Advanced Glycation Endproducts and Rat Dental Implant Osseointegration

David G. Quintero, BS
Julia N. Winger, BS
Rania Khashaba, DMD, PhD
James L. Borke, PhD
*

Advanced glycation endproducts (AGEs) are a diverse group of molecular adducts formed in environments high in reducing sugars that accumulate with aging and in diabetes. This study tests the hypothesis that AGEs inhibit the stable osseointegration of dental implants through tissue interactions that interfere with bone turnover and compromise the biomechanical properties at the bone-implant interface. Maxillary first molars were extracted from 32 rats and allowed to heal for 4 weeks. Titanium implants (1 mm × 3 mm) were placed in the healed sockets of 2 groups of 16 rats consisting of 8 rats injected 3 times/wk for 1 month with AGE (prepared from glucose and lysine) and 8 rats injected with vehicle as a control. AGE injections continued for an additional 14 or 28 days before sacrifice. X-ray images, blood, and tissues were collected to examine bone/implant contact ratio, serum pyridinoline ([PYD] a collagen breakdown marker), osteocalcin ([OSC] a bone formation marker), and for immunohistochemistry with antibodies to AGE and the bone turnover-marker protein matrix metalloproteinase1. Compared with the AGE-treated groups, the controls showed significantly higher bone/implant contact at both 14- and 28-day time points. PYD (P < .05) and OSC (trend) levels from controls showed decreases at 28 days when compared with AGE-treated groups. Immunohistochemistry with AGE-specific and bone turnover marker antibodies showed stronger staining associated with the implant/tissue interface in AGE-treated rats. Our studies indicate an association between AGE and inhibition of bone turnover, suggesting that the formation of AGE in high glycemic conditions, such as diabetes, may contribute to a slower rate of osseointegration that negatively affects implant stability.

Key Words: hyperglycemia, osseointegration, bone turnover

INTRODUCTION

Advanced glycation endproducts

Aldehyde and ketone structures within the tissues and fluids of the body condense with lysine side chains on proteins to form a reversible Schiff’s base. This structure isomerizes to a ketoamine (Amadori product) that is highly reactive. Amadori products may undergo oxidation reactions, dehydration, condensation, or additional
rearrangement. These result in protein cross-links and protein modifications called advanced glycation end products, or AGEs.\(^1\)

AGEs accumulate in tissues during aging, but actually are formed throughout life. However, AGEs are found in significantly higher levels in the elderly.\(^2,3\) In the aging individual, the formation of AGEs is considered to be a major causative factor of tissue failure.\(^4,5\) The development of many age-related chronic diseases, including Alzheimer’s disease, periodontal disease, nephropathy, osteoarthritis, cataract formation, and myocardial dysfunction all appear to be related to the formation of AGEs.\(^6–15\) In addition, the hyperglycemia associated with diabetes has been linked to elevations in AGE formation. Of particular interest to dental implantology is the finding that AGEs also impair osseous wound healing.\(^16\) Some of the effects associated with elevated AGEs result from the cross-linking of matrix and other proteins. Although this is physiologically relevant, glycation can also alter proteins so that they gain signaling properties that were not present before glycation. Cell surface receptors and binding proteins for AGEs have now been identified. Of these, RAGE (receptor for advanced glycation end-products) is the most thoroughly investigated. RAGE is a member of the immunoglobulin superfamily of cell surface receptors which binds many ligands. Prominent in this group are those of the S100 family.\(^17\) Other receptors and binding proteins include macrophage scavenger receptor types I and II, and AGE-R1, R2, and R3 that are expressed on many cell types including endothelial cells, astrocytes, microglia, smooth muscle cells, monocytes, macrophages, podocytes, and fibroblasts. Of the receptors for AGEs found on cells, the scavenger receptors are thought to regulate removal of AGEs, while most of the biologic activities associated with AGEs appear to be dependent on binding to RAGE.\(^18,19\) In addition, AGEs can affect inflammatory events by promoting chemotaxis, activation of monocytes and macrophages, production of reactive oxygen species, and stimulation of IL-1 and TNF formation.\(^20–25\) We speculate that both osteoblasts and osteoclasts interact with AGEs through RAGE and that this interaction will negatively affect dental implant osseointegration. These molecules have the potential of compromising the stability of dental implants by binding to structures and inhibiting the activity of bone cells that are necessary for stabilizing the implant in the bone.

The purpose of this study is to characterize the effects of advanced glycation end products on dental implant osseointegration in an animal model (rat). The use of this small animal model for osseointegration research limits some of the issues related to sample size when using larger research models.

**Materials and Methods**

Thirty-two male retired breeder rats were purchased for this study (Harlan, Indianapolis, Ind) and housed in the small animal facility at the Medical College of Georgia Research and Education Building with free access to food and water. These rats were chosen because most implant patients are not young and also because retired breeder rats are larger in size (300+ grams) than younger rats which, therefore, facilitates intraoral procedures.

Before surgery, animals were placed under general anesthesia by intramuscular injection of 1 mg/kg of a solution containing 100 mg/mL ketamine, 20 mg/mL xylazine, and 10 mg/mL acepromazine in accordance with a Medical College of Georgia Institutional Animal Care and Use Committee–approved protocol. Implant sites were prepared by extraction of one maxillary first molar. The extraction sites were allowed one month to heal. Pilot holes were drilled at the extraction sites to use as guides for installing 1 mm (D) × 3 mm (L) titanium miniscrews.
(Stryker Leibinger, Kalamazoo, Mich). Each screw was installed to a point where the top of the head appeared grossly in occlusion with the mandibular first molar. Articulating paper was used to make a final adjustment of occlusion. Figure 1 demonstrates the placement technique as previously described.\(^{26}\)

Two groups of 16 rats consisted of 8 rats injected 3 times/wk with AGE (prepared as described below) and 8 rats injected with normal saline during the socket healing period for 14 or 28 days. The healing times given were selected based on previous published studies using miniature titanium screws inserted into rat tibias which described highly organized lamellar bone around the screws after 3 to 4 weeks.\(^{27–29}\)

After each time period the rats were anesthetized as above and 200 \(\mu\)l of blood was removed from each rat for pyridinoline (PYD) and osteocalcin (OSC) assays (as described below). After harvesting the blood, each rat maxilla containing an implant was perfusion-fixed with 10% buffered formalin. This specimen was dissected from each rat and characterized at the implant site using soft x-ray microradiography. Following radiography, harvested tissue was decalcified for one month. The implants were removed before tissue embedding in paraffin and sectioning for histology by routine methodology. Tissue sections were used for routine histology (hematoxylin and eosin staining) and immunohistochemistry. Decalcification and paraffin embedding were used for this study rather than methacrylate embedding in order to accommodate the immunohistochemical localization of markers for AGE and matrix metalloproteinase1 (MMP-1).

### Preparation of AGE

AGE was prepared by incubating lysine (Sigma, St Louis, Mo) and 1.67 M glucose in 0.5 M phosphate buffer (pH 7.4) for 12 weeks at 37°C. AGE formation was confirmed by measurement of fluorescence at an emission wavelength of 440 nm after excitation at 365. Endotoxin levels were checked using an endotoxin test kit (Limulus Single Test, Wako Pure Chemicals Inc, Osaka, Japan).

### PYD and osteocalcin assays

The serum PYD assay gives a quantitative measure of the excretion of pyridinoline crosslinks released into the blood due to collagen degradation. Collagens such as type I and type II that are present in bone and cartilage (respectively) are crosslinked within their \(\alpha\)-chains and between molecules to provide strength to the collagen fibrils. When the collagen is degraded, the PYD is released into the bloodstream. We used the Metra Serum PYD assay kit from Quidel Corp (San Diego, Calif) for this determination. Twenty five ul of filtered serum were used for each determination.

A rat OSC EIA kit (sandwich type assay from BTI, Stoughton, Mass) was used for serum OSC determinations. Levels of OSC were measured in duplicate directly from rat serum using 10 ul of serum per determination.

### Results

Rats were injected with AGE or saline for 14 and 28 days. The estimated bone/implant...
Contact ratio shows a significantly lower contact ratio for implants in AGE-injected rats (3.5 μm bone contact/implant length) than for implants placed in saline-injected controls (5.5 μm bone contact/implant length) (Figure 2).

Serum PYD levels in the AGE-treated rats were equivalent to controls at 14 days but became elevated (~2.62 ng/mL) at 28 days as compared to the control rats which declined (~2.45 ng/mL) (Figure 4).

Immunohistochemistry with an antibody to AGE showed greater AGE localization (++++) in the fibrous tissue at the implant tissue interface after 28 days than in the control tissue from the same area (++) (Figure 5). In addition, intense intranuclear localization of MMP-1 was seen in cells associated with the implant-tissue interface in the AGE-treated animals (++++) but not in the controls (Figure 6).

**DISCUSSION**

This study characterized the effects of AGEs on the tissues associated with osseointegration of titanium dental implants (miniscrews) in a rat model.

Estimated bone/implant contact ratios were found to be less in AGE-treated rats than in controls (Figure 2C). Studies in diabetes-induced animals have shown that, although the amount of bone formed is similar when compared with controls, there is a reduction in the bone-implant contact in diabetics. One study that analyzed the placement of implants in the femurs of diabetic rodents observed new bone formation comparable to that of the control group in the region of the periosteum, whereas it was significantly lower in the endosteum and medullar canal, and bone bridges between the endosteum and the implant surface were only observed in a small number of cases.

The reduction in the levels of bone-implant contact observed in the present study confirms that diabetes inhibits osseointegration. This situation may be reversed by treating the hyperglycemia and maintaining near-normal glucose levels.

The decrease in PYD in serum from control rats relative to AGE-treated rats over
time (Figure 3) was indicative of reduced bone breakdown in the control rats but not in the AGE-treated rats as the implant osseointegrated.

The observed trend toward a decrease in OSC in serum from control rats relative to AGE-treated rats over time was supportive of an interpretation of reduced bone formation in the control rats relative to the AGE-treated rats as the bone healed.

Immunohistochemistry showed MMP-1 (a bone resorption marker) and AGE localization at the bone/implant interface in AGE-treated rats (Figures 5 and 6).

Together these data suggest an extended period of bone turnover in the AGE-treated rats. These findings suggest that AGE promotes prolonged bone turnover, delayed bone healing, and suggest delayed osseointegration of titanium implants.

These studies demonstrate an association between AGEs and prolonged bone turnover suggesting that the formation of AGEs in high glycemic conditions such as diabetes and aging may contribute to a decrease in bone/implant contact, a delay in bone healing around dental implants, and an increase in implant failure.
ABBREVIATIONS

AGE: advanced glycation end product
MMP-1: matrix metalloproteinase1
OSC: osteocalcin
PYD: pyridinoline
RAGE: receptor for advanced glycation end-products

ACKNOWLEDGMENTS

This study was supported by a grant from the American Academy of Implant Dentistry. The authors are also grateful to Ms Michelle Barnes for secretarial support.

REFERENCES


