SHORT REPORT: CYCLOOXYGENASE 2 EXPRESSION IN VESSELS AND NERVES IN REVERSAL REACTION LEPROSY

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Abstract. Tissue expression of cyclooxygenase (COX)2, an inducible enzyme synthesizing eicosanoids in inflammation, was studied in reversal reaction (RR) leprosy in comparison with nonreactionary leprosy. COX2 was consistently expressed in cells of the mononuclear-macrophage lineage across the leprosy spectrum. Only in RR, the following two additional sites showed COX2 expression in the dermis and subcutis: 1) microvessels and 2) nerve bundles and isolated nerve fibers. The same sites also express vascular endothelial growth factor (VEGF). This is in keeping with experimental models relating VEGF to COX2 expression, with VEGF enhancing prostaglandin production through COX2 stimulation and prostaglandin synthase expression. We postulate that selective COX2 inhibitors, which are currently used in several inflammatory conditions, could be considered for RR treatment to reduce acute symptoms caused by tissue edema and possibly prevent long-term nerve damage, the main complication of RR.

Reversal reaction (RR, type-1 reaction) can occur in the borderline (BT-BL) area of the spectrum of leprosy to alter the chronic, uneventful course of the disease. It consists of acute episodes, which may involve skin and/or nerves and often amount to medical emergencies demanding immediate treatment to prevent progression of nerve damage to the irreversible stage. The underlying immunologic alteration is a localized Th1 cell-mediated immune reaction against mycobacterial antigens. In a previous work,1 we have shown that vascular endothelial growth factor (VEGF) is a major player in phophate-buffered saline was applied to define the leprosy diagnosis across the leprosy spectrum. Only in RR, the following two additional sites showed COX2 expression in the dermis and subcutis: 1) microvessels and 2) nerve bundles and isolated nerve fibers. The same sites also express vascular endothelial growth factor (VEGF). This is in keeping with experimental models relating VEGF to COX2 expression, with VEGF enhancing prostaglandin production through COX2 stimulation and prostaglandin synthase expression. We postulate that selective COX2 inhibitors, which are currently used in several inflammatory conditions, could be considered for RR treatment to reduce acute symptoms caused by tissue edema and possibly prevent long-term nerve damage, the main complication of RR.

Skin biopsies from seven patients with RR (six BT and one BL) were retrieved from the files of the Italian Reference Center for Leprosy of Genoa. Additional leprosy biopsies without histologic signs of RR were retrieved from seven patients with BT, three with BL, and four with LL. The above 21 biopsies were subsequent entries in our Registry. The specimens had been fixed in 10% formaldehyde for 24 hours. Sections were stained with hematoxylin and eosin for general microscopic examination and a modified Fite-Faraco procedure for M. leprae. The Ridley-Jopling leprosy classification scheme2 was applied to define the leprosy diagnosis across the spectrum of the disease. In particular, RR was recognized histologically when conspicuous edema with dispersion of the granuloma and foreign body type giant cells were present.

After routine deparaffinization and rehydration, immunochemistry was performed on 5-μm sections of paraffin-embedded tissue mounted onto slides coated with polylysine and subjected to microwave antigen retrieval in sodium citrate 10 mmol/L, pH 6/0.05% Tween 20. Endogenous peroxidase was blocked with 3% H2O2 in phosphate-buffered saline (PBS) 10 mmol/L followed by immersion in 10% goat serum. The antibody was anti-COX2 monoclonal (Cayman Chemical, Ann Arbor, MI) at 1:80 dilution (starting at 0.5 mg/mL) in PBS 10 mmol/L/5% goat serum. The antibody was layered onto the sections at 4°C overnight. Detection was performed with polyclonal goat antimouse antibody biotinylated and diluted 1:300 in PBS 10 mmol/L using the StreptABC/HRP kit and diaminobenzidine (Dako Cytomation, Glostrup, Denmark) as substrate solution. Sections were counterstained with Mayer’s hematoxylin. Sections without primary antibody were prepared as negative controls. Sections of lichen planus were used as positive controls for COX2 expression.

COX2 was consistently expressed in cells of the mononuclear–macrophage lineage, especially macrophages and epithelioid cells. The granuloma cells were positive across the spectrum of leprosy, from the vacuolated macrophages of the lepromatous area to the predominantly epithelioid cell infiltrate of the tuberculoid area. RR specimens showed positivity of cells of mononuclear–macrophage lineage in a fashion similar to that of specimens pertaining to the rest of the leprosy spectrum. The accompanying lymphocytes were negative. COX2 positivity in the cells of mononuclear–macrophage lineage of the leprosy granuloma has already been reported by Kiszewski and others,4 who claim that it is more pronounced in lepromatous cases.

In our experience, the characteristic finding in cases of RR was COX2 expression in the vessels and nerves of the dermis and subcutis. Microvessels, especially the tall-endothelium ones implied in vascular dilation and tissue edema, were distinctly positive in RR, in contrast to the vessels encountered in nonreactionary leprosy (Figure 1). In addition, nerve bundles and isolated nerve fibers were distinctly positive for COX2 (Figure 2). In cases of RR, our group previously described positivity for VEGF in the vessels and, at a lower degree, in the nerves.1 Vascular changes, leading to tissue edema, characterize RR at both early and conlamate stages of development. Edema, which is minimal in nonreactionary leprosy, is a major diagnostic marker of RR. It starts in the early phase and is most conspicuous in the conlamate phase of RR.5 With progression of RR, edema occurring at nerve fibers and bundles may lead to permanent nerve damage, the most important, long-term sequela of RR.

Experimental models support the view that VEGF and COX2 expression are related events. The VEGF enhancement of PG production through COX2 stimulation and PG

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synthase expression is associated with a dose-dependent increase in the expression of COX2 and a parallel membrane-associated PG synthase mRNA increase in cell culture. High PG levels induce vasodilation, a main feature of RR, pain, and inflammation. COX2 is an immediate-early gene product, which is undetectable in normal tissues, but can be readily induced in response to cell activation by cytokines, growth factors, and tumor promoters. Furthermore, treatment with the COX2 inhibitor NS-398 abolishes the VEGF-enhanced prostaglandin synthase expression. Finally, PG2 stimulates VEGF expression. These considerations suggest that selective COX2 inhibitors, which are currently used in several inflammatory conditions, could be considered for RR treatment, particularly at its early stage, to reduce acute symptoms and possibly prevent long-term nerve damage. Furthermore, they could be useful to prevent RR recurrence in unstable forms of the disease.

Note: Supplemental figures of non-reactional BT leprosy can be found online at www.ajtmh.org.

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Figure 1. Tall-endothelium microvessels of the granuloma show positivity for COX2. The leprosy granuloma is disrupted by edema in this case of conclamate RR. Magnification, ×400. This figure appears in color at www.ajtmh.org.

Figure 2. In addition to microvessels, two edematous nerve bundles, associated with the granuloma, also show COX2 positivity. Magnification, ×400. This figure appears in color at www.ajtmh.org.