Role of keratinocytes GPR109A and COX-2 in nicotinic acid and monomethyl fumarate induced flushing

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Introduction

The anti-diabetic drug nicotinic acid and the anti-psoriatic drug monomethyl fumarate induce cutaneous flushing through the activation of the G-protein-coupled receptor GPR109A. Flushing is a troublesome side effect of nicotinic acid, but may be a direct reflection of the wanted effects of monomethyl fumarate. Here, we analysed the mechanisms underlying GPR109A-mediated flushing and show that both Langerhans cells and keratinocytes express GPR109A. Using cell ablation approaches and transgenic cell-type-specific expression of GPR109A in Gpr109a–/– mice, we provide evidence that the early phase of flushing depends on GPR109A expressed on Langerhans cells, whereas the late phase is mediated by GPR109A expressed on keratinocytes. Interestingly, the first phase of flushing is blocked by a selective cyclooxygenase-1 (COX-1) inhibitor, and the late phase is sensitive to a selective COX-2 inhibitor. Both, monomethyl fumarate and nicotinic acid, induce PGE2 formation in isolated keratinocytes through activation of GPR109A and COX-2.

Fig. 1. Keratinocytes express GPR109A. A, scheme of the Gpr109a reporter transgene. B, Gpr109a expression in epidermis. Shown are sections through the epidermis of wild-type and Gpr109a–/– mice. C, Langerhans cells were visualized by immunohistochemical labelling with antibodies directed against cytokera-121 and MHC-II. The mRFP-fluorescence was detected in parallel to visualizing GPR109A expression. Dermal tissue, basal membrane, S. Stratum basale, Se, Stratum spinosum, Sp, Stratum granulosum. E, effect of 100 µM nicotinic acid (NA) and fetal bovine serum (FBS) on ERK1/2 activation of GPR109A and COX-2 in human keratinocytes. Data are presented as mean ± S.E.M. (n = 6).

Fig. 2. Gpr109a-expressed by Langerhans cells mediated only the early phase of nicotinic acid-induced flushing. A, experimental scheme. One day after induction, Langerhans (DTR) mice were pre-treated with bupivacaine from wild-type (Gpr109a+) or Gpr109a-deficient mice (Gpr109a–/–) carrying in both cases the Gpr109a expression reporter transgene (Gpr109a-mRFP). Three months after the transplants, both groups were treated with diethylstilbestrol, and these mice later on, nicotinic acid-induced flushing was evaluated. B, nicotinic acid (NA) induced flushing three months after diethylstilbestrol treatment was determined by flow cytometry. C, fluorescence image of epidermal sheet from template 30 minutes after flushing or diethylstilbestrol treatment compared with an anti-MHC-II antibody and stained with mRFP-expression.

Fig. 3. Expression of GPR109A in keratinocytes is sufficient to mediate the late phase of nicotinic acid-induced flushing. A, scheme of the Krt14Gpr109a reporter transgene. B, effect of nicotinic acid (NA) and monomethyl fumarate (MF) on flushing in Gpr109a–/– mice carrying the Krt14Gpr109a reporter transgene. Data are presented as mean ± S.E.M. (n = 6). C, fluorescence image of epidermal sheets pre-treated with wild-type and Krt14Gpr109a–/– mice. Shown are the four views of epidermal sheets stained with anti-MHC-II antibodies and analyzed for expression of mRFP.

Fig. 4. Roles of COX-1 and COX-2 in GPR109A-mediated flushing. A, analysis of COX-2 expression in the epidermis. Shown are transversal sections of the skin specimens stained with DAPI and with endothelin specific to COX-2 and MHC-II. Dermal tissue, basal membrane, Sb, Stratum basale, Se, Stratum spinosum, Sp, Stratum granulosum, B-D, flushing responses induced by nicotinic acid (NA) and monomethyl fumarate (MF) in wild-type mice treated in the presence of 5 µg of the COX-2 inhibitor BM22057 (B), 10 µg of the COX-2 inhibitor ND09 (C), or pre-treated with both inhibitors (D). Shown are representative traces as well as the quantification of at least four experiments. Data are presented as mean ± S.E.M. (n = 4).

Fig. 5. GPR109A-mediated stimulation of prostaglandin release from keratinocytes. A, time course effect of nicotinic acid (NA) and the release of PGE2 from human keratinocytes. Keratinocytes were left untreated or were pre-treated with the COX-2 inhibitor ND09 (10 µg) or COX-1 inhibitor HIB222057 (10 µM). Data shown are mean values (n = 5, 6). C, proposed model for the local mechanism underlying GPR109A-mediated flushing. Application of GPR109A agonists results in the release of PGE2 as a result of direct blood flow, which results from activation of GPR109A on Langerhans cells, which is responsible for the first phase and from the activation of GPR109A on keratinocytes, which is responsible for the late phase of the response.

Conclusions

Here we show that nicotinic acid and monomethyl fumarate induced flushing results from two GPR109A-mediated mechanisms which involve Langerhans cells and keratinocytes as well as different prostanooid forming enzymes. These data will help to further improve flush-reducing strategies in patients taking GPR109A agonists like nicotinic acid by combined inhibition of COX-1 and COX-2 or PDI, and PGE2, activities. In addition, the presented data shed new light on the mechanisms of action of the anti-psoriatic drug monomethyl fumarate and suggest that GPR109A expressed on epidermal cells but also on other cells mediates anti-inflammatory effects.