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ORIGINAL RESEARCH

ASSESSMENT OF OXIDATIVE STRESS GENERATED BY ORTHODONTIC ARCHWIRES- AN IN VITRO STUDY

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Abstract

Introduction: *Numerous metals go through redox cycling, which results in the production of free radicals and oxidative stress. This study looked into the in-vitro oxidative stress placed on orthodontic archwires comprised of different alloys.*

Methods: *The quantity of the oxidative stress marker 8-hydroxy-20-deoxyguanosine in DNA was measured after exposing mouse fibroblast cells L929 to 6 different types of archwires. Cell vitality and number were determined using trypan blue dye.*

Results: *Coated nickel-titanium, copper-nickel-titanium, and cobalt-chromium produced oxidative stress levels that were lower than those of standard nickel-titanium archwires (P <0.05), but higher than those of titanium-molybdenum and the negative and absolute controls (P <0.05). Standard nickel-titanium archwires generated the highest oxidative stress, significantly higher than all other wires and the controls (P The least tension was produced by stainless steel and titanium-molybdenum. The lowest viability was caused by nickel-titanium, which was lower than the negative and absolute controls, all other wires, and titanium-molybdenum (P< 0.05). The material with the highest viability was stainless steel. The largest suppression of cell growth was caused by nickel-titanium, which was higher than all other samples (P< 0.05) excluding the positive control and cobalt-chromium. The positive control, cobalt-chromium, nickel-titanium, and titanium-molybdenum showed the lowest inhibition (P< 0.05), followed by stainless steel.*

Conclusions: *Every orthodontic archwire causes oxidative stress in a test tube. The maximum biocompatibility is found in stainless steel archwires, and the lowest is found in nickel-titanium.*

Keywords: *Orthodontic archwires, oxidative stress, Ni-Ti archwires*

Introduction

During orthodontic therapy, orthodontic appliances and their corrosion products come into sustained, 1- to 2-year-long contact with the oral mucosa; this places a significant demand on biocompatibility and stability. There hasn't been much research done on the involvement of orthodontic archwire alloys as mediators in the generation of free radicals and oxidative stress. Free radicals are produced directly by several heavy metals employed in the production of archwires, such as iron, chromium, copper, and cobalt, whereas they are produced indirectly by nickel. [1] Free radicals are always present in live cells as a result of cell metabolism or the influence of exogenous physical or chemical sources. These radicals take the form of reactive oxygen species. [2] Cellular DNA repair mechanisms thwart reactive oxygen species-induced DNA damage. Oxidative stress, which is associated to the mechanism of carcinogenesis, emerges when these defence mechanisms are outperformed by an increase in the synthesis of reactive oxygen species or a decrease in their removal. [1,3]

Since free radicals have a short half-life, it is more difficult to identify them, therefore attention is focused on the by-products of oxidative stress. These are mutated nucleosides, with 8-hydroxy-20-deoxyguanosine (8-OHdG) being the most intriguing one. This mutation, which can cause the conversion of guanine into thymine, is one of the most common ones seen in malignancies, and it is biologically quite relevant. [2,4] The effects of long-term exposure to different environmental conditions on the content of 8-OHdG in leukocytes and urine have been studied in the past with varying degrees of success. While independent studies mainly confirm DNA harm, research funded by the business frequently dispute it. [4] Studies have been done so far on chronic exposure to nanoparticles, nicotine, metals, asbestos, azo dyes, benzene, polycyclic aromatic hydrocarbons, silicates, styrene, toluene, xylene, and rubber manufacture. [2] According to the information available, there is currently no evidence of a dose-response association between chronic exposure and the induction of 8-OHdG.

This study's objective was to compare the levels of in-vitro induced oxidative stress among several types of archwires.

Materials and methods

Six different orthodontic archwire types were tested, including stainless steel (steel archwires; Forestadent, Pforzheim, Germany), nickel-titanium (Titanol; Forestadent), copper-nickel-titanium (Copper Ni-Ti; Ormco, Orange, Calif.), rhodium-coated nickel-titanium (BioForce High Aesthetic; Dentsply GAC, Bohemia, NY). Commercial artificial saliva (Glandosane; Stada Arzneimittel, Vienna, Austria) was utilised to mimic the circumstances in the oral cavity, and the pH of 6.75 to 0.15 was adjusted with 10 mol/L of sodium hydroxide buffer. Each archwire type was present in one sample of each, and all tests were conducted three times. Each archwire was divided into pieces that were each 1 cm long, weighed, autoclaved (at 121C and 1 bar for 35 minutes, and then put in a sealed bag), submerged in synthetic saliva with a pH-adjusted solution in sterile hermetic polyethylene tubes containing 1 mL of saliva per 0.2 g of archwire, and then kept at 37C in stationary conditions for 30 days. As a negative control, the synthetic saliva with sodium hydroxide buffer was kept in the same circumstances. The archwire pieces were taken out after 30 days, and the saliva samples were kept at 4C until additional examination.

Mouse fibroblast L929 cell cultures were used in the investigation. All cells were cultured in Dulbecco's modified eagle medium with 10% foetal bovine serum, 11 mmol/L sodium bicarbonate, 2 mmol/L L-glutamine, 100 mg/mL streptomycin, and 100 U/mL penicillin as supplements. To create a cell monolayer, cells were grown in 100-mm cell culture dishes at 37°C for 24 hours in a humidified atmosphere containing 5% carbon dioxide.

To previously prepared samples of mouse fibroblast cultures in cell culture dishes, we introduced 200 mL of each saliva sample (each, 2 x 10^6 cells). The fibroblasts were given 200 mL of hydrogen peroxide at 0.1 mmol/L as the positive control. As an absolute control, the cell culture medium without the test substance was employed. 48 hours were spent incubating the samples. 20 mL of cell suspension and 80 mL of 10% trypan blue solution, which particularly colours dead cells blue, were combined in order to count the number of cells and assess their vitality. Utilizing an optical microscope and a hemocytometer, the cells were counted.

Genomic DNA was extracted from the cells using a commercial kit. In each sample, 5 3 106 cells were extracted since they had grown from 2 to more than 5 x 10^6 in just 2 days. After being trypsinized, the cells underwent a 5-minute, 300-fold gravity centrifugation. RNA was removed from the cells by resuspending them in 200 mL of resuspension solution (PBS), which also contained 20 mL of RNase A solution. The cells were then incubated at room temperature for 2 minutes. The cells were resuspended and lysed in accordance with the manufacturer's instructions. DNA was able to adhere to the silicate membrane of the column thanks to the addition of 95% ethanol. Rinsing out the leftover contaminants and testing the DNA with a tris-EDTA buffer.

Using a spectrophotometer, the quality and quantity of the isolated DNA were assessed at 260 and 280 nm wavelengths. For subsequent analysis, the extracted DNA at 0.1 mg per millilitre was employed. By incubating the material at 95C for 5 minutes and quickly cooling it on ice, single-stranded DNA was produced from the sample. The DNA was then treated with 10 U of nuclease P1 for 2 hours at 37C in 20 mmol/L of sodium hydroxide to achieve a pH of 5.2, followed by 5 U of alkaline phosphatase for 1 hour at 37C in 100 mmol/L of tris buffer to get a pH of 7.5, to digest it into

nucleosides. The reaction mixture was centrifuged at 6000 times gravity for 5 minutes, and the concentration of the oxidative stress marker 8-OHdG was determined in the supernatant.

In accordance with the manufacturer's recommendations, the competitive enzyme immunoassay was used to measure the oxidative DNA damage. The microplate reader with a 620-nm filter was used to take the absorbance readings right away. Each sample's 8-OHdG content was quantified by comparing its absorbance to a standard curve that had been previously created using a series of standards that ranged in concentration from 0 to 20 ng per millilitre.

In statistical analysis, analysis of variance (ANOVA) and the StudentNewman-Keuls post-hoc test was utilised, and Chi square in statistical software was used to determine the effect size (version 10.0; SPSS, Chicago, Ill)

Results

High vitality was shown in every sample, and no signs of acute toxicity were found. The standard nickel-titanium archwire's viability (94.13 ±1.23) was the closest to the positive control's (89.93 ± 3.07) and much lower than all other wires ($P < 0.05$) with the exception of titanium-molybdenum. The most viable material was stainless steel, which outperformed nickel-titanium and the positive control ($P < 0.05$) with a viability of 98.97 ± 1.09. The effect size was 75%, indicating that changes across the examined groups accounted for a significant percentage of the diversity in viability.

With the exception of the positive control and the cobalt-chromium sample, nickel-titanium exhibited the lowest increase in cell counts and was considerably lower than all other samples ($P < 0.05$). Only statistically substantially higher than nickel-titanium, cobalt-chromium, and the positive control did stainless steel and titanium molybdenum show the biggest rise in cell counts ($P < 0.05$). 53.3% of the variability was due to variations in the increase of cell counts between samples.

The highest level of oxidative stress was produced by nickel-titanium (2.39 ± 0.82), which was higher than all wires and controls ($P < 0.05$). Coated nickel-titanium, copper nickel-titanium, and cobalt-chromium produced levels of oxidative stress that were lower than those of nickel-titanium ($P < 0.05$), but higher than those of titanium molybdenum and the negative and absolute controls. The lowest stresses were found in titanium-molybdenum and stainless steel (0.74 ± 0.20 and 1.25 ± 0.56, respectively), which were not significantly different from the positive control (1.10 ± 0.50). Oxidative stress variations between samples were responsible for 66.8% of the variability.

Table 1: I. Distribution of the viability, cell growth, and oxidative stress marker values in samples

Group	Viability index (%)	Cell growth (N-fold increase)	OHdG (ng/mL)
Stainless steel	98.97 ± 1.09	7.25 ± 1.14	1.25 ± 0.56
Titanium-molybdenum	96.02 ± 2.35	7.58 ± 0.56	0.74 ± 0.20
Cobalt-chromium	96.64 ± 1.42	4.76 ± 1.07	1.53 ± 0.25
Nickel-titanium	94.13 ± 1.23	3.75 ± 1.14	2.39 ± 0.82
Copper-nickel-titanium	98.55 ± 1.48	5.68 ± 0.83	1.73 ± 0.21
Coated nickel-titanium	97.55 ± 1.41	5.82 ± 0.84	1.63 ± 0.08
Control positive	89.93 ± 3.07	4.57 ± 0.10	1.10 ± 0.50
Control negative	97.75 ± 1.94	6.17 ± 2.90	0.65 ± 0.22
Control absolute	97.65 ± 0.63	6.28 ± 1.06	0.87 ± 0.26

Discussion

This study shown that in simulated in-vitro oral cavity circumstances with regulated pH, all wires are causes of oxidative stress. Standard nickel-titanium archwires produced the most oxidative stress, whereas titanium-molybdenum and stainless steel produced the least. Compared to nickel-titanium, stainless steel has a higher biocompatibility, which is in line with a previous study on old orthodontic wires. [5] Orthodontic devices are exposed to a variety of potentially harmful chemical, biologic, and physical stimuli in the mouth cavity, which can lead to the oxidation of their metal constituents. [6-8] The most frequent type of oxidation is uniform attack corrosion, in which metals interact with their

surroundings to produce hydroxides or organometallic compounds, disrupting the equilibrium between cellular oxidation and reduction. [6]

The conventional nickel-titanium arch wire contains 50% to 60% nickel, which is probably what causes its poor biocompatibility, as evidenced by the study's lowest cell viability, greatest concentrations of oxidative stress markers, and suppression of cell growth. Because of increased intracellular lactate dehydrogenase synthesis, nickel is known to have cytotoxic effects [9–11], which have been demonstrated in an in-vitro model of human oral epithelium cells by altering the redox balance and inciting apoptosis. [12] In terms of the 8-OHdG marker, all arch wires except titanium molybdenum caused more oxidative stresses than did the positive control, but only nickel-titanium demonstrated a statistically significant difference, resulting in the highest stress. Reactive oxygen species generation is increased, most likely due to fenton-line processes. [2,13] Reactive oxygen species in cells function as secondary messengers in intracellular signalling cascades by activating the nuclear factor kappa B and activator protein-1 signal transduction pathways, which in turn trigger the transcription of genes involved in the cell's growth regulating mechanisms. [14] The genotoxic effects of nickel have been extensively studied [15–17], and it has been noted that nickel ions inhibit the protective enzymes that prevent 8-oxo-20-deoxyguanosine 50-triphosphate pyrophosphatase,[18], leading to multiple types of DNA damage, including the production of 8-OHdG. Our findings support this. [19] With the exception of titanium molybdenum, all studied archwires include some nickel, which likely explains why they cause oxidative stress.

In this experiment, only brand-new cables were utilised to avoid confounding variables in the analyses. Wires that were once therapeutically employed in fixed appliances were analysed, which raises issues with managing their irregular intraoral exposure to various biochemical, mechanical, and thermal circumstances.

Our research shows that nickel-titanium archwires have low in vitro biocompatibility. In the levelling and alignment stage of orthodontic treatment, these archwires are utilised. It is crucial that the archwires have a good level of biocompatibility because that phase lasts, on average, between 6 and 12 months. It looks that copper-nickel-titanium and rhodium-coated nickel-titanium may be the sole substitutes for traditional nickel-titanium archwires because no other material is good enough to replace nickel-titanium archwires that have the same biomechanical properties and great biocompatibility. Nickel-titanium, titanium-molybdenum, and stainless steel archwires are frequently utilised during the final stage of orthodontic therapy, which lasts around six months. Stainless steel is the best material on the market in terms of biology.

Conclusion

This study adds to the body of knowledge regarding the biocompatibility of orthodontic archwires by offering recommendations for archwire types that minimise cytotoxic and genotoxic effects. Oxidative stress is a factor in all arch wires. Regarding cell survival, cell growth inhibition, and oxidative stress induction, nickel titanium archwires are the least biocompatible materials and stainless steel archwires are the most biocompatible. The sole alternatives for the same biomechanical uses are copper-nickel-titanium and rhodium-coated nickel-titanium since they exhibit relatively lower toxicity than normal nickel-titanium.

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