Chronic Akt blockade aggravates pathological hypertrophy and inhibits physiological hypertrophy

Sebastian J. Buss,* Johannes H. Riffel,* Pratima Malekar, Marco Hagenmueller, Christina Asel, Min Zhang, Celine Weiss, Hugo A. Katus, and Stefan E. Hardt
Department of Cardiology, University of Heidelberg, Heidelberg, Germany

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Buss SJ, Riffel JHI, Malekar P, Hagenmueller M, Asel C, Zhang M, Weiss C, Katus HA, Hardt SE. Chronic Akt blockade aggravates pathological hypertrophy and inhibits physiological hypertrophy. Am J Physiol Heart Circ Physiol 302: H420–H430, 2012. First published November 11, 2011; doi:10.1152/ajpheart.00211.2011.—The attenuation of adverse myocardial remodeling and pathological left ventricular (LV) hypertrophy is one of the hallmarks for improving the prognosis after myocardial infarction (MI). We investigated the effect of chronic Akt blockade with deguelin on the development of pathological [MI and aortic banding (AB)] and physiological (controlled treadmill running) hypertrophy. Primary cardiomyocyte cultures were incubated with 10 μmol deguelin for 48 h, and Wistar rats were treated orally with deguelin (4.0 mg·kg
1·day


In vivo, we observed reduced phosphorylation of Akt and glycogen synthase kinase (GSK)-3β after an incubation with deguelin, whereas MAPK signaling was not significantly affected. In vitro, treatment with deguelin led to attenuated phosphorylation of Akt and GSK-3β 4 wk after MI. These animals showed significantly increased heart weights and impaired LV function with increased end-diastolic diameters (12.0 ± 0.3 vs. 11.1 ± 0.3 mm, P < 0.05), end-diastolic volumes (439 ± 8 vs. 388 ± 18 μl, P < 0.05), and cardiomyocyte sizes (+20%, P < 0.05) compared with MI animals receiving vehicle treatment. Furthermore, activation of Ca²⁺/calmodulin-dependent kinase II in deguelin-treated MI animals was increased compared with the vehicle-treated group. Four wk after AB, we observed an augmented phosphorylation of pathological hypertrophy in the deguelin-treated group with a significant increase in heart weights and cardiomyocyte sizes (>20%, P < 0.05). In contrast, the development of physiological hypertrophy was inhibited by deguelin treatment in exercise-trained animals. In conclusion, chronic Akt blockade with deguelin aggravates adverse myocardial remodeling and antagonizes physiological hypertrophy.

cardiac remodeling; exercise training

ISCHEMIC HEART DISEASE is still one of the leading causes of mortality worldwide (34). Myocardial ischemia and myocardial infarction (MI) lead to contractile dysfunction and remodeling even after sufficient reperfusion due to early coronary artery intervention. A major cost problem for public health is the development of heart failure in these patients (13). One of the most important factors for improving the prognosis after MI is the attenuation of adverse myocardial remodeling and pathological left ventricular (LV) hypertrophy (16, 36).

Despite the widespread use of agents such as angiotensin-converting enzyme inhibitors, β-blockers, and aldosterone antagonists, the occurrence of heart failure as the end stage of LV remodeling still remains high (15, 37). On the other hand, the physiological form of cardiac hypertrophy, which is found in highly trained athletes and during pregnancy, is associated with a good prognosis and has not yet been associated with cardiac arrhythmias or the development of congestive heart failure (33, 45). The protein kinase Akt (also known as PKB) belongs to a family of serine/threonine protein kinases and is highly conserved in mammals (11). Several growth factors and hormones are known to activate Akt in the heart, which are mediated via the action of phosphatidylinositol 3-kinase (PI3K) and a number of receptor tyrosine kinases, including growth hormone, IGF-I, FGF, and EGF. Activation of Akt is regulated by several stimuli in the heart, e.g., insulin, exercise training, and pressure overload (30, 40, 45). Akt signaling regulates myocyte size, at least in part, through the activation of mammalian target of rapamycin (mTOR)-dependent progrowth signaling and suppression of FOXO- and glycogen synthase kinase (GSK)-3β-dependent pathways (20, 21, 39).

Mortality due to cancer is the other topic of great economic and medical weight. Many natural compounds, especially plant products and dietary constituents, have recently been found to exhibit antimalignant activities both in vitro and in vivo (17, 25). The mechanisms of action include different effects on cell growth suppression, modulation of cell differentiation, and induction of apoptosis. Rotenoids, which are plant derived from the flavonoid family, deliver chemopreventive activity in vitro (17). One of these rotenoids, deguelin, is a cancer-protective substance that selectively blocks the PI3K/Akt pathway (8, 27). The aim of our study was to investigate the influence of chronic Akt inhibition by the oral application of deguelin on the development of pathological hypertrophy and cardiac remodeling after aortic banding (AB) and MI. Additionally, we investigated the role of Akt blockade by deguelin on the development of physiological hypertrophy after exercise training.

MATERIALS AND METHODS

This investigation conformed with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996) and was approved by the authorities of the Regierungspäsidium (Karlsruhe, Germany). For all animal models, we used male Wistar rats with a weight of ~200 g (Charles River Laboratories).

MI. Animals were anesthetized by an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (2 to 5 mg/kg). After orotraehal intubation and ventilation, the thorax was opened left parasternally,
and MI was induced by ligating the left anterior descending coronary artery just below the left atrial appendage, as previously described in detail (4). The quality of the infarction was confirmed visually by the change in the color of the myocardium.

**AB.** Animals were anesthetized as described above. After orotracheal intubation and ventilation, the thorax was opened left parasternally, and AB was induced by clipping the aorta ascendens, as previously described in detail (1, 26, 44).

**Exercise training.** Physiologic cardiac hypertrophy was generated by a vigorous exercise regime for 5 wk. Rats of approximately the same weight were randomly divided into three groups: the exercise-trained group, the exercise-trained group with deguelin treatment, and the sedentary control group. Rats in the exercise-trained group were trained on a rodent treadmill (TSE Treadmill Model Advanced, TSE Systems, Bad Homburg, Germany), where four rats could be trained simultaneously. The training duration consisted of a 1-wk run-in period and a 4-wk training period with increasing velocities and duration. Animals trained 5 days/wk for 4 wk. Speed, grade, and duration were increased progressively. Rats began training at 0.2 cm/s and 5% grade for 20 min/day for the first week. The speed and grade were gradually increased such that by the end of the second week, animals ran at least 0.4 cm/s at 10% grade for 50 min/day. After the duration was further increased such that by the end of the second week, animals ran at least 0.4 cm/s at 10% grade for 50 min/day. After the duration was further increased to 60 min/day, speed and grade were maintained until the end of the study.

**Study design.** Animals from the different interventions were treated with deguelin [4.0 mg·kg⁻¹·day⁻¹], as previously described (43)] or vehicle by oral gavage, starting after the recovery from surgery, within 24 h after the operation. Exercise-trained animals were treated 4 wk throughout the treadmill training. One group of animals received deguelin alone without any intervention for 48 h and 28 days. Animals were killed 28 days after MI, AB, and exercise training for further characterization. Anesthesia was induced by an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (2–5 mg/kg); afterward, echocardiographic and invasive measurements were performed. The heart was arrested in diastole by an injection of saturated KCl solution, and heart weight was measured. After LV was dissected, LV weight was measured, and myocardial samples from the different regions of the LV (infarction, border zone, and remote area) were snap frozen for biochemical measurements or fixed in formalin for further histological evaluation. Immunoblot analyses were performed using tissue homogenates from the remote area to the infarction.

**Echocardiography.** Transthoracic echocardiography was performed in a modified setting as previously described in detail (19). Experiments were recorded using a dynamic focused 10-MHz probe with an ATL 5000 echocardiography machine. The investigator who conducted the echocardiography was blinded to the treatment status.

**LV pressure-volume measurements.** For the invasive assessment of pressure-volume relationships, some of the rats that received MI underwent catheterization as previously described (4) and were therefore anesthetized in the above-described manner. The LV was catheterized retrogradely via the right carotid artery using a 2.0-Fr impedance micrometer catheter (Millar Instruments). For the subsequent analysis of pressure-volume loops, PVAN software (Millar Instruments) was used.

**Primary cultures of ventricular cardiac myocytes.** Primary cultures of ventricular cardiac myocytes were prepared from 1- to 3-day-old Wistar rats (Charles River Laboratories) and purified using a discontinuous Percoll gradient. Cells were cultured in cardiac myocyte culture medium containing DMEM and F-12 supplemented with 5% horse serum, 4 μg/ml transferrin, 0.7 ng/ml sodium selenite, 2 g/l BSA (fraction V), 3 mM pyruvic acid, 15 mM HEPES, 100 μM ascorbic acid, 100 μg/ml ampicillin, 5 μg/ml linoleic acid, and 100 μM 5-bromo-2′-deoxyuridine. We obtained cultures in which >95% of cells were myocytes. Culture medium was changed to serum-free medium at 24 h. Deguelin was dissolved in absolute ethanol, and myocytes were treated with 10 μmol deguelin for 48 h.

**Western blot analysis.** Myocytes were washed twice with ice-cold PBS and scraped from the culture dish. Cells were lysed and incubated for 30 min in ice-cold RIPA buffer containing 150 mM NaCl, 50 mM Tris (pH 7.5), 0.5% deoxycholic acid, 1% Nonidet P-40, 0.1% SDS, 1 mM NaVO₄, 10 mM NaF, and the following protease inhibitors: 0.5 mM ABEFS, 5 μg/ml aprotinin, and 5 μg/ml leupeptin. Protein concentrations were measured using a BCA protein assay (Intercham). Equal amounts of protein were separated with SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore). Membranes were incubated overnight at 4°C with primary antibody. Anti-rabbit IgG and anti-mouse IgG horseradish peroxidase-conjugated antibodies (Cell Signaling Technology) were used as secondary antibodies.

For further protein analysis, animals were killed 28 days after MI. LV protein lysates (100 μg) were prepared from the LV tissue of the remote area. Western blot analysis of the rat heart lysates was performed as previously described (22). In brief, protein extracts were prepared by homogenization of frozen rat LV tissue in RIPA buffer. Proteins were resolved on SDS-PAGE depending on their molecular masses and transferred to PVDF membranes. Secondary antibody was visualized using chemiluminescence techniques.

**Gene expression.** Primers and specific probes were chosen as follows: Akt (Cell Signaling Technology), phospho-(p)-Akt (Ser²⁷³), Cardiac Signaling Technology, GSK-3β (BD Biosciences), p-GSK-3β (Ser²⁷), Cell Signaling Technology, β-p70 S6 kinase (S6K; Thr³⁸⁷), Cell Signaling Technology, p70 S6 kinase (Cell Signaling Technology), ERK1/2 (Cell Signaling Technology), Ca²⁺/calmodulin-dependent kinase II (CaMKII; Millipore), p-CaMKII (Promega), and p-ERK1/2 (Santa Cruz Biotechnology). Bands were quantified by densitometry using ImageJ software (NIH).

**Quantitative real-time PCR.** Total RNA was isolated from the LV using TRIzol reagent (Invitrogen). cDNA was synthesized with a Revert Aid first-strand cDNA synthesis kit (Fermentas). Copy numbers of the hypoxanthine-guanine phosphoribosyltransferase (HPRT) housekeeping gene, atrial natriuretic factor (ANF), and brain natriuretic peptide (BNP) were determined using the LightCycler system (Roche Diagnostics). Primers and specific probes were designed using the Rat Universal Probe Library from Roche Diagnostics. The following primers were used: HPRT, 5′-GTCAAGGGGGGACATAAAAG-3′ and 5′-TGCAATTGGTTT-TACCAGTGCA-3′, probe 22; ANF, 5′-CAACAACAGATCTGTG- GATTCA-3′ and 5′-CCCTACCTCTCTACCCGAGC-3′, probe 25; and BNP, 5′-GTCAAGGGGGGACATAAAAG-3′ and 5′-AGAGC-TGGGGAAAGAGACGC-3′, probe 13. All real-time PCRs were normalized to HPRT mRNA expression. A standard curve was run with the dilution series of the amplified fragment allowing for the calculation of mRNA copy numbers.

**Histopathological evaluation.** Histological experiments of the hearts were conducted in formaline-fixed hearts from animals from all groups. Myocardial connective tissue was analyzed quantitatively in a cross section of the LV obtained midway between the base and apex and stained with hematoxylin-eosin and Masson’s trichrome (to detect collagen deposition). Myocyte size was measured in cross sections of the LV using Imaging software (NIH). Evidence of fibrosis was evaluated in a blinded manner by two independent investigators who used light microscopy according to the following scoring system: grade 0, no fibrosis; grade 1, cardiac infiltration in up to 5% of the cardiac sections; grade 2, infiltration in 6–10% of the cardiac sections; grade 3, infiltration in 11–30% of the cardiac sections; grade 4, infiltration in 31–50% of the cardiac sections; and grade 5, infiltration in >50% of cardiac sections, as previously described in detail (18). To confirm an equal distribution of MI size among the infarcted groups, in a subgroup of animals, triphenyltetrazolium chloride (TTC) staining was performed to assess infarct size, as determined by planimetric measurements. Thus, LVs of the excised hearts were frozen, cut into six to eight slices, and then incubated at 37°C for 20 min in a well plate in TTC buffer. LVs were then photographed, and the infarct area was determined as a percentage of the whole LV using ImageJ software.

**Statistics.** Results are expressed as means ± SE. Differences between groups were tested by one-way ANOVA with post hoc
RESULTS

In vitro effects of deguelin. In a first step, the in vitro effects of deguelin in primary cardiomyocyte cultures were evaluated. After 48 h, we observed a clear reduction of p-Akt levels, whereas total Akt levels remained unchanged (Fig. 1A). The levels of p-GSK-3β were significantly reduced, whereas those for total GSK-3β remained unchanged. Furthermore, there were no differences in the phosphorylation levels of ERK1/2 in cardiomyocytes after

Fig. 1. A, left: Western blot analysis revealed a significant decrease of phosphorylation of Akt and glycogen synthase kinase (GSK)-3β in cardiomyocytes after an incubation with 10 μmol deguelin (DEG) for 48 h. Phosphorylation levels of ERK1/2 remained unaffected. Right, quantification of Western blots of cardiomyocytes after the incubation with DEG. B: representative protein levels of Akt and phosphorylated (p)Akt in control (Co) animals and animals receiving DEG alone (Co + DEG) for 48 h. Phosphorylation of Akt was reduced after DEG administration. C: representative protein levels of Akt, pAkt, GSK-3β, pGSK-3β, p70 S6 kinase (S6K), and pp70 S6K after myocardial infarction (MI). Western blot analysis revealed a decrease of phosphorylation of Akt (Ser473), GSK-3β (Ser9), and p70 S6K (Thr389) after the administration of DEG after MI. Veh, vehicle. D: representative protein levels of Ca²⁺/calmodulin-dependent kinase II (CaMKII) and pCaMKII after MI. Western blot analysis showed an augmented activation of pCaMKII after the administration of DEG after MI. Data are means ± SE; n ≥ 4 in all groups. *P < 0.05 vs. the control group. #P < 0.05 vs. the sham.
deguelin incubation (Fig. 1A), indicating that MAPK signaling was not significantly affected.

**In vivo effects of deguelin.** The dosage of 4 mg/kg leads to a high cardiac tissue distribution of deguelin in rats (43). After oral treatment for 48 h, we observed a clear reduction of p-Akt levels, whereas total Akt levels remained unchanged (Fig. 1B). In animals with MI treated deguelin for 28 days, we found similar results compared with the in vitro data (Fig. 1C). Moreover, a clear reduction of the phosphorylation levels of p70 S6K, a downstream target of Akt, was observed (Fig. 1C). Activation of CaMKII in deguelin-treated MI animals was increased compared with the vehicle-treated group (Fig. 1D).

**Akt inhibition by deguelin aggravates myocardial remodeling after MI.** To elucidate the role of chronic Akt blockade in post-MI remodeling, the left anterior descending coronary artery was ligated in male Wistar rats permanently for 28 days. In MI animals treated with vehicle for this period, the ejection fraction (EF), as measured by echocardiography, was significantly reduced (38 ± 1.8% vs. 32 ± 1.8%; Fig. 2C). Furthermore, deguelin treatment led to an increase of LVEDD (12.0 ± 0.32 vs. 11.1 ± 0.27 mm; Fig. 2B) and LVESD (10.6 ± 0.33 vs. 9.5 ± 0.27 mm; Fig. 2A). EF was reduced from 19 ± 1.1% in deguelin-treated MI animals versus 27 ± 2.4% in vehicle-treated MI animals (P < 0.01; Table 1). Values of LV end-diastolic pressure were significantly increased after deguelin treatment (7.9 ± 0.7 vs. 14.5 ± 1.8 mmHg; Fig. 3B). In addition, there was a further reduction of other hemodynamic parameters, such as dP/dt min, dP/dt max, and the time constant (Table 1). Deguelin treatment led to an obvious rightward shift of LV pressure-volume curves (Fig. 3) and markedly increased the LV end-diastolic volume (439 ± 8 μl, P < 0.05; Fig. 3A), end-systolic volume (375 ± 9 μl, P < 0.01; Table 1), and cardiac output (41,576 ± 3,317 vs. 28,246 ± 1,190 μl/min; Fig. 3C) compared with the vehicle-treated MI group.

In summary, echocardiography and invasive measurements consistently demonstrated a significant increase of adverse myocardial remodeling compared with vehicle-treated animals after MI.

**Effects of deguelin on infarct size, myocardial hypertrophy, and fibrosis.** Treatment with deguelin after the induction of MI did not lead to a relevant increase of the infarct size compared
with vehicle treatment after 28 days, as assessed by TTC staining (38 ± 0.7% vs. 44 ± 1.1%, not significant; Fig. 4A). Absolute and relative heart weights were significantly elevated compared with the vehicle-treated group after 28 days (Table 2 and Fig. 4, B and C). mRNA levels of ANF and BNP between the vehicle-treated MI group and sham group presented significant changes, with a slight increase in the deguelin-treated group (Fig. 4, D and E). We additionally evaluated the effects of deguelin on cardiac myocyte size in the remote area of the infarction. There was a significant increase in relative myocyte size in the vehicle-treated MI group compared with the sham group. Deguelin-treated animals showed augmented cardiomyocyte sizes compared with vehicle-treated animals with an increase in cardiomyocyte size of >20% (P < 0.05; Fig. 5A).

Finally, deguelin-treated MI animals showed a higher amount of fibrosis in the remote area compared with vehicle-treated MI animals (Fig. 5B).

Akt inhibition aggravates pathological hypertrophy after AB. In the second animal model, where AB was used as another pathological stimulus, chronic Akt inhibition also lead to an augmentation of pathological hypertrophy. In detail, the heart weight-to-body weight ratio and heart weight-to-tibia length ratio were significantly enhanced in the deguelin-treated group (Fig. 6, A and B). Echocardiographic parameters revealed an increase in end-diastolic anterior and posterior wall thicknesses in deguelin-treated animals compared with vehicle-treated animals (end-diastolic anterior wall thickness: 2.08 ± 0.05 vs. 1.89 ± 0.06 mm, P < 0.05; end-diastolic posterior thickness: 2.4 ± 0.13 vs. 2.0 ± 0.11 mm, P < 0.05; end-systolic thickness: 1.9 ± 0.06 vs. 1.6 ± 0.05 mm, P < 0.05).

Table 1. Hemodynamic parameters of Veh- and DEG-treated animals 28 days after MI

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>MI + Veh</th>
<th>MI + DEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals/group</td>
<td>10</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>402 ± 12</td>
<td>395 ± 17</td>
<td>356 ± 11</td>
</tr>
<tr>
<td>End-systolic volume, μl</td>
<td>135 ± 16</td>
<td>305 ± 21*</td>
<td>375 ± 9†</td>
</tr>
<tr>
<td>End-diastolic volume, μl</td>
<td>307 ± 18</td>
<td>388 ± 18*</td>
<td>439 ± 8†</td>
</tr>
<tr>
<td>End-systolic pressure, mmHg</td>
<td>135.3 ± 12</td>
<td>120.8 ± 5</td>
<td>109.0 ± 3</td>
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<tr>
<td>End-diastolic pressure, mmHg</td>
<td>3.75 ± 0.5</td>
<td>7.94 ± 0.68*</td>
<td>14.50 ± 1.79†</td>
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<tr>
<td>Stroke volume, μl</td>
<td>194 ± 10</td>
<td>106 ± 8*</td>
<td>85 ± 5</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>61 ± 3.8</td>
<td>27 ± 2.4*</td>
<td>19 ± 1.1†</td>
</tr>
<tr>
<td>Cardiac output, μl/min</td>
<td>77,794 ± 4,069</td>
<td>41,576 ± 3,317*</td>
<td>28,246 ± 1,190†</td>
</tr>
<tr>
<td>Stroke work, mmHg·μl</td>
<td>22,196 ± 2,272</td>
<td>8,204 ± 1,145*</td>
<td>5,333 ± 318†</td>
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<td>Arterial elastance, mmHg/μl</td>
<td>0.71 ± 0.06</td>
<td>1.21 ± 0.08*</td>
<td>1.4 ± 0.13</td>
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<tr>
<td>dP/dtmax, mmHg/s</td>
<td>10,300 ± 705</td>
<td>7,210 ± 683*</td>
<td>6,069 ± 201</td>
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<td>dP/dtmin, mmHg/s</td>
<td>−9,769 ± 706</td>
<td>−6,100 ± 567*</td>
<td>−4,234 ± 257†</td>
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<td>Time constant, ms</td>
<td>9.05 ± 0.26</td>
<td>13.42 ± 0.76*</td>
<td>17.46 ± 1.01†</td>
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<tr>
<td>Preload adjusted maximal power, mW/l²</td>
<td>14.04 ± 1.84</td>
<td>5.94 ± 1.1*</td>
<td>3.75 ± 0.59</td>
</tr>
</tbody>
</table>

Results are presented as means ± SE. MI, myocardial infarction; Veh, vehicle; DEG, deguelin. *P < 0.05, Veh-treated group vs. sham group; †P < 0.05 vs. the sham and Veh-treated groups.
wall thickness: 2.08 ± 0.05 vs. 1.87 ± 0.05 mm, *P < 0.01). There were no differences in EF between both groups (Fig. 6C and Supplemental Fig. S2). Cardiomyocyte sizes were increased in deguelin-treated animals compared with vehicle-treated animals (20%, *P < 0.05; Fig. 6D). mRNA levels of ANF between the vehicle-treated group and the sham group were significantly elevated, with slightly higher levels in deguelin-treated animals. Furthermore, BNP expression was increased in deguelin-treated animals compared with vehicle-treated animals (Supplemental Fig. S1).

Akt inhibition antagonizes the development of physiological hypertrophy in exercise-trained animals. After 4 wk of exercise training, vehicle-treated animals developed physiological hypertrophy, with a significant increase in cardiomyocyte size (>30%, *P < 0.01) and an elevated heart weight-to-body weight ratio compared with the control group. Treatment with deguelin during the training period completely abrogated the development of physiological hypertrophy (Fig. 6, E, F, and H).

The deguelin-treated group and the sedentary control group showed almost similar cardiomyocyte sizes and heart weight body-to-weight ratios, suggesting that deguelin may be able to antagonize the strong physiologic stimulus of exercise training (Fig. 6H). No significant differences in EF between all three groups were found (Fig. 6G and Supplemental Fig. S3).

Adverse side effects. Finally, we examined potential negative effects on growth, wound healing, and cardiac function. We did not find an increased propensity for wound infections. Deguelin treatment did not cause a change in other organ weights, such as those of the liver or brain. There were no differences in body weights between the groups (Supplemental Table S1 and Supplemental Fig. S4). Treatment with deguelin alone in sedentary animals for over 4 wk did not result in a deterioration of heart function, differences in heart weights, or any other serious side effects. No animals died due to deguelin treatment.

DISCUSSION

We thoroughly investigated the influence of a chronic oral pharmacological blockade of the PI3K/Akt axis via deguelin in two in vivo models of pathological hypertrophy caused by MI and AB and in one in vivo model of physiological hypertrophy due to extensive exercise training. Even though the role of PI3K/Akt signaling has previously been examined in several knockout and transgenic mouse models, there are no chronic in vivo studies using the orally available Akt inhibitor deguelin after either a pathological or physiological stimulus over several weeks.

The main finding of this study was that targeting the Akt pathway in a chronic manner leads to the progression of adverse LV remodeling after a pathologic stimulus. The effects of a physiologic stimulus were counteracted. Therefore, deguelin acts as an inhibitor of physiological hypertrophy and aggravates pathological hypertrophy.

The anti-cancer substance deguelin is a plant product derived from several plant species (17, 25, 43). Its potential lies in inhibiting malignant growth in several in vitro tumor cell lines. The positive effects are mainly attributed to blocking

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1 Supplemental Material for this article is available at the American Journal of Physiology-Heart and Circulatory Physiology website.
tumor growth by disrupting the PI3K/Akt axis (3, 8). Previously, it has been reported that the tissue distribution of deguelin is known to be the highest in the heart after intragastric administration (43).

The protein kinase Akt plays a key role in regulating physiological cellular growth and development (5, 23). Activation of PI3K leads to the recruitment of Akt. Three subforms of Akt exist, but only Akt1 and Akt2 are highly expressed in the heart. Interestingly, Akt1-null mice have a ~20% reduction in body and heart size, but Akt2-null mice do not show any reductions in body weights or growth (6, 7). Akt itself is needed for physiological cardiac growth (5, 12, 35). Mice without active Akt (Akt1−/− mice) develop cardiac dilation and dysfunction due to a pathological stimulus induced by aortic constriction (12). On the other hand, activated Akt leads into a kind of physiological hypertrophy as a gain of function with preserved myocardial contractility (10). However, deleterious effects of chronic overexpression of Akt are known, as it also leads into cardiac dysfunction over time in some animal models (29, 39).

Transgenic mice with nuclear-targeted Akt overexpression show no cardiac hypertrophy and are protected from ischemia (41, 42), and transgenic mice expressing a constitutively active mutant of PI3K in the heart are protected against MI-induced heart failure (28). Thus, Akt signaling seems to be advantageous to the heart, when it is activated under physiological conditions or when it functions in the nucleus.

Akt1 knockout mice were found to be resistant to swimming training-induced cardiac hypertrophy in a study by deBosch et al. (12). In accordance with these findings, exercise-trained rats treated with deguelin did not develop physiological hypertrophy. Cardiomyocyte size was significantly decreased and almost equal to the group that did not exercise at all. These findings confirm that Akt is essential for the development of physiological hypertrophy and that chronic oral Akt blockade antagonizes this physiological process. More than that, supraphysiological activity of the PI3K/Akt axis seems to be beneficial in the context of heart

Table 2. Gross pathology of Veh- and DEG-treated animals 28 days after MI

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI + Veh</th>
<th>MI + DEG</th>
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<td>Number of animals/group</td>
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<tr>
<td>BW, g</td>
<td>374 ± 12</td>
<td>345 ± 8</td>
<td>343 ± 10</td>
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<tr>
<td>HW, g</td>
<td>0.87 ± 0.03</td>
<td>0.99 ± 0.02*</td>
<td>1.11 ± 0.04†</td>
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<tr>
<td>TL, mm</td>
<td>37.7 ± 0.43</td>
<td>37.3 ± 0.28</td>
<td>36.6 ± 0.3</td>
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<tr>
<td>HW/BW, mg/g</td>
<td>2.32 ± 0.03</td>
<td>2.88 ± 0.06*</td>
<td>3.25 ± 0.13†</td>
</tr>
<tr>
<td>HW/TL, mg/mm</td>
<td>23.0 ± 0.82</td>
<td>26.5 ± 0.45*</td>
<td>30.2 ± 1.1†</td>
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<tr>
<td>LV, g</td>
<td>0.71 ± 0.03</td>
<td>0.76 ± 0.02</td>
<td>0.84 ± 0.03</td>
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<tr>
<td>LV/TL, mg/mm</td>
<td>18.7 ± 0.71</td>
<td>20.5 ± 0.54</td>
<td>23.0 ± 0.67†</td>
</tr>
<tr>
<td>LV/BW, mg/g</td>
<td>1.88 ± 0.04</td>
<td>2.22 ± 0.05*</td>
<td>2.46 ± 0.07†</td>
</tr>
<tr>
<td>RV, g</td>
<td>0.18 ± 0.01</td>
<td>0.23 ± 0.02*</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>RV/BW, mg/g</td>
<td>0.49 ± 0.01</td>
<td>0.67 ± 0.06*</td>
<td>0.79 ± 0.06</td>
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<tr>
<td>RV/TL, mg/mm</td>
<td>4.85 ± 0.22</td>
<td>6.15 ± 0.5*</td>
<td>7.28 ± 0.52</td>
</tr>
</tbody>
</table>

Results are presented as means ± SE. BW, body weight; HW, heart weight; TL, tibia length; LV, left ventricle; RV, right ventricle. *P < 0.05, Veh-treated group vs. sham group; †P < 0.05 vs. MI + Veh-treated group.
In the MI model, cardiac function was decreased and relative heart weights were significantly elevated. Blockade of Akt led to aggravated myocardial damage due to the pathological stimulus. Cardiomyocyte size was significantly increased and fibrosis was highly elevated in deguelin-treated animals with MI compared with vehicle-treated animals. In the present study, we observed an increase in cardiac hypertrophy in animals with MI treated with deguelin, but we also found a reduction of p70 S6K in these animals. Activation of p70 S6K is associated with enhanced protein synthesis, which leads to myocardial hypertrophy (2, 9, 32). We (4) have previously shown that inhibition of mTOR after MI leads to decreased phosphorylation of p70 S6K, resulting in a reduction of cardiac hypertrophy. Others (32) have reported that deletion of ribosomal S6K1 and S6K2 does not change the myocardial growth response of physiological or pathological stimuli. Even if the activation of p70 S6K seems to be a critical factor for the development of cardiac hypertrophy in response to MI, other pathways may take part in compensating for the effect of Akt inhibition. Previously, Chun et al. (8) reported that deguelin treatment had only minimal effects on the MAPK pathway. In our study, we also found no significant differences in phosphorylation levels of ERK1/2, suggesting that deguelin application does not have a sufficient effect on MAPK signaling in our experimental setting.

One potential mechanism of our findings could be the effects on GSK-3β. The role of GSK-3β in the heart is still controversial. On one hand, GSK-3β is known to be a negative regulator of hypertrophy mainly regulated via Akt signaling (19, 21). On the other hand, overexpression of wild-type GSK-3β in mice induces cardiac dysfunction. Hirotani et al. (24) showed that overexpression of GSK-3β in the heart led to depressed cardiac function, the development of prominent fibrosis, and an increase in the heart weight-to-tibia length ratio, suggesting that cardiac hypertrophy developed even in the presence of GSK-3β overexpression. Moreover, inhibition of GSK-3β during heart failure is protective. In transgenic mice with cardiac-specific expression of dominant negative GSK-3β, LV systolic function was significantly better and less fibrosis was observed compared with control mice after a pathological stimulus (aortic constriction) (24).

In our study, phosphorylation of GSK-3β was significantly attenuated in animals with MI after treatment with deguelin. This led to an increase of activated GSK-3β over expression. Moreover, inhibition of GSK-3β during heart failure is protective. In transgenic mice with cardiac-specific expression of dominant negative GSK-3β, LV systolic function was significantly better and less fibrosis was observed compared with control mice after a pathological stimulus (aortic constriction) (24).

In summary, our hypothesis is that by blocking the prosurvival kinase Akt in the setting of a pathological stimulus, a dysbalance toward pathological pathways occurs and leads to a deterioration of cardiac remodeling and function.

Recently, more concern has been raised about the cardiotoxicity of new anticancer substances, especially the newer ones acting on the PI3K/Akt pathway (31). Anticancer sub-
stances (especially tyrosine kinase inhibitors) are well known to mediate, at least in part, cardiotoxic effects (14, 31, 35). Unfortunately, versatile physiological conditions are maintained via the PI3K/Akt pathway (35). Thus, blockade of physiological growth pathways could lead to a disruption of normal growth and development (14, 31).

Under basal conditions, inhibition of Akt through deguelin did not have deleterious effects on cardiac function. The same has been seen in transgenic animals overexpressing Akt (12). However, after a pathologic stimulus or stressor such as MI or AB, the missing “protective effect” of the prosurvival kinase Akt led to a deterioration of myocardial function. PI3K/Akt is therefore essential for preserving cardiac function in response to a pathological stimulus or under stress.

The PI3K/Akt/mTOR pathway is a complex signaling cascade. Inhibition of Akt and mTOR can lead to divergent pathologies and effects on myocardial function (4).

Fig. 6. A and B: In animals with aortic banding (AB), HW/BW and HW/TL were significantly increased in DEG-treated animals compared with Veh-treated animals. C: there were no significant differences in EF, as estimated from transthoracic echocardiography, in animals treated with DEG compared with Veh-treated animals. D, left: exemplary H&E-stained sections of myocardial tissue in sham, Veh-treated, and DEG-treated animals after AB after 28 days. Right: cardiomyocyte sizes were significantly increased in DEG-treated animals compared with Veh-treated animals. *P < 0.05 vs. the sham group; #P < 0.05 vs. the AB + Veh-treated group. E and F: in exercise-trained (Ex) animals, HW/BW and HW/TL were significantly increased compared with the control group. There were no increases in HW/BW and HW/TL in Ex animals treated with DEG compared with the control group. G: there were no significant differences in EF, as estimated from transthoracic echocardiography, in all groups. H: exemplary H&E-stained sections of myocardial tissue in control, Ex, and Ex animals treated with DEG. Right: cardiomyocyte sizes were significantly increased in Ex animals. DEG-treated animals showed no increases in cardiomyocyte size after exercise training compared with control animals. *P < 0.05 vs. the control group.
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REFERENCES


