

EXPRESSION AND MODIFICATION OF NO SYNTHASE IN HUMAN DENTAL PULPS DURING ORTHODONTIC TREATMENT

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RESUME

Au cours des dernières années notre laboratoire d'histologie a travaillé en coopération avec Winchmore Hill Dental Practice à Londres dans le domaine de la détection du NOS et ses variations sur la pulpe dentaire (1) (2) (3). Cette étude a été menée sur des échantillons provenant de dents traitées du point de vue orthodontique et dents non-traitées. Les résultats suggèrent une stricte corrélation entre la durée de la traction orthodontique et l'expression du susmentionné neurotransmetteur (NO).

ABSTRACT

In the last few years our histology laboratory has worked in collaboration with Winchmore Hill Dental Practice in London in studying nitric oxide synthase (NOS) protein expression by the use of immunohistochemistry in dental pulps following orthodontic treatment (A. Gerbino et al. 2000; A. Gerbino et al 2001; A. Gerbino et al 2001.) The study has been carried out on samples taken from orthodontically treated and non-orthodontically treated teeth. The results suggest a close correlation between the duration of the orthodontic traction and the expression of the above-mentioned neurotransmitter (NO).

INTRODUCTION

Our laboratory, in collaboration with orthodontics research groups, has focused on examining whether orthodontic treatment involving the application of forces of varying intensity could somewhat affect the histophysiology of the dental pulp. To this end, we have previously studied the expression of a variety of enzymes and neurotransmitters using both histochemical and immunohistochemical analysis.

Our published results showed (Buscemi et al. 1993) that AchE did not vary according to the duration and intensity of applied orthodontic forces. As far as substance P, endorphins, enkephalins and CGRP are concerned, they were detected only after short-term treatment but disappeared after seven days, showing that the pulp could adjust itself to the effects of the orthodontic practice. We then decided to investigate the expression of nNOS and iNOS in order to develop our understanding of NOS immunoreactivity in pulp during orthodontic treatment.

NOS (Degnim et al. 1996) is the intracellular constitutive enzyme for the synthesis of NO. Four different isoforms of this enzyme have been detected, isolated, cloned and sequenced so far: neuronal NOS (nNOS) and endothelial NOS (eNOS) are found in all cell types; hepatic NOS (hepNOS) and macrophageal NOS (macNOS/iNOS) are inducible forms produced in response to inflammation (M. Harren et al. 1998).

MATERIAL AND METHODS

The pulps have been divided into two groups:

GROUP A: control pulps removed from bicuspid extracted for orthodontic reasons;

GROUP B: pulps removed from teeth subjected to orthodontic forces using the STRAIGHT WIRE technique, with Nickel Titanium and stainless steel archwires. The teeth, all upper or lower bicuspid, were extracted from patients of both sexes, aged 11-13, under anaesthesia with carbocaine.

The extractions were performed 7 days, 14 days, 3 months, 6 months and 14 months after the beginning of the orthodontic treatment.

All pulps were then placed in Bouin's fixative for immunohistochemical analysis and stained using haematoxylin-eosin for morphological control. After fixation the pulps were embedded in paraffin and 7 μ m-thick sections were cut. We used anti-nNOS and anti-iNOS polyclonal antibodies (Transduction Laboratories) for the immunohistochemical assay and the Ultrastain Polyvalent Strept ABC-HRP Kit for the detection work. All sections were mounted using Dako Faramount Aqueous Mounting Medium.

RESULTS

Immunohistochemical reactivity for nNOS:

Control pulps (i.e., pulps of untreated, intact teeth extracted for orthodontic reasons):

nNOS was detected in odontoblasts, vessels walls and in the pulp cells as well (Fig. 1).

Treated pulps:

– after six-month traction nNOS was detected both in vessels and in the parenchymal tissue (Fig. 2);

– after 14-month traction nNOS had the same localisation as in previous cases (Fig. 3).

Immunohistochemical reactivity for iNOS:

Control pulps:

the absence of iNOS expression (Fig. 4) was related to the well-preserved pulpal structure (Fig. 5).

Treated pulps (i.e., subjected to orthodontic forces):

– after three-month traction vessel walls were found positive for iNOS (Fig 6);

– after six-month traction iNOS immunohistochemical reactivity was detected in nervous fibres (Fig. 7);

– after 14-month traction increased reactivity was found, reaching its peak in odontoblasts (Fig. 8) and at the vascular and nervous levels (Fig. 9). The pulpal structure appears well-preserved on optical microscopy (Fig. 10).

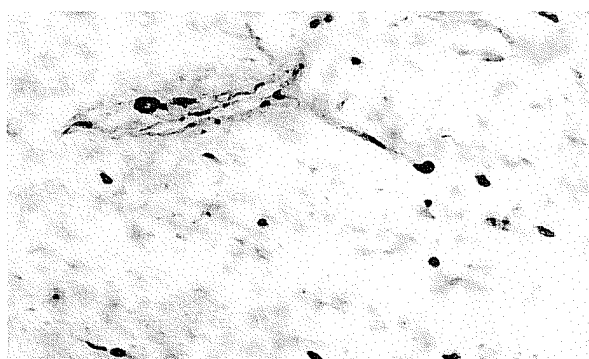


Fig. 1

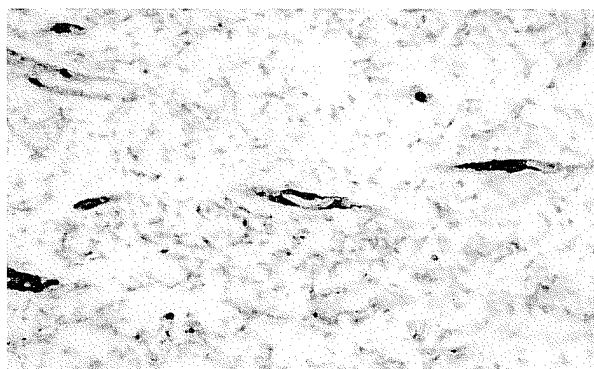


Fig. 2

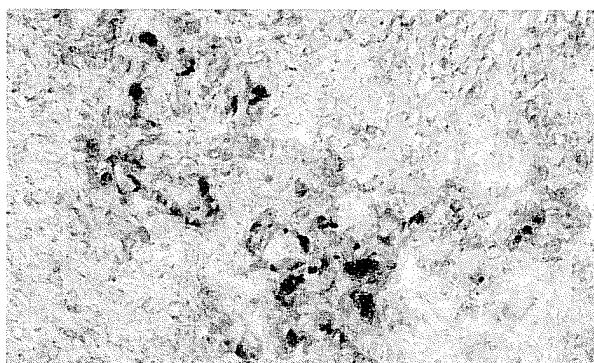


Fig. 3

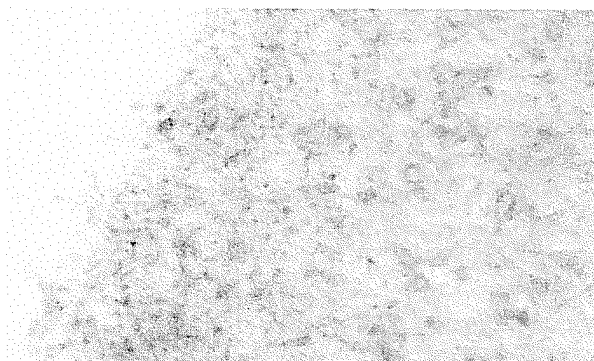


Fig. 4

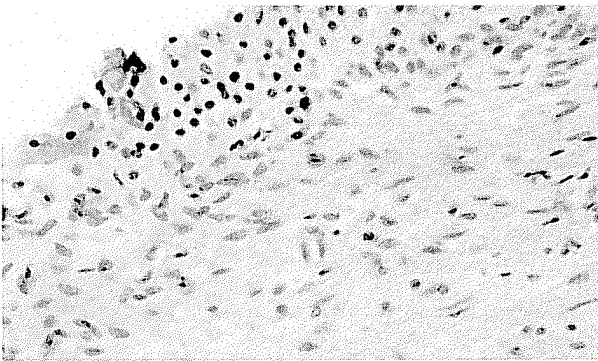


Fig. 5

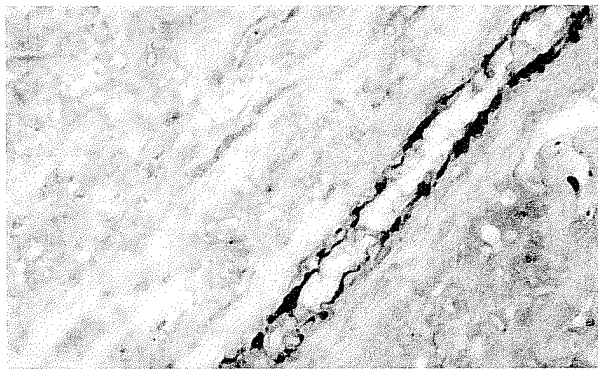


Fig. 6

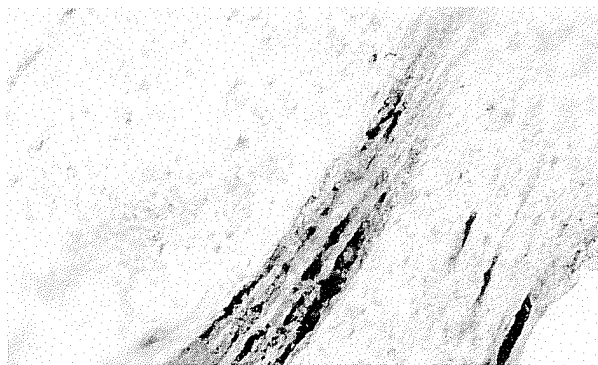


Fig. 7

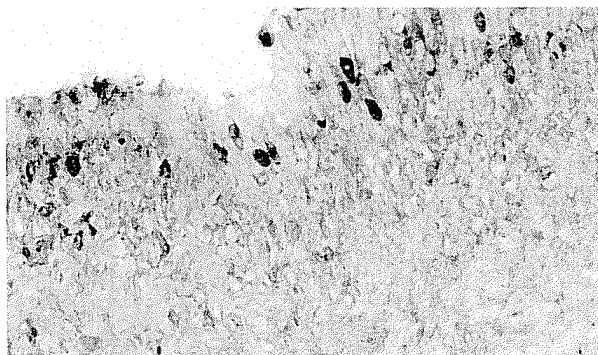


Fig. 8

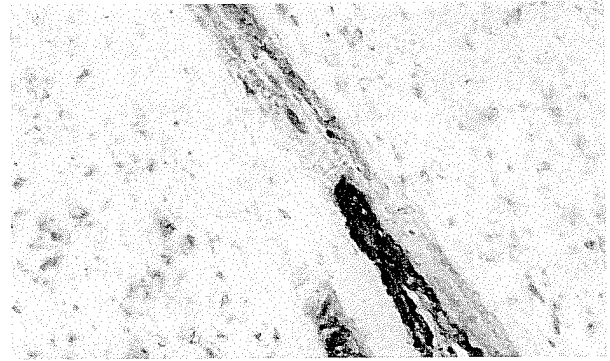


Fig. 9

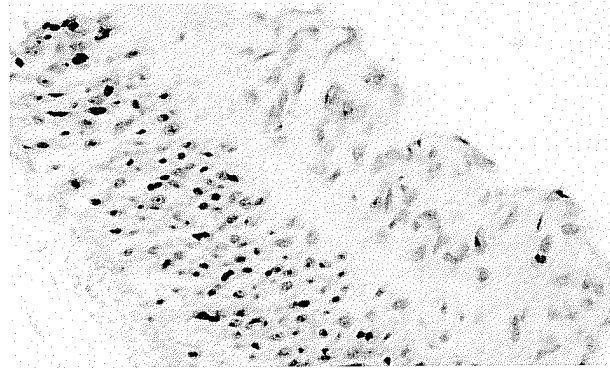


Fig. 10

Fig. 1: Pulp not subjected to orthodontic forces: nNOS is expressed in parenchymal cells and vessel walls. 40x

Fig. 2: Pulp after 6-month orthodontic traction: nNOS is expressed in vessel walls and parenchymal cells. 20x

Fig. 3: Pulp after 14-month orthodontic traction: nNOS is expressed in the pulp parenchyma. 20x

Fig. 4: Pulp not subjected to orthodontic forces: no nNOS expression was found. 20x

Fig. 5: E.E. Pulp not subjected to orthodontic forces: odontoblastic layer and cell-free zone of Weil. 40x

Fig. 6: Pulp after 3-month orthodontic traction: iNOS expression in a vessel wall. 40x

Fig. 7: Pulp after 6-month orthodontic traction: iNOS expression in a nervous fibre. 20x

Fig. 8: Pulp after 14-month orthodontic traction: iNOS expression in odontoblasts. 40x

Fig. 9: Pulp after 14-month orthodontic traction: iNOS is markedly expressed in vascular structures. 40x

Fig. 10: E.E. Pulp after 14-month orthodontic traction: the structures are perfectly preserved. 40x

CONCLUSIONS

The immunohistochemical analysis of treated and untreated pulps showed that nNOS was expressed in all pulps, including control and treated samples. This finding is consistent with what other authors have already observed in several tissues where the nNOS enzyme is an essential metabolite in NO synthesis.

On the other hand, an increase in iNOS immunohistochemical detection was apparent in pulps from orthodontically treated teeth, indicative of pulp stress. After three months a fairly small quantity of enzyme indicated mild inflammation, which became more and more apparent with the increase of orthodontic forces in time, as shown by the images and results shown here.

These findings would easily lead us to the conclusion that the application of increased orthodontic forces causes growing stress in treated pulps.

Our aim is to evaluate whether this stress is mainly due to the application of forces of greater intensity rather than to the duration of orthodontic traction itself.

As a future direction, we plan to check the pulp response to long-term weak orthodontic traction and compare it to the resistance of the pulpal tissue to stronger forces applied for a shorter amount of time. In case of no pulp injury after weak force application for a long time, teeth movement should also be evaluated to establish whether a longer treatment using weak traction would be a more suitable option, avoiding pulp problems due to the application of high-intensity orthodontic forces.

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