BRIEF REPORT

Enhancing capillary blood collection: The influence of nicotinic acid and nonivamide

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Background: Nicotinic acid and nonivamide are often applied topically during capillary blood collection to induce vasodilation. These molecules may have an influence on immune effector cell activity in nearby tissues. This study investigates whether the induction of flushing by nicotinic acid and nonivamide causes an inflammatory response that influences the composition of immune cells present in a capillary blood sample.

Methods: Females aged between 18 and 30 years old provided capillary blood samples. Experimental samples were taken from an earlobe treated with nicotinic acid and nonivamide with controls obtained from the untreated earlobe. Immunophenotypic analyses were conducted using polychromatic flow cytometry to determine whether any changes occurred in leucocyte subpopulations (CD3, CD4, CD8, CD19, CD56, and CD14) and granulocytic functional-related surface antigen markers (CD11b, CD18, CD16b, and CD66b).

Results: No significant differences were observed between experimental and control samples in the mean percent of parent for the lymphocyte, monocyte, or granulocyte subpopulations, or in the median fluorescence intensity of particular surface markers expressed on these leucocytes.

Conclusion: The topical application of nicotinic acid and nonivamide is a possible method to improve capillary blood collection for immunological assessments. The use of these agents may increase the safety and compliance of patients who suffer from needle phobia or are unable to provide venous blood samples.

KEYWORDS

B cells, blood testing, capillary blood, Finalgon, flow cytometry, leukocytes, nicotinic acid, nonivamide, T cells, Venous Blood

Modern laboratory techniques require decreasing blood volumes for tests. For example, a biosensor test strip is able to measure β -hydroxybutyrate at very high accuracy in diabetes patients using only 1.5 μ L of capillary blood,¹ and a multiplexed volumetric bar-chart chip (V-Chip) is able to complete an ELISA reaction using a 10 μ L sample to detect serum biomarkers.² As technology develops, the number of tests that use miniscule amounts of blood will likely continue to increase.

Currently, the fingertips, earlobes, and heels are the most common regions for capillary blood collection although rapid clotting mechanisms and poor circulation may make capillary blood collection difficult

in some patients. Techniques for enhancing the extraction of blood from these sites are therefore often utilized, including the application of rubefacient cream, in order to induce vasodilation and increase local blood flow.

The topical cream used in this study contains butoxyethyl nicotinate and nonivamide.^{3,4} Nicotinate is the anionic form of nicotinic acid (niacin).⁵ The blanket term vitamin B3 is used for both nicotinic acid and niacinamide, and these can be converted into each other within an organism.⁶ Nicotinic acid induces skin flushing and itching, which is thought to be induced by the release of prostaglandin D₂ (PGD₂) since this prostaglandin is elevated following nicotinic acid administration.⁷

Papaliodis et al.⁸ report that a derivative of nicotinic acid is capable of stimulating release of PGD₂ from mast cells. During allergy states, this prostanoid induces a vasodilatory response, increases vessel permeability, and mediates the activity of effector cells such as helper T cells, eosinophils, basophils, mast cells, and dendritic cells.⁹ Furthermore, in combination with cysteinyl leukotriene E₄ (LTE₄), PGD₂ is capable of altering adhesion, migration, and cytokine production by helper T cells.¹⁰

Many topical creams used to enhance capillary blood collection also contain the capsaicinoid organic compound nonivamide, which promotes vasodilation by inducing the release of both polypeptides substance P (SP) and calcitonin-gene-related peptide.¹¹ The latter promotes increased concentration of inflammatory cells in the inflamed area and enhances neutrophilic adhesion to the endothelium.¹² SP is an important element of the immune system and is synthesized by macrophages and lymphocytes. Synthesis of SP by these immune cells is dependent on immune regulation.¹³ In mice, SP is responsible for the induction of granulocyte infiltration in skin through degranulation of mast cells and activation of vascular endothelial cells.¹⁴ Specifically, it has been found that subcutaneous injection of SP in mice results in neutrophil and eosinophil infiltration in the skin, along with increased vascular permeability of endothelial cells.¹⁵

Previous studies have investigated the effects of nicotinic acid on altering blood composition in relation to oxygen saturation testing and fibrinolytic activity.^{4,16,17} For example, Canetti et al.¹⁸ recently demonstrated a trend in greater production of reactive oxygen species by neutrophils obtained from a capillary sample compared to the gold standard venous blood sample. This study employed the use of nicotinic acid and nonivamide and highlighted that the observed increase, while non-significant, could be attributed to the use of nicotinic acid to obtain the capillary samples. A significant increase in neutrophilic CD11b and CD18 expression in capillaries after application of nicotinic acid and nonivamide compared to the venous samples was observed.

However, it is not yet clear whether the flushing response induced by nicotinic acid and nonivamide causes an inflammatory response that influences the composition of immune cells present in a capillary blood sample. We therefore investigated whether nicotinic acid induces immunological changes in capillary blood samples, hypothesizing that there would be a greater migration of leucocytes into the capillary bed following application of nicotinic acid and nonivamide.

Capillary blood was collected from female students 18-30 years old, using a sterile, single-use safety lancet (BD Bioscience). An experimental sample and a control sample were collected from each participant. The experimental sample was collected from an earlobe treated for 5 minutes with nicotinic acid and nonivamide and the control sample collected from the opposite, clean, untreated earlobe. A sample of 100 µL was collected from experimental and control earlobes using a heparin-coated capillary blood collection system (Kabe Labortechnik). Informed consent was obtained from all participants. The study was approved by Bond University Human Research Ethics Committee.

Capillary-obtained whole blood was aliquoted into a polystyrene tube (BD Biosciences, cat: 352052) for the analysis of lymphocyte subpopulations and into a separate tube for analysis of functional-related

surface antigens expressed on mono- and polymorphonuclear subpopulations. In total, 10 control and 10 experimental samples were investigated in the study, with surface antigen markers CD3, CD4, CD8, CD19, CD56, CD14, CD11b, CD18, CD16b, and CD66b analyzed from each.

Staining was conducted as per manufacturer instructions, with measurements adjusted for 20 µL. Whole blood samples were processed to maintain the appropriate concentration and ensure antibody saturation. Following staining with selective antibodies, each tube was treated with an NH₄Cl lysing solution (500 µL at 1:10 dilution) and incubated in the dark for 10 minutes. Once red blood cells were lysed, the tubes were washed with phosphate buffered saline (PBS), centrifuged to remove red cell fragments, and supernatant removed. The leucocytes were resuspended in 300 µL PBS and immediately processed. Flow cytometry was performed on a FACSVerse Flow Cytometer with FACSuite software (Becton-Dickinson, Erembodegem, Belgium), after performance quality control was performed.

A manufacturer-recommended isotype control for each antibody was run prior to sampling, and gates for the experiment were subsequently defined. Furthermore, a non-stained blood sample was run as a negative control. Leucocyte subpopulations were gated based on their forward scatter (FSC) vs side scatter (SSC) properties. In Tube 1, CD marker-dependent quadrant-gating determined the percentage of each lymphocyte subpopulation and a histogram was used to determine changes in surface antigen expression (measured through changes in median fluorescence intensity) between control and experimental samples. In tube 2, granulocyte and monocyte populations were gated and analyzed as detailed previously.

Differences between the values were determined using a Student's paired *t*-test. Significance was set at *P* < .05.

The population of lymphocytes, granulocytes, and monocytes as a percentage of total events collected was evaluated by flow cytometric analysis. No significant differences were observed between experimental samples and controls in expression of surface markers CD18, CD16, CD66b, CD11b, CD14, and CD71 on leucocytes in median fluorescence intensity (MFI); the mean percent of parent for granulocytes expressing the same surface markers; or the mean percent of parent for monocytes expressing CD18, CD11b, CD14, and CD7.

Treatment of earlobes with nicotinic acid and nonivamide also did not induce significant changes in the mean percent of parent for helper T cell (CD3⁺CD4⁺), cytotoxic T cell (CD3⁺CD8⁺), NK cell (CD3⁺CD56⁺), and B cell (CD3⁺CD19⁺) subpopulations of lymphocytes, as compared with control samples. The CD71⁺ lymphocyte subpopulations likewise showed no significant differences between the experimental and control samples in the mean percent of parent (Table 1).

It has previously been suggested that nicotinic acid and nonivamide topical cream may modulate the activity of local immune cells, including granulocytes and TH₂ cells.^{9,10} However, our study identified no influence of this mechanism of enhancing capillary flow on either the local leucocyte subpopulations or granulocytic functional-related surface antigen markers.

Moreover, no significant differences were detected between the MFI of surface markers expressed on lymphocytes, monocytes, and

TABLE 1 Median fluorescence intensity (AU) for granulocytes, monocytes, and lymphocytes. Values expressed as mean ($\pm$ SD), n=10 for each

	Control sample	Experimental sample	P value
Granulocytes			
CD18+	3410.3 $\pm$ 1335.3	3616.2 $\pm$ 1332.1	.249
CD16+	94 387.7 $\pm$ 25 806.3	98 013.6 $\pm$ 25 519.1	.161
CD66b+	1410.4 $\pm$ 444.7	1558.6 $\pm$ 547.5	.121
CD11b+	22 643.7 $\pm$ 9262.2	24 327 $\pm$ 10 000.7	.204
CD14+	203.2 $\pm$ 125.0	194.1 $\pm$ 98.8	.605
CD71+	339.8 $\pm$ 141.9	375.4 $\pm$ 207.9	.208
Monocytes			
CD18+	7942.1 $\pm$ 2719.8	7622.3 $\pm$ 2559.9	.429
CD11b+	42 708.4 $\pm$ 13 465.5	38 916.9 $\pm$ 15 597.7	.088
CD14+	1864.1 $\pm$ 837.1	1913.8 $\pm$ 819.2	.361
CD71+	722.8 $\pm$ 228.7	683.4 $\pm$ 286.0	.386
Lymphocytes			
CD18+	980.5 $\pm$ 260.4	1008.2 $\pm$ 213.0	.389
CD11b+	1140 $\pm$ 407.9	1116.2 $\pm$ 464.6	.543

granulocytes in control and experimental samples. Because the surface antigens included in the MFI analysis are associated with cellular activity, it was expected that any changes in MFI (expression) of these markers would also represent changes in cellular activity. The absence of changes was surprising since Xue et al.¹⁰ studied the role of PGD₂ and LTE₄ in activation of helper T cells and reported the subsequent downstream activation of effectors such as neutrophils. It was expected that increases in expression of markers including CD71, CD18, and CD11b might occur as CR3 and transferrin receptor expression are known to increase in activated leucocytes, particularly lymphocytes. In addition, it was thought that up-regulation of host defense by particular leucocytes, especially via antigenic expression of CD14, CD16, and CD66b, might occur.

Side effects of using nicotinic acid and nonivamide may include burning at the application site, hypersensitivity (eg, swelling of the face or localized tingling, rashes, pain, or blistering), anaphylaxis, paraesthesia, cough, and dyspnea.^{7,19} However, none of the participants in the study either reported adverse events or withdrew their participation due to experiencing adverse events.

Our study was limited to female subjects and investigated 11 cell surface markers. As immunological differences exist between genders,²⁰ further studies would be recommended. Nevertheless, the study suggests that in situations where venous sampling is impractical (eg, due to resource limitations) or unnecessary (low blood volume required), the use of nicotinic acid to increase the yield of blood collected during capillary extraction for use in immunological investigations is effective and viable. This is further supported by absence of adverse effects in participants following the application of nicotinic acid and nonivamide.

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