Original Article

The effect of red wine extract, resveratrol, on the degree and rate of orthodontic tooth movement in guinea pigs

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Abstract

Objective: An animal trial, its protocol approved by the Institutional Animal Care and Use Committee of the U.P. National Institutes of Health (IACUC Protocol No. 2010-008), was employed to investigate the effects of resveratrol on the degree and rate of orthodontic tooth movement in guinea pigs. **Materials and Methods:** Eighteen male adult guinea pigs were randomly allocated into 3 groups: low dose, high dose, and control groups. A 0.016" titanium molybdenum alloy wire formed into a helical torsion spring with a coil, with the loops cemented onto the maxillary incisors of the animals, served as the orthodontic appliance. Daily oral administration of resveratrol was provided to the low dose (0.047 mg/kg) and high dose (0.47 mg/kg) groups, while water was provided to the control group. Measurements were taken everyday at the interproximal area at the level of the incisal edge using a measuring caliper. **Results:** The results of the ANOVA showed no statistically significant differences in the mean measurements of tooth separation among the three groups from day 2 (P=0.966) to day 8 (P=0.056). However, starting from day 9 (P=0.049) until day 18 (P=0.000), there was a significant difference in the mean tooth separation among the test groups. **Conclusion:** Using the LSD, it was noted that the low dose and the high dose groups have similar degrees of mean tooth separation, with the control group being significantly different from the two.

Keywords: Tooth movement, orthodontic appliance, resveratrol

INTRODUCTION

Orthodontic treatment is based on the premise that when force is delivered to a tooth, certain structural alterations take place within the supporting alveolar bone which allow for tooth movement. The specific changes which occur in the investing bone that surrounds the root of an orthodontically moving tooth are commonly described as follows: Resorption of the bone on the pressure side clears a path, or makes space available, ahead of the advancing tooth; while deposition of bone on the tension side maintains a

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progressively advancing socket wall behind the moving tooth.[1]

Hence, the efficiency of alveolar bone resorption may be considered as a limiting factor in the rate of tooth movement.[2] An important mediator of alveolar bone resorption is prostaglandin (PG).[3] An increase in the number of osteoclasts was observed in response to local injections of PG in the alveolar bone during experimental tooth movement in rats.[4] Likewise, tooth movement was accelerated by local injections of prostaglandin E (PGE) in the buccocervical subgingival tissue of human subjects.[5]

Studies have shown that some medications affect orthodontic tooth movement by indirectly affecting PG levels. Indomethacin, a potent PG inhibitor, was found to slow down the rate of tooth movement in cats.[6] Ibuprofen significantly inhibited PG production in the periodontal ligament (PDL) of guinea pigs causing a marked decrease in the degree and rate of orthodontic tooth movement.[7]

It is not only drugs that have an inhibitory effect on alveolar bone resorption. In an effort to find an inexpensive means to prevent loss of bone mass in postmenopausal women affected by osteoporosis, studies were done in order to develop a nutritional approach to the problem. Certain vegetables and fruits, varieties of mushrooms and red wine, all common components of the adult diet, were shown to inhibit bone resorption.[8,9]

Of particular interest in the adult diet that shows an inhibitory effect on bone resorption is red wine. Resveratrol, one of the compounds in red wine extracts, has been studied extensively in the scientific literature due to its profound effect in many biological systems.[10] It is a polyphenol that has several pharmacological effects, including anti-inflammatory properties.[11] Since orthodontic tooth movement involves an inflammatory process,[12] it was the aim of this study to investigate the effects of resveratrol on the degree and rate of orthodontic tooth movement in guinea pigs.

Review of literature

Local mediators of alveolar bone resorption that promote inflammatory responses, such as PGs and interleukins are essential to tooth movement. The mechanism of bone resorption may be related to the release of inflammatory mediators, such as PGE which is a potent stimulator of bone resorption,[13] and interleukin-lß that stimulates bone resorption and concomitantly inhibits bone formation.[14]

Many agents used in medicines affect PG levels and other messenger compounds.[15] Nonsteroidal anti-inflammatory drugs (NSAIDs) exhibit analgesic, antipyretic, and anti-inflammatory actions as a result of the inhibition of PG biosynthesis from arachidonic acid (AA). Aspirin-like drugs inhibited PG synthesis in cell-free homogenates of guinea pig lungs.[16] Indomethacin indirectly inhibited PG synthesis and resulted in delayed tooth movement.[6] Ibuprofen significantly inhibited PG production in the PDL of guinea pigs resulting in a marked decrease in orthodontic tooth movement.[7]

Apart from drugs, certain substances in the diet may also have effects on the alveolar bone. The anti-inflammatory effects of flavonoids or polyphenols present in commonly consumed beverages and foods have been widely reported in the literature.[17,18] These effects are based on the inhibition of the cyclooxygenase-2 (COX-2)-mediated transformation of AA into PGs and are analogous to those of NSAIDs.

Resveratrol is a natural polyphenolic molecule that also has antioxidant and anti-inflammatory activity attributed to suppression of PG biosynthesis.[17] Also known as trans-3,4'5-trihydroxystilbene, resveratrol is a phytoalexin produced naturally by several plants when under attack by pathogens such as bacteria or fungi.[19] It is a phytochemical synthesized in various plant species such as grapes, peanuts, and wine.[20,21]

The low incidence of cardiovascular mortality among the French despite their high fat diet was correlated with their daily consumption of red wine that supposedly decreased cardiovascular mortality by over 30% for those who regularly consumed at least two glasses of red wine per day. Resveratrol was implicated in this beneficial action of red wine because of its ability to act as an antioxidant and an inhibitor of platelet aggregation.[22] Consumption of resveratrol through drinking moderate amounts of red wine has been suggested to be beneficial to health.[23]

The anti-inflammatory actions of resveratrol were observed in a study that reduced lung tissue neutrophilia to a similar magnitude as that achieved by treatment with budesonide. The effect was associated with a reduction in pro-inflammatory cytokines and prostanoid levels.[24]

Even at high doses (HDs), resveratrol does not have harmful effects. HDs of trans-resveratrol have been evaluated in rats, and the results showed that even with a dose 1000 times the amount consumed by a 70 kg person, there were no detrimental effects.[25] It was extrapolated that assuming that the average concentration trans-resveratrol in wine is 5 mg/L and if the moderate daily consumption of wine is 250 ml, the mean daily intake of trans-resveratrol under these conditions is ~0.02 mg/kg.

In another study,[26] which aims to determine the effect of resveratrol on spontaneously hypertensive rats, the researchers used resveratrol doses that were within the range reported in the previous study.[25] The doses employed were designed to mimic chronic resveratrol intake from moderate red wine consumption (low) and 10 times this level (high), weight adjusted to the rat from the human. The target low dose (LD) was 0.047 mg/kg body weight per day for 28 days. This is equivalent to a low 3.3 mg resveratrol for a 70 kg human per day and for a high human dose (10 times) of 33 mg resveratrol per day.

MATERIALS AND METHODS

The protocol of this study (IACUC Protocol # 2010-008) was approved by the Institutional Animal Care and Use Committee of the National Institutes of Health of the University of the Philippines, Manila, Philippines.

Male guinea pigs ranging in weight from 340 g to 680 g were used in this study. Maturation was determined by initial body weight at 8-10 weeks of age.[7] The animals were purchased from a local breeder in Los Baños, Laguna. The oral cavities of the animals were examined to ensure that the two maxillary incisors were present, with no measurable space existing between the incisors.

The sample size was determined using the operating characteristic curve, with the level of significance $\alpha = 0.05$ and the power of the test set at 0.90. Using this formula, the sample size was determined to be a minimum of five per group. Taking into account possible dropouts or failure, one animal was added per group, making the final sample size at six per group for a total of 18 subjects for the three groups. To evaluate the accuracy and precision of appliance design and placement, two more guinea pigs were fastened with the orthodontic appliance for pretesting. Data gathered from these two animals were not included in the results of this study.

The guinea pigs were housed in the animal room of the National Institutes of Health Building of the University of the Philippines Manila. The environmental requirements were maintained at temperatures of 18-22°C, with an 8-20 air change/hour, a relative humidity 45-70%, and a 12-16 h light/day cycle.

The metal cages had solid floors and mesh panels for walls and top, in order to allow the animals to see people approaching and prevent agitation of the animals.[27,28] Each cage housed three guinea pigs. The groupings of the guinea pigs in the cages were dependent on their social compatibility with each other, to prevent display of hostility and antagonism. Prior to the start of the experiment, the guinea pigs were dewormed and quarantined for a period of 1-week.

Commercially pelleted food consisting of protein and fiber, and water with Vitamin C (PedCee, 500 mg/5 ml), at 1 g/L concentration, were available for consumption *ad libitum* and were replenished daily to sustain the animals' dietary needs.

The 18 ($n = 18$) guinea pigs were randomly assigned into three groups:

- Group 1 (LD resveratrol group) was given 0.047 mg/kg of trans-resveratrol in 10 g/L food grade carboxymethylcellulose at a of 5 ml/kg body weight, for 28 days.
- Group 2 (HD resveratrol group) was given 0.47 mg/kg of transresveratrol in 10 g/L food grade carboxymethylcellulose at a volume of 5 ml/kg body weight, for 28 days.
- Group 3 (control group [CG] was given 5 ml/kg of 10 g/L food grade carboxymethylcellulose for 28 days.

The animals were identified through a code determined by letter code to indicate their group (LD group, HD group, and CG group) and a number was assigned by the researcher (e.g., CG1, LD1, HD1). Each identification code had an accompanying descriptive remark of the fur markings of the guinea pig assigned with that identification code.

This study made use of an ultra-pure pharmaceutical grade resveratrol powder from polygonum cuspidatum extract with 99.92% purity. Due to its low solubility in water, trans-resveratrol was suspended in 10 g/L food grade carboxymethylcellulose at a volume of 5 ml/kg body before each administration.[25] The solution was administered orally using a 10 ml syringe and needle. The dose was adjusted according to the animals' weight.

On day 11, after a hands-on demonstration of a qualified veterinarian on several animal subjects, the guinea pigs were anesthetized by the investigator prior to the placement of the appliance. A dose of 20 mg/kg of Zoletil (Tiletamine HCL, Zolazepam HCL) was injected intramuscularly.

Tooth preparation involved placing a 0.5 mm undercut between the maxillary incisors, with the aid of a micro-motor handpiece and a #1 round diamond bur. The undercut served to stabilize the appliance during the bonding procedure and to resist occlusogingival displacement. The mandibular incisal edges were reduced using a diamond fissure bur to prevent occlusal interference with the appliance.

The labial surfaces of the maxillary incisors were debrided and dried. Following etching, the enamel surfaces were rinsed with water for 20 s and dried until chalky in appearance. The primer was applied to the etched surfaces; this was light cured for 20 s. Then the arms of the fixed orthodontic appliance were inserted through the interproximal contact until engaged in the undercut.

The design of the fixed orthodontic appliance consisted of a 0.016" titanium molybdenum alloy wire (TMA, Ormco Corp; Glendora, Calif) formed into a helical torsion spring with a coil (4 turns, 2 mm in diameter) with arms (15 mm in length). Horizontal V-bends 10 mm anterior to the coil were placed on both arms to prevent labial displacement of the appliance [Figure 1]. The arms are bent 90° outward [Figure 2]. The arms are extended into loops that fit the incisors [Figure 3]. The initial force exerted by the orthodontic appliance prior to insertion was determined with a Universal Testing Machine (Tinnius Olsen, USA), low capacity, at the UP Diliman Civil Engineering Construction Materials and Structures Laboratory. It was capable of exerting a reciprocal lateral force of 25 ± 0.1 g when both arms are touching each other.

The orthodontic appliance was retained in position with light cured flowable composite and several layers of light cured composite (3M ESPE Filtek™ Z250), to compensate for the adhesive wear that will be brought about by the animals' continuous gnawing behavior.

No measurable space existed between the maxillary incisors prior to the placement of the orthodontic appliance. The average of three direct linear measurements on the animals was computed and recorded as intraoral measurements. Measurements were taken everyday at the interproximal area at the level of the incisal edge using a measuring caliper (Mitutoyo Corp, Japan) accurate to 0.01 mm from days 2 to 18 after placement of the orthodontic appliance. On completion of data collection, each specimen's maxilla was radiographed to verify the continuity of the interpremaxillary suture and separation of the incisors.

After completion of the experiment, the appliance was removed from each guinea pig. All the animals were sacrificed guinea pigs and properly disposed by the personnel of the National Institutes of Health.

Levene's test of equality of error variances was used to test the homogeneity of the variances to see if analysis of variance (ANOVA) was good to use. After determining that the variances were homogenous, ANOVA was done to determine whether or not statistically significant

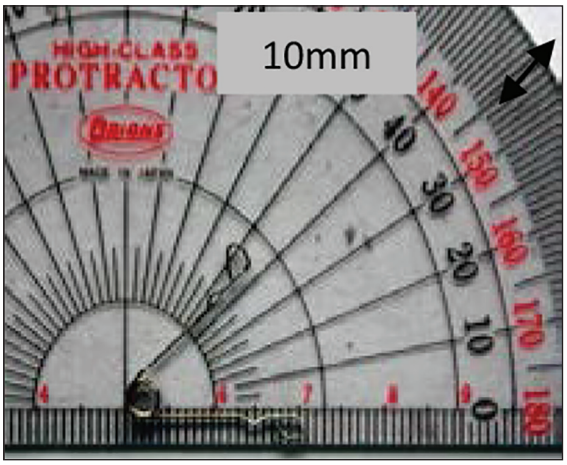

Figure 1: Titanium molybdenum alloy spring to scale

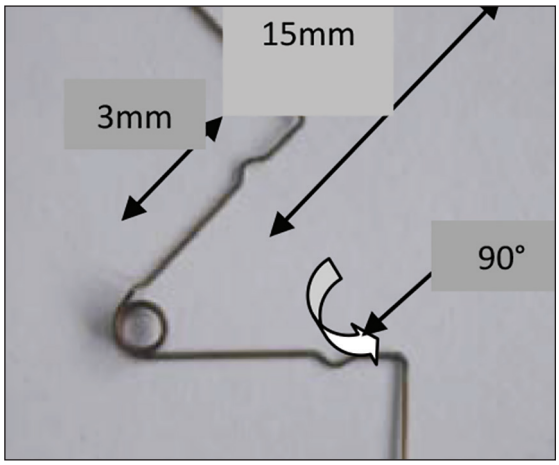

Figure 2: Dimensions of Spring

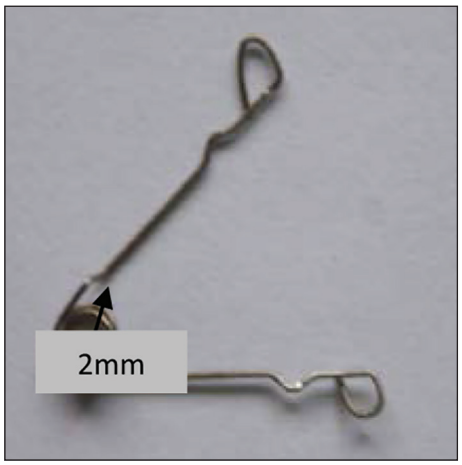

Figure 3: Final design of orthodontic appliance

differences existed between and within groups. When it was determined that a significant difference existed among the three groups, a *post-hoc* test, Fisher's least significant difference (LSD), was then used to establish which groups differ from each other. ANOVA was also done to determine if there are significant differences between the degree and

rate of maxillary incisor separation, and the comparative rate of change among the three groups.

RESULTS AND DATA ANALYSIS

Levene's test of equality of error variances [Table 1] was used to test the homogeneity of the variances. Results showed that variances are homogeneous, and there is a normal distribution of data.

The ANOVA table [Table 2] revealed that there are significant differences between the treatment groups (LD, HD, and control) and the time (different intervals of measurement) since *P* values are both 0.000. Table also shows that the power of the test is high, mostly 100%.

After determining from the ANOVA that there was a significant difference between the three groups, a *post-hoc* test was done for multiple comparisons. Using Fisher's LSD [Table 3], the groups that differ from each other were determined. From the table (significant column), one could see that the LD and the HDs are not significantly different from each other, but they are both statistically different from the CG.

Descriptive statistics of the mean degree of tooth separation and the mean rate of tooth movement were summarized in Tables 4 and 5, respectively. There was no statistically significant difference in the mean measurements of tooth separation among the three groups from day 2 of placement of appliance ($P = 0.966$) to day 8 ($P = 0.056$) [Table 4]. However, starting from day 9 of appliance placement ($P = 0.049$) until the last day of the experiment, day 18 of appliance placement ($P = 0.000$), there was a significant difference between the mean tooth separation among the test groups. Using LSD, it was noted that the LD and the HD have a similar degree of mean tooth separation with the CG significantly different from the two. Furthermore, the *post-hoc* test indicated that tooth movement was significantly different from days 9 to 18 as compared to tooth movement from days 2 to 8. It was also shown that there was a highly significant difference between the mean rates of tooth separation among the three treatment groups on days 7 and 8 of appliance placement with the two resveratrol groups being similar and both being different from the CG [Table 5].

The radiographs of the maxilla of the subjects at day 11 of appliance placement revealed the continuity of the

Table 1: Levene's test of equality of error variances

Dependent variable: SEPRTN			
F	df1	df2	Sig.
0.976	50	222	0.525

Tests the null hypothesis that the error variance of the Dependent variable is equal across groups. a. Design: Intercept+TX=TIME=TX*TIME

Table 2: ANOVA table: Tests of between-subjects

Dependent variable: SEPRTN							
Source type	Type III sum of squares	df	Mean square	F	Sig	Noncent parameter	Observed power[a]
Corrected model	247.551[b]	50	4.951	29.613	0.000	1480.667	1.000
Intercept	3009.944	1	3009.44	180003.242	0.000	18003.242	1.000
Treatment (Tx)	26.796	2	13.398	80.137	0.000	160.273	1.000
Time	215.77	16	13.486	80.661	0.000	1290.576	1.000
TX*TIME	6.699	32	0.209	1.252	0.177	40.068	0.949
Error	37.116	222	0.167				
Total	3205.072	273					
Corrected total	284.667	272					

a-Computed using alpha = 0.05; b-R Squared = 0.870 (Adjusted R Squared = 0.840)

Table 3: Fisher's least significant difference

Dependent variable: SEPRTN						
					95% Confidence interval	
(I) TX	(J) TX	Mean difference (I-J)	Std. error	Sig	Lower bound	Upper bound
Low dose	High dose	-0.0154	0.06062	0.800	-0.1348	0.1041
	Control	-0.6505*	0.06062	0.000	-0.7700	-0.5311
High dose	Low dose	0.0154	0.06062	0.800	-0.1041	0.1348
	Control	-0.6352*	0.06062	0.000	0.7546	-0.5157
Control	Low dose	0.6505*	0.06062	0.000	0.5311	0.7700
	High dose	0.6352*	0.06062	0.000	0.5157	0.7546

Based on observed means; * The mean difference is significant at the 0.05 level

Table 4: Comparison of degree of tooth separation in mm

Day	*n*	Degree of tooth separation (mean ± SD)			*P*	LSD
		Group 1 (low dose)	Group 2 (high dose)	Group 3 (control group)		
2	6	1.80167±0.19156	1.79167±0.27448	1.75833±0.38097	0.966	
3	6	2.06833±0.27169	2.105±0.47243	2.09667±0.40352	0.988	
4	6	2.245±0.3235	2.21167±0.47089	2.53167±0.35707	0.315	
5	6	2.38667±0.36811	2.485±0.53031	2.87±0.39799	0.164	
6	6	2.48333±0.39707	2.58333±0.55648	3±0.35214	0.131	
7	6	2.63333±0.4244	2.66±0.56413	3.26333±0.37564	0.055	
8	5	2.624±0.43998	2.688±0.61654	3.38±0.37068	0.056	
9	5	2.758±0.48226	2.774±0.63779	3.538±0.34564	0.049	(1=2) 3
10	5	2.894±0.42998	2.92±0.61577	3.834±0.33344	0.016	(1=2) 3
11	5	3.14±0.50299	3.18±0.61806	4±0.37417	0.034	(1=2) 3
12	5	3.468±0.34946	3.352±0.52855	4.244±0.38468	0.012	(1=2) 3
13	5	3.77±0.21131	3.524±0.47522	4.484±0.36801	0.004	(1=2) 3
14	5	3.876±0.16817	3.74±0.44654	4.672±0.35074	0.002	(1=2) 3
15	5	3.96±0.19596	4.022±0.44617	4.838±0.32105	0.002	(1=2) 3
16	5	4.08±0.19235	4.18±0.47645	5.02±0.31145	0.002	(1=2) 3
17	5	4.212±0.19677	4.304±0.43478	5.196±0.29788	0.001	(1=2) 3
18	5	4.382±0.18833	4.444±0.46166	5.496±0.17813	0.000	(1=2) 3

LSD – Least significant difference; SD – Standard deviation

Table 5: Comparison of rate of tooth movement in mm/day

Day	*n*	Rate of tooth movement (mean ± SD)			*P*	LSD
		Group 1 (low dose)	Group 2 (high dose)	Group 3 (control group)		
2	6	1.80±0.19	1.79±0.27	1.76±0.38	0.965	
3	6	0.27±0.14	0.31±0.19	0.34±0.15	0.779	
4	6	0.18±0.09	0.09±0.06	0.43±0.19	0.262	
5	6	0.14±0.04	0.27±0.15	0.34±0.12	0.372	
6	6	0.10±0.06	0.10±0.03	0.13±0.23	0.910	
7	6	0.15±0.06	0.08±0.07	0.26±0.04	0.000	(1=2) 3
8	5	0.07±0.03	0.09±0.02	0.19±0.09	0.018	(1=2) 3
9	5	0.13±0.08	0.09±0.03	0.16±0.05	0.223	
10	5	0.14±0.07	0.22±0.17	0.30±0.15	0.296	
11	5	0.25±0.10	0.19±0.05	0.17±0.06	0.338	
12	5	0.33±0.16	0.17±0.12	0.24±0.17	0.376	
13	5	0.30±0.10	0.17±0.06	0.24±0.10	0.337	
14	5	0.11±0.06	0.21±0.09	0.19±0.11	0.224	
15	5	0.08±0.06	0.28±0.10	0.17±0.07	0.061	
16	5	0.12±0.07	0.16±0.08	0.18±0.06	0.453	
17	5	0.13±0.05	0.12±0.08	0.18±0.07	0.519	
18	5	0.17±0.03	0.17±0.09	0.30±0.14	0.100	

LSD – Least significant difference; SD – Standard deviation

interpremaxillary suture [Figure 4]. This verified that the tooth separation that occurred was due to the force exerted by the fixed orthodontic appliance on the maxillary incisors.

DISCUSSION

Resveratrol has been studied extensively in the different fields of medicine because of its different effects in many biological systems. Studies have shown these effects as an antioxidant, an inhibitor of platelet aggregation, a modulator of lipid and lipoprotein metabolism, a vasorelaxing agent, anticancer, and anti-inflammatory. These studies have also shown that in exhibiting these effects, resveratrol inhibited local inflammatory mediators such as PGs, interleukins, neurosecretory agents, and growth factors,[17,22,29-33] which were previously identified to be necessary in orthodontic tooth movement and bone resorption.

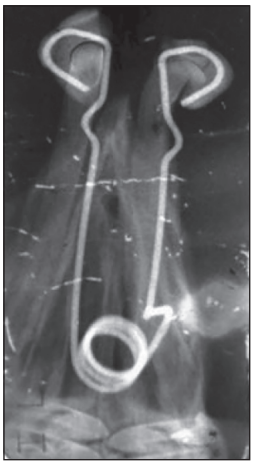

Figure 4: Radiograph of premaxillary suture at day 21

The aim of this study was to determine whether resveratrol would have an effect on the degree and rate of experimental orthodontic tooth movement in guinea pigs. It was presumed that chronic resveratrol intake would reduce the degree of tooth separation and decrease the rate of orthodontic tooth movement with its aforementioned inhibitory effect on the inflammatory mediators.

This study made use of an ultra-pure pharmaceutical grade resveratrol powder from polygonum cuspidatum extract with 99.92% purity available as a dietary supplement instead of the dry residue extracted from red wine. Extraction of dry residue from 1-l of red wine produces only approximately 2.9 g and this is prepared according to the following procedure: Briefly, phenolic compounds were adsorbed on a preparative column, then alcohol desorbed; the alcoholic-eluent was gently evaporated; the concentrated residue was lyophilized and finely sprayed to obtain the dry powder.

The doses of resveratrol were derived from the studies of Rush *et al*.[26] and Juan *et al*.[25] These were designed to mimic chronic resveratrol intake from moderate red wine consumption (low) and 10 times this level (high). The target LD was 0.047 mg/kg body weight per day for 28 days. This is equivalent to a low human dose of 3.3 mg resveratrol per day and to a high human dose (10 times) of 33 mg resveratrol per day. This modest dose mimicking moderate red wine consumption results in absorption of a sufficient quantity of resveratrol to inhibit platelet aggregation, one clinical index of the beneficial effects of red wine.[34]

The anti-inflammatory property of resveratrol is attributed to the suppression of PG biosynthesis. These effects are based on the inhibition of COX-2-mediated transformation of AA into PGs and these are analogous to those of NSAIDs.[35] These abilities of resveratrol to inhibit PG synthesis and affect the whole AA cascade may explain results from the present study, wherein there is a lesser degree of tooth separation seen in subjects given the low and HDs of resveratrol compared to the CG. This could have been further substantiated if a PGE_2 Radioimmunoassay Kit[7] was used to analyze samples of inflammatory exudate from the PDL space of the guinea pigs subjected to orthodontic tooth movement, in order to detect for the presence of PGE_2.

Many of these inflammatory biomarkers found during orthodontic tooth movement have been reported to be affected by resveratrol. Resveratrol has been demonstrated to inhibit the expression of interleukin-6,[36,37] tumor necrosis factor-α,[38] and matrix metalloproteinase-9.[39,40] With the different roles of these biomarkers in orthodontic tooth movement, from osteoclast migration to bone resorption, inhibiting their expression may explain the significant lesser degree of tooth separation of the resveratrol groups (LD and HD) compared to the CG.

The HD resveratrol group despite having 10 times more dose than the LD did not significantly differ with the LD in the degree of tooth separation. This may be based on the kinetics of absorption of resveratrol administered over a prolonged period of time. After a sufficiently high tissue concentration of resveratrol has been attained, the equilibrium between the absorbed and eliminated resveratrol can prevent further increases, with the amount of resveratrol in plasma leveling-off.[34] This may point to similar amounts of resveratrol in guinea pigs in the LD and HD, accounting for the similar degree of tooth separation between the two resveratrol groups. However, another study observed that some of the biochemical parameters on spontaneously hypertensive rats appeared to have a dose-dependent effect.[26]

Results from the current study showed that the greatest degree of mean rate of tooth movement occurred during day 2 after placement of appliance (0.88-0.90 mm). On day 3 onward, the rate of tooth movement decreased. The initial rate of tooth displacement was not different for the three groups ($P = 0.965$). However, on days 7 and 8, there was a highly significant difference in mean rate of tooth movement between the groups ($P = 0.000$, $P = 0.018$ respectively), with the LD and HDs having similarly decreased rates of tooth movement than the CG. Furthermore, the three groups from this study were not different in the degree of tooth separation from days 2 to 8. However, from day 9 onward, there was a highly significant difference in the degree of tooth separation between the three groups, with the LD and HD resveratrol group having comparable lesser degree of tooth separation compared to the CG. Tooth movement from days 9 to 18

was also significantly different from days 2 to 8. This is consistent with what Profit has discussed, that from 7 to 14 days after placement of appliance, as undermining resorption removes the lamina dura adjacent to compressed PDL, tooth movement occurs.[41] This can be further corroborated by a study on the effect of acetaminophen, ibuprofen, and misoprostol on the degree and rate of orthodontic tooth movement in guinea pigs, which also revealed a highly significant difference between the mean rates of tooth separation among the various groups investigated after day 8. This difference in the mean rates of tooth movement after day 8 was attributed to enhanced osteoclastic activity.[7]

The possible effects of the two resveratrol groups on osteoclast proliferation, on days 7 and 8, during which time these cells are supposed to have enhanced activity, might have contributed to the decrease in the rates of tooth movement of the two groups compared to the CG. This decreased rate of tooth movement on days 7 and 8 might have also caused the significant decrease in the degree of tooth separation of the two resveratrol groups from day 9 onward compared to the CG. This explains the significant difference in tooth movement from days 9 to 18 as compared to days 2-8.

The presence of resveratrol in the treatment groups should have been determined as described in a study by Juan *et al*.[42] At 24 h after the last oral administration, the trans-resveratrol concentration should have been measured using the method described by Juan *et al*.[43] With this procedure, excellent separation of trans-resveratrol will be achieved, thus allowing for a rapid analysis of the sample for absorption, distribution, and metabolism studies.

A force of 25 g was selected because this was found to be the optimal force necessary for orthodontic separation without creating separation of the interpremaxillary suture or transfer of nonphysiologic forces to the teeth or supporting periodontal tissues in the guinea pig.[7,44]

Upon completion of data collection at day 21, each specimen's maxilla was radiographed at the Restorative Laboratory of the UP College of Dentistry. One-third of a periapical x-ray film (Kodak) was used to verify the continuity of the interpremaxillary suture and the linear separation of the incisors. Results of the radiographs showed that in all of the three groups, separation of the maxillary incisors of the guinea pigs are primarily due to the orthodontic tooth movement caused by the delivery of force through the fixed orthodontic appliance.

CONCLUSION

In this study, it was demonstrated that resveratrol, both in low and HDs, significantly decreased the degree of orthodontic tooth movement as compared to the CG. There is no statistically significant difference in the low and HDs.

In addition, there was also a statistically significant difference in the mean rate of tooth movement in the resveratrol group (low and HD) as compared to the CG. Both the LD and HD have a significantly lesser mean rate of tooth movement compared to the CG on days 7 and 8.

From these results, it may be concluded that resveratrol, both at low and HDs, has an inhibitory effect on the degree and rate of orthodontic tooth movement in guinea pigs.

These findings indicate that the inhibitory properties of trans-resveratrol may be utilized for anchorage purposes. Care should also be observed in orthodontically treating patients who drink red wine on a daily basis, since resveratrol, even at LDs, equivalent to two glasses of red wine, can already exhibit its inhibitory effect.

RECOMMENDATIONS

It is recommended that further studies should include the effect of resveratrol on PGE_2 production in the PDL during orthodontic tooth movement. Results may be more conclusive if the effect of resveratrol on inflammatory mediators during orthodontic tooth movement can be determined. A radioimmunoassay technique can be employed to quantitatively analyze samples of inflammatory exudates from the PDL space for the presence of some inflammatory mediators associated with orthodontic tooth movement and whether resveratrol would inhibit their expression concomitant with an inhibitory effect on tooth movement.

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Conflicts of interest

There are no conflicts of interest.

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