A Pilot Investigation into the Effect of Ammonium Chloride and Sodium Bicarbonate on Enamel Hardness in Wistar Rats. An *In Vivo* Study

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Abstract

Tooth erosion is a surface dissolution of the dental hard tissues by acids without the involvement of microorganisms. Microhardness test is suitable for determining small changes in surface microhardness, demonstrating the effects of acids, acidic beverages and bleaching products on enamel. The *in vitro* erosive potential of food and drink on teeth has been documented by using the immersion method. ***Aim:*** The aim of this study was to investigate the *in vivo* effects of ammonium chloride (NH_4Cl) and sodium bicarbonate ($NaHCO_3$) on the enamel surface microhardness of rat maxillary incisors. ***Methods:*** Twenty-one Wistar rats were divided into three groups: control, ammonium chloride-treated (NH_4Cl), and sodium bicarbonate-treated ($NaHCO_3$). Ammonium chloride and sodium bicarbonate were administered via drinking water. After eight weeks, blood pH levels and enamel surface microhardness of the rats' maxillary incisors were assessed using a blood gas analyser and microhardness measuring-machine, respectively. ***Results:*** Blood pH levels and bicarbonate (HCO_3^-) concentrations significantly increased in $NaHCO_3$ group compared with the control group; however, the decrease in blood pH of NH_4Cl group was not significant. In NH_4Cl group, incisal, middle, and cervical hardnesses slightly decreased. In $NaHCO_3$ group, a slight decrease and a slight increase were observed in the hardness of the incisal and middle regions, respectively. ***Conclusions:*** This pilot study suggests the erosive potential of two agents of which the public should be made aware, because NH4CI is used as a systemic and urinary acidifying agent, diuretic, expectorant, and as food additive, and $NaHCO_3$ has particular significance in dentistry as an ingredient in dentifrices and mouth rinses.

Key Words: Ammonium Chloride, Sodium Bicarbonate, Enamel, Hardness, Tooth, Rat

Introduction

Tooth wear is considered to be of great importance in oral health. Dental tissue loss can occur in different ways, including through caries and trauma [1,2]. Abrasion, attrition, abfraction, resorption, and erosion are described as additional forms of chronic destructive processes that can lead to irreversible tooth-surface loss [3-6]. Tooth erosion is defined as a surface dissolution of the dental hard tissues by acids, a chemical process, without the involvement of microorganisms [4].

It is well established that diet and certain food components have a clear impact on the acid-base balance [5]. After digestion, absorption, and metabolism, nearly all foods release either acid or bicarbonate (base) into the systemic circulation [6,7]. Carbonated drinks, acidic salts such as ammonium chloride (NH_4Cl), uncontrolled diabetes, starvation, hypoaldosteronism, toxic metabolites such as methanol and ethylene glycol, and high doses of salycilates are the major causes of metabolic acidosis. On the other hand, excessive intake of alkalines such as sodium bicarbonate ($NaHCO_3$), prolonged vomiting, diarrhoea with chloride loss, hyperparathroidism, various neoplasms, some diuretics, and excessive mineralocorticoids may cause metabolic alkalosis [8].

The erosive potential of food and drinks on teeth has been well documented [9,10]. Most investigations report an increase in defects after the consumption of fruits, juices, and soft drinks. In general, as a result of *in vitro* studies it has been concluded that, after immersion in beverages with a low pH, the surface microhardness of the teeth is

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reduced [11-13]. It would therefore be useful to replicate a more realistic consumption pattern under *in vivo* experimental conditions.

Aims

In contrast to *in vitro* studies investigating the erosive potential of food and drinks, the aim of the present study is to determine differences in enamel microhardness of rat maxillary incisors in relation to an acidic drink and an alkaline drink consumed for eight weeks.

Material and Methods

1. Animals and treatments

All animal protocols were approved by the committee on the use of live animals in teaching and research at the University of Marmara, Istanbul, Turkey. Twenty-one Wistar rats aged eight weeks at the beginning of the experiment (initial body weight 200-250g) were used. The animals were obtained from the Marmara University Animal Laboratory. Animals were housed in cages in an environment-controlled room (room temperature, 22 ±20°C; relative humidity, light/dark cycle, 12h/12h) with free access to food and water. In the experimental design, 21 rats were divided into three groups of seven rats each: control, ammonium chloride-treated (NH_4Cl), and sodium bicarbonate-treated ($NaHCO_3$). All rats were fed on standard rat chow containing 20% crude protein, 2.85% crude oil, 5.96% cellulose, 8% crude ash, 0.97% calcium, 0.5% phosphorous, 1.03% lycine, 0.33% methionine, 0.65% methionine+cysteine, 0.14% sodium, and 1.13% linoleic acid. The control group was given normal tap water, the ammonium chloride-treated group was given drinking water supplemented with 0.25 mol/L NH_4Cl, and the sodium bicarbonate-treated group was given drinking water supplemented with 0.25 mol/L $NaHCO_3$ for eight weeks. The consumption of food and water by the three groups was determined every day throughout the experiment period.

2. Blood collection and tooth extraction

After eight weeks, the rats were anaesthetised by urethane (1.25 g/kg) given intraperitoneally. While the rats were unconscious, blood samples were taken from the cut tips of their tails. Maxillary incisals were extracted and tooth crowns were divided into thirds as incisal, middle, and cervical. To minimise diurnal variations, the rats were routinely killed between 07:00 and 08:00 hours.

3. Assay of blood gases

Blood samples taken into capillary tubes containing heparin and an "iron flea" for stirring were used for the measurement of pH, bicarbonate (HCO_3^-), oxygen and carbon dioxidepartial pressures (pCO_2 and pO_2), with a blood gas analyser (Corning 168 pH/Blood Gas Analyzer, Corning Medical, USA.).

4. Microhardness analysis

The enamel surface microhardness of the rat maxillary incisors was assessed using a microhardness measuring machine (HMV-2000/Shimadzu Corporation, Japan). A Knoop indenter was used, with static load of 100 g, applied for 10 seconds. In each sample, five hardness measurements were carried out and the mean Vickers hardness (VH) was calculated.

5. Statistical analysis

Data are presented as mean ± standard deviation. The differences between the values of the groups were tested by Student t-test. Differences with P values of 0.05 or less were considered significant. All analyses were performed using statistical software (SPSS for Windows Version 10.0; SPSS Inc, Chicago, USA).

Results

The blood pH levels of the control, NH_4Cl, and $NaHCO_3$ groups were found to be 7.30±0.06, 7.27±0.07 and 7.37±0.02, respectively. Blood bicarbonate (HCO_3^-) concentrations of the control, NH_4Cl, and $NaHCO_3$ groups were found to be 18.43±2.3, 17.82±2.70, and 20.89±1.55, respectively. Significant increases in blood pH levels and bicarbonate (HCO_3^-) concentrations were observed in the $NaHCO_3$ group when compared with those of the control ($P<0.05$); however, the decrease in the blood pH of NH4Cl group was not significant.

When the enamel hardness levels of the NH_4Cl group were compared with those of the control group, the hardnesses levels of the incisal, middle, and cervical regions were found to be decreased by 7.13%, 3.78%, and 6.68%, respectively, and the total hardness levels decreased by 5.78%; however, these decreases were not significant *(Table 1).*

When the enamel hardness levels of the $NaHCO_3$ group were compared with those of the control group, the hardness levels of the middle, cervical, and the total hardness were found to be increased by 4.97%, 1.69%, and 2.17%, respectively, and the hardness levels of the incisal region were found to be decreased by 2.84%; however, these changes were not significant *(Table 2).*

Table 1. *Mean values of the parameters and significances of the differences between NH_4Cl and control groups*

	Control group (n=7) Mean	**NH_4Cl group (n=7) Mean**
Body weight (g)	175.71±43.92	158.57 ±38.48
Hardness incisal 1/3 (VH)	336.71±50.33	312.71 ±26.94
Hardness middle 1/3 (VH)	313.57±21.81	301.71 ±53.23
Hardness cervical 1/3 (VH)	296.14 ±32.11	277.29±39.08
Hardness total (incisal+middle+ cervical) (VH)	315.47±34.75	297.24±39.75
pH (blood)	7.30 ±0.06	7.27±0.07

Key: NH_4Cl = ammonium chloride,VH = Vickers hardness
Values are mean ±SD

Table 2. *Mean values of the parameters and significances of the differences between $NaHCO_3$ and gontrol groups*

	Control group (n=7) Mean	**$NaHCO_3$ group (n=7) Mean**
Body weight (g)	175.71±43.92	160±57.74
Hardness incisal 1/3 (VH)	336.71±50.33	327.14± 29.82
Hardness middle 1/3 (VH)	313.57±21.81	329.14± 46.44
Hardness cervical 1/3 (VH)	296.14 ±32.11	301.14± 40.33
Hardness total (incisal+middle+ cervical) (VH)	315.47±34.75	322.33±45.7
pH (blood)	7.30 ±0.06	7.37±0.02*

Key: $NaHCO_3$ = sodium bicarbonate, VH = Vickers hardness
Values are mean ±SD for seven rats
* Indicates a significant difference from the control group (<0.05)

Discussion

Microhardness changes are related to a loss or gain of mineral (demineralisation or remineralisation) of the dental structure [14]. It has been shown that the microhardness test is suitable for determining small changes in surface microhardness that demonstrate the effects of acids [15], acidic beverages [16,17], and bleaching products [18] on enamel.

Dental erosion directly affects enamel, and acidic food and beverages are the most common extrinsic factors that cause dental erosion [12,19]. Some *in vitro* studies have reported the relationship between dental erosion and acidic foodstuffs such as soft drinks, fruit juices, and sour food [11-13,20]. On the other hand, both the factors relating to the properties of the drink itself [21] as well as associated factors relating to the method of drinking, frequency of consumption, salivary parameters and dental plaque play a role in the development of dental erosion [17]. The interplay of these factors is complex and *in vitro* studies fail to obtain a realistic pattern of enamel hardness due to variation in acidic drink intake. Consequently, the present experimental study aimed to investigate *in vivo* effects of NH_4Cl consumption for eight weeks on enamel hardness levels of rats' teeth.

Several congenital chronic diseases causing acid-base disturbances result in changes in dental health and affect the development and the structure of the teeth [22]. Chronic metabolic acidosis (exposure to NH_4Cl) has been shown to result in major disturbances in the rat incisal enamel [23]. In metabolic acidosis, in addition to osteoclastic stimulation osteoblastic bone formation is inhibited. Collagen synthesis is reduced. Calcium and protein metabolism are impaired [24]. In the present study, exposure of rats to NH_4Cl for eight weeks did not change blood pH and blood gases significantly. This might be due to the physiological adaptation to induced acidosis or to the short-term exposure to

NH_4Cl. However, lower blood pH and body weights compared with those of the control group may show impairment of bone calcium and protein metabolism. Slight decreases in the enamel hardness levels support this finding. With our experimental model, it is important to simulate the effects of consuming an acidic drink for eight weeks before a chronic low-grade metabolic acidosis occurs. Accordingly, in the present study, the enamel hardness levels of the incisal, middle, and cervical and total hardness were found to be decreased insignificantly by 7.13%, 3.78%, 6.68%, and 5.78%, respectively, in NH_4Cl group when compared with the control group. Whitford *et al.* (1995) concluded that the fluorosis-like effects of acidosis on the structure and composition of enamel were due to acidosis rather than to exposure to high levels of NH_4 [23]. Consequently in our present study, because acidosis was not induced in NH_4Cl group, we did not observe severe and significant decreases in enamel hardness levels due to disturbances in enamel mineralisation that resemble severe fluorosis. This may explain the slight decreases in the enamel hardness levels of the rats exposed to NH_4Cl, resembling the effects of acidic soft drinks on enamel.

The capacity of human saliva to stabilise acids is essential for maintaining pH in the oral environment above critical levels for hydroxyapatite to protect the teeth from demineralisation. The system responsible for the buffering capacity of human saliva includes bicarbonate, phosphate and proteins, with the bicarbonate buffer system being the most important [25]. Metabolic alkalosis, which is recognised by increases in both arterial blood pH-alkalaemia- and plasma bicarbonate concentration, decreases bone calcium efflux by stimulating the osteoblasts and suppressing the osteoclasts. Alkalosis causes a decrease in the release of osteoclastic enzyme beta-glucuronidase, which has an important role in bone resorption. Also, the osteoblastic collagen synthesis is induced [26]. In several clinical studies, metabolic alkalosis has decreased bone resorption and even increased bone formation [27,28].

In our current study, exposure of rats to 0.25 M $NaHCO_3$ for eight weeks significantly increased blood pH and bicarbonate (HCO_3^-) concentrations. When the enamel hardness levels of the $NaHCO_3$ group were compared with the control group, the hardness levels of the middle and cervical regions and the total hardness were found to be increased by 4.97%, 1.69, and 2.17%, respectively. On the other hand, the hardness level of the incisal region decreased by 2.84%. Both the significant increase of pH levels and insignificant increases of enamel hardness levels in $NaHCO_3$ group may reflect positive effect of $NaHCO_3$ on rat enamel. Increased enamel hardness is consistent with the beneficial effects of $NaHCO_3$ in dental health. Sodium bicarbonate has particular significance in dentistry because of its increasing use in dentifrices and mouth rinses due to its safety, low cost, low abrasivity, water solubility, acid buffering properties, compatibility with fluoride, and, in high concentrations, antibacterial properties [29].

On the other hand, metabolic alkalosis enhances the excretion rate of fluoride by the kidneys, which is reflected in reduced fluoride levels in both soft and hard tissues. The enamel is severely damaged both macroscopically and microradiographically and uniformly bleached to the color of chalk [30]. Through its interaction with the surface of enamel, fluoride in saliva and dental plaque inhibits demineralisation and promotes remineralisation taking place at the surface of the tooth [31]. Consequently, the decreased hardness of the incisal region observed in the present study may be due to reduced fluoride levels in experimentally metabolic alkalosis-induced rats.

This experimental study aimed to investigate the effects of ammonium chloride and sodium bicarbonate in drinking water for eight weeks on the hardness of rat enamel. In contrast to other experimental *in vitro* studies that examined the effects of soft drinks on enamel hardness by immersion method, this study used an *in vivo* model by administering NH_4Cl and $NaHCO_3$ via drinking water in order to replicate a more realistic consumption pattern under *in vivo* experimental conditions. Accordingly, the changes observed were slight due to associated factors relating to the method of drinking, frequency of consumption, salivary parameters, and dental plaque that play a role in the development of dental erosion. The limitations of this study include a small sample size and relatively short time period; however, this is a pilot study and further studies are necessary to strengthen the present findings.

Conclusion

As a result, this pilot study suggests the erosive potential of two agents of which the public should be made aware of because NH_4CI is used as a systemic and urinary acidifying agent, diuretic, expectorant, and as a food additive, and $NaHCO_3$ has particular significance in dentistry [32, 33].

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