GDP, GDP/AlF_4^- or GTPγS (corresponding to the inactive, transition, or active state of Gα subunit, respectively. In control experiments, GST protein itself did not bind Gαq at all (Fig. 7C).

Essentially the same results were obtained in the experiments on the binding property of His_6-RGS domain or GST-C2S to *C. elegans* Gα subunits. His_6-RGS bound to EGL-30 and GOA-1 in the presence of GDP/AlF_4^-, while GST-C2S bound them in both the presence and the absence of AlF_4^- (Fig. 8).

GAP activity of RGS domain of C2-RGS

We measured GAP activity of RGS domain of C2-RGS and RGS5 toward Gαi3 subunit. RGS domain of C2-RGS accelerated the catalytic rate of GTP hydrolysis of Gαi3 subunit (Fig. 9), as strongly as RGS5, a typical mammalian RGS protein.

Discussion

Sequence determination

In this study, we determined the cDNA sequence of the full length of a novel *C. elegans* RGS protein, C2-RGS. The results revealed the unique feature that C2-RGS contains a C2 domain with the type I topology, in addition to an RGS domain. RGS domain was found to belong to the type B of the RGS protein family (for example, RGS1, 2, 3, 4, 5, 8, and 16) (Fig. 2A) (Zheng et al., 1999). On the other hand, C2 domain of C2-RGS protein has extremely low structural similarity to other known C2 domain-containing proteins and is thought to be a Ca^{2+}-independent type, based on the number of aspartic acids essential for Ca^{2+} binding (Fig. 2B). Recently, C2PA-RGS3, a long isoform of human RGS3 with a C2 domain and an RGS domain, was reported (Kehrl et al., 2002). The sequences of C2 domain and RGS domain of C2PA-RGS3 are 26% and 48% identical to those of C2-RGS, respectively. However, the function of C2PA-RGS3 is not characterized yet.

Characterization of C2-RGS protein

The results in this paper showed that RGS domain of C2-RGS binds to not only Gαq and Gαi/o subunits, but also EGL-30 and GOA-1 in their transition state (GDP/AlF_4^- bound state), and acts as a GAP, like RGS4 (Figs. 7–9). It was also shown that proteins containing the RGS domain (the full-length protein, RGS domain, and ΔC2) suppressed ET-1-induced Ca^{2+} responses (Figs. 4 and 5). Taken together, these results indicated that RGS domain of C2-RGS regulates G protein signaling negatively like known typical mammalian RGS proteins.

On the other hand, C2L, C2S and ΔRGS, all of which contain C2 domain of C2-RGS, suppressed ET-1-induced Ca^{2+} transients, although the inhibitory effects were moderate, compared to those of the full-length protein of C2-RGS and RGS domain (Figs. 4 and 5). The effects of C2 domains (C2L and C2S) were concentration-dependent (Fig. 5A and B). These results indicated that the full-length C2-RGS suppressed potently intracellular Ca^{2+} responses by not only RGS domain, but also C2 domain, suggesting that these two domains work together in negative regulation of Gαq-mediated signaling. The finding of the inhibitory effect of *C. elegans* C2 domain tempted us to examine whether another

mammalian C2 domain also has an inhibitory effect. C2 domain of PLC-β1, with the type II C2 topology, also exhibited the inhibitory effects in a concentration-dependent manner (Fig. 5D).

Next, to identify the target sites of C2 domains of C2-RGS and PLC-β1, we performed binding experiments. The results demonstrated that both C2 domains bound strongly to Gαq subunit (Fig. 7), and that C2 domain of C2-RGS bound strongly to EGL-30, Gαq homologue of *C. elegans* (Fig. 8), indicating specific interaction between these C2 domains and Gαq subunits. As to PLC-β1, it has been well known that the C-terminal domain (residues ~ 840 to the C-terminal end) is required for activation by Gαq and for the GAP activity on Gαq subunit (Paulssen et al., 1996; Ilkaeva et al., 2002). However, Wang et al. has revealed that C2 domains of PLC-β1 and PLC-β2 interact specifically with Gαq subunit not only at the activated state (in the presence of GTPγS) but also at the inactive state (in the presence of GDP) (Wang et al., 1999). Thus, their report and our results in the present study may add a new member, C2 domain, as a binding module for Gαq, in addition to the C-terminal portion of PLC-β1.

Inhibition of Ca^{2+} responses by C2 domains of C2-RGS and PLC-β1, and their binding to Gαq subunit, strongly suggest some roles of these C2 domains in Gαq signaling, but the actual function in each full-length protein is not fully understood yet. Although the Ca^{2+} responses were reduced by overexpression of the C2 domain fragment, it remains unsolved whether the inhibition is due to a direct action of C2 domain itself on Gαq subunit (such as GAP action), or to C2 domain-induced exclusion of PLC-β1 from the binding interface on Gαq. At least, the present results suggest that C2 domain of C2-RGS protein serves as a functional domain with an inhibitory effect, which is in accordance with the effects of C2 domain of PLC-β1 by Wang et al. (Wang et al., 1999). It might also be possible that this C2 domain tunes the character of the whole C2-RGS protein more specific to Gαq, than that of the RGS domain, which shows the specificity to Gαq and Gαi/o.

Furthermore, it is noteworthy that these C2 domains bound to Gαq subunits almost equally under all the experimental condition used; the presence of GDP, GDP/AlF_4^- or GTPγS (Figs. 7 and 8). If C2 domain of PLC-β1 binds with Gαq subunits, irrespective of the activation states, the persistent association between the C2 domain and Gαq subunits may help the entire PLC-β1 molecule to keep its proper location close to Gαq subunit at any stage during GTPase cycle. Thus, the C2 domain may act as a scaffold module (Pawson and Scott, 1997) to organize Gαq and PLC-β1 in a stable signaling complex both in the absence and presence of stimulus. It is also possible that the C2 domain of C2-RGS has such a role as a scaffold module, as well as its own inhibitory activity in Gαq signaling. The further study is in progress to clarify the detail mechanism and the functional roles of these C2 domains in Gαq-mediated intracellular signal transduction.

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References

Brose, N., Petrenko, A.G., Sudhof, T.C., Jahn, R., 1992. Synaptotagmin: a calcium sensor on the synaptic vesicle surface. Science 256 (5059), 1021–1025.

Carman, C.V., Parent, J.L., Day, P.W., Pronin, A.N., Sternweis, P.M., Wedegaertner, P.B., Gilman, A.G., Benovic, J.L., Kozasa, T., 1999. Selective regulation of $G\alpha_{q/11}$ by an RGS domain in the G protein-coupled receptor kinase, GRK2. The Journal of Biological Chemistry 274 (48), 34483–34492.

De Vries, L., Mousli, M., Wurmser, A., Farquhar, M.G., 1995. GAIP, a protein that specifically interacts with the trimeric G protein $G\alpha_{i3}$, is a member of a protein family with a highly conserved core domain. Proceedings of National Academy of Sciences U.S.A. 92 (25), 11916–11920.

De Vries, L., Zheng, B., Fischer, T., Elenko, E., Farquhar, M.G., 2000. The regulator of G protein signaling family. Annual Review of Pharmacological Toxicology 40, 235–271.

Dohlman, H.G., Song, J., Ma, D., Courchesne, W.E., Thorner, J., 1996. Sst2, a negative regulator of pheromone signaling in the yeast *Saccharomyces cerevisiae*: expression, localization, and genetic interaction and physical association with Gpa1 (the G-protein α subunit). Molecular Cell Biology 16 (9), 5194–5209.

Fukuda, M., Mikoshiba, K., 2000. Doc2γ, a third isoform of double C2 protein, lacking calcium-dependent phospholipid binding activity. Biochemical Biophysical Research Communications 276 (2), 626–663.

Fukuda, M., Saegusa, C., Kanno, E., Mikoshiba, K., 2001. The C2A domain of double C2 protein γ contains a functional nuclear localization signal. The Journal of Biological Chemistry 276 (27), 24441–24444.

Hart, M.J., Jiang, X., Kozasa, T., Roscoe, W., Singer, W.D., Gilman, A.G., Sternweis, P.C., Bollag, G., 1998. Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by $G\alpha_{13}$. Science 280 (5372), 2112–2114.

Hepler, J.R., Berman, D.M., Gilman, A.G., Kozasa, T., 1997. RGS4 and GAIP are GTPase-activating proteins for Gqα and block activation of phospholipase Cβ by γ-thio-GTP-Gqα. Proceedings of National Academy of Sciences U.S.A. 94 (2), 428–432.

Ilkaeva, O., Kinch, L.N., Paulssen, H., Ross, E.M., 2002. Mutations in the carboxyl-terminal domain of phospholipase C-β1 delineate the dimer interface and a potential $G\alpha_q$ interaction site. The Journal of Biological Chemistry 277 (6), 4294–4300.

Kehrl, J.H., Srikumar, D., Harrison, K., Wilson, G.L., Shi, C.S., 2002. Additional 5′ exons in the RGS3 locus generate multiple mRNA transcripts, one of which accounts for the origin of human PDZ-RGS3. Genomics 79 (6), 860–868.

Koelle, M.R., Horvitz, H.R., 1996. EGL-10 regulates G protein signaling in the C. elegans nervous system and shares a conserved domain with many mammalian proteins. Cell 84 (1), 115–125.

Kovoor, A., Chen, C.-K., He, W., Wensel, T.G., Simon, M.I., Lester, H.A., 2000. Co-expression of Gβ5 enhances the function of two Gγ subunit-like domain-containing regulators of G protein signaling proteins. The Journal of Biological Chemistry 275 (5), 3397–3402.

Kozasa, T., Jiang, X., Hart, M.J., Sternweis, P.M., Singer, W.D., Gilman, A.G., Bollag, G., Sternweis, P.C., 1998. p115 RhoGEF, a GTPase activating protein for $G\alpha_{12}$ and $G\alpha_{13}$. Science 280 (5372), 2109–2111.

Li, C., Ullrich, B., Zhang, J.Z., Anderson, R.G., Brose, N., Sudhof, T.C., 1995. Ca^{2+}-dependent and -independent activities of neural and non-neural synaptotagmins. Nature 375 (6532), 594–599.

Nalefski, E.A., Falke, J.J., 1996. The C2 domain calcium-binding motif: structural and functional diversity. Protein Science 5 (12), 2375–2390.

Nishizuka, Y., 1988. The molecular heterogeneiety of protein kinase C and its implications for cellular regulation. Nature 334 (6184), 661–665.

Paulssen, R.H., Woodson, J., Liu, Z., Ross, E.M., 1996. Carboxyl-terminal fragments of phospholipase C-β1 with intrinsic Gq GTPase-activating protein (GAP) activity. The Journal of Biological Chemistry 271 (43), 26622–26629.

Pawson, T., Scott, J.D., 1997. Signaling though scaffold, anchoring, and adaptor proteins. Science 278 (28), 2075–2080.

Ponting, C.P., Parker, P.J., 1996. Extending the C2 domain family: C2s in PKCs δ, ε, η, ϑ, phospholipases, GAPs, and perforin. Protein Science 5 (1), 162–166.

Rizo, J., Sudhof, T.C., 1998. C2-domains, structure and function of a universal Ca^{2+}-binding domain. The Journal of Biological Chemistry 273 (26), 15879–15882.

Ross, E.M., Wilkie, T.M., 2000. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. Annual Review of Biochemistry 69, 795–827.

Sutton, R.B., Davletov, B.A., Berghuis, A.M., Sudhof, T.C., Sprang, S.R., 1995. Structure of the first C2 domain of synaptotagmin I: a novel Ca^{2+}/phospholipid-binding fold. Cell 80 (6), 929–938.

The C. elegans Sequencing Consortium, 1998. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. Science 282 (5396), 2012–2018.

Usui, H., Nishiyama, M., Moroi, K., Shibasaki, T., Zhou, J., Ishida, J., Fukamizu, A., Haga, T., Sekiya, S., Kimura, S., 2000.

RGS domain in the amino-terminus of G protein-coupled receptor kinase 2 inhibits Gq-mediated signaling. International Journal of Molecular Medicine 5 (4), 335–340.

Wang, T., Pentyala, S., Elliott, J.T., Dowal, L., Gupta, E., Rebecchi, M.J., Scarlata, S., 1999. Selective interaction of the C2 domains of phospholipase C-β1 and -β2 with activated $G\alpha_q$ subunits: An alternative function for C2-signaling modules. Proceedings of National Academy of Sciences U.S.A. 96 (14), 7843–7846.

Zheng, B., De Vries, L., Farquhar, M.G., 1999. Divergence of RGS proteins: evidence for the existence of six mammalian RGS subfamilies. Trends in Biochemical Sciences 24 (11), 411–414.

Zhou, J., Moroi, K., Nishiyama, M., Usui, H., Seki, N., Ishida, J., Fukamizu, A., Kimura, S., 2001. Characterization of RGS5 in regulation of G protein-coupled receptor signaling. Life Sciences 68 (13), 1457–1469.

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IMAGES IN CARDIOLOGY

Fibromuscular dysplasia in renovascular hypertension demonstrated by multislice CT: comparison with conventional angiogram and intravascular ultrasound

A 36 year old woman with a history of a gradual increase in blood pressure presented at our hospital. A renogram was performed and a decreased blood flow in the first and second phases was observed in the right kidney, with a significantly higher activity of serum renin in the right renal vein compared with the left. Furthermore, a bruit could be heard at the right lateroabdominal site, so right sided renovascular hypertension was suspected.

To evaluate the renal arteries, enhanced multislice computed tomography (CT) (Light Speed Ultra, General Electric, Milwaukee, Wisconsin, USA) was performed with a 1.25 mm slice thickness, helical pitch 7. Following intravenous injection of 100 ml of iodinated contrast material (350 mgI/ml), CT scanning was performed and volume data were transferred to a workstation (M900, Zio, Tokyo, Japan). The curved planar reconstruction image indicated a moniliform irregularity in the mid portion of the right renal arterial lumen (arrowhead in the upper right hand panel), suggesting renovascular hypertension caused by fibromuscular dysplasia.

Selective renal arteriography was therefore performed revealing the moniliform lumen of the mid portion of the right renal artery in the same position as the CT image (arrowhead in panel A). Intravascular ultrasound revealed four segmental luminal stenotic sites, approximately 2 mm in diameter, with an echolucent area just outside the intima (arrowhead in panel B), suggesting a thickening of smooth muscle cells. These thickenings were spotty, located among an average 4.5 mm diameter's normal lumina (panel C), the typical configuration of fibromuscular dysplasia in renovascular hypertension. Percutaneous transluminal angioplasty was therefore performed with good results.

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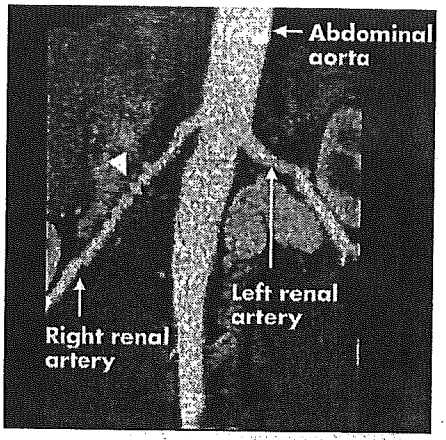

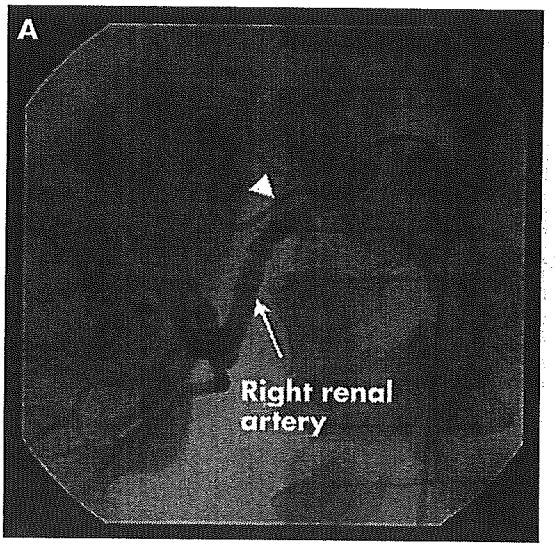

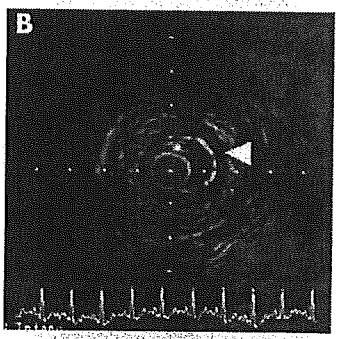

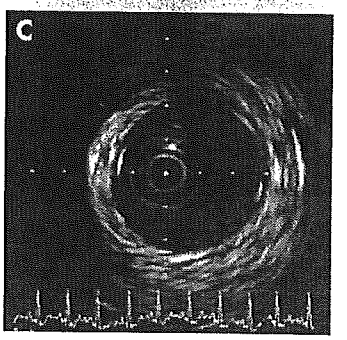

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Impact of Change in the Price of Percutaneous Coronary Intervention Devices on Medical Expenses

Nakabumi Kuroda, MD; Yoshio Kobayashi, MD*; Kartik Desai, MD*; Costantino Costantini, MD*; Mika Kobayashi, RN*; Issei Komuro, MD**

Percutaneous coronary intervention (PCI) devices are much more expensive in Japan than in the United States, but their prices were reduced in April 2002. This study evaluated the impact of that change in the price of PCI devices on medical expenses. In-hospital costs of 22 consecutive patients who underwent elective single-vessel PCI without a debulking procedure before April 2002 were collected and the in-hospital cost of each patient was recalculated by applying the current prices of the PCI devices and those in the USA. For patients treated with PCI before April 2002, the in-hospital cost was ¥1,456,375±358,781, but when the current price is used, the in-hospital cost is estimated to be ¥1,355,812±313,237 (7% reduction). If the prices of the devices were reduced to those in USA, there would be a 53% reduction (¥689,417±99,139). Although the change in the price of PCI devices in April 2002 has reduced in-hospital costs, the devices are still much more expensive in Japan than in the USA. Further reduction of the price is required to make PCI more cost-effective. (*Circ J* 2003; **67:** 576–578)

Key Words: Angioplasty; Cost analysis; Stent

Because of the prolonged recession, structural reform has been implemented in Japan and the Government sponsored, Japanese health insurance system has not been exempted. Heated debate on reform of the medical insurance system was held in the last Diet session. Because of increasing medical expenses as a result of the growing elderly population, the medical insurance system is mired in red ink, with almost all health insurance associations currently in the red. In this situation, physicians must be more cost-conscious, but little attention has been paid to cost analysis in Japan!

It has been known for a long time that medical devices are much more expensive in Japan than in the United States (USA)[1–7] so in April 2002 the prices of medical devices, such as pacemaker and percutaneous coronary intervention (PCI) devices, were reduced, in an attempt to save medical expenses. This study evaluated the impact of that change in the price of PCI devices on medical expenses.

Methods

The in-hospital costs of 22 consecutive patients who underwent elective single-vessel PCI without debulking procedure at Sawara Hospital in Chiba, Japan before April 2002 were collected. Coronary stenting was performed in 18 of the 22 patients and procedural success was achieved in all patients except 1 with chronic total occlusion. There were no major in-hospital complications. Non-Q-wave myocardial infarction occurred in 1 patient. None had a vascular complication that required a longer hospital stay. When patients had other diseases such as diabetes mellitus, the medical cost of those diseases was excluded. Changes in the technique of PCI and modification of the devices affect catheterization laboratory resource utilization, and subsequently in-hospital cost. To evaluate the pure reduction of in-hospital cost by a change in the prices of the PCI devices catheterization laboratory resource utilization, such as the number of balloon catheters and stents used, must be the same. Thus we recalculated the in-hospital cost of each patient by applying the current prices of PCI devices! In addition, to evaluate the difference in the in-hospital cost between Japan and USA because of the different prices of the PCI devices, it was recalculated using the USA prices ($1=¥120)[2–4]

Results

In patients treated by PCI before April 2002, the in-hospital cost was ¥1,456,375±358,781. The costs of PCI and PCI devises were ¥1,314,159±357,301 and ¥1,069,819±357,010, respectively, which were 90% and 73% of the total in-hospital cost. Table 1 presents the number of devices used, previous and current prices of PCI devices in Japan and those in the USA. When the current prices of PCI devices are used in the recalculation of the in-hospital cost of those patients, there is a 7% reduction (ie, in-hospital cost=¥1,355,812±313,237). The cost of the PCI device is ¥935,256±311,494 (13% reduction). If the prices of the devices were reduced to those in USA, in-hospital and PCI device costs would be ¥689,417±99,139 (53% reduction) and ¥268,861±96,534 (75% reduction), respectively.

Discussion

Because of snowballing medical expenses in an aging society, the nation's health insurance system is on the verge

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Table 1 Catheterization Laboratory Resource Utilization, Unit Cost and Procedural Fee for-PCI

	No. of devices used	*Previous cost*	*Current cost*	*United States ($1=¥120)*	*United Kingdom (£1=¥187)*	*the Netherlands (1 euro=¥126)*	*Argentine ($1=¥120)*
Stent	1.0±0.6	¥338,000	¥324,000	¥126,000 ($1,050)*	¥108,834 (£582)	¥102,942 (817 euro)	¥360,000 ($3,000)
Balloon catheter	1.7±0.8	¥263,000**	¥197,000	¥34,800 ($290)	¥36,652 (£196)	¥46,368 (368 euro)	NA
Intravascular ultrasound	0.8±0.4	¥219,000	¥214,000	¥55,200 ($460)	NA	¥68,670 (545 euro)	NA
Guidewire (0.014")	1.7±0.9	¥32,700†	¥30,800	¥12,000 ($100)	¥11,220 (£60)	¥11,970 (95 euro)	NA
Guiding catheter	1.3±0.6	¥39,900††	¥38,000	¥6,240 ($52)	¥6,732 (£36)	¥10,332 (82 euro)	NA
Procedural fee	–	¥205,000‡/¥199,000#	¥239,000	NA	NA	NA	NA

*PCI, percutaneous coronary intervention. *Average price of the Penta, BX Belocity, NIR, and S7 stent. **general I, †standard II, ††standard I, ‡conventional balloon angioplasty, and #stenting. NA, not available or applicable (the procedural fee in the US was not used to recalculate the in-hospital cost).*

of financial crisis. Medical expenses in Japan increased from 20 trillion yen in the fiscal year 1989 to 31 trillion yen in the fiscal year 1999 (the statistical reports of the Health, Labor and Welfare Ministry in Japan). More than 1,700 corporate insurance societies are making losses, the combined total of which is likely to amount to 500 billion yen this fiscal year. Heated discussion was held in the last Diet session, and reforms of the medical insurance system, such as increase in a salaried worker's payment for a medical expense and a decrease in medical insurance reimbursements, were decided. However, it might be difficult to improve the economic situation of the insurance system and thus fundamental reform of the medical insurance system may be inevitable. In that situation, physicians are required to be more cost-conscious and to treat patients efficiently, although there must not be any impairment of quality of care. Although considerable attention has been paid to cost analysis in the USA[2–7] it has not been fully discussed in Japan!

Increasing demand for PCI is placing a large financial burden on limited healthcare resources. The Japanese Coronary Intervention Study Group conducted a nationwide survey on coronary revascularization procedures during 1997 and reported that a total of 109,788 PCI and 17,667 coronary artery bypass procedures were performed in Japan[8] The ratio of PCI to coronary artery bypass surgery was 6.2, which is much higher than in the USA[8] Thus reduction in the cost of PCI in Japan, especially of the PCI device, which constitutes 73% of the total in-hospital cost, is important. Even though some of patients may have multivessel coronary intervention, it can be roughly estimated that the change in the prices of PCI devices in April 2002 saves ¥11 billion/year [¥(1,456,375–1,355,812) ×109,788].

PCI devices are much more expensive in Japan than in USA or Europe (Table 1)[1–4,9,10] and although the change in the prices in April 2002 reduces the gap slightly, the devices are still much expensive in Japan, even though most are imported. If the prices were reduced to those in the USA, it would save medical expenses of ¥73 billion/year [¥(1,355,812–689,417) ×109,788]. The numbers of balloon catheters and stents for reimbursement have been less since April 2002, but there are cases with complex lesions that require more devices. If the prices of the devices were reduced to those in the USA, we might be allowed to use the devices as required. Deregulation of the competition in the interventional products is necessary to reduce stent and angioplasty equipment prices.

It has been reported that the difference in overall (in-hospital + follow-up) cost between conventional balloon angioplasty and stenting would be ¥74,016 in the USA (¥1,098,807 vs ¥1,172,823), whereas it is ¥375,655 in Japan (¥1,188,583 vs ¥1,564238)! Few modern medical advances are truly cost-saving, and many wide-spread practices, including bypass surgery for left main disease![11] β-blocker therapy after acute myocardial infarction[12] and thrombolytic therapy for acute myocardial infarction[13] prolong life only at the expense of increased healthcare costs. Nevertheless, such treatments are viewed as cost-effective because their benefits to the patient are 'worth the additional cost'. From this viewpoint, stenting is considered to be 'worth the additional cost' (¥74,016) in the USA, but the cost difference in Japan (¥375,655) may be too high for such justification. Recently, a dramatic reduction in the rate of restenosis has been demonstrated with drug-eluting stents![14] The projected cost of a drug-eluting stent is likely to be approximately $3,200, which is almost 3-fold more expensive than a bare stent![15] Thus it might cost one million yen in Japan and all patients might not be able to receive the benefit of drug-eluting stents.

Recent randomized studies demonstrated long-term outcomes in patients with multivessel disease treated with multivessel stenting compared with CABG![16–18] The long-term mortality was controversial, although target revascularization rates were higher in the stent group than in the surgery group![16–18] Cost analysis of multivessel stenting and coronary bypass surgery in the USA and Europe showed a lower overall (in-hospital and follow-up) cost in the stent group![16,19] but a study in Argentine where stenting is much more expensive ($3,000) than in the USA and Europe, as in Japan, demonstrated no difference in the overall cost between the 2 techniqes![17]

There has been a comparison of the long-term outcome of medical therapy and PCI, particularly in patients with single-vessel disease, which showed that long-term mortality, myocardial infarction, and target lesion revascularization were similar between the 2 therapy groups, but that PCI was superior to medical treatment for the alleviation of angina[20] Overall cost was less in the medical therapy group than in the PCI group? Thus, particularly in the face of constraints on healthcare spending, it may be recommended that physicians reserve PCI for patients with single-vessel disease whose symptoms of angina are not well controlled by medical treatment?[20]

Because of the Government sponsored, Japanese health

insurance system, all Japanese citizens are able to receive the best treatment equally. However, the nation's health insurance system is on the verge of a financial crisis and so physicians must be more cost-conscious, and furthermore everyone in Japan must consider reforms to the medical insurance system. Otherwise physicians may not be able to provide the best treatment for all patients equally.

Study Limitations

The in-hospital cost of patients treated before April 2002 was recalculated by applying the current device prices and those in the USA to estimate those after the change in the prices and in USA! The difference in catheterization laboratory resource utilization affects in-hospital cost. Furthermore, the method of calculating in-hospital cost and the reimbursement system are completely different between Japan and the USA![-7] That is why we used this method to evaluate the pure difference in the in-hospital cost because of the different device prices! Reimbursement prices of the devices were used to calculate the in-hospital cost in Japan. On the other hand, market prices are used in the USA, because there are no reimbursement prices. Thus the difference in the prices of the PCI devices may be less; however, even considering this possible disagreement, PCI devices are much more expensive in Japan!

Conclusions

The change in the prices of PCI devices in April 2002 reduces the in-hospital cost by 7%. However, the devices are still much more expensive in Japan than in USA and further reduction of the prices is required to make PCI more cost-effective.

References

1. Kobayashi Y, De Gregorio J, Yamamoto Y, Komiyama N, Miyazaki A, Masuda Y. Cost analysis between stent and conventional balloon angioplasty. *Jpn Circ J* 2000; **64:** 161–164.
2. Cohen DJ, Taira DA, Berezin R, Cox DA, Morice MC, Stone GW, et al. Cost-effectiveness of coronary stenting in acute myocardial infarction: Results from the stent primary angioplasty in myocardial infarction (stent-PAMI) trial. *Circulation* 2001; **104:** 3039–3045.
3. No authors listed. Stent prices expected to decline. *Hosp Mater Manage* 2001; **26:** 1, 12–13.
4. Choi JW, Goodreau LM, Davidson CJ. Resource utilization and clinical outcomes of coronary stenting: A comparison of intravascular ultrasound and angiographical guided stent implantation. *Am Heart J* 2001; **142:** 112–118.
5. Cohen DJ, Krumholz HM, Sukin CA, Ho KKL, Siegrist RB, Cleman M, et al. In-hospital and one-year economic outcomes after coronary stenting or balloon angioplasty: Result from a randomized clinical trial. *Circulation* 1995; **92:** 2480–2487.
6. Cohen DJ, Breall JA, Ho KKL, Weintraub RM, Kuntz RE, Weinstein MC, et al. Economics of elective coronary revascularization: Comparison of costs and charges for conventional angioplasty, directional atherectomy, stenting and bypass surgery. *J Am Coll Cardiol* 1993; **22:** 1052–1059.
7. Cohen DJ, Breall JA, Ho KKL, Kuntz RE, Goldman L, Baim DS, et al. Evaluating the potential cost-effectiveness of stenting as a treatment for symptomatic single-vessel coronary disease: Use of a decision-analytic model. *Circulation* 1994; **89:** 1859–1874.
8. Shihara M, Tsutsui H, Tsuchihashi M, Shigematsu H, Yamamoto S, Koike G, et al. Coronary revascularization in Japan. *Jpn Circ J* 2001; **65:** 1005–1010.
9. Sculpher MJ, Smith DH, Clayton T, Henderson RA, Buxton MJ, Pocock SJ, et al. Coronary angioplasty versus medical therapy for angina: Health service costs based on the second Randomized Intervention Treatment of Angina (RITA-2) trial. *Eur Heart J* 2002; **23:** 1291–1300.
10. Serruys PW, de Bruyne B, Carlier S, Sousa JE, Piek J, Muramatsu T, et al. Randomized comparison of primary stenting and provisional balloon angioplasty guided by flow velocity measurement: Doppler Endpoints Balloon Angioplasty Trial Europe (DEBATE) II Study Group. *Circulation* 2000; **102:** 2930–2937.
11. Weinstein MC, Stason WB. Cost-effectiveness of coronary artery bypass surgery. *Circulation* 1982; **66**(Suppl III): III-56–III-66.
12. Goldman L, Sia STB, Cook EF, Rutherford JD, Weinstein MC. Costs and effectiveness of routine therapy with long-term beta-adrenergic antagonists after acute myocardial infarction. *N Engl J Med* 1988; **319:** 152–157.
13. Krumholz HM, Pasternak RC, Weinstein MC, Friesinger GC, Ridker PM, Tosteson ANA, et al. Cost effectiveness of thrombolytic therapy with streptokinase in elderly patients with suspected acute myocardial infarction. *N Engl J Med* 1992; **327:** 7–13.
14. Morice MC, Serruys PW, Sousa JE, Fajadet J, Ban Hayashi E, Perin M, et al. Randomized study with the sirolimus-coated Bx velocity balloon-expandable stent in the treatment of patients with de novo native coronary artery lesions: A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. *N Engl J Med* 2002; **346:** 1773–1780.
15. Sharma S, Bhambi B, Nyitray W. Sirolimus-eluting coronary stents. *N Engl J Med* 2002; **347:** 1285.
16. Serruys PW, Unger F, Sousa JE, Jatene A, Bonnier HJ, Schonberger JP, et al. Comparison of coronary-artery bypass surgery and stenting for the treatment of multivessel disease. *N Engl J Med* 2001; **344:** 1117–1124.
17. Rodriguez A, Bernardi V, Navia J, Baldi J, Grinfeld L, Martinez J, et al. Argentine Randomized Study: Coronary angioplasty with stenting versus coronary bypass surgery in patients with multiple-vessel disease (ERACI II): 30-day and one-year follow-up results: ERACI II Investigators. *J Am Coll Cardiol* 2001; **37:** 51–58.
18. SoS Investigators. Coronary artery bypass surgery versus percutaneous coronary intervention with stent implantation in patients with multivessel coronary artery disease (the Stent or Surgery trial): A randomised controlled trial. *Lancet* 2002; **360:** 965–970.
19. Reynolds MR, Neil N, Ho KK, Berezin R, Cosgrove RS, Lager RA, et al. Clinical and economic outcomes of multivessel coronary stenting compared with bypass surgery: A single-center US experience. *Am Heart J* 2003; **145:** 334–342.
20. Bucher HC, Hengstler P, Schindler C, Guyatt GH. Percutaneous transluminal coronary angioplasty versus medical treatment for non-acute coronary heart disease: Meta-analysis of randomised controlled trials. *BMJ* 2000; **321:** 73–77.

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THE ROLE OF PPARγ -DEPENDENT PATHWAY IN THE DEVELOPMENT OF CARDIAC HYPERTROPHY

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Summary

Peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the nuclear receptor superfamily. PPARs have three isoforms, α, β (or δ) and γ. It has been conceived that PPARγ is expressed predominantly in adipose tissue and promotes adipocyte differentiation and glucose homeostasis. Recently, synthetic antidiabetic thiazolidinediones and natural prostaglandin D_2 (PGD_2) metabolite, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), have been identified as ligands for PPARγ. Following demonstration that PPARγ is present in a variety of cell types, further study of PPARγ has been conducted. Although activation of PPARγ appears to have beneficial effects on atherosclerosis and heart failure, it is still largely uncertain whether PPARγ ligands prevent the development of cardiovascular diseases. Recent evidence suggests that some benefit from the antidiabetic agents known as thiazolidinediones may occur through PPARγ-independent mechanisms. In this review, we report on the latest developments concerning the study of PPARs and summarize the roles of the PPARγ-dependent pathway in cardiovascular diseases.

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Introduction

PPARs are transcription factors belonging to the nuclear receptor superfamily that heterodimerize with the retinoid X receptor (RXR) and bind to specific response elements termed PPAR responsive elements in target gene promoters. The PPAR responsive elements are direct repeats of hexam-

eric consensus sequence AGGTCA, separated by one nucleotide. These nuclear receptors are ligand-dependent transcription factors and activation of target gene transcription depends on the binding of the ligand to the receptor. PPARs have three isoforms, α, β (or δ) and γ. PPARα regulates genes involved in the β-oxidative degradation of fatty acids, whereas PPARγ promotes adipocyte differentiation and glucose homeostasis. The main function of PPARδ has yet to be ascertained, but it seems to be implicated in epidermal maturation and skin wound repair. PPARα is present in the liver, kidney and muscle, whereas PPARγ is expressed predominantly in adipose tissue. PPARδ is expressed almost ubiquitously. Recently, it has been demonstrated that PPARα and PPARγ are also expressed in a variety of cell types. Following reports that the activation of PPARγ suppresses the production of inflammatory cytokines in activated macrophages (1, 2), medical interest in PPARs have grown and a huge effort has been concentrated on related research. The thiazolidinediones, novel insulin-sensitizing agents and high-affinity ligands for PPARγ, have been reported to prevent growth factor-induced proliferation and migration of vascular smooth muscle cells (3, 4). The thiazolidinediones have also been reported to inhibit cytokine-mediated proliferation of endothelial cells (5) and to suppress endothelin-1 secretion from vascular endothelial cells (6). These observations suggest that PPARγ ligands may influence the growth of vascular cells (7). Furthermore, we recently reported that PPARγ plays an important role in the inhibition of cardiac hypertrophy. Here, we present current trends in PPARγ research and discuss the roles of PPARγ in cardiovascular diseases.

PPARγ

The peroxisome is a subcellular organelle which plays a crucial role in cellular metabolism. Peroxisome enzymes are implicated in a broad range of catabolic and anabolic enzymatic pathways such as fatty acid oxidation, biosynthesis of both glycerolipids and cholesterol, and metabolism of reactive oxygen species. The induction of peroxisome proliferation in rodents is associated with cellular responses to a range of chemical compounds. In 1990, Issemann and Green (8) reported that peroxisome proliferators activate a member of the steroid hormone receptor superfamily in mouse liver. This nuclear receptor was named PPAR, and shortly thereafter three major types of PPAR (α, β/δ and γ) were recognized. While PPARγ1 is abundantly expressed in various tissues (*e.g.*, liver, kidney, spleen, intestine, muscle, brain and lung), PPARγ2 is predominantly expressed in adipose tissue (9–13). Several lines of evidence have demonstrated the functional significance of PPARγ in atherosclerotic lesions (14, 15). Recently, PPARγ has also been shown to be expressed in macrophages, vascular smooth muscle cells, endothelial cells and cardiac myocytes (2, 3, 16–18).

PPARα regulates genes involved in the β-oxidative degradation of fatty acids (19, 20), whereas PPARγ promotes adipocyte differentiation and glucose homeostasis (21–25). In addition, it was recently reported that PPARα and PPARγ are important immunoinflammatory mediators (1, 2, 26). The function of PPARβ/δ is still relatively unknown. Two different PPARγ splice variants, PPARγ1 and PPARγ2, have been cloned. These two forms differ only in their N-terminal 30 amino acids. Both PPARγ isoforms are derived from the same gene by alternative promoter usage and differential splicing. Like other members of the nuclear receptor superfamily, PPARs have several modular domains. The N-terminal A/B domain, which is the least conserved region, contains a ligand-independent activating function-1. The C domain, which is the best conserved domain, is DNA-binding. The D domain allows for bending or conformational alteration of PPAR. The E/F domain is ligand-binding and ligand-dependent transcription requires an activating function-2, located at the C-terminus of the ligand-binding domain. Ligand binding by PPARγ is regulated by intracellular communication between its N-terminal A/B domain and its C-terminal ligand-binding domain. Activity of PPARγ is depressed by phosphorylation of a serine residue (Ser^{112}) in the A/B domain, mediated by a member of the mitogen-activated protein kinase family, extracellular signal-regulated protein kinase (27, 28). In addition, another member of the mitogen-activated protein kinase family, c-Jun N-terminal kinase (JNK), also phosphorylates PPARγ at Ser^{82} and reduces the transcriptional activity of PPARγ (29). These modifications may control interactions between PPARγ and coactivators or corepressors that have been described to interact with many members of the nuclear receptor family. Recently, several lines of evidence have implicated the functional significance of interaction between nuclear receptors and coactivators in transcriptional activation. cAMP response element binding protein (CREB)-binding protein (CBP)/p300 is a transcriptional coactivator of PPARα, PPARγ and nu-

clear factor-κB (NF-κB) (30–32). Steroid receptor coactivator-1 (SRC-1) also functions as a coactivator for PPAR and NF-κB (33–35). It has been reported that both CBP and SRC-1 interact with the PPARγ: RXR heterodimer and that the interaction is mediated by initial binding of PPAR coactivator-1 (PGC-1) (36). PGC-1 interacts with several other nuclear hormone receptors including the glucocorticoid receptor, the mineralcorticoid receptor, the estrogen receptor and PPARα. These observations raise the possibility that nuclear competition for limiting amounts of CBP/p300, SRC-1 or PGC-1 may occur between PPARs and other transcription factors.

PPARγ ligands

The PGD_2 metabolite, 15d-PGJ_2, is the first endogenous ligand for PPARγ to be discovered (37, 38). Although 15d-PGJ_2 is the most potent natural ligand of PPARγ, the extent to which its effects are mediated through PPARγ *in vivo* remains to be determined. Two components of oxidized low-density lipoprotein (LDL), 9-hydroxy and 13-hydroxy octadecadienoic acids (HODE), are also potent endogenous activators of PPARγ (39, 40). Activation of 12/15-lipoxygenase induced by interleukin-4 (IL-4) also produces the endogenous ligands for PPARγ ligands (41). The antidiabetic thiazolidinediones (*e.g.*, troglitazone, pioglitazone, ciglitazone and rosiglitazone) are pharmacological ligands of PPARγ (22, 42, 43). They bind PPARγ with various affinities and it is conceivable that their insulin-sensitizing and hypoglycemic effects are exerted by activating PPARγ. The mechanism by which thiazolidinediones improve insulin resistance has been not fully understood. Okuno *et al.* (44) demonstrated that troglitazone increases the number of small adipocytes and decreases the number of large adipocytes in obese Zucker rats. This action of troglitazone appears to be an important mechanism by which increased expression levels of tumor necrosis factor-α (TNF-α) and plasma lipids are normalized, leading to alleviation of insulin resistance. The principal insulin-sensitive glucose transporter in muscle is GLUT4, which is recruited to the sarcolemma in response to elevated levels of insulin. It was reported that PPARγ ligands induce expression of GLUT4 during conversion of fibroblasts into adipocytes (45). Michael *et al.* (46) have demonstrated that PGC-1 increases expression of GLUT4 in part by binding to and coactivating myocyte enhancer factor-2C. In contrast with these results, heterozygous PPARγ-deficient mice show more insulin-sensitive phenotype compared with wild-type mice on a high-fat diet (47, 48). The function of PPARγ in improving insulin sensitivity is controversial. Several nonsteroidal anti-inflammatory drugs (NSAIDs), including ibuprofen, indomethacin and fenoprofen, also bind to PPARγ and have PPARγ activity at high drug concentrations (49). Their anti-inflammatory effects stem from their ability to inhibit cyclooxygenase-1 (COX-1) and COX-2. Because activation of both PPARα and PPARγ suppress induction of COX-2 (50, 51), PPARs may contribute to the anti-inflammatory responses produced by NSAIDs. PPARγ is master regulator of adipocyte differentiation and thiazolidinediones enhance adipocyte differentiation through activation of PPARγ (21, 24, 37, 38). As enhanced PPARγ expression induces the differentiation of many cell types, thiazolidinedione-mediated PPARγ activation suppresses the growth of colon cancer cells *in vitro*. However, the effects of thiazolidinediones on colon cancer are controversial and further investigations are needed to clarify the roles of PPARγ in colon carcinogenesis (52–55). RXR, which interacts with PPARs, is activated by 9-cis retinoic acid. When combined as a PPAR: RXR heterodimer, PPAR ligands and 9-cis retinoic acid can act synergistically on PPAR responses (56–58).

PPARγ in cardiovascular diseases

The formation of atherosclerosis is a complex process to which many different factors contribute. The injury of endothelium, the proliferation of smooth muscle cells, the migration of monocytes/macrophages, and the regulatory network of growth factors and cytokines are important in the development of atherosclerosis. In addition, chronic inflammation of the vascular wall is also involved in the process. As mentioned above, PPARγ is implicated in inflammation. PPARγ ligands have been shown to reduce production of inflammatory cytokines such as IL-1β, IL-6, inducible nitric oxide synthase (iNOS) and TNF-α by inhibiting the activity of transcription factors such as activator protein-1 (AP-1), signal transducers and activators of transcription (STATs) and NF-κB in monocytes/macrophages (1, 2). These results suggest that PPARγ activation may have beneficial effects in modulating inflammatory responses in atherosclerosis. It has been reported that PPARγ ligands ameliorate other inflammatory diseases such as arthritis and colitis in animal models (59, 60). On the other hand, the in-

flammatory activation of aortic smooth muscle cells, which is a hallmark of atherosclerosis, is inhibited by PPARα ligands but not by PPARγ ligands (26). Macrophages affect the vulnerability of plaques to undergo rupture in atherosclerotic lesions. The role of macrophages in plaque rupture is implicated in the secretion of matrix metalloproteinases (MMPs), enzymes that are important in the degradation of extracellular matrix. In macrophages and smooth muscle cells, PPARγ ligands have been shown to reduce expression of MMP-9, which is implicated in both migration of vascular smooth muscle cells and plaque destabilization (2, 3).

Vascular smooth muscle cell proliferation and migration are also critical events in atherosclerosis and restenosis. Thiazolidinediones inhibit these changes of vascular smooth muscle cells and neointimal thickening after vascular injury (4, 61–63). Furthermore, thiazolidinediones induce apoptosis of smooth muscle cells via p53 and Gadd45 (64). Angiotensin II plays an important role in vascular remodeling via angiotensin II type 1 receptor (AT_1R). As activation of PPARγ inhibits AT_1R gene expression at a transcriptional level in vascular smooth muscle cells, PPARγ ligands may prevent angiotensin II-induced vascular remodeling by reduced expression of AT_1R (65). Expression of adhesion molecule by endothelial cells, leading to adhesion of leukocytes to endothelial cells, is a critical early step in atherosclerosis. PPARγ ligands inhibit expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) in HUVECs (66, 67) and decrease production of chemokines such as IL-8, and monocyte chemotactic protein-1 (MCP-1) in human aortic endothelial cells (68). PPARγ ligands also inhibit MCP-1-induced monocyte migration (69). Endothelin-1 is involved in the regulation of vascular tone and endothelial functions. In addition, endothelin-1 is expressed in atherosclerotic lesions and induces proliferation of smooth muscle cells. PPARγ ligands suppress the transcription of the endothelin-1 promoter by interfering with AP-1 in bovine aortic endothelial cells (BAECs) (6).

PPARγ activation by 9-HODE and 13-HODE, the major oxidized lipid components of oxLDL, plays an important role in the development of lipid-accumulating macrophages through transcriptional induction of CD36, a scavenger receptor (39, 40, 70). It has been reported that CD36-deficient mice are protected from atherosclerosis (71). These data suggest that atherogenic oxLDL particles could induce their own uptake through activation of PPARγ and expression of CD36, leading to atherosclerosis. On the other hand, PPARγ ligands could suppress induction of the class A scavenger receptor by TPA (2). Although it is uncertain whether increased expression of CD36 enhances foam cell formation, there is the possibility that PPARγ ligands may promote foam cell formation and have proatherogenic effects. However, Li *et al.* (72) reported that PPARγ ligands (*i.e.*, rosiglitazone and GW7845, a nonthiazolidinedione tyrosine analog) significantly inhibit the development of atherosclerosis in spite of increased expression of CD36 in the arterial wall in LDL receptor-deficient male, but not female, mice. Chen *et al.* (73) demonstrated that thiazolidinediones significantly inhibit the development of atherosclerotic foam cell lesions in apolipoprotein E-knockout mice and suggested that the anti-inflammatory effects of thiazolidinediones may predominate over the induced expression of CD36 in macrophage foam cells. In addition, PPARγ has been shown to induce expression of ABCA1, a transporter that controls apoAI-mediated cholesterol efflux, and cholesterol removal from macrophages through a transcriptional cascade mediated by the nuclear receptor α LXR (74, 75). Very recently, Jiang *et al.* (76) reported that PPARγ ligands induce hepatocyte growth factor (HGF) mRNA and protein expression in fibroblasts. Because hepatocyte growth factor is among the pleiotropic polypeptides, which have mitogenic and antiapoptotic effects in endothelial cells (77), PPARγ ligands may play beneficial roles in vascular disease via hepatocyte growth factor expression.

The effects of PPARs on heart tissue have not been fully understood. PPARα has been demonstrated to play an important role in mitochondrial fatty acid β-oxidation (FAO) in the heart. Pressure overload-induced cardiac hypertrophy results in deactivation of PPARα and subsequent reduction of FAO enzymes (*e.g.*, carnitine palmitoyltransferase I, medium-chain acyl-CoA dehydrogenase, acyl-CoA oxidase) gene expression (78). These results suggest that cardiac hypertrophy induces abnormalities in cardiac lipid homeostasis and energy production via reduction of PPARα activity. Cardiac-specific PGC-1 transgenic mice display uncontrolled mitochondrial biogenesis, abnormalities of sarcomeric structure and dilated cardiomyopathy (79). PGC-1 appears to control the number and function of cardiac mitochondria. Recently, we demonstrated that PPARγ ligands inhibit cardiac expression of TNF-α at the transcriptional level in

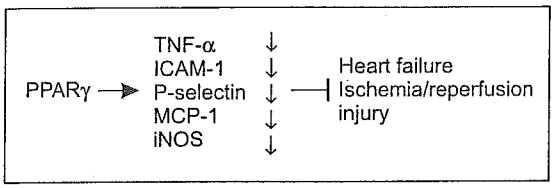

Fig. 1. PPARγ inhibits heart failure and ischemia/reperfusion injury. The effects of PPARγ are associated with decreases in TNF-α, ICAM-1, P-selectin, MCP-1 and iNOS.

part by antagonizing NF-κB activity (80). Because TNF-α expression is elevated in the failing heart and has a negative inotropic effect on cardiac myocytes, treatment with PPARγ ligands may prevent the development of congestive heart failure. Diabetic cardiomyopathy, which is characterized by systolic and diastolic dysfunctions, is a major complication of diabetes; however, the antidiabetic agents known as the thiazolidinediones appear to be beneficial to cardiac function impairment in patients with diabetes mellitus.

Following our study, the role of PPARγ in myocardial ischemia/reperfusion injury has also been elucidated (80–82). PPARγ ligands reduced myocardial infarction size and improved contractile dysfunction after ischemia/reperfusion injury in rats. The beneficial effects of PPARγ ligands were associated with reduction in the expression of ICAM-1, P-selectin, MCP-1 and iNOS (Fig. 1). Moreover, the activation of JNK, AP-1 and NF-κB was inhibited by PPARγ ligands in the animal model.

PPARγ in cardiac hypertrophy

Cardiac hypertrophy is recognized as an adaptive increase in heart size characterized by a growth of each cardiomyocyte rather than an increase in cell number (83). Cardiac hypertrophy is induced by a variety of diseases such as hypertension, valvular diseases, myocardial infarction and endocrine disorders. Although cardiac hypertrophy may initially be a beneficial response that normalizes wall stress and maintains normal cardiac function, prolonged hypertrophy becomes a leading cause of heart failure and sudden death (84). Therefore, it is very important to understand the precise mechanisms and mediators of cardiac hypertrophy in order to prevent its development. Although numerous signal transduction pathways that promote cardiac hypertrophy have been characterized, only a few studies have focused on inhibitory regulators of hypertrophic response. The modification of antihypertrophic regulators is expected to be a novel therapeutic strategy for cardiac hypertrophy.

Recently, we examined the effects of the thiazolidinediones such as troglitazone, pioglitazone and rosiglitazone on angiotensin II-induced hypertrophy in neonatal rat cardiac myocytes and on pressure overload-induced cardiac hypertrophy in mice (85, 86). To further elucidate the role of PPARγ in the development of cardiac hypertrophy, we examined pressure overload-induced cardiac hypertrophy using heterozygous PPARγ-deficient ($PPAR\gamma^{+/-}$) mice. In the study, we demonstrated that the PPARγ ligands troglitazone, pioglitazone and rosiglitazone inhibit angiotensin II-induced hypertrophy of neonatal rat cardiac myocytes. The pressure overload-induced cardiac hypertrophy was more prominent in $PPAR\gamma^{+/-}$ mice than in WT mice. Treatment with the PPARγ ligand pioglitazone inhibited the pressure overload-induced cardiac hypertrophy strongly in WT mice and moderately in $PPAR\gamma^{+/-}$ mice. These results suggest that the PPARγ-dependent pathway inhibits the development of cardiac hypertrophy. Yamamoto *et al.* (87) have also reported that PPARγ ligands such as troglitazone and 15d-PGJ_2 inhibit angiotensin II-, phenylephrine- or mechanical strain-induced cardiac hypertrophy.

However, the molecular mechanism by which PPARγ suppresses cardiac hypertrophy remains to be determined. A variety of transcription factors may be implicated in the development of cardiac hypertrophy. c-Fos and c-jun make the heterodimer complex called AP-1, which transactivates many genes that have a TPA responsible element in their promoter such as atrial natriuretic peptide and endothelin-1 genes (88, 89). STATs are known to play important roles in cytokine signaling pathways (90). Recently, it was reported that STAT3 is activated in cardiac myocytes by the IL-6 family of cytokines and plays a crucial role in generating cardiac hypertrophy through gp130 (91). The cardiac-restricted zinc finger transcription factor GATA4 has also been shown to be required for transcriptional activation of the genes for AT_1R, β-myosin heavy chain and endothelin-1 during cardiac hypertrophy (92–94). The calcium-dependent phosphatase calcineurin dephosphorylates the transcription factor NF-AT3, and NF-AT3 translocates to the nucleus and interacts with GATA4, resulting in the development of cardiac hypertrophy (95). It was recently reported that PPARγ ligands could downregulate inflammatory responses in monocytes by interfering with AP-1, STAT and NF-κB signaling pathways

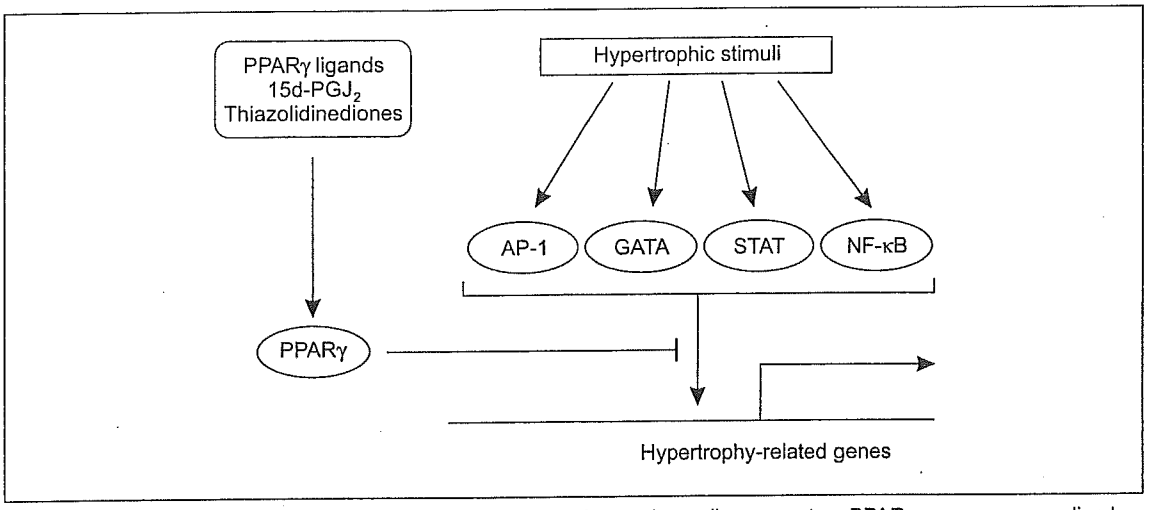

Fig. 2. PPARγ negatively regulates hypertrophic signaling pathways in cardiac myocytes. PPARγ suppresses cardiac hypertrophy by antagonizing the activities of transcription factors such as AP-1, GATA, STAT and NF-κB.

via competition for essential cofactors (2). Furthermore, our laboratory and others have demonstrated that PPARγ ligands inhibit NF-κB activity in cardiac myocytes (18, 87). NF-κB activation is involved in G-protein-coupled receptor agonist- and mechanical strain-induced cardiac hypertrophy (96, 97). Therefore, PPARγ may suppress the development of cardiac hypertrophy by antagonizing the activities of transcription factors such as AP-1, STAT3, GATA4 and NF-κB (Fig. 2).

Conclusions

Unfortunately, troglitazone was shown to have hepatotoxic effects in some diabetic patients, leading to the withdrawal of this agent from the market. It is hoped that new generations of nonthiazolidinedione PPARγ ligands, which exert antiatherogenic effects and have neither hepatotoxic nor proatherogenic effects, will be explored. Further studies using tissue-specific gene targeting mice are necessary to address the effects of PPARγ on atherosclerosis *in vivo*. Once the beneficial activities of the PPARs have been determined, their modulation may become a promising therapeutic strategy for cardiovascular diseases. Recently, many reports have suggested that insulin resistance and hyperinsulinemia are involved in cardiac hypertrophy (98, 99). Because cardiac hypertrophy can be seen even in normotensive diabetic patients and given the fact that diabetic cardiomyopathy is a major complication of diabetes, antidiabetic agents such as the thiazolidinediones would appear to be beneficial for the treatment of cardiac hypertrophy and dysfunction in patients with diabetes mellitus. Our study suggests the potential clinical efficacy of the thiazolidinediones for the prevention of cardiac hypertrophy, but further studies are necessary to elucidate whether or not the inhibition of cardiac hypertrophy by the PPARγ ligand improves prognosis.

References

1. Jiang, C., Ting, A. T., Seed, B. *PPAR-γ agonists inhibit production of monocyte inflammatory cytokines.* Nature 1998, 391: 82-6.
2. Ricote, M., Li, A.C., Willson, T.M., Kelly, C.J., Glass, C.K. *The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation.* Nature 1998, 391: 79-82.
3. Marx, N., Schonbeck, U., Lazar, M.A., Libby, P., Plutzky, J. *Peroxisome proliferator-activated receptor γ activators inhibit gene expression and migration in human vascular smooth muscle cells.* Circ Res 1998, 83: 1097-103.
4. Law, R.E., Meehan, W.P., Xi, X.P. et al. *Troglitazone inhibits vascular smooth muscle cell growth and intimal hyperplasia.* J Clin Invest 1996, 98: 1897-1905.
5. Gralinski, M.R., Rowse, P.E., Breider, M.A. *Effects of troglitazone and pioglitazone on cytokine-mediated endothelial cell proliferation in vitro.* J Cardiovasc Pharmacol 1998, 31: 909-13.
6. Delerive, P., Martin-Nizard, F., Chinetti, G. et al. *Peroxisome proliferator-activated receptor activators inhibit thrombin-induced endothelin-1*

production in human vascular endothelial cells by inhibiting the activator protein-1 signaling pathway. Circ Res 1999, 85: 394-402.

7. Rosen, E.D., Spiegelman, B.M. *Peroxisome proliferator-activated receptor γ ligands and atherosclerosis: Ending the heartache.* J Clin Invest 2000, 106: 629-31.
8. Issemann, I., Green, S. *Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators.* Nature 1990, 347: 645-50.
9. Kliewer, S.A., Forman, B.M., Blumberg, B. et al. *Differential expression and activation of a family of murine peroxisome proliferator-activated receptors.* Proc Natl Acad Sci USA 1994, 91: 7355-9.
10. Braissant, O., Foufelle, F., Scotto, C., Dauca, M., Wahli, W. *Differential expression of peroxisome proliferator-activated receptors (PPARs): Tissue distribution of PPAR-α, -β, and -γ in the adult rat.* Endocrinology 1996, 137: 354-66.
11. Auboeuf, D., Rieusset, J., Fajas, L. et al. *Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-α in humans.* Diabetes 1997, 46: 1319-27.
12. Vidal-Puig, A.J., Considine, R.V., Jimenez-Linan, M. et al. *Peroxisome proliferator-activated receptor gene expression in human tissues.* J Clin Invest 1997, 99: 2416-22.
13. Cullingford, T.E., Bhakoo, K., Peuchen, S., Dolphin, C.T., Patel, R., Clark, J.B. *Distribution of mRNAs encoding the peroxisome proliferator-activated receptor α, β, and γ and the retinoid X receptor α, β, and γ in rat central nervous system.* J Neurochem 1998, 70: 1366-75.
14. Marx, N., Sukhova, G., Murphy, C., Libby, P., Plutzky, J. *Macrophage in human atheroma contain PPARγ: Differentiation-dependent peroxisomal proliferator-activated receptor γ (PPARγ) expression and reduction of MMP-9 activity through PPAR activation in mononuclear phagocytes in vitro.* Am J Pathol 1998, 153: 17-23.
15. Ricote, M., Huang, J., Fajas, L. et al. *Expression of the peroxisome proliferator-activated receptor γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein.* Proc Natl Acad Sci USA 1998, 95: 7614-9.
16. Iijima, K., Yoshizumi, M., Ako, J. et al. *Expression of peroxisome proliferator-activated receptor γ (PPARγ) in rat aortic smooth muscle cells.* Biochem Biophys Res Commun 1998, 247: 353-6.
17. Benson, S., Wu, J., Padmanabhan, S., Kurtz, T.W., Pershadsingh, H.A. *Peroxisome proliferator-activated receptor (PPAR)-γ expression in human vascular smooth muscle cells: Inhibition of growth, migration, and c-fos expression by the peroxisome proliferator-activated receptor (PPAR)-γ activator troglitazone.* Am J Hypertens 2000, 13: 74-82.
18. Takano, H., Nagai, T., Asakawa, M. et al. *Peroxisome proliferator-activated receptor activators inhibit lipopolysaccharide-induced tumor necrosis factor-α expression in neonatal rat cardiac myocytes.* Circ Res 2000, 87: 596-602.
19. Keller, H., Dreyer, C., Medin, J., Mahfoudi, A., Ozato, K., Wahli, W. *Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers.* Proc Natl Acad Sci USA 1993, 90: 2160-4.
20. Gulick, T., Cresci, S., Caira, T., Moore, D.D., Kelly, D.P. *The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression.* Proc Natl Acad Sci USA 1994, 91: 11012-6.
21. Tontonoz, P., Hu, E., Spiegelman, B.M. *Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor.* Cell 1994, 79: 1147-56.
22. Lehmann, J.M., Moore, L.B., Smith-Oliver, T.A., Wilkinson, W.O., Willson, T.M., Kliewer, S.A. *An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ).* J Biol Chem 1995, 270: 12953-6.
23. Zhang, B., Berger, J., Hu, E. et al. *Negative regulation of peroxisome proliferator-activated receptor-γ gene expression contributes to the antiadipogenic effects of tumor necrosis factor-α.* Mol Endocrinol 1996, 10: 1457-66.
24. Rosen, E.D., Sarraf, P., Troy, A.E. et al. *PPARγ is required for the differentiation of adipose tissue in vivo and in vitro.* Mol Cell 1999, 4: 611-7.
25. Wu, Z., Rosen, E.D., Brun, R. et al. *Cross-regulation of C/EBPα and PPARγ controls the transcriptional pathway of adipogenesis and insulin sensitivity.* Mol Cell 1999, 3: 151-8.
26. Staels, B., Koenig, W., Habib, A. et al. *Activation of human aortic smooth-muscle cells is inhibited by PPARα but not by PPARγ activators.* Nature 1998, 393: 790-3.

27. Hu, E., Kim, J.B., Sarraf, P., Spiegelman, B.M. *Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ.* Science 1996, 274: 2100-3.
28. Zhang, B., Berger, J., Zhou, G. et al. *Insulin- and mitogen-activated protein kinase-mediated phosphorylation and activation of peroxisome proliferator-activated receptor γ.* J Biol Chem 1996, 271: 31771-4.
29. Camp. H.S., Tafuri, S.R., Leff, T. *c-Jun N-terminal kinase phosphorylates peroxisome proliferator-activated receptor-γ1 and negatively regulates its transcriptional activity.* Endocrinology 1999, 140: 392-7.
30. Dowell, P., Ishmael, J.E., Avram, D., Peterson, V.J., Nevrivy, D.J., Leid, M. *P300 functions as a coactivator for the peroxisome proliferator-activated receptor α.* J Biol Chem 1997, 272: 33435-43.
31. Gerritsen, M.E., Williams, A.J., Neish, A.S., Moore, S., Shi, Y., Collins, T. *CREB-binding protein/p300 are transcriptional coactivators of p65.* Proc Natl Acad Sci USA 1997, 94: 2927-32.
32. Mizukami, J., Taniguchi, T. *The antidiabetic agent thiazolidinedione stimulates the interaction between PPARγ and CBP.* Biochem Biophys Res Commun 1997, 240: 61-4.
33. Zhu, Y., Qi, C., Calandra, C., Rao, M.S., Reddy, J.K. *Cloning and identification of mouse steroid receptor coactivator-1 (mSRC-1), as a coactivator of peroxisome proliferator-activated receptor γ.* Gene Expr 1996, 6: 185-95.
34. Nolte, R.T., Wisely, G.B., Westin, S. et al. *Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ.* Nature 1998, 395: 137-43.
35. Sheppard, K.A., Phelps, K.M., Williams, A.J. et al. *Nuclear integration of glucocorticoid receptor and nuclear factor-κB signaling by CREB-binding protein and steroid receptor coactivator-1.* J Biol Chem 1998, 273: 29291-4.
36. Puigserver, P., Adelmant, G., Wu, Z. et al. *Activation of PPARγ coactivator-1 through transcription factor docking.* Science 1999, 286: 1368-71.
37. Forman, B.M., Tontonoz, P., Chen, J., Brun, R.P., Spiegelman, B.M., Evans, R.M. *15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ.* Cell 1995, 83: 803-12.
38. Kliewer, S.A., Lenhard, J.M., Willson, T.M., Patel, I., Morris, D.C., Lehmann, J.M. *A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation.* Cell 1995, 83: 813-9.
39. Nagy, L., Tontonoz, P., Alvarez, J.G.A., Chen, H., Evans, R.M. *Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ.* Cell 1998, 93: 229-40.
40. Tontonoz, P., Nagy, L., Alvarez, J.G.A., Thomazy, V.A., Evans, R.M. *PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL.* Cell 1998, 93: 241-52.
41. Huang, J.T., Welch, J.S., Ricote, M. et al. *Interleukin-4-dependent production of PPAR-γ ligands in macrophages by 12/15-lipoxygenase.* Nature 1999, 400: 378-82.
42. Camp, H.S., Li, O., Wise, S.C., et al. *Differential activation of peroxisome proliferator-activated receptor-γ by troglitazone and rosiglitazone.* Diabetes 2000, 49: 539-47.
43. Sakamoto, J., Kimura, H., Moriyama, S. et al. *Activation of human peroxisome proliferator-activated receptor (PPAR) subtypes by pioglitazone.* Biochem Biophys Res Commun 2000, 278: 704-11.
44. Okuno, A., Tamemoto, H., Tobe, K. et al. *Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats.* J Clin Invest 1998, 101: 1354-61.
45. Wu, Z., Xie, Y., Morrison, R.F., Bucher, N.L.R., Farmer, S.R. *PPARγ induces the insulin-dependent glucose transporter GLUT4 in the absence of C/EBPα during the conversion of 3T3 fibroblasts into adipocytes.* J Clin Invest 1998, 101: 22-32.
46. Michael, L.F., Wu, Z., Cheatham, R.B. et al. *Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1.* Proc Natl Acad Sci USA 2001, 98: 3820-5.
47. Kubota, N., Terauchi, Y., Miki, H. et al. *PPARγ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance.* Mol Cell 1999, 4: 597-609.
48. Miles, P.D.G., Barak, Y., He, W., Evans, R.M., Olefsky, J.M. *Improved insulin-sensitivity in mice heterozygous for PPAR-γ deficiency.* J Clin Invest 2000, 105: 287-92.
49. Lehmann, J.M., Lenhard, J.M., Oliver, B.B., Ringold, G.M., Kliewer, S.A. *Peroxisome proliferator-activated receptors α and γ are activated by indomethacin and other non-steroidal anti-inflammatory drugs.* J Biol Chem 1997, 272: 3406-10.

50. Inoue, H., Tanabe, T., Umesono, K. *Feedback control of cyclooxygenase-2 expression through PPARγ.* J Biol Chem 2000, 275: 28028-32.
51. Subbaramaiah, K., Lin, D.T., Hart, J.C., Dannenberg, A.J. *Peroxisome proliferator-activated receptor γ ligands suppress the transcriptional activation of cyclooxygenase-2.* J Biol Chem 2001, 276: 12440-8.
52. Kubota, T., Koshizuka, K., Williamson, E.A. et al. *Ligand for peroxisome proliferator-activated receptor γ (troglitazone) has potent antitumor effect against human prostate cancer both in vitro and in vivo.* Cancer Res 1998, 58: 3344-52.
53. Lefebvre, A.M., Chen, I., Desreumaux, P. et al. *Activation of the peroxisome proliferator-activated receptor γ promotes the development of colon tumors in C57BL/6J-APCMin/+ mice.* Nat Med 1998, 4: 1053-7.
54. Saez, E., Tontonoz, P., Nelson, M.C. et al. *Activators of the nuclear receptor PPARγ enhance colon polyp formation.* Nat Med 1998, 4: 1058-61.
55. Sato, H., Ishihara, S., Kawashima, K. et al. *Expression of peroxisome proliferator-activated receptor (PPAR)γ in gastric cancer and inhibitory effects of PPARγ agonists.* Br J Cancer 2000, 83: 1394-400.
56. Kliewer, S.A., Umesono, K., Noonan, D.J., Heyman, R.A., Evans, R.M. *Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors.* Nature 1992, 358: 771-4.
57. Benson, S., Padmanabhan, S., Kurtz, T.W., Pershadsingh, H.A. *Ligands for the peroxisome proliferator-activated receptor-γ and the retinoid X receptor-α exert synergistic antiproliferative effects on human coronary artery smooth muscle cells.* Mol Cell Biol Res Commun 2000, 3: 159-64.
58. Uchimura, K., Nakamuta, M., Enjoji, M. et al. *Activation of retinoic X receptor and peroxisome proliferator-activated receptor-γ inhibits nitric oxide and tumor necrosis factor-α production in rat Kupffer cells.* Hepatology 2001, 33: 91-9.
59. Su, C.G., Wen, X., Bailey, S.T. et al. *A novel therapy for colitis utilizing PPAR-γ ligands to inhibit the epithelial inflammatory response.* J Clin Invest 1999, 104: 383-9.
60. Kawahito, Y., Kondo, M., Tsubouchi, Y. et al. *15-deoxy-delta(12,14)-PGJ (2) induces synoviocyte apoptosis and suppresses adjuvant-induced arthritis in rats.* J Clin Invest 2000, 106: 189-97.
61. Goetze, S., Xi, X.P., Kawano, H. et al. *PPAR γ-ligands inhibit migration mediated by multiple chemoattractants in vascular smooth muscle cells.* J Cardiovasc Pharmacol 1999, 33: 798-806.
62. Hsueh, W.A., Jackson, S., Law, R.E. *Control of vascular cell proliferation and migration by PPAR-γ: A new approach to the macrovascular complications of diabetes.* Diabetes Care 2001, 24: 392-7.
63. Takata, Y., Kitami, Y., Okura, T., Hiwada, K. *Peroxisome proliferator-activated receptor-γ activation inhibits interleukin-1β-mediated platelet-derived growth factor-α receptor gene expression via CCAAT/enhancer-binding protein-δ in vascular smooth muscle cells.* J Biol Chem 2001, 276: 12893-7.
64. Okura, T., Nakamura, M., Takata, Y., Watanabe, S., Kitami, Y., Hiwada, K. *Troglitazone induces apoptosis via the p53 and Gadd45 pathway in vascular smooth muscle cells.* Eur J Pharmacol 2000, 407: 227-35.
65. Sugawara, A., Takeuchi, K., Uruno, A. et al. *Transcriptional suppression of type 1 angiotensin II receptor gene expression by peroxisome proliferator-activated receptor-γ in vascular smooth muscle cells.* Endocrinology 2001, 142: 3125-34.
66. Jackson, S.M., Parhami, F., Xi, X.P. et al. *Peroxisome proliferator-activated receptor activators target human endothelial cells to inhibit leukocyte-endothelial cell interaction.* Arterioscler Thromb Vasc Biol 1999, 19: 2094-104.
67. Pasceri, V., Wu, H.D., Willerson, J.T., Yeh, E.T.H. *Modulation of vascular inflammation in vitro and in vivo by peroxisome proliferator-activated receptor-γ activators.* Circulation 2000, 101: 235-8.
68. Lee, H., Shi, W., Tontonoz, P. et al. *Role of peroxisome proliferator-activated receptor α in oxidized phospholipid-induced synthesis of monocyte chemotactic protein-1 and interleukin-8 by endothelial cells.* Circ Res 2000, 87: 516-21.
69. Kintscher, U., Goetze, S., Wakino, S. et al. *Peroxisome proliferator-activated receptor and retinoid X receptor ligands inhibit monocyte chemotactic protein-1-directed migration of monocytes.* Eur J Pharmacol 2000, 401: 259-70.
70. Han, J., Hajjar, D.P., Tauras, J.M., Feng, J., Gotto, A.M. Jr., Nicholson, A.C. *Transforming growth factor-β1 (TGF-β1) and TGF-β2 decrease expression of CD36, the type B scavenger receptor, through mitogen-activated protein kinase*

phosphorylation of peroxisome proliferator-activated receptor-γ. J Biol Chem 2000, 275: 1241-6.
71. Febbraio, M., Podrez, E.A., Smith, J.D. et al. *Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice.* J Clin Invest 2000, 105: 1049-56.
72. Li, A.C., Brown, K.K., Silvestre, M.J., Willson, T.M., Palinski, W., Glass, C.K. *Peroxisome proliferator-activated receptor γ ligands inhibit development of atherosclerosis in LDL receptor-deficient mice.* J Clin Invest 2000, 106: 523-31.
73. Chen, Z., Ishibashi, S., Perrey, S. et al. *Troglitazone inhibits atherosclerosis in apolipoprotein E-knockout mice: Pleiotropic effects on CD36 expression and HDL.* Arterioscler Thromb Vasc Biol 2001, 21: 372-7.
74. Chawla, A., Boisvert, W.A., Lee, C.H. et al. *A PPARγ-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis.* Mol Cell 2001, 7: 161-71.
75. Chinetti, G., Lestavel, S., Bocher, V. et al. *PPAR-α and PPAR-γ activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway.* Nat Med 2001, 7: 53-8.
76. Jiang, J.G., Johnson, C., Zarnegar, R. *PPARγ-mediated transcriptional upregulation of the hepatocyte growth factor gene promoter via a novel composite cis-acting element.* J Biol Chem 2001, 276: 25049-56.
77. Taniyama, Y., Morishita, R., Aoki, M. et al. *Therapeutic angiogenesis induced by human hepatocyte growth factor gene in rat and rabbit hindlimb ischemia models: Preclinical study for treatment of peripheral arterial disease.* Gene Ther 2001, 8: 181-9.
78. Barger, P.M., Brandt, J.M., Leone, T.C., Weinheimer, C.J., Kelly, D.P. *Deactivation of peroxisome proliferator-activated receptor-α during cardiac hypertrophic growth.* J Clin Invest 2000, 105: 1723-30.
79. Lehman, J.J., Barger, P.M., Kovacs, A., Saffitz, J.E., Medeiros, D.M., Kelly, D.P. *Peroxisome proliferator-activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis.* J Clin Invest 2000, 106: 847-56.
80. Yue, T., Chen, J., Bao, W. et al. *In vivo myocardial protection from ischemia/reperfusion injury by the peroxisome proliferator-activated receptor-γ agonist rosiglitazone.* Circulation 2001, 104: 2588-94.
81. Khandoudi, N., Delerive, P., Berrebi-Bertrand, I., Buckingham, R.E., Staels, B., Bril, A. *Rosiglitazone, a peroxisome proliferator-activated receptor-γ, inhibits the Jun NH(2)-terminal kinase/activating protein 1 pathway and protects the heart from ischemia/reperfusion injury.* Diabetes 2002, 51: 1507-14.
82. Wayman, N.S., Hattori, Y., McDonald, M.C. et al. *Ligands of the peroxisome proliferator-activated receptors (PPAR-γ and PPAR-α) reduce myocardial infarct size.* FASEB J 2002, 16: 1027-40.
83. Zou, Y., Takano, H., Akazawa, H., Nagai, T., Mizukami, M., Komuro, I. *Molecular and cellular mechanisms of mechanical stress-induced cardiac hypertrophy.* Endocr J 2002, 49: 1-13.
84. Levy, D., Garrison, R.J., Savage, D.D., Kannel, W.B., Castelli, W.P. *Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study.* N Engl J Med 1990, 322: 1561-6.
85. Asakawa, M., Takano, H., Nagai, T. et al. *Peroxisome proliferator-activated receptor γ plays a critical role in inhibition of cardiac hypertrophy in vitro and in vivo.* Circulation 2002, 105: 1240-6.
86. Takano, H., Zou, Y., Akazawa, H. et al. *Inhibitory molecules in signal transduction pathways of cardiac hypertrophy.* Hypertens Res 2002, 25: 491-8.
87. Yamamoto, K., Ohki, R., Lee, R.T., Ikeda, U., Shimada, K. *Peroxisome proliferator-activated receptor γ activators inhibit cardiac hypertrophy in cardiac myocytes.* Circulation 2001, 104: 1670-5.
88. Kovacic-Milivojevic, B., Gardner, D.G. *Divergent regulation of the human atrial natriuretic peptide gene by c-jun and c-fos.* Mol Cell Biol 1992, 12: 292-301.
89. Lee, M-E., Bloch, K.D., Clifford, J.A. et al. *Functional analysis of the endothelin-1 gene promoter.* J Biol Chem 1990, 265: 10446-50.
90. Kishimoto, T., Taga, T., Akir, A.S. *Cytokine signal transduction.* Cell 1994, 76: 253-62.
91. Kunisada, K., Tone, E., Fujio, Y. et al. *Activation of gp130 transduces hypertrophic signals via STAT3 in cardiac myocytes.* Circulation 1998, 98: 346-52.
92. Herzig, T.C., Jobe, S.M., Aoki, H. et al. *Angiotensin II type1a receptor gene expression in the heart: AP-1 and GATA-4 participate in the response to pressure overload.* Proc Natl Acad Sci USA 1997, 94: 7543-8.

93. Hasegawa, K., Lee, S.J., Job, E.S.M. et al. *cis-Acting sequences that mediate induction of β-myosin heavy chain gene expression during left ventricular hypertrophy due to aortic constriction.* Circulation 1997, 96: 3943-53.
94. Morimoto, T., Hasegawa, K., Kaburagi, S. et al. *Phosphorylation of GATA-4 is involved in α1-adrenergic agonist-responsive transcription of the endothelin-1 gene in cardiac myocytes.* J Biol Chem 2000, 275: 13721-6.
95. Molkentin, J.D., Lu, J.R., Antos, C.L. et al. *A calcineurin-dependent transcriptional pathway for cardiac hypertrophy.* Cell 1998, 93: 215-28.
96. De Keulenaer, G.W., Wang, Y., Feng, Y. et al. *Identification of IEX-1 as a biomechanically controlled nuclear factor-κB target gene that inhibits cardiomyocyte hypertrophy.* Circ Res 2002, 90: 690-6.
97. Hirotani, S., Otsu, K., Nishida, K. et al. *Involvement of nuclear factor-κB and apoptosis signal-regulated kinase 1 in G-protein-coupled receptor agonist-induced cardiomyocyte hypertrophy.* Circulation 2002, 105: 509-15.
98. Watanabe, K., Sekiya, M., Tsuruoka, T. et al. *Effect of insulin resistance on left ventricular hypertrophy and dysfunction in essential hypertension.* J Hypertens 1999, 17: 1153-60.
99. Paternostro, G., Pagano, D., Gnecchi-Ruscone, T. et al. *Insulin resistance in patients with cardiac hypertrophy.* Cardiovasc Res 1999, 42: 246-53.

Leukemia Inhibitory Factor Enhances Survival of Cardiomyocytes and Induces Regeneration of Myocardium After Myocardial Infarction

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Background—Myocardial infarction (MI) is a leading cause of cardiac morbidity and mortality in many countries; however, the treatment of MI is still limited.

Methods and Results—We demonstrate a novel gene therapy for MI using leukemia inhibitory factor (LIF) cDNA. We injected LIF plasmid DNA into the thigh muscle of mice immediately after inducing MI. Intramuscular injection of LIF cDNA resulted in a marked increase in circulating LIF protein concentrations. Two weeks later, left ventricular remodeling, such as infarct extent and myocardial fibrosis, was markedly attenuated in the LIF cDNA–injected mice compared with vehicle-injected mice. More myocardium was preserved and cardiac function was better in the LIF-treated mice than in the vehicle-injected mice. Injection of LIF cDNA not only prevented the death of cardiomyocytes in the ischemic area but also induced neovascularization in the myocardium. Furthermore, LIF cDNA injection increased the number of cardiomyocytes in cell cycle and enhanced mobilization of bone marrow cells to the heart and their differentiation into cardiomyocytes.

Conclusions—The intramuscular injection of LIF cDNA may induce regeneration of myocardium and provide a novel treatment for MI. (***Circulation.*** **2003;108:748-753.)**

Key Words: gene therapy ■ muscles ■ leukemia inhibitory factor ■ myocardial infarction ■ regeneration

The loss of cardiomyocytes in myocardial infarction (MI) is a major cause of heart failure. Many cardiomyocytes are dead by apoptosis and necrosis in MI,[1] and the myocardium in the infarcted area is gradually replaced by collagen tissue. It has long been believed that adult cardiomyocytes do not proliferate; however, it was recently reported that adult cardiomyocytes could enter the cell cycle and increase the cell number.[2,3] Moreover, it has been reported that cardiomyocytes can be generated from marrow stromal cells from mice in vitro[4] and that undifferentiated stem cells in the bone marrow may be transported to the heart and differentiate into cardiomyocytes and vascular endothelial cells in vivo.[5–8] Given these possibilities, MI could be treated by enhancing the ability of cardiomyocytes to divide or of bone marrow cells (BMCs) to differentiate into cardiomyocytes.

The interleukin 6 (IL-6) family of cytokines, including IL-6, leukemia inhibitory factor (LIF), ciliary neurotrophic factor, and cardiotrophin-1, have a variety of biological functions not only in the hematopoietic and immune systems but also in other organs, including the nervous and cardiovascular systems.[9,10] The IL-6 family regulates growth and differentiation of many types of cells. Furthermore, these cytokines contribute to the regeneration of many tissues, such as nerves, skeletal muscle, liver, and bone.[11–14] LIF has been reported to ameliorate denervation-induced muscle atrophy and improve regeneration of muscle and nerves.[11] Locally administered LIF cDNA plasmid in a gelatin carrier can increase bone density and subsequent bone formation.[14] In the heart, gp130, the common receptor of the IL-6 family, is expressed abundantly and has been reported to be critically involved in the growth and survival of cardiomyocytes.[15–18] It has also been reported that LIF receptor is expressed abundantly in cardiomyocytes and that LIF induces marked cardiomyocyte hypertrophy.[19,20] All these findings suggest that LIF may promote survival of cardiomyocytes and regeneration of myocardium. We thus examined the potential usefulness of LIF to treat MI.

Methods

Murine MI Model

MI was produced in 12-week-old male C57BL/6 mice by permanent ligation of the left coronary artery.[21] A total of 40 mice were operated on to induce MI and randomly divided into 2 groups, LIF cDNA–injected and vehicle-injected groups (20 mice each). At 2

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