

## LOCAL APPLICATION OF IGF1 ON DENTAL PULP MECHANICALLY EXPOSED; IN VIVO STUDY ON RABBIT.

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**MOTS CLES:** *Dentinoblastes, IGF1, Protéines liantes*

### RESUME

Dans ce travail, L'IGF1 a été appliqué dans le but de provoquer une régénération dentinaire après avoir effectué iatrogéniquement 72 brèches pulpaire sur des molaires de lapin ; différentes concentrations d'IGF1 ont été utilisées. Les études histo et anatomopathologiques ont montré la persistance de la vitalité pulpaire et l'absence de nécrose, même 6 semaines après application ; les couches de dentinoblastes ont été décomptées, selon un protocole préétabli, à J=7, 14, 22, 28 et 42. L'IGF1 appliqué est résistant aux binding protéines afin d'éviter leur effet inhibiteur (Hochscheid et coll.) Les résultats suggèrent la possibilité d'utiliser l'IGF1 comme produit potentiel de coiffage pulpaire.

### ABSTRACT

IGF1 (Insulin Growth Factor, 1) was intentionally applied onto pulp tissues, aiming to provoke a dentine regeneration process through the stimulation of the dentinoblasts' potentials. 72 cavities were hence performed on rabbit molars, intentionally exposing the dental pulp. Different concentrations of IGF1 were then applied; The histo and anatomo-pathological observations showed persistent vitality of the pulp without any sign of necrosis, even 6 weeks after the IGF1 application. Dentinoblasts layers (as an indication of the regeneration activity) were counted, according to a pre-established protocol, at days 7, 14, 22, 28 and 42. The type of the applied IGF1, was carefully selected to be "Binding Protein Resistant" (IGF-BPR), so to avoid any inhibition of the IGF1 action by the endogenous binding proteins (Hochscheid and coll.). The results were conclusive in indicating the IGF1 as an efficient dental pulp capping product.

### INTRODUCTION

Pulp reaction to aggression caused by decay, trauma, mechanical or thermal variation, can lead to either a reversible inflammation, or to apoptosis, degeneracy, necrosis or even to a neo-dentine formation.

Odontoblast cells, preferably called dentinoblasts,

produce the dentine.

According to Hart and Korman, the dentinoblasts have insulin-like growth factors receptors (IGF R), onto which, the extrinsic IGF1 can bind.

Since the IGF-R is a tyrosine kinase receptor type, the IGF 1 will stimulate the proliferation of

dentinoblasts, while enhancing their secretion of collagenous matrix, and mobilizing the calcium ions present within the endoplasmic reticulum;

The higher is the proliferation rate of dentinoblasts, the faster will be rebuilt a protective dentine wall via neo-formation.

One of the IGF1 drawbacks was its extreme affinity to react with a multitude of soluble IGF-binding proteins (IGFBPs), abundantly present in the extracellular space, preventing it from reaching the targeted receptors on the cell's membrane. For such reason, the IGFBPs are considered to be inhibitors of IGF1.

Six distinct "binding proteins" types have been identified and cloned to date (Shimasaki and Ling ).

In order to avoid the interaction IGF1-Binding protein in the extra-cellular zone, a special type of IGF1 was selected, for its ability to resist to binding proteins.

Blunck has conducted a comparative study, between calcium hydroxide and adhesive systems on pulp capping; his conclusion was that neither showed sufficient results.

In this study our aim is to show the effect of IGF1 as a dental pulp capping agent.

## MATERIAL AND METHODS

### *Selection criterias:*

Rabbits were selected as optimal lab animals, due to the practical size of their teeth.

Their incisors were disregarded as they are in a continuous growth status, leaving enough space to suppose more autocrine secretion of IGF1 interfering with our exogenous application.

Cavities were performed on three upper left molars, always unilateral, leaving the possibility for the rabbits to eat on their controlateral side.

Anaesthesia: Chanazine and Ketamine were used as an intraperitoneal injection; Twenty minutes were sufficient to reach the relaxation of jaws mussels and complete sedation;

- 2 cc of chanazine; 10 minutes
- 2 cc of ketamine; 10 minutes

Under such sedation and relaxation, pulp exposures were performed.

The pulp chambers were previously localised by X rays. 1 mm diameter burs were used to drill, under generous irrigation, all 3 left upper molars for each rabbit, until a drop of blood appeared.

### *Application:*

IGF1 was stored in vials at  $-20^{\circ}\text{C}$ .

Each vial was dosed at 5 $\mu\text{l}$  of liquid containing 10 ng of IGF1;

5 $\mu\text{l}$  pipettes were used to apply the product onto the exposed pulps.

Cavities were then sealed with glass ionomer (glass polyalkenoate ISO 9917:1991 class4.2.2 Shofu)

Applied IGF1 had these characteristics:

- IGF1 ( IGFBP-resistant),
- recombinant,
- does not react with "IGF binding proteins";
- lyophilised powder;
- rehydrated : 3 months at  $-20^{\circ}$  or  $-80^{\circ}\text{C}$

Source: mutant IGF1, expressed in E.Coli ; catalogue: 01-189 Euromedex.

### *Methods:*

24 six months aged, 2 kg weight rabbits were distributed in 4 groups of six each.

All rabbits were of albinos kind:

- group 1: 10 ng IGF1
- group 2: 20 ng IGF1
- group 3: twice 10 ng IGF1
- group 4: comparative

### *Operative procedure:*

#### *First intervention:*

6 rabbits of group 3 + 2 rabbits of group 4 (comparative).

3 molars excavated on each rabbit under general anaesthesia

- 6 x 3 = 18 cavities treated with 10 ng IGF1
- 2 x 3 = 6 cavities without IGF1

All in all, 24 teeth excavated on 8 rabbits.

These teeth were reopened 14 days later for another intervention.

*Nomenclature:*

1, 2, 3, 4, 5, 6 LF (1, 2, 3) LF for lapin fois  
1, 2 LFT (1, 2, 3) LFT for lapin fois témoin

Rabbits were marked by piercing their ears with different colors of earrings.

*Second intervention:*

6 rabbits of group 1 + 2 rabbits of group 4 (comparative)

Same procedure as above.

*Nomenclature:*

1, 2, 3, 4, 5, 6 L10 (1, 2, 3); L10 for lapin 10ng  
1, 2 L10T (1, 2, 3); L10T for lapin 10ng témoin

*Third intervention:*

J=1 sacrifice of 1, 2, L 10 and 1 L 10 T; conservation of 9 molars in liquid nitrogen .

*Fourth intervention :*

6 rabbits of group 2 + 2 rabbits of group 4 (comparative)

*Nomenclature:*

1, 2, 3, 4, 5, 6 L20 (1, 2, 3). L20 for lapin 20ng  
1, 2 L20T (1, 2, 3). L20T for lapin 20 ng témoin

*Fifth intervention:*

J=1 sacrifice of 1, 2 L20 and 1L20T; conservation of 9 other molars in liquid nitrogen.

*Sixth intervention:*

J=7 sacrifice of 3, 4 L10 and 2L10T; conservation of 9 molars in formol.

*Seventh intervention:*

J=14 Second application of 10ng IGF1 on group 3

*Eighth intervention:*

J=15 sacrifice of 1, 2 LF and 3LFT; conservation of 9 molars in liquid nitrogen.

*Ninth intervention:*

J=21 sacrifice of 3, 4 LF and 4 LFT; conservation of 9 molars in formol.

*Tenth intervention:*

J=30 sacrifice of 5L10, 5 L20, 5L10T and 5L20T; conservation of 12 molars in formol.

*Eleventh intervention:*

J= 42 sacrifice of 6L10, 6L20, 6L10T and 6L20T; conservation of 12 molars in formol.

*Twelfth intervention:*

J=42 sacrifice of 5LF and 5 LFT; conservation of 6 molars in formol .

*Preparation of molars for analysis:*

*Histopathological:*

Upper left jaws were cut and immersed in formol at 10 %, demineralised with nitric acid at 7%, then rinsed, dehydrated in ethanol and embedded in paraffin.

Tissue blocks were cut into 5 µm-thick serial sections; sections were then deparaffinized and stained with hematoxylin-eosin.

*Immunohistochemical:*

Specimens were stored in liquid nitrogen at -180°C, and then cut with cryotome.

Antibody was antihuman IGF1 receptor, chicken polyclonal Ig γ (Upstate Biotechnology incorporated); 250 µg/250 µl of Na<sub>2</sub>CO<sub>3</sub> at 0, 1M .

## RESULTS

Specimens analysed at day 1 and 15, with antibodies, anti IGF1 receptor, showed no significant results, due to probable internalisation of these receptors .

Histopathology of teeth perforated without IGF1 application showed inflammatory reaction in the pulp, consistant call for fibroblasts and a little "coagulum"(as described by histologist) with many polynuclears.

Thickness of the dentinoblast layer was 0, 25mm at day 7 and 0, 3 mm at day 14, then 0, 25 mm at day 28 ; at day 42 vacuolisation in dental pulp was noticed.

Whereas on teeth treated with IGF1, dentinoblast layer had 0, 4 mm at day 7, and inflammatory reaction was discreet (Fig. 1).

At day 14, Von Korf fibres were seen (Goldberg) (Fig. 2).

At day 22 ( teeth treated with twice 10 ng of IGF1 ) 0, 6 and 0, 8 mm were noticed (Fig. 3).

At day 28 there was still 0,6 mm, without inflammation, nor necrosis; at day 42, "everything

returned to previous order": thickness of layers was normal, without congestion; fibbers were seen near dentinoblasts layers (Fig. 4).

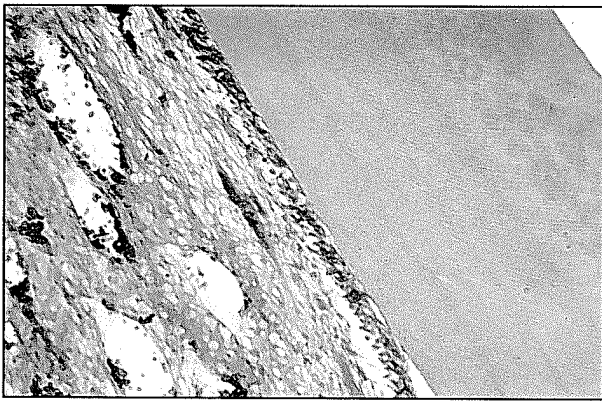
Clinically, iatrogenic hole in dental pulp was not evident anymore, whereas it was always present on teeth untreated with IGF1, 6 weeks after .

Although, treated rabbits had normal alimentation at the opposite of those untreated, which seemed more agitated.

## DISCUSSION

Did application of IGF1 lead to multiplication of dentinoblasts or differentiation of other cells into dentinoblasts? Did it enhance collagen synthesis with mobilization of intracellular calcium, generating new dentine ? Did IGF1 act as a ligand and bring about nuclear reaction of dentinoblasts?

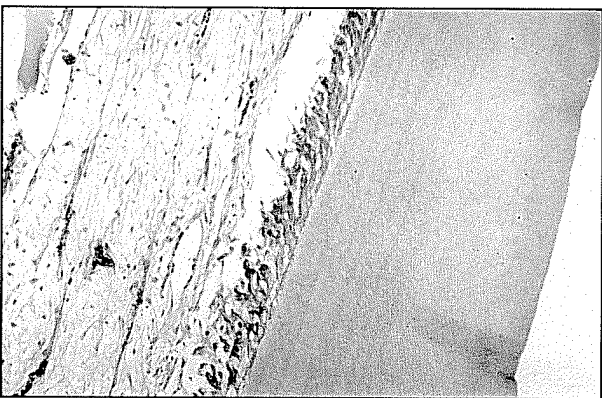
The IGF receptor is present on essentially all cell types and is critical for proliferation ( Baserga, Rubin).The addition of IGF or IGF-R antibodies or



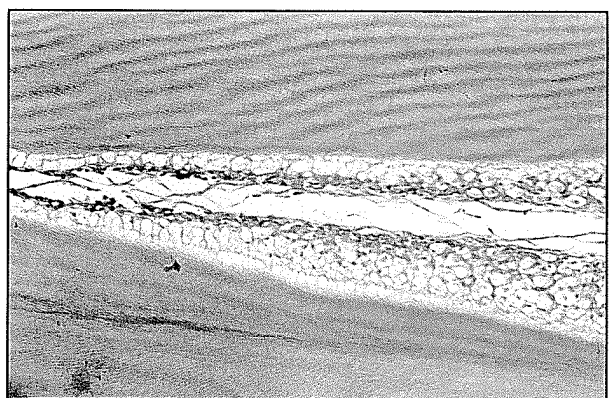
**Fig. 1:** day=7; 4 layers of dentinoblasts; discrete inflammation; many fibroblasts. magnification x 200.



**Fig. 3:** day=22; twice treated rabbits; observation at day 7 after second application of 10ng of IGF1; 6 to 8 layers of dentinoblasts; magnification x 200



**Fig. 2:** day=14; still 4 layers of dentinoblasts; more discrete inflammation; Von Korf fibres; magnification x 200



**Fig. 4:** day=42; normal thickness of dentinoblasts layers; no congestion; no necrosis; many fibres; magnification x 100

antisense oligomers blocks cell growth in culture ( Porcu and coll ).

IGFs mediate their mitogenic effects by binding the IGF-R and initiating a cascade of phosphorylation via the tyrosine kinase activity of b-subunit of the receptor.

Further steps in this cascade involve phosphorylation of IRS-1, a substrate for both the IGF and the insulin receptor (Valentinis and coll).

Results showed increased number of dentinoblast layers with applied IGF1; return to normal state six weeks after application, signed reversibility of process. Presence of Von Korf fibres denoted secretive activity of dentinoblasts; these fibres were intensively seen when there was increased number of dentinoblast layers, which involves dentinoblasts in their secretion.

However, no critical differences were noticed between application of 10ng and 20ng of IGF1.

Yet, difference was significant on twice treated rabbits.

Furthermore, minor and transitory inflammation was noticed on treated teeth, whereas it was more intense and irreversible on teeth without IGF application.

Did six weeks were sufficient to declare teeth "safe"? Shall apoptosis, degeneracy or necrosis appear later in the pulp?

Is IGF1-binding protein resistant, more efficient?

Experimentation in vitro on human fibroblasts with IGF1 not BP-resistant, had no significant results in leading cells into S phase (flow cytometry), even on cells deprived of serum bovine in culture medium ( we did this experimentation at the CRBM-CNRS Montpellier).

## CONCLUSION

Our findings have shown that applied IGF1 binding protein resistant after perforating dental pulp enhanced new dentine generation, with little reversible inflammation; in particular,

We observed multiplication of dentinoblasts and increased Von Korf fibres.

Furthermore, no necrosis occurred in the 60 teeth treated, six weeks after application .

Perspectives : we suggest using IGF1 for dental pulp cupping ; we found a new galenic form and are intending to apply it .

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