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Soluble epoxide hydrolase disruption as therapeutic target for wound healing

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ABSTRACT

Background: Cytochrome P450 (CYP)-derived epoxyeicosatrienoic acids (EETs) possess angiogenic effects. However, the effect of CYP-derived EETs and soluble epoxide hydrolase (sEH) deletion on wound healing *in vivo* has not been rigorously investigated. In this study, we measured the effect of exogenous CYP-derived EETs and targeted disruption of sEH in an *in vivo* wound model.

Materials and methods: Standardized full-thickness dermal wounds were created on the dorsum of mouse ears. Wound epithelialization was directly viewed and measured using intravital microscopy and computerized planimetry every second day until healing was complete. Wound sections were analyzed by immunostaining for metalloproteinase (MMP) 2, MMP7, MMP9, tissue inhibitor of metalloproteinases (TIMP) 1, and tumor necrosis factor (TNF) α on days 2, 4, and 12.

Results: Treatment with 11,12-EETs, 14,15-EETs, and sEH deletion significantly accelerated wound closure. This effect was attenuated by the EET antagonist 14,15-epoxyeicosa-5(Z)-enoic acid (14,15-EEZE) in $sEH^{-/-}$ mice. Neither 11,12- nor 14,15-EETs caused significant alterations in MMP9 expression in wounds. In contrast, MMP2 and MMP7 were significantly upregulated in the EET-treated groups, whereas TIMP1 and TNF-α were downregulated.

Conclusions: Collectively, these data demonstrated that potentiation of the CYP epoxygenase pathway by either exogenous CYP-derived EETs or sEH deletion significantly accelerated wound epithelialization *in vivo*. This beneficial effect might be due to downregulation of TNF-α production and, to a lesser degree, to the release of MMPs and could be used as a viable angiogenic therapeutic strategy.

1. Introduction

Successful cutaneous wound healing requires coordinated, complex cellular and molecular processes, including cell migration, proliferation, inflammation, angiogenesis, granulation tissue formation, and extracellular matrix (ECM) deposition. Determining the molecular mechanisms that regulate these processes may provide novel targets for the development of effective therapy strategies for impaired wound healing.

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It is now clear that extrahepatic cytochrome P450 (CYP) epoxygenases, long thought to be biologically important only as xenobiotic metabolizing enzymes, can affect vascular tone, modulate inflammation, and stimulate multiple steps in angiogenesis, including endothelial cell migration, proliferation, and tube formation [1]. CYP epoxygenases metabolize a range of endogenous substrates, including arachidonic acid to regiospecific and stereospecific epoxyeicosatrienoic acids (EETs) [1]. Epoxidation can occur at any of the four double bonds of arachidonic acid, giving rise to the four regioisomers 5,6-, 8,9-, 11,12-, and 14,15-EETs [2]. Intracellular levels of EETs are tightly regulated and metabolism occurs rapidly by the activity of the CYP epoxygenases that generate them as well as by the soluble epoxide hydrolase (sEH), which converts the EETs to their corresponding dihydroxyeicosatrienoic acids, showing generally less biological activity than the parent epoxides [3].

Targeting CYP epoxygenases *in vivo* is difficult because these enzymes are involved in the metabolism of several currently used therapeutic agents. However, targeting sEH, which metabolizes fatty acid epoxides to their corresponding diols, is a highly effective way of manipulating levels of these lipid mediators *in vivo* [3]. Indeed, sEH inhibitors have been shown to lower blood pressure in different hypertension models. Moreover, these compounds decreased the inflammatory response in endotoxemia and acute lung injury models, as well as ischemic injury in stroke models in rats [4]. Although studies using sEH inhibitors demonstrate generally antiinflammatory/protective effects, this is not always the case when $sEH^{-/-}$ mice were studied. In fact, sEH deletion reduced survival after cardiac arrest, and genetic variation in the sEH gene (EPHX2) has been linked to a higher incidence of stroke in rats and humans [5]. Such reports suggest that sEH deletion could induce negative rather than beneficial effects.

We recently reported that 11,12-EETs, 14,15-EETs, and the selective sEH inhibitor *trans*-4-[4-(3-adamantan-1-ylureido)-cyclohexyloxy]-benzoic acid (t-AUCB) accelerated wound epithelialization and closure in the hairless mouse ear wound model by activating signaling cascades that promote angiogenesis [6]. With regard to the apparently contradictory findings between wild-type animals treated with sEH inhibitors and $sEH^{-/-}$ mice, the aim of the present study was to analyze the role of sEH deletion and its inflammatory response in wound healing. As $sEH^{-/-}$ mice were available only with C57BL/6 background, we used our wound model—for the first time—at the C57BL/6 mouse ear. Moreover, we compared the effects of exogenous CYP-derived EETs with sEH deletion, which have not been investigated to date.

2. Materials and methods

2.1. Animals and wound model

All procedures were performed in accordance with the National Research Council's guide for the care and use of laboratory animals and the experiments were approved by the State Animal Review Committee (reference number: F 3/15). C57BL/6 mice were purchased from Charles River Laboratories (Sulzfeld, Germany). The $sEH^{-/-}$ mice were obtained from Frank Gonzalez (National Institutes of Health, Bethesda, MD) and then cross-bred for eight generations onto the C57BL/6 background. All mice (20–30 g, 8–12 wk) were housed in separate cages in temperature- (24°C), light- (12 h/d), and airflow-regulated rooms and fed a balanced rodent diet and water *ad libitum*.

All manipulation and measurements of the mouse ear wounds were performed with the animals anesthetized with a 100 μL solution containing 2.215 mg ketamine and 0.175 mg xylazine hydrochloride delivered intraperitoneally. After shaving and disinfection of the ears, animals were placed on a specially designed Plexiglas platform with their ears extended on a microscope slide by placing three permanent suture loops (9-0, nylon) at opposite poles of their ears. Standardized, circular wounds (2.25 mm in diameter, 125 μm in depth) were created on the dorsum of the ears using a specially designed punch. Wounds were positioned between the ears' anterior and middle principal neurovascular bundles. After the punch incision was performed, a full-thickness layer of skin was dissected away down to the underlying cartilage layer using careful microsurgical technique. Because of the structure of the mouse ear, in which the dorsal skin is firmly bound to the central cartilage layer of the ear, contraction does not contribute to healing in this model. Therefore, wound closure is almost entirely due to epithelialization and neovascularization. The day of wounding was designated as day 0 [7–11].

Methylcellulose discs containing the different substances under study were used to standardize their local application to the wounds. Accordingly, vehicle or substances—described in the Results section—were mixed with a 2.5% methylcellulose solution. Aliquots (10 μL) of the resulting 1.25% methylcellulose solution were applied to Parafilm (Pechiney Plastic Packaging, Inc, Chicago, IL), dried for 20 min on a warming plate, and then placed onto the wound [6].

Wounds and the entire ears were covered with a bioadhesive dressing (OpSite; Smith & Nephew Medical Limited, Hull, UK) to prevent wound desiccation and create a moist wound healing environment. Elizabethan mouse collars were used to protect the ears from mechanical irritation. Wound dressings and discs were changed every second day.

2.2. Wound measurement

Wound epithelialization was directly visualized and measured using intravital microscopy and computerized planimetry. Wound surface area measurements were performed immediately after wounding and every second day thereafter until complete wound closure. When epithelialization was near completion, the wounds were observed daily to determine the exact day wound closure was completed. Measurements were performed by placing the anesthetized mice on a Plexiglas platform to the stage of an intravital microscope (Carl Zeiss, Oberkochen, Germany). The microscope image was captured with a low-light camera (DXC-390P, 3CCD color video camera; Sony, Tokyo, Japan) and transferred through a digital converter (ADVC-100; Canopus, Ruppach-Goldhausen, Germany) to a monitor.

The wound surface images were analyzed using ImageJ software (NIH, Bethesda, Maryland, US) by tracing the wound