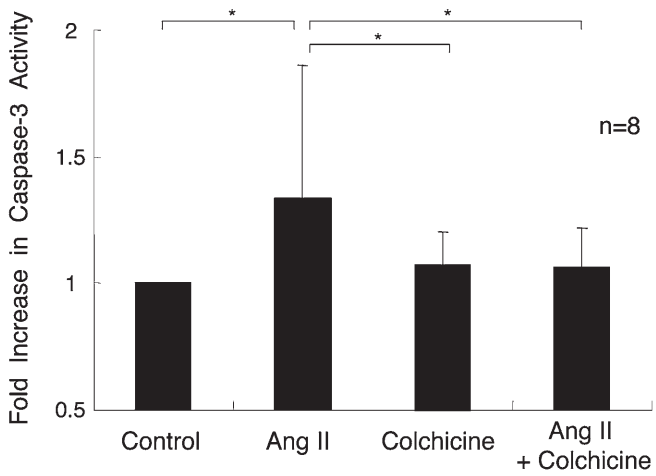

Fig. 6. Cardiac caspase-3 activities in rats in vivo.
AngII, angiotensin II. Results are expressed as means ± S.D. $*p < 0.05$.

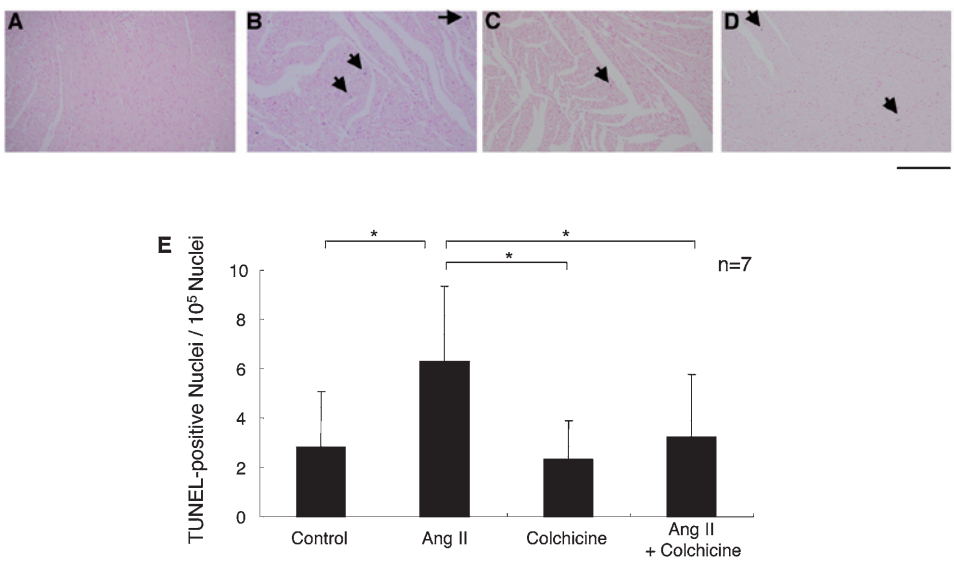

Fig. 7. TUNEL assay in rat left ventricle in vivo.
A-D. Representative photographs for myocardial TUNEL staining in control (A), angiotensin II (AngII)-treated (B), colchicine-treated (C), and angiotensin II- and colchicine-treated (D) rats. Arrows indicate TUNEL postive cells. Scale bar, 200 μm. E. Quantitative analysis of TUNEL-positive cells in the 4 groups. Results are expressed as means ± S.D. $*p < 0.05$.

Discussion

The novel finding of the present study is that a microtubule-depolymerizing agent can prevent myocardial apoptosis in rats in vitro and in vivo. Because loss of cardiomyocytes due to apoptosis can accelerate the development of heart failure (Liu et al. 1995; Sharov et al. 1996), a microtubule-depolymerizing agent could be a potential therapeutic strategy to prevent the progression of heart failure.

Apoptotic death pathway

In apoptotic death pathway, caspases are

activated by death receptor- and/or mitochondrion-dependent mechanisms, followed by subsequent cleavage of multiple cellular target proteins and resultant apoptotic cell death (Haunstetter and Izumo 1998). In the death receptor pathway, the binding of extracellular death signal proteins, such as TNF-α or Fas ligand, to their cognate cell surface receptors is a well-known initiation of apoptosis (Haunstetter and Izumo 1998). The mitochondrial apoptotic pathway requires the presence of cytochrome *c*, apoptotic protease-activating factor (APAF)-1, and 2′-deoxyadenosine-5′-triphosphate (dATP) (Haunstetter and Izumo 1998). This pathway is modulated by pro-apoptotic members of the Bcl-2 family of proteins, which enhance membrane permeability and thereby favor the release of molecules from mitochondrial intermembrane space to the cytoplasm (Yang et al. 1997; Tsujimoto 2003; Breckenridge and Xue 2004). Specifically, cytochrome *c* initiates ATP-dependent formation of apoptosome by interacting with APAF-1 (Cesselli et al. 2001; Adams and Cory 2002; Ferraro et al. 2003). This complex recruits and cleaves procaspase 9 into the active enzyme, which in turn activates the executioner caspases-3 and -7 (King et al. 1998). The degradation of cellular substrates proceeds culminating in apoptotic cardiomyocyte death (Martin and Green 1995).

Role of microtubule polymerization in apoptotic death pathway

Paclitaxel is an enhancer of apoptosis and microtubule polymerization, and is also used as a chemotherapy agent against cancers as it polymerizes microtubule network and inhibits microtubule dynamics in cancer cells, resulting in cell cycle arrest and activation of the intrinsic mitochondrial apoptotic pathway (Rowinsky 1997; Mekhail and Markman 2002). The present study demonstrates that paclitaxel, but not angiotensin II, isoproterenol, or TNF-α, increased microtubule network (as shown as micrographic density) in cardiomyocytes, whereas paclitaxel, angiotensin II, isoproterenol, and TNF-α induce their apoptosis. Angiotensin II is known to activate the intrinsic mitochondrial apoptotic pathway through angiotensin II type 1 receptor (AT_1) in cardiomyocytes (Haunstetter and Izumo 1998; Matsusaka et al. 2006). The present study indicates that the combination of paclitaxel and angiotensin II further increases apoptosis, suggesting that microtubule polymerization enhances cardiomyocyte apoptosis in rats.

By contrast, in the present study, colchicine, a microtubule-depolymerizing agent, prevented cardiomyocyte apoptosis in response to angiotensin II, isoproterenol and TNF-α, which was associated with the decreased micrographic density of microtubules. Colchicine binds to free tubulin heterodimers, which are incorporated into microtubules, inhibiting addition of further subunits that increase GTPase activity of tubulin (David-Pfeuty et al. 1979). It also inhibits the stretch-induced increase in microtubule polymerization and secretion of angiotensin II from hypertrophic cardiomyocytes (Yutao et al. 2006). Microtubule disruption by colchicine influences Ca^{2+} signaling to attenuate the response to β-adrenergic stimulation (Gomez et al. 2000; Calaghan et al. 2001). Therefore, our present results suggest that microtubule depolymerization by colchicine inhibits apoptotic cardiomyocyte death.

As the present study indicated, microtubule polymerization can induce apoptosis; however, apoptosis does not always require its polymerization. In fact, angiotensin II, isoproterenol, or TNF-α can cause apoptosis independent of microtubule polymerization. We consider that the appropriate microtubule depolymerization inhibits myocardial apoptosis, probably because the microtubule depolymerization may delay the apoptotic death signaling.

Limitations of the study

Several limitations should be mentioned for the present study. First, although we confirmed that colchicine could decrease the density of microtubules and inhibit myocardial apoptotic cell death, experiments with genetically engineered animals are required to further confirm the direct link between myocardial polymerization/depolymerization of microtubules and apoptosis. Second, although colchicine might be a potential

therapeutic agent for heart failure, the higher doses of colchicine may not be suitable for clinical use because of its toxicity (Wilbur and Makowsky 2004). Furthermore, colchicine may have a pro-apoptotic effect by itself in neuronal cells or cancer cells (Goh et al. 1998). Thus, anti-polymerizing agents with reasonable safety need to be developed for clinical use. Third, we did not examine the long-term effects of colchicine on myocardial apoptosis and heart failure in vivo, and this point needed to be addressed in a future study.

Conclusions

The possible role of increased microtubule polymerization has been implicated in animal models of heart failure and in patients with severe aortic stenosis (Zile et al. 2001). In the present study, we were able to demonstrate that a microtubule-depolymerizing agent, colchicine, inhibits myocardial apoptosis in rats both in vitro and in vivo. Thus, inhibition of microtubule polymerization in cardiomyocytes could be a potential therapeutic strategy to prevent the progression of heart failure.

Acknowledgements

The authors wish to thank F. Hase for excellent technical assistance. This work was supported in part by the grants-in-aid (Nos. 15256003, 16209027, 16659192) from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan, the Japanese Ministry of Health, Labor, and Welfare, Tokyo, Japan, the Program for Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical Safety and Research of Japan, and Technology Agency, CREST, Tokyo, Japan.

References

Adams, J.M. & Cory, S. (2002) Apoptosomes: engines for caspase activation. *Curr. Opin. Cell Biol.,* **14**, 715-720.

Ashkenazi, A. & Dixit, V.M. (1998) Death receptors: signaling and modulation. *Science,* **281**, 1305-1308.

Bishopric, N.H., Andreka, P., Slepak, T. & Webster, K.A. (2001) Molecular mechanisms of apoptosis in the cardiac myocyte. *Curr. Opin. Pharmacol.,* **1**, 141-150.

Breckenridge, D.G. & Xue, D. (2004) Regulation of mitochondrial membrane permeabilization by BCL-2 family proteins and caspases. *Curr. Opin. Cell Biol.,* **16**, 647-652.

Calaghan, S.C., Le Guennec, J.Y. & White, E. (2001) Modulation of Ca(2+) signaling by microtubule disruption in rat ventricular myocytes and its dependence on the ruptured patch-clamp configuration. *Circ. Res.,* **88**, E32-E37.

Cesselli, D., Jakoniuk, I., Barlucchi, L., Beltrami, A.P., Hintze, T.H., Nadal-Ginard, B., Kajstura, J., Leri, A. & Anversa, P. (2001) Oxidative stress-mediated cardiac cell death is a major determinant of ventricular dysfunction and failure in dog dilated cardiomyopathy. *Circ. Res.,* **89**, 279-286.

Chen, Q.M. & Tu, V.C. (2002) Apoptosis and heart failure: mechanisms and therapeutic implications. *Am. J. Cardiovasc. Drugs,* **2**, 43-57.

David-Pfeuty, T., Simon, C. & Pantaloni, D. (1979) Effect of antimitotic drugs on tubulin GTPase activity and self-assembly. *J. Biol. Chem.,* **254**, 11696-11702.

De Angelis, N., Fiordaliso, F., Latini, R., Calvillo, L., Funicello, M., Gobbi, M., Mennini, T. & Masson, S. (2002) Appraisal of the role of angiotensin II and aldosterone in ventricular myocyte apoptosis in adult normotensive rat. *J. Mol. Cell Cardiol.,* **34**, 1655-1665.

de Jonge, N., van Wichen, D.F., van Kuik, J., Kirkels, H., Lahpor, J.R., Gmelig-Meyling, F.H., van den Tweel, J.G. & de Weger, R.A. (2003) Cardiomyocyte death in patients with end-stage heart failure before and after support with a left ventricular assist device: low incidence of apoptosis despite ubiquitous mediators. *J. Heart Lung Transplant.,* **22**, 1028-1036.

Ferraro, E., Corvaro, M. & Cecconi, F. (2003) Physiological and pathological roles of Apaf1 and the apoptosome. *J. Cell Mol. Med.,* **7**, 21-34.

Fukumoto, Y., Libby, P., Rabkin, E., Hill, C.C., Enomoto, M., Hirouchi, Y., Shiomi, M. & Aikawa, M. (2001) Statins alter smooth muscle cell accumulation and collagen content in established atheroma of watanabe heritable hyperlipidemic rabbits. *Circulation,* **103**, 993-999.

Goh, E.L., Pircher, T.J. & Lobie, P.E. (1998) Growth hormone promotion of tubulin polymerization stabilizes the microtubule network and protects against colchicine-induced apoptosis. *Endocrinology,* **139**, 4364-4372.

Gomez, A.M., Kerfant, B.G. & Vassort, G. (2000) Microtubule disruption modulates Ca(2+) signaling in rat cardiac myocytes. *Circ. Res.,* **86**, 30-36.

Gonzalez, A., Fortuno, M.A., Querejeta, R., Ravassa, S., Lopez, B., Lopez, N. & Diez, J. (2003) Cardiomyocyte apoptosis in hypertensive cardiomyopathy. *Cardiovasc. Res.,* **59**, 549-562.

Haunstetter, A. & Izumo, S. (1998) Apoptosis: basic mechanisms and implications for cardiovascular disease. *Circ. Res.,* **82**, 1111-1129.

Hein, S., Kostin, S., Heling, A., Maeno, Y. & Schaper, J. (2000) The role of the cytoskeleton in heart failure. *Cardiovasc. Res.,* **45**, 273-278.

Howarth, F.C., Calaghan, S.C., Boyett, M.R. & White, E. (1999) Effect of the microtubule polymerizing agent taxol on contraction, Ca^{2+} transient and L-type Ca^{2+} current in rat ventricular myocytes. *J. Physiol.,* **516**, 409-419.

Ishibashi, Y., Tsutsui, H., Yamamoto, S., Takahashi, M., Imanaka-Yoshida, K., Yoshida, T., Urabe, Y., Sugimachi, M. & Takeshita, A. (1996) Role of microtubules in myocyte contractile dysfunction during cardiac hypertrophy in the rat. *Am. J. Physiol.,* **271**, H1978-H1987.

Ishibashi, Y., Takahashi, M., Isomatsu, Y., Qiao, F., Iijima, Y., Shiraishi, H., Simsic, J.M., Baicu, C.F., Robbins, J., Zile, M.R. & Cooper, G. (2003) Role of microtubules versus myosin heavy chain isoforms in contractile dysfunction of hypertrophied murine cardiocytes. *Am. J. Physiol. Heart*

Circ. Physiol., **285**, H1270-H1285.
Kajstura, J., Cigola, E., Malhotra, A., Li, P., Cheng, W., Meggs, L.G. & Anversa, P. (1997) Angiotensin II induces apoptosis of adult ventricular myocytes in vitro. *J. Mol. Cell Cardiol.,* **29**, 859-870.
King, D., Pringle, J.H., Hutchinson, M. & Cohen, G.M. (1998) Processing/activation of caspases, -3 and -7 and -8 but not caspase-2, in the induction of apoptosis in B-chronic lymphocytic leukemia cells. *Leukemia,* **12**, 1553-1560.
Koide, M., Hamawaki, M., Narishige, T., Sato, H., Nemoto, S., DeFreyte, G., Zile, M.R., Cooper, G.I. & Carabello, B.A. (2000) Microtubule depolymerization normalizes in vivo myocardial contractile function in dogs with pressure-overload left ventricular hypertrophy. *Circulation,* **102**, 1045-1052.
Li, H.L., Suzuki, J., Bayna, E., Zhang, F.M., Dalle Molle, E., Clark, A., Engler, R.L. & Lew, W.Y. (2002) Lipopolysaccharide induces apoptosis in adult rat ventricular myocytes via cardiac AT(1) receptors. *Am. J. Physiol. Heart Circ. Physiol.,* **283**, H461-H467.
Liu, Y., Cigola, E., Cheng, W., Kajstura, J., Olivetti, G., Hintze, T.H. & Anversa, P. (1995) Myocyte nuclear mitotic division and programmed myocyte cell death characterize the cardiac myopathy induced by rapid ventricular pacing in dogs. *Lab. Invest.,* **73**, 771-787.
Martin, S.J. & Green, D.R. (1995) Protease activation during apoptosis: death by a thousand cuts? *Cell,* **82**, 349-352.
Matsusaka, H., Kinugawa, S., Ide, T., Matsushima, S., Shiomi, T., Kubota, T., Sunagawa, K. & Tsutsui, H. (2006) Angiotensin II Type 1 Receptor Blocker Attenuates Exacerbated Left Ventricular Remodeling and Failure in Diabetes-Associated Myocardial Infarction. *J. Cardiovasc. Pharmacol.,* **48**, 95-102.
Mekhail, T.M. & Markman, M. (2002) Paclitaxel in cancer therapy. *Expert. Opin. Pharmacother,* **3**, 755-766.
Nicholson, D.W. & Thornberry, N.A. (1997) Caspases: killer proteases. *Trends Biochem. Sci.,* **22**, 299-306.
Redfield, M.M., Jacobsen, S.J., Burnett, J.C., Jr., Mahoney, D.W., Bailey, K.R. & Rodeheffer, R.J. (2003) Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *JAMA,* **289**, 194-202.
Rowinsky, E.K. (1997) Paclitaxel pharmacology and other tumor types. *Semin. Oncol.,* **24**, S19-1-S19-12.
Sato, H., Hori, M., Kitakaze, M., Iwai, K., Takashima, S., Kurihara, H., Inoue, M. & Kamada, T. (1993) Reperfusion after brief ischemia disrupts the microtubule network in canine hearts. *Circ. Res.,* **72**, 361-375.
Sharma, A. & Singh, M. (2000) Possible mechanism of cardioprotective effect of angiotensin preconditioning in isolated rat heart. *Eur. J. Pharmacol.,* **406**, 85-92.
Sharov, V.G., Sabbah, H.N., Shimoyama, H., Goussev, A.V., Lesch, M. & Goldstein, S. (1996) Evidence of cardiocyte apoptosis in myocardium of dogs with chronic heart failure. *Am. J. Pathol.,* **148**, 141-149.
Suzuki, J., Bayna, E., Dalle Molle, E. & Lew, W.Y. (2003) Nicotine inhibits cardiac apoptosis induced by lipopolysaccharide in rats. *J. Am. Coll. Cardiol.,* **41**, 482-488.
Tada, H., Kagaya, Y., Takeda, M., Ohta, J., Asaumi, Y., Satoh, K., Ito, K., Karibe, A., Shirato, K., Minegishi, N. & Shimokawa, H. (2006) Endogenous erythropoietin system in non-hematopoietic lineage cells plays a protective role in myocardial ischemia/reperfusion. *Cardiovasc. Res.,* **71**, 466-477.
Tabet, J.Y., Lopes, M.E., Champagne, S., Su, J.B., Merlet, P. & Hittinger, L. (2002) Inflammation, cytokines and anti-inflammatory therapies in heart failure. *Arch. Mal. Coeur Vaiss.,* **95**, 204-212.
Tsujimoto, Y. (2003) Cell death regulation by the Bcl-2 protein family in the mitochondria. *J. Cell Physiol.,* **195**, 158-167.
Tsutsui, H., Ishibashi, Y., Takahashi, M., Namba, T., Tagawa, H., Imanaka-Yoshida, K. & Takeshita, A. (1999) Chronic colchicine administration attenuates cardiac hypertrophy in spontaneously hypertensive rats. *J. Mol. Cell. Cardiol.,* **31**, 1203-1213.
Wilbur, K. & Makowsky, M. (2004) Colchicine myotoxicity: case reports and literature review. *Pharmacotherapy,* **24**, 1784-1792.
Yang, J., Liu, X., Bhalla, K., Kim, C.N., Ibrado, A.M., Cai, J., Peng, T.I., Jones, D.P. & Wang, X. (1997) Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science,* **275**, 1129-1132.
Yutao, X., Geru, W., Xiaojun, B., Tao, G. & Aiqun, M. (2006) Mechanical stretch-induced hypertrophy of neonatal rat ventricular myocytes is mediated by beta(1)-integrin-microtubule signaling pathways. *Eur. J. Heart Fail.,* **8**, 16-22.
Zile, M.R., Green, G.R., Schuyler, G.T., Aurigemma, G.P., Miller, D.C. & Cooper, G. (2001) Cardiocyte cytoskeleton in patients with left ventricular pressure overload hypertrophy. *J. Am. Coll. Cardiol,* **37**, 1080-1084.