Identification of Skin as a Major Site of Prostaglandin D\textsubscript{2} Release Following Oral Administration of Niacin in Humans

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Oral administration of niacin (nicotinic acid) at pharmacologic doses that reduce serum cholesterol levels induces intense flushing in humans. We have recently shown that the vasodilation following ingestion of niacin is due to the release of prostaglandin (PG) D\textsubscript{2}. However, the site from which PGD\textsubscript{2} is released is not known. It has previously been shown that topical application of methyl nicotinate causes local cutaneous erythema. Thus, we investigated whether topical methyl nicotinate causes a release of PGD\textsubscript{2} locally from skin and the possibility that skin may be a major contributor to the release of PGD\textsubscript{2} when niacin is administered by mouth.

Topical administration of methyl nicotinate (10\textsuperscript{-4} M) to the forearms of human volunteers resulted in 58- to 122-times increases in levels of PGD\textsubscript{2} and 25- to 33-times increases in levels of the metabolite of PGD\textsubscript{2}, 9\alpha,11\beta-PGF\textsubscript{2}, in blood drawn from the antecubital vein draining the treated sites. Increased levels of PGD\textsubscript{2} and 9\alpha,11\beta-PGF\textsubscript{2} were not found in blood drawn simultaneously from veins in the contralateral arm, indicating that the PGD\textsubscript{2} was released from the site of methyl nicotinate application. The release of PGD\textsubscript{2} in response to topical application of methyl nicotinate occurred in a dose-dependent manner over the concentration range of 10\textsuperscript{-3} to 10\textsuperscript{-1} M. The release of PGD\textsubscript{2} was not accompanied by a release of histamine, suggesting that the release of PGD\textsubscript{2} was not from the mast cell. Following oral ingestion of niacin, levels of PGD\textsubscript{2} in superficial venous blood draining the skin were 14 to 1200 times higher than the level in arterial blood supplying the skin of the same arm. This finding indicates that the skin is a major site from which PGD\textsubscript{2} is released following oral ingestion of niacin.

These studies thus indicate that the cutaneous vasodilation that occurs following oral administration of niacin is primarily due to a release of PGD\textsubscript{2} from a niacin responsive cell that resides in the skin. *J Invest Dermatol* 98:812–815, 1992

Nicotinic acid (niacin) is a water soluble B vitamin. It is also recognized as one of the most effective hypolipidemic agents available. However, when administered at pharmacologic doses required to lower serum lipid levels (2–8 grams/d), its use is severely limited by a high incidence of adverse side effects, the most prominent of which is intense flushing. Flushing occurs in most people with dosages as small as 100 mg orally [1]. Thus, understanding the cause and mechanism of niacin-induced vasodilation can have important clinical relevance with regard to the use of niacin as a hypolipidemic agent. Previous studies had shown that niacin-induced vasodilation can be greatly attenuated by pretreatment with cyclooxygenase inhibitors, implicating a role for prostaglandins. However, the prostaglandin responsible for the vasodilation had not been clearly established until we recently reported that the ingestion of niacin in humans selectively resulted in 400 to 800-times increases in the endogenous release of prostaglandin (PG) D\textsubscript{2}, a potent vasodilator [2].

The above finding implicated PGD\textsubscript{2} as the mediator responsible for niacin-induced vasodilation. However, the site from which the PGD\textsubscript{2} is released is not known. We previously had demonstrated that the human mast cell produces large quantities of PGD\textsubscript{2} and that PGD\textsubscript{2} is released along with histamine following mastocyte activation [3]. However, we found that the release of PGD\textsubscript{2} after ingestion of niacin is not accompanied by a release of histamine, suggesting that the origin of the PGD\textsubscript{2} is unlikely to be the mast cell [2].

Previous studies have shown that topical administration of the methyl ester and other esters of niacin cause marked local cutaneous vasodilation that can be inhibited by inhibitors of the cyclooxygenase enzyme [4,5]. Thus we investigated the possibility that niacin activates a cell or cells in the skin that releases PGD\textsubscript{2}, and explored the contribution of the skin to the total endogenous release of PGD\textsubscript{2}, which occurs following oral administration of niacin. The results of these studies suggest that the skin is a major site from which PGD\textsubscript{2} is released following ingestion of niacin.

**MATERIALS AND METHODS**

**Clinical Protocols** Healthy volunteers who were taking no medication (ages 31 to 46 years old) were recruited for the study and gave informed consent. Each volunteer had a circumferential 6-inch wide patch of coarse porosity, ashless grade filter paper saturated with 10\textsuperscript{-4} M aqueous methyl nicotinate applied to a forearm 2 inches below the elbow. This concentration of methyl nicotinate has been found to produce maximal cutaneous vasodilation [4]. The filter paper was removed after 5 min. Blood was obtained before methyl nicotinate application and at 5, 10, 15, 20, and (in one case) 30 min after methyl nicotinate application from the antecubital vein of the treated arm. Blood also was obtained after 10 min from the...
antecubital vein of the contralateral untreated arm in two of the volunteers. In two other volunteers, blood was withdrawn from treated and contralateral arms 10 min after methyl nicotinate application only. The blood was immediately placed in ice cold tubes containing EDTA and 10^-6 M indomethacin. Following centrifugation, the plasma was removed and subsequently assayed for prostaglandins and histamine.

In the dose-response study, concentrations of methyl nicotinate ranging from 10^-5 to 5 x 10^-4 M were applied to the forearm of three volunteers using the technique described above. Blood was obtained from the antecubital vein after 10 min for prostaglandin determinations. At least 3 d were allowed to elapse between applications.

In three volunteers, 500 mg of niacin was administered orally and blood was obtained for prostaglandin measurements simultaneously from the brachial artery and antecubital vein of the same arm before and 30 min after ingestion.

Materials Nicotinic acid methyl ester (methyl nicotinate) was purchased from Sigma Chemical Co. (St. Louis, MO). Niacin for oral ingestion was supplied by Vanderbilt Hospital Pharmacy. [H3]PGD2 and [H3]9α,11β-PGF2α were prepared as previously described [6]. [α,β,γ,δ,Ψ,Ψ]Histamine dihydrochloride was purchased from MSD Isotopes (Montreal, Canada).

Quantification of Prostaglandins and Histamine PGD2 and 9α,11β-PGF2α were quantified in 3 ml of plasma by stable isotope dilution assays using gas chromatography negative ion chemical ionization mass spectrometry (GC NICI/MS) as previously described [6]. Histamine was quantified in plasma (2 ml) by mass spectrometric assay as described [7].

RESULTS

Clinical Symptoms and Signs Topical administration of 10^-1 M methyl nicotinate to the forearms of three volunteers resulted in the rapid onset (within 2 min) of intense cutaneous erythema, which was limited to the site of application. The erythema became maximal between 10 and 20 min and then began to subside after approximately 30 min, similar to what had been described previously [4]. When varying doses of methyl nicotinate were tested, no cutaneous erythema was apparent below doses of 10^-3 M, which caused slight erythema. More intense vasodilation occurred with a dose of 10^-2 M and the erythema appeared maximal at the dose of 10^-1 M.

The individuals to whom niacin (500 mg) was administered by mouth experienced the onset of intense flushing within 10 min after ingestion. The flushing was maximal at approximately 30 min and subsided within 90 minutes.

Endogenous Prostaglandin and Histamine Release Following Topical Methyl nicotinate Figure 1A shows the results of serial measurements of PGD2 in blood obtained from the antecubital vein draining the site of methyl nicotinate application in the three volunteers studied. Circulating PGD2 levels in normal humans are in the sub-picogram/ml range [8]. Topical methyl nicotinate administration evoked a rapid and dramatic increase in blood levels of PGD2 that correlated temporally with the cutaneous vasodilation. In the three individuals, the peak levels of PGD2 measured were increased 58, 59, and 122 times above baseline. Concomitant measurements were made of the levels of the PGD2 metabolite 9α,11β-PGF2α, which reached a maximum approximately 5 min later than the peak levels of PGD2 (Fig 1B).

The above results suggested that topical application of methyl nicotinate to the skin causes a release of PGD2 from the site of application. However, the possibility remained that the PGD2 levels measured in the venous blood draining the treated arm might arise from systemic absorption of the methyl nicotinate and subsequent release from distal sites. Although this seemed very unlikely in view of the fact that the cutaneous erythema is limited to the site of application of methyl nicotinate, we addressed this possibility by measuring PGD2 levels in blood obtained from the antecubital vein of the untreated arm contralateral to the arm treated with methyl nicotinate in four volunteers. In contrast to the markedly increased levels of PGD2 and 9α,11β-PGF2α measured in the plasma draining the treated site, no increases in the levels of these prostaglandins were found in blood obtained from the untreated arm (Table 1). These results also indicated that the increased levels of 9α,11β-PGF2α measured in plasma obtained simultaneously from the antecubital veins of an arm treated with topical methyl nicotinate and the contralateral arm.

Table 1. Levels of PGD2 and 9α,11β-PGF2α in Plasma Obtained Simultaneously from the Antecubital Veins of an Arm Treated with Topical Methyl nicotinate and the Contralateral Arm

<table>
<thead>
<tr>
<th></th>
<th>Treated Skin</th>
<th>Untreated Skin</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>PGD2</td>
<td>9α,11β-PGF2α</td>
</tr>
<tr>
<td>Volunteer 1</td>
<td>430</td>
<td>131</td>
</tr>
<tr>
<td>Volunteer 2</td>
<td>21,500</td>
<td>287</td>
</tr>
<tr>
<td>Volunteer 3</td>
<td>112</td>
<td>45</td>
</tr>
<tr>
<td>Volunteer 4</td>
<td>25,000</td>
<td>468</td>
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*Prostaglandin levels are expressed as pg/ml plasma. Detection limits of assays are 5 pg/ml for PGD2 and 4 pg/ml for 9α,11β-PGF2α.
Figure 2. Relationship between different concentrations of methyl nicotinate applied topically to the forearm of three volunteers and the concentration of 9α,11β-PGF₂ measured in venous blood draining the treated site. Each individual is represented by a different line.

...indicated did not arise from metabolism of PGD₂ at sites distant to the skin. This implies that human skin contains substantial 11-ketoreductase activity that converts PGD₂ to 9α,11β-PGF₂.

We then examined the dose-response relationship between topically applied methyl nicotinate and PGD₂ release. Topical application of varying concentrations of methyl nicotinate resulted in a dose-dependent increase in the release of PGD₂ in three volunteers, indicated by increasing plasma levels of 9α,11β-PGF₂ (Fig 2).

Although the human mast cell produces large quantities of PGD₂ and releases histamine following mastocyte activation [3], we previously demonstrated that the release of PGD₂ following oral administration of niacin was not accompanied by a release of histamine [2]. This finding suggested that the mast cell is unlikely to be the cellular source of PGD₂ release following the ingestion of niacin. Further support for this notion was obtained from measurements of histamine concentrations in venous blood draining the site of topically applied methyl nicotinate in three of the volunteers. There was no increase in the histamine content of venous plasma (pretreatment level 0.37 to 0.61 ng/ml compared to 0.34 to 0.51 ng/ml at the peak of erythema, normal less than 1.00 ng/ml) despite 97 to 5000-times increases in PGD₂ (pretreatment level less than 5 pg/ml in all volunteers compared to 487 to 25,000 pg/ml at the peak of erythema).

Cutaneous Release of PGD₂ Following Oral Administration of Niacin Because the results of the above studies indicated that topical methyl nicotinate results in the release of large quantities of PGD₂ from the skin, we examined the possibility that the skin might also be a major site from which PGD₂ is released following oral administration of niacin. This possibility was addressed in the following way. Three volunteers were given 500 mg of niacin orally and blood was obtained 30 min later from an antecubital vein. Simultaneously, blood was obtained from the brachial artery of the same arm. If the skin is a major site from which PGD₂ is released following oral ingestion of niacin, then a large arteriovenous concentration gradient in levels of PGD₂ measured should be found with much higher concentrations present in venous blood draining the skin compared to arterial blood. Levels of PGD₂ were found to be approximately 14 to 1200 times higher in the venous circulation than in the arterial circulation (Fig 3A). Less than 5 to 750 pg/ml of PGD₂ were present in arterial blood at 30 min when the level measured in venous blood ranged from 3,100 to 10,600 pg/ml. A large arteriovenous difference in levels of 9α,11β-PGF₂ was also present ranging from 17 to 83 times (Fig 3B), consistent with the results discussed previously using topical methyl nicotinate, which indicated that substantial 11-ketoreductase activity is present in skin.

DISCUSSION

In recent studies, we showed that PGD₂ is the mediator responsible for the cutaneous vasodilatation that occurs following ingestion of niacin. However, the tissue source of the PGD₂ release was not known. Others had previously reported that topical methyl nicotinate also causes cutaneous erythema at the site of application [4]; therefore, we examined whether niacin activates a cell or cells in...
human skin to release PGD₂. We found that indeed topical methyl-nicotinate evoked a release of large quantities of PGD₂ from the site of application in a dose-dependent fashion. Knowing that some cell(s) in the skin is activated by niacin to produce PGD₂ led us then to examine the degree to which the skin may contribute to the total endogenous release of PGD₂ when niacin is ingested orally. From these studies emerged the intriguing finding that whereas extremely high concentrations of PGD₂ (several thousand pg/ml) were detected in superficial venous blood draining skin following oral ingestion of niacin, comparatively trivial quantities (14 to 1200 times less) were present in the arterial circulation supplying the skin. These results indicate that the skin is a major site from which PGD₂ is released following oral administration of niacin and that the flushing that occurs following oral ingestion of niacin results from a local release of PGD₂ in skin.

This information provides the impetus to pursue the identity of the cell(s) in the skin that is activated by niacin to produce PGD₂, and explore the cellular mechanism by which this occurs. We previously reported that the mast cell is unlikely to be the cell from which PGD₂ is released because histamine is not also released [2]. This was further confirmed in the present study by finding that histamine levels did not increase in venous blood draining the arm on which methyl nicotinate had been topically applied.

Although the cell in the skin that is activated by niacin to release PGD₂ is not known, the recent findings reported by Urade and colleagues are of some interest [9]. They demonstrated that the cells in the skin of rats that contain the highest concentration of PGD synthase and thus have the greatest capacity for production of PGD₂ are the dermal macrophages and Langerhans cells. However, whether human skin is analogous to the skin of the rat in this regard is not known. Regardless, in the search for the niacin-responding cell, the finding that the release of PGD₂ appears highly selective for skin may provide a valuable clue. This selectivity would suggest that the niacin-responding cell is either a cell that is unique to the skin and is not found in other tissues or a cell that in the skin is functionally different than its counterpart present in other organs.

In summary, we report the finding that the skin is a major site from which PGD₂ is released following oral ingestion of niacin. Future studies aimed at identifying the cell in the skin that responds to niacin and the mechanism by which it is activated by niacin will be of interest and of potential clinical relevance regarding the use of niacin as a hypolipidemic agent in humans.

We wish to thank Tanya Minton and William Zackert for their technical assistance and Amanda Simpson for her secretarial contribution.

REFERENCES

MEETING ANNOUNCEMENT

The 4th Meeting of the European Society for Pigment Cell Research will be held September 17–20, 1992 at the University Medical Center Steglitz, The Free University of Berlin, Hindenburgdamm 30, 1000 Berlin 45, Germany. Congress Organization: Docent C. Garbe, M.D., Department of Dermatology, University Medical Center Steglitz, The Free University of Berlin, Hindenburgdamm 30, 1000 Berlin 45, Germany. Tel., 030-7982769 or 7982808; Fax, 030-798 41 41.