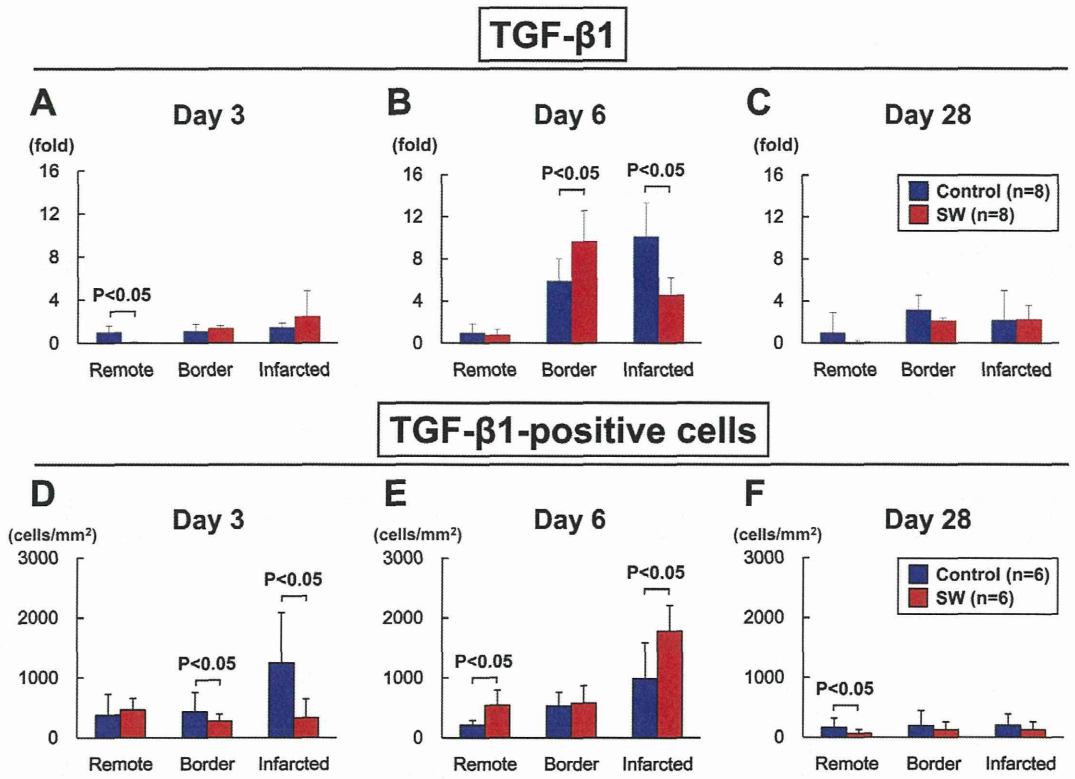

Figure 7. Shock-wave (SW) therapy suppresses transforming growth factor (TGF)-β1 expression after acute myocardial infarction. (**A**–**C**) The mRNA expression levels of TGF-β1 on day 3 (**A**), day 6 (**B**) and day 28 (**C**). (**D**–**F**) Immunohistochemical staining of TGF-β1-positive cells on day 3 (**D**), day 6 (**E**) and day 28 (**F**). Results are expressed as mean±SEM. P<0.05 for the control vs. the SW group.

flammatory responses might enhance tissue degradation and LV fibrosis with a resultant LV remodeling in the chronic phase.[15–17] Also, Nahrendorf et al reported the functional consequence of orchestrated mobilization of monocytes/macrophage subtypes during the healing of AMI.[19] In the present study, infiltration of neutrophils and macrophages early after AMI was significantly attenuated by SW therapy, while the number of infiltrated M2 macrophages was higher in the SW group than in the control group early after AMI. These results suggest that SW therapy exerts anti-inflammatory effects not only by suppressing the infiltration of inflammatory cells but also by inducing the shift of the macrophage phenotypes to anti-inflammatory M2 subtype. SW therapy significantly suppressed the production of pro-inflammatory cytokines at day 6. Also, the production of pro-inflammatory cytokines increased with time (day 3 vs. day 6) in the control group, which was suppressed by SW therapy. Indeed, anti-inflammatory effects of low-energy SW therapy has also been reported in cultured cells,[13] murine skin isografts[12] and patients with inflammatory orthopedic diseases, such as tendinitis and plantar fasciitis.[14] Stojadinovic et al reported that low-energy SW therapy affects the expression of a variety of chemokines (CXCL1, CXCL2, CXCL5, CCL2, CCL3 and CCL4) and cytokines towards an anti-inflammatory direction in murine skin isografts.[12] Although we did not examine these chemokines in the present study, it is conceivable that SW therapy affected not only the cytokines mentioned above but also these chemokines. Taken together, these results raise the possibility that SW therapy suppresses post-MI LV remodeling, at least in part, by suppressing inflammatory responses early after AMI. Further studies are needed to elucidate the inhibitory effects of SW therapy on the infiltration of inflammatory cells after AMI.

Attenuation of LV Fibrosis After AMI by SW Therapy

TGF-β1, which is known to promote LV fibrosis, is released from fibroblasts and infiltrated macrophages after myocardial injury, and excessive infiltration of macrophages might promote LV fibrosis and thus deteriorate LV function.[15–17,20] In the present study, SW therapy attenuated macrophage infiltration, TGF-β1 expression and LV fibrosis. These results suggest that the anti-fibrotic effects of SW therapy are related to the suppression of macrophage infiltration and TGF-β1 expression. However, it remains to be examined whether the reduced expression of TGF-β1 is attributed to the reduction of macrophage infiltration and TGF-β1 production from macrophages and other cells.

Mechanisms for the Inhibitory Effects of SW Therapy on LV Remodeling After AMI

We and others have previously demonstrated angiogenic effects of low-energy SW therapy in several animal models,[7,10–12,14,16,21–25] as well as in humans.[8,9,26–32] In the present study, we have demonstrated that SW therapy attenuates inflammatory responses and LV fibrosis in a rat model of AMI.

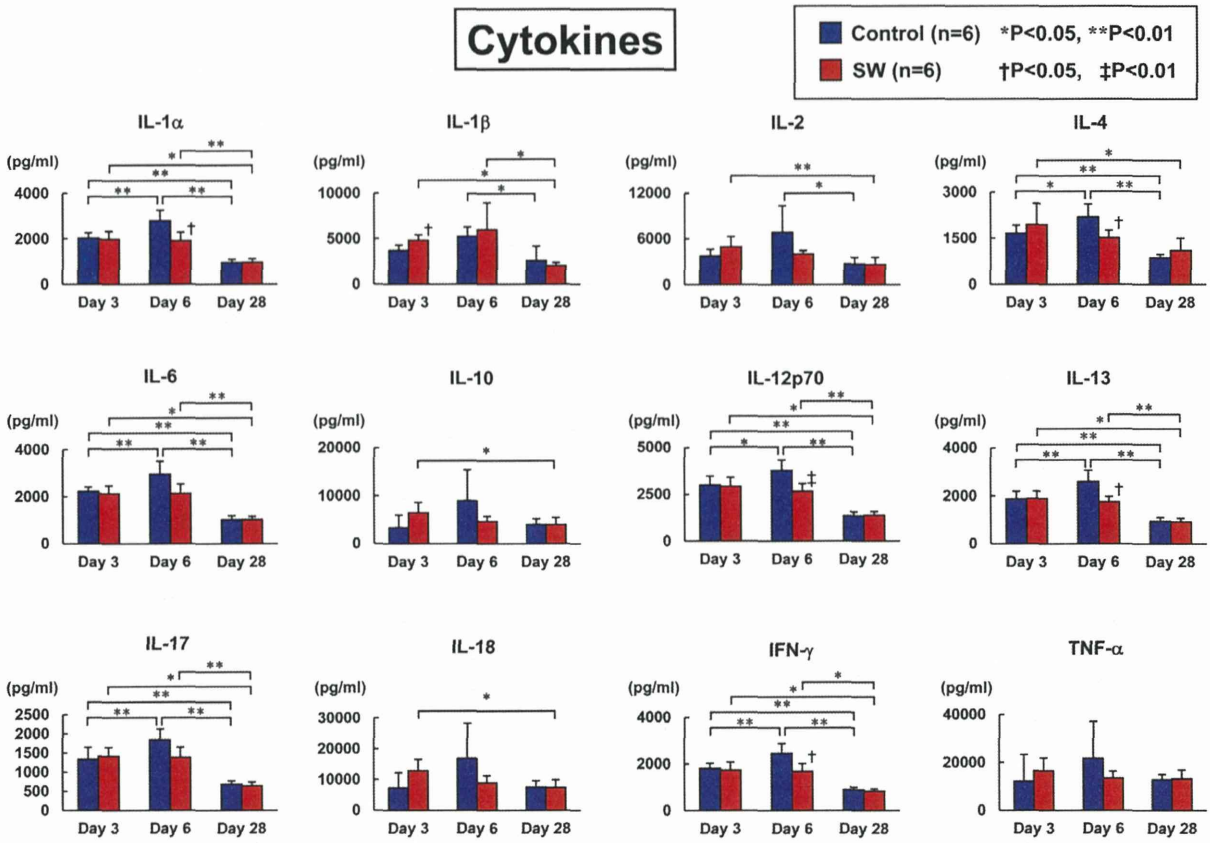

Figure 8. Shock-wave (SW) therapy upregulates anti-inflammatory cytokines and downregulates pro-inflammatory cytokines after acute myocardial infarction. The production of inflammation-related cytokines in left ventricular homogenates from the border zone. Results are expressed as mean±SEM. *P<0.05, **P<0.01; among different observation days in each group by Tukey's honest significant difference multiple comparison test. †P<0.05, ‡P<0.01; the control group vs. the SW group by Bonferroni type multiple comparisons.

These results suggest that SW therapy ameliorates post-MI LV remodeling not only through angiogenesis but also through suppression of inflammatory responses and LV fibrosis (**Figure S2**). The low-energy SW therapy, when applied to ischemic tissues, has been reported to enhance the expression of stromal-derived factor 1, a key regulator of stem cell migration to the site of tissue injury during the process of tissue repair.[33–37] In addition, SW therapy has also been reported to promote migration and differentiation of bone marrow-derived mononuclear cells (BMDMC).[38,39] Furthermore, macrophages could modulate the activity of stem cells.[40] In the present study, we also showed that macrophage infiltration was ameliorated by SW therapy. Thus, SW therapy might directly and/or indirectly affect the function of stem cells, such as BMDMCs, residential cardiac stem cells, and multilineage-differentiating stress-enduring (Muse) cells.[38,39,41,42] Additional studies are needed to clarify the contribution of stem cells to the beneficial effects of SW therapy.

Study Limitations

Several limitations should be mentioned for the present study. First, in the present study, we chose the condition of SW therapy (eg, energy levels, number of shots) based on our previous studies[7–11,18] and did not test other therapeutic conditions. It is unknown whether different levels, numbers and protocols of SW therapy could be more effective than that used for the present study. Second, in the present study, we had to apply SW to the whole rat heart due to the focus size of the SW machine, whereas we were able to selectively apply SW to the border area in our previous studies in pigs.[10,11] Interestingly, however, in the present study, the SW therapy increased capillary density only in the border area. Thus, SW therapy might enhance angiogenesis and exert anti-inflammatory effects mainly in the border area in an AMI model even when the SW was applied to the whole heart. The detailed molecular mechanisms for the different effects of SW therapy between ischemic and non-ischemic areas remain to be examined. Third, the detailed molecular mechanisms of the anti-inflammatory effects of SW therapy also remain to be elucidated in future studies. Fourth, in the present study, we did not show whether the anti-inflammatory action mediates the beneficial effects of SW therapy on LV remodeling. To clarify this issue, an additional approach such as gene deletion or selective inhibition of candidate molecules might provide further insights into the effects of SW therapy. Finally, in the present study, we focused on neutrophils and macrophages as inflammatory cells; however, other types of cells, such as fibroblasts, myofibroblasts, natural killer T cells and regulatory T cells, have been

reported to affect the inflammatory state and LV remodeling after AMI.[43–47] Also, we did not examine the effects of SW therapy on functional aspects of inflammatory cells. Further studies are needed to clarify these issues.

Conclusions

In the present study, we demonstrated that low-energy SW therapy suppresses post-MI LV remodeling in rats in vivo, which is associated with anti-inflammatory effects in addition to its angiogenic effects, thus demonstrating a novel aspect of the therapy for AMI (**Figure S2**). Because SW therapy is non-invasive and safe, it could be a novel option for the prevention of LV remodeling after AMI in humans.

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Supplementary Files

Supplementary File 1

Figure S1. No effects of shock-wave (SW) therapy on left ventricular (LV) function in the sham-operated group.

Figure S2. Summary of the present findings and proposed mechanisms of the inhibitory effects of shock-wave (SW) therapy on post-myocardial infarction (MI) left ventricular (LV) remodeling.

Please find supplementary file(s);
http://dx.doi.org/10.1253/circj.CJ-14-0230