Transforming Growth Factor-beta 1 (TGF-beta 1) expression in normal healthy pulps and in those with irreversible pulpitis

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Abstract

Piattelli A, Rubini C, Fioroni M, Tripodi D, Strocchi R. Transforming Growth Factor-beta 1 (TGF-beta 1) expression in normal healthy pulps and in those with irreversible pulpitits. *International Endodontic Journal,* **37**, 114–119, 2004.

Aim To evaluate the Transforming Growth Factor-beta 1 (TGF-beta 1) expression in normal healthy pulps and in those with irreversible pulpitis.

Methodology Twenty-three normal, healthy pulps were removed from mandibular third molars, and 20 pulps were retrieved from teeth with irreversible pulpitis. TGF-beta 1 was evaluated in the odontoblastic and subodontoblastic layers, in the stromal cells (fibroblasts), and in the blood vessels. TGF-beta 1 expression was determined by evaluating 500 cells in the odontoblastic and subodontoblastic layers and 500 fibroblasts in the stroma for each specimen, and counting the number of positive cells. The number of the positive vessels was evaluated in 10 high power fields (HPF). In almost all cases, the cellular positivity was cytoplasmatic. Statistical analysis was performed using Mann–Whitney *U*- and Student's *t*-tests.

Results A higher expression of TGF-beta 1 was found in the odontoblastic–subodontoblastic layer of the irreversible pulpitis specimens; this difference was statistically significant ($P = 0.0002$). No statistically significant difference was observed between the two groups in TGF-beta 1 expression in the stromal cells ($P = 0.54$) or in the vascular component ($P = 0.94$).

Conclusions The higher and statistically significant expression of TGF-beta 1 found in the odontoblastic–subodontoblastic layer of irreversible pulpitis specimens may indicate a role for TGF-beta 1 in the dentinal repair processes after pulp inflammation.

Keywords: inflammation, irreversible pulpitis, Transforming Growth Factor-beta.

Received 19 February 2002; accepted 10 September 2003

Introduction

Members of the Transforming Growth Factor-beta 1 (TGF-beta 1) superfamily have been implicated in many aspects of the regulation of cell growth, differentiation and function (Smith *et al.* 1998, Tziafas & Papadimitriou 1998). These molecules have mitogenic effects and a regulatory role in matrix biosynthesis, and they are chemotactic for fibroblasts, neutrophils and monocytes (Tziafas & Papadimitriou 1998). Coordinated expression of TGF-beta members in pulp may be important in tooth development and repair (Toyono *et al.* 1997a, Smith *et al.* 1998, Nakashima *et al.* 1998). It has been shown that TGF-beta 1 as a pulp-capping medicament enhances reparative dentine formation in rat molars (Hu *et al.* 1998, Tziafas *et al.* 1998), and exerts dentine-specific effects inducing differentiation of odontoblast-like cells and stimulating primary odontoblasts (Tziafas *et al.* 1998).

TGF-beta 1 plays a role in the differentiation of pulp cells into odontoblasts during reparative dentinogenesis (Toyono *et al.* 1997b). TGF-beta 1 is significantly up-regulated in fully differentiated odontoblasts during primary dentine formation (D'Souza *et al.* 1998). It is a mitogen for human pulp cells (Shirakawa *et al.* 1994), and is expressed in the developing tooth from the initiation stage through adulthood (Thyagarajan *et al.* 2001).

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Expression of TGF-beta in the odontoblasts continues throughout life; the precise biological function of this growth factor in the odontoblasts is, however, still not clearly understood (Thyagarajan *et al.* 2001). The effects exerted by TGF-beta in reparative dentinogenesis are unknown (Tziafas & Papadimitriou 1998).

The aim of this study was to evaluate and compare TGF-beta 1 expression in healthy pulps and in those with irreversible pulpitis.

Materials and methods

Twenty-three normal healthy pulps were removed from mandibular third molar teeth extracted from 23 patients (mean age: 21 years, range 18–23 years). Twenty specimens were retrieved from teeth having irreversible pulpitis from 20 patients (mean age 25 years; range 19–31 years); these patients presented with spontaneous pain, and sudden temperature changes induced prolonged episodes of pain. All patients gave their informed consent. The healthy pulp specimens were removed with an excavator from extracted mandibular third molars after sectioning them in half with a diamond bur; the irreversible pulpitis specimens were obtained in the same way from extracted mandibular third molars with gross caries.

All the biopsies were fixed in formalin (10% neutral buffered formalin) and embedded in paraffin. Sections of 4 μm were cut and mounted on poly-L-lysine-coated slides and then heated in a microwave oven (700 W) for 10 min. Endogenous peroxidase was blocked by the incubation of samples in 3% hydrogen peroxide in methanol.

After being washed with phosphate-buffered saline (PBS), the samples were incubated overnight with anti-TGF-beta 1 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The sections were then incubated with Envision-labelled polymer reagent (Dako, Copenhagen, Denmark) for 60 min at room temperature. Envision-labelled polymer reagent is a peroxidase-labelled polymer conjugated to goat antirabbit and goat antimouse immunoglobulins in Tris–HCl buffer. The reaction products were visualized with diaminobenzidine as the chromogen, and the sections were counterstained with haematoxylin. Normal rabbit immunoglobulin G was used instead of the primary antibodies for negative controls.

TGF-beta 1 was evaluated in the odontoblastic and subodontoblastic layers, in the stromal cells (fibroblasts), and in the blood vessels. TGF-beta 1 expression was determined by evaluating 500 cells in the odontoblastic and subodontoblastic layers and 500 fibroblasts in the stroma for each specimen, and counting the number of positive cells. The number of the positive vessels was evaluated in 10 HPF. In almost all cases the cellular positivity was cytoplasmatic. All measurements were made by a blinded examiner with no prior knowledge of the experimental design, and without knowledge of control and test specimens.

Statistical analysis was performed using Mann–Whitney *U*- and Student's *t*-tests.

Results

Healthy pulps

In 5 cases, the number of positive cells in the odontoblastic-subodontoblatic layer was more than 50. In 6 cases, the positivity was between 10 and 50 cells (Figs 1 and 2), whilst 12 cases were negative. In 7 cases the number of the positive stromal cells was between 1 and 5.

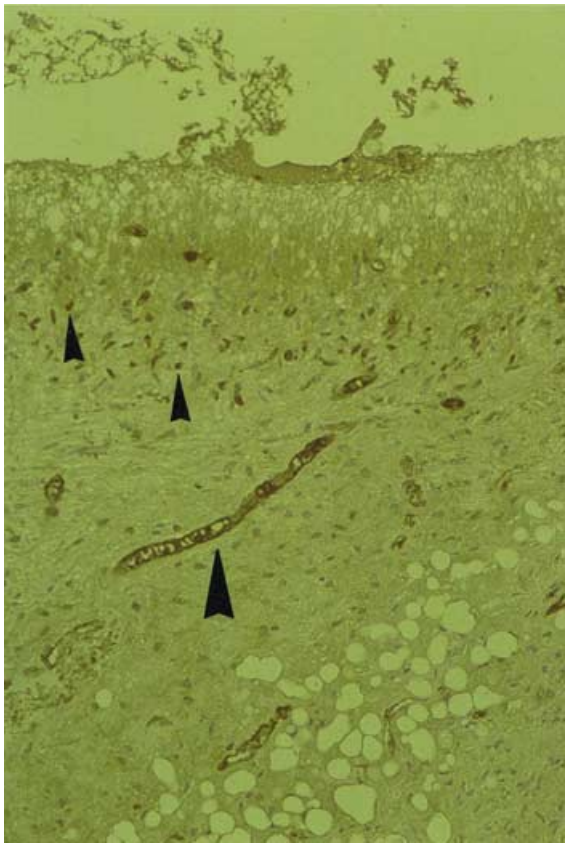

Figure 1 Healthy pulp. Focal positivity of the cells of the odontoblast-subodontoblast layer (small arrows); moderate positivity of the vascular component (large arrow). TGF-beta 1, APAAP, ×100.

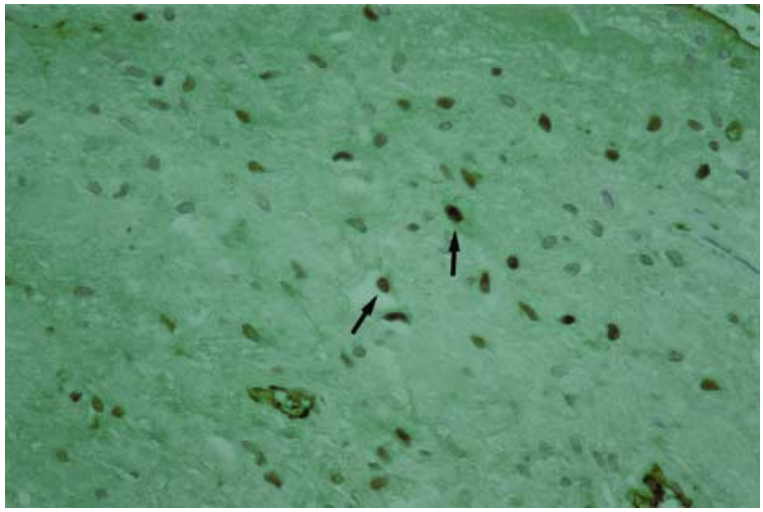

Figure 2 Healthy pulp. Nuclear positivity of the cells of the subodontoblastic layer (arrows). TGF-beta 1, APAAP, ×200.

Table 1 Camparison of TGF-beta 1 expression in normal healthy pulps versus irreversible pulpitis

	Stromal cells		Odontoblastic layer		Vessels	
Patients	Normal	Pulpitis	Normal	Pulpitis	Normal	Pulpitis
1	0	0	58	0	48	65
2	0	1	0	57	82	45
3	0	0	0	62	57	34
4	1	1	12	53	29	82
5	0	0	62	0	0	49
6	3	2	0	75	63	61
7	0	0	23	28	48	8
8	0	3	0	54	19	0
9	1	0	68	0	41	11
10	0	5	8	72	28	12
11	0	0	0	63	65	48
12	1	0	0	49	82	18
13	0	3	72	62	0	14
14	0	0	12	78	32	0
15	0	1	0	67	57	27
16	0	0	81	63	0	9
17	3	1	0	56	0	42
18	0	0	0	79	0	29
19	5	1	0	59	68	67
20	1	0	14	64	81	87
21	0	–	18	–	0	–
22	0	–	11	–	0	–
23	0	–	9	–	0	–
MV	0.65	0.90	19.47	52.05	34.78	35.40

The vessels were positive in 15 cases; in 7 cases, the positivity was between 10 and 50 and more than 50 in 8 cases, whilst 8 cases were negative (Table 1).

Irreversible pulpitis

In 15 cases the number of positive cells in the odontoblastic–subodontoblastic layer was greater than 50 (Fig. 3). In 2 cases, the number of positive cells was between 10 and 50, whilst 3 cases were negative. The vessels were positive in 18 cases (Fig. 4); in 11 cases, the positivity was between 10 and 50 and more than 50 in 5 cases (Table 1). The stromal cells were positive in 9 cases with a positivity between 1 and 5 (Fig. 5).

In healthy pulps, the mean value of positive cells in the odontoblastic-subodontoblatic layer was 19.47 (SD 27.34; median 9.0), whilst, in irreversible pulpitis the mean value of positive cells was 52.05 (SD 25.06; median 60.50). The mean value of stromal positive cells was 0.65 (SD 1.30; median 0.0) in healthy pulps, whilst in irreversible pulpitis the mean value of positive cells was 0.90 (SD 1.37; median 0.0).

The mean value of the positive vessels was 34.78 (SD 30.74; median 32.0) in healthy pulps, whilst the mean value was 35.40 (SD 26.96; median 31.50) in irreversible pulpitis.

Statistical analysis

A higher expression of TGF-beta 1 was found in the odontoblastic–subodontoblastic layer of the irreversible pulpitis specimens and this difference was statistically significant ($P = 0.0002$; confidence interval: −48.820 to −16.324). No statistically significant difference was observed between the two groups in the TGF-beta 1 expression in the stromal cells ($P = 0.5469$; confidence interval: 1.072–0.576). No statistically significant difference was observed between the two groups in the TGF-beta 1 expression in the vascular component ($P = 0.9496$; confidence interval: 18.558–17.323).

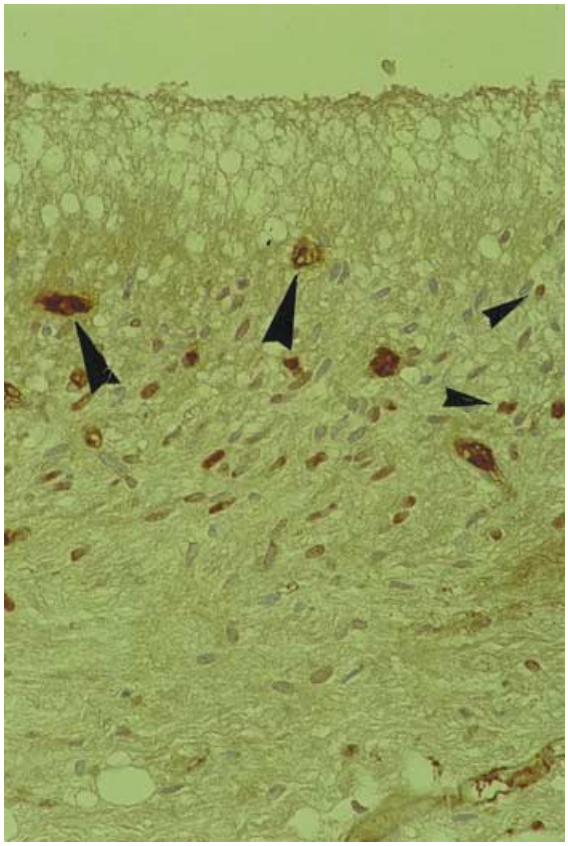

Figure 3 Irreversible pulpitis. Positivity of the cells of the odontoblastic–subodontoblastic layer (small arrows) and of vascular structures (large arrows). TGF-beta 1, APAAP, ×160.

Discussion

Caries, trauma and operative procedures can produce an injury of the dentine with subsequent pulp inflammation and necrosis (D'Souza *et al.* 1998). After resolution of the inflammatory processes, a protective layer of reparative dentine at the injured dentine–pulp interface is deposited (D'Souza *et al.* 1998). TGF-beta 1 is a potent modulator of tissue repair in various tissues (Melin *et al.* 2000). TGF-betas are multifunctional cytokines with biologic effects that depend upon the type of target cells, local concentration and the interaction with other molecules (Tziafas & Papadimitriou 1998). TGF-beta 1 stimulates matrix secretion and type I collagen production (Sloan *et al.* 1999, Melin *et al.* 2000); it also initiates odontoblast cytodifferentiation and a local increase in predentine secretion (Sloan *et al.* 1999, Melin *et al.* 2000). Such activities might be important during reparative processes in the dentine–pulp complex after tissue injury (Sloan & Smith 1999). Moreover, TGF-beta 1 is a potent immunosuppressant (D'Souza *et al.* 1998).

Odontoblasts and other cells of the pulp show the presence of both TGF-beta receptors I and II in health and disease with odontoblasts showing the strongest expression (Sloan *et al.* 1999). Such findings may be important in the response of these cells to tissue injury (Nakashima *et al.* 1994, Sloan *et al.* 1999). TGF-beta 1 could be directly involved in the regulation of the cell proliferation, migration and extracellular matrix production in the human dental pulp and eventually in the repair processes occurring after tooth injury (D'Souza *et al.* 1998, Melin *et al.* 2000).

An improved understanding of the role played by these growth factors in the repair of dental tissues, either via a reparative or reactionary dentinogenesis, may lead to

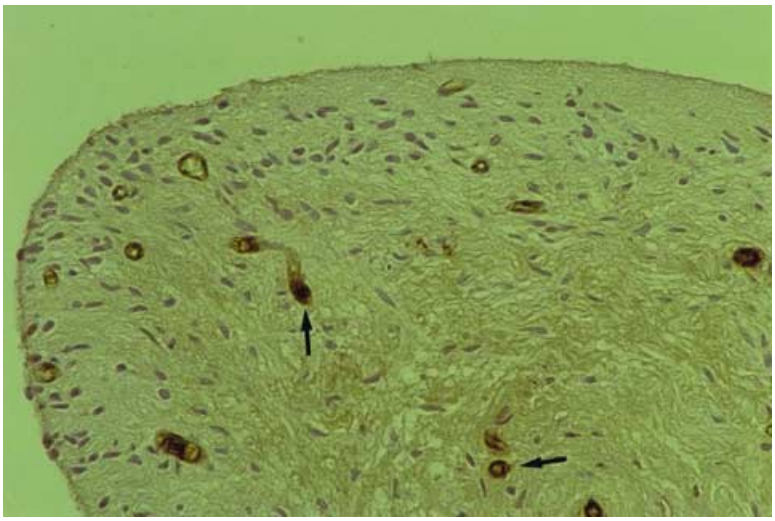

Figure 4 Irreversible pulpitis. Strong positivity of the vessels (arrows) located in the central and peripheral portions of the pulp. TGF-beta 1, APAAP, ×100.

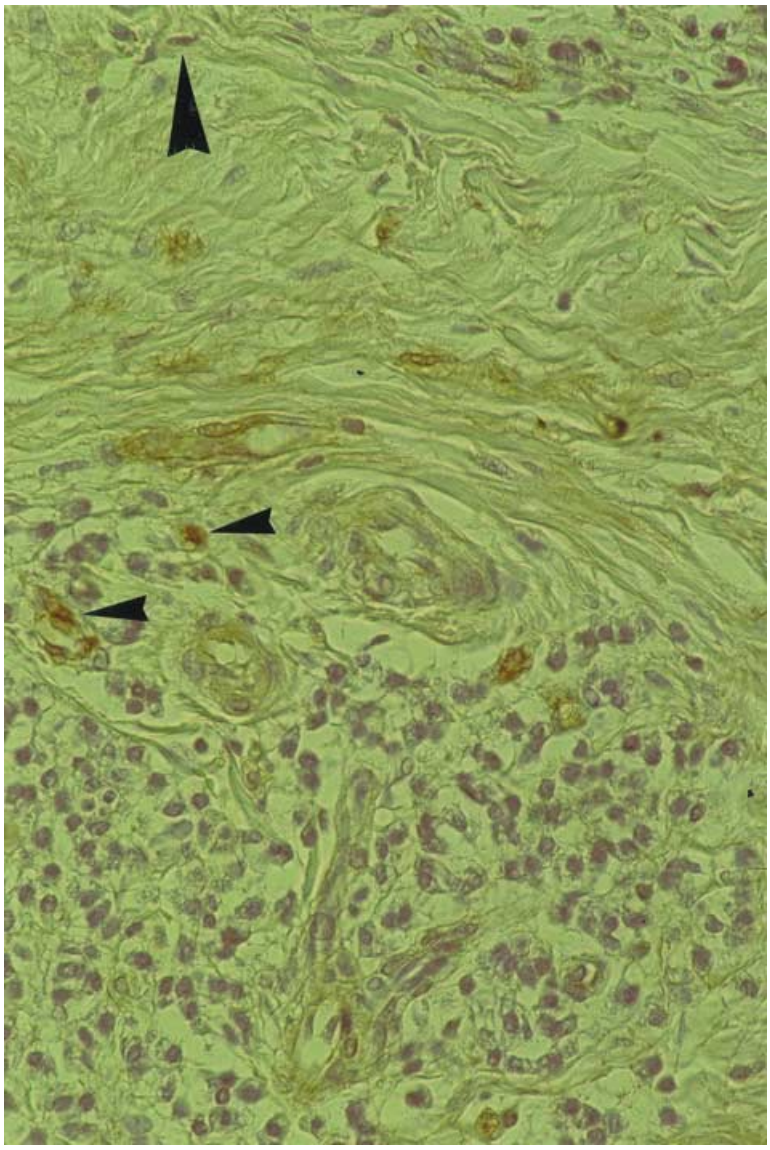

Figure 5 Irreversible pulpitis. Mild positivity of the inflammatory component (small arrows) and of the stromal cells (large arrows). TGF-beta 1, APAAP, ×200.

new avenues of approach to the knowledge and treatment of dental disease (Smith *et al*. 1998). The results demonstrate a higher and statistically significant positivity in the cells of the odontoblastic and subodontoblastic layers in the specimens with irreversible pulpitis. These data are likely related to a role performed by TGF-beta 1 in the tissue injury and repair events after pulp inflammation.

TGF-beta 1 may play a role in the differentiation of pulp cells into preodontoblasts and odontoblasts and in the stimulation of odontoblasts in the reparative dentinogenesis processes, after tissue injury. Moreover, TGF-beta 1 induces Type I collagen production by the odontoblastic/subodontoblastic pulp cells, and these processes help the soft and hard tissue repair after pulp injury, enhancing reparative dentine formation.

Acknowledgements

This work was partially supported by the National Research Council (C.N.R), Rome, Italy, and by the Ministry of Education, University and Research (M.I.U.R), Rome, Italy.

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