EFFECT OF CYTOCHROME P450 INHIBITION ON BASELINE BLOOD FLOW AND ON ACETYLCHOLINE-INDUCED VASODILATION IN THE HUMAN SKIN MICROCIRCULATION

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Background

Cytochrome P450 (CYP) epoxygenase expressed in endothelial cells has been shown to be involved in the generation of epoxyeicosatrienoic acids (EETs). EETs are known to induce endothelial cell hyperpolarization and as such are suggested to be one of the mechanisms for the action of yet undefined endothelium-derived hyperpolarizing factor (EDHF). EDHF induces vasodilatation, independent of nitric oxide (NO) and prostacyclin (PGI_2), of numerous vessels from different species. The identity of EDHF in the human circulation remains controversial. In coronary arterioles, EDHF appears to be CYP 2C9-derived metabolite, whereas there are no data for human skin microcirculation. Skin microcirculation is of interest because it is easily accessible and might reflect functional changes of other parts of the circulation. Therefore, the aim of our study was to determine whether or not a CYP-dependent mechanism contributes to the regulation of blood flow in skin microcirculation. To this end, we studied the effects of sulphaphenazole, a selective CYP 2C9 inhibitor, on baseline skin blood flow (SkBF) and on endothelium-dependent vasodilatation, in the presence and absence of combined eNOS and COX inhibition, respectively.

Methods and results

We measured SkBF at four independent skin sites on the volar aspect of the forearm in 12 healthy subjects using laser-Doppler fluxmetry (LDF). Endothelium-dependent vasodilatation was assessed by an iontophoretical application of acetylcholine (ACh), whereas we used sodium-nitroprusside (SNP) to assess endothelium-independent vasodilatation. To study the NO/PGI_2-independent vasodilatation, potentially attributable to an EDHF, we inhibited endothelial NO synthase (eNOS) and cyclooxygenase (COX) by an intradermal injection (10 µl) of the eNOS inhibitor, L-NMMA (40 mM) and the COX inhibitor, diclofenac (30 mM); saline (10 µl) was injected as a control. To test the involvement of CYP in the EDHF-mediated vasodilatation, sulfaphenazole (10mM) was applied intradermally.
Combined eNOS and COX inhibition had no effect on baseline LDF (10.8±2.1 PU in control vs. 8.3±1.2 PU in treated site). On the other hand, the ACh-stimulated increase in LDF was significantly attenuated after eNOS and COX inhibition (35.7±5.5 PU), compared to the control (60.5±8.2 PU, $p < 0,05$). Nevertheless, at least 60 % of ACh-mediated vasodilatation was preserved after combined eNOS and COX inhibition. On the contrary, eNOS and COX inhibition had no impact on the SNP-stimulated increase in LDF (71.1±8.3 PU in control vs. 63.5±5.8 PU increase in the treated site). Sulfaphenazole had no significant effect on baseline LDF (10.8±2,1 PU for saline and 11.6±2.6 PU for sulfaphenazole) as well as on the ACh-induced LDF increase (60.5±8.2 PU in saline and 63.6±6.9 PU in treated site). Also, when sulfaphenazole was coinjected to sites, pretreated with L-NMMA and diclofenac, there were no statistical differences between saline and sulfaphenazole-treated sites either in baseline LDF values (8.3±1.2 PU for saline vs. 10.4±2.7 PU for the sulfaphenazole-treated site) or in the responses to ACh. (35.7±5.5 PU for saline vs. 34.2±5.6 PU for the sulfaphenazole-treated site).

Conclusion

Our investigation showed that a non-NO-, non-prostanoid-dependent mechanism, potentially attributable to EDHF, contributes substantially to the regulation of baseline blood flow as well to the ACh-provoked vasodilatation in the human skin microcirculation. Furthermore, the results indicate that CYP 2C9 is probably not involved in the generation of the putative EDHF in cutaneous microcirculation. Further studies are needed to clarify the exact nature of EDHF in human skin.