Aspirin Delays Skin Wound Healing

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Aspirin, the most famous drug in the world, has been used to kill pain and fever as a representative nonsteroidal anti-inflammatory drug (NSAID). In addition to its beneficial effects, aspirin has several side effects including bleeding, damage to gastrointestinal tracts, and delayed onset of labor. In the present study, our group found aspirin-dependent delay in skin wound healing and its precise mechanism, which was recently published as an article of *Journal of Experimental Medicine* 1.

I am a biochemist and molecular biologist and known worldwide as an expert of G–protein coupled receptors (GPCRs) for lipid mediators, especially arachidonic acid–derivatives leukotrienes. In addition to our cloning of BLT1, a high affinity leukotriene B4 receptor 2, we cloned another GPCR, namely BLT2 3 with a sequence homology to BLT1. After the intensive research for ligand fishing, we finally purified and determined the structure of an endogenous ligand for BLT2, 12-HHT (12-hydroxyheptadecatrienoic acid) from rat small intestine 4. Activated platelets have been known to produce 12-HHT (12-hydroxyheptadecatrienoic acid) is produced from arachidonic acid by sequential reactions by COX (cyclooxygenases) and TXA2S (thromboxane A2 synthase). Despite the well known functions of BLT1 receptor, the roles of BLT2 receptor has not been established.

**Figure 1** Biosynthesis of 12-HHT

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and release 12-HHT during blood coagulation (Figure-1), however, the role of 12-HHT *in vivo* remained unclear until this report.

Based on their finding that BLT2 is highly expressed in mouse skin, they explored the roles of BLT2 in skin function. RT-PCR and immunohistochemical staining revealed that BLT2 is exclusively expressed in keratinocytes but not in fibroblasts in mouse and human skin. As we reported in the previous publication that a synthetic BLT2 agonist induced keratinocyte migration *in vitro*, we examined the skin wound healing process after skin punching using BLT2-deficient (BLT2-KO) mice. As expected, BLT2-KO mice exhibited much delayed wound closure when compared with BLT2-WT mice (Figure-2). Aspirin also delayed wound closure in WT mice, however, this aspirin effect was completely lost in BLT2-KO mice, suggesting that aspirin delays wound healing in a BLT2-dependent fashion. Detailed histological examination revealed that only re-epithelialization, which is a hallmark of keratinocyte migration, was attenuated by aspirin and BLT2-deficiency, and keratinocyte proliferation or myofibroblast transformation was not affected either. Mice lacking thromboxane synthase, a responsible enzyme for production of 12-HHT and thromboxane A2, also showed delayed wound healing, and this was not observed in mice lacking TP, a receptor for thromboxane A2. These results clearly showed that aspirin (and other NSAIDs) delays skin wound healing by reducing the skin content of 12-HHT that binds to BLT2 and induces keratinocyte migration after skin injury.

We also addressed the detailed molecular mechanism of BLT2-dependent keratinocyte migration. Stimulation of keratinocyte BLT2 by 12-HHT induced transcription of various molecules including TNFα, IL-1β, and metalloproteinases (MMPs), all of which had been reported to accelerate keratinocyte migration. Time course studies using primary and BLT2-transfected keratinocytes (HaCaT cells) revealed that BLT2 stimulation initially induces TNFα expression, which then induces mRNA of MMP9. Treatment of keratinocytes with either Infliximab, a neutralizing antibody for TNFα or MMP inhibitors inhibited BLT2-dependent keratinocyte migration *in vitro*, supporting 12-HHT/BLT2 accelerates keratinocyte migration in a TNFα-MMP dependent fashion.

![Figure-2](image-url)

**Figure-2** Aspirin delays skin wound healing in a BLT2-dependent manner
(A) A representative photo of mouse skin punching. BLT2-deficiency (B) and aspirin administration (C) delay skin wound healing. Aspirin-dependent delay in wound healing was cancelled by BLT2-deficiency (D).
Finally we tried to evaluate the therapeutic effect of a synthetic BLT2 agonist CAY10583 in skin wound healing in vivo. Topical application of CAY10583 containing vaseline to the punched skin clearly accelerated wound closure in WT C57Black mice, and this effect was more obvious in a diabetic db/db mice, a model of intractable wounds (Figure-3).

Many patients are suffering from bedsore and diabetic skin ulcer, however, there are no specific medicine for these intractable wounds. Drugs for wound repair target fibroblasts and vessels, and no drugs are available to directly activate keratinocytes to close the wounds. Our group is now willing to develop more potent BLT2 agonists to accelerate wound healing in human. This work also provides a completely novel mechanism of aspirin side effect by reducing 12-HHT production, but not prostaglandins.

References