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ORIGINAL ARTICLE

An animal model of intrinsic dental erosion caused by gastro-oesophageal reflux disease

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OBJECTIVES: To explore the association between dental erosion and gastro-oesophageal reflux disease (GORD), we used an animal model of GORD.

MATERIALS AND METHODS: We performed an operation to force gastro-duodenal contents reflux in male Wistar rats, and examined the teeth in the reflux rats at 15 or 30 weeks postoperatively. Dental erosion was evaluated based on a slightly modified index from a previous report. Estimation of pH was employed in the oesophageal and gastric contents.

RESULTS: Macroscopically, dental erosion was only detected in the reflux rats. Histopathologically, dentin exposure was detected in three of the seven cases after 30 weeks. Alveolar bone destruction and osteomyelitis were also noted in severe cases. The pH of the oesophageal and stomach contents was 6.93 ± 0.15 and 3.7 ± 0.39, respectively.

CONCLUSIONS: We confirmed the relationship between dental erosion and GORD. First step of dental erosion caused by GORD is the loss of surface enamel induced by regurgitation of an acidic liquid and acidic gas. Subsequently, further destruction of dental hard tissues and tooth supporting structure is accelerated by mixed juice with gastric and duodenal contents. The reflux animal model is a useful tool to examine the mechanism of dental erosion in GORD.

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Keywords: dental erosion; rat reflux model; extra-oesophageal manifestation

Introduction

Gastro-oesophageal reflux disease (GORD) refers to the varied clinical manifestations of stomach and duodenal reflux into the oesophagus and has been implicated as a causative factor in Barrett's oesophagus and oesophageal adenocarcinoma (Wild and Hardie, 2003). In recent years, GORD has increased (Dent *et al*, 2005), and its potential involvement in a range of extra-oesophageal lesions has been a subject of considerable debate (Vakil, 2006; Farrokhi and Vaezi, 2007). It is associated with a variety of extra-oesophageal manifestations including laryngitis, glossitis and sinusitis, as well as dental erosion (Schroeder *et al*, 1995; Bartlett *et al*, 1996; Muñoz *et al*, 2003; Benages *et al*, 2006; Vakil *et al*, 2006). Dental erosion is the irreversible loss of tooth substance that does not involve bacteria, ranging from a minimal loss of surface enamel to the partial or complete exposure of dentine by a chemical process (Pace *et al*, 2008). The association between acid reflux and dental erosion was first described by Howden (1971) and was later confirmed in the adult population (Benages *et al*, 2006). Moreover, the recently published Montreal definition deals with a global classification of GORD and states: 'The prevalence of dental erosion, especially on the lingual and palatal tooth surfaces, is increased in patients with GORD'. This statement was approved by 98% of the Montreal Group panellists and is reportedly based on a high level of evidence (Vakil *et al*, 2006). Other authors have denied, at least in children, that dental erosion is relevant to GORD patients (O'Sullivan *et al*, 1998). This discrepancy may be because of the difficulty of making a clear distinction between tooth abrasion and erosion in human cases. Establishing an animal model is an excellent way to confirm the relationship between dental erosion and GORD and to further the mechanistic and chemo-preventive study of human dental erosion. Using a rat gastro-duodenal reflux model, we reported that laryngitis is induced by gastro-duodenal reflux (Ling *et al*, 2007). This model has also been used to investigate Barrett's oesophagus and its relationship to oesophageal adenocarcinoma (Kumagai *et al*, 2003, 2004; Chen *et al*, 2007a,b). Thus, the reflux animal model is useful for examining the mechanism of oesophageal lesions and the extra-oesophageal manifestations of GORD. In this study, we used the reflux model to examine the relationship between dental erosion and oesophageal refluxate in rats.

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Materials and methods

All procedures complied with the ethical guidelines for animal experimentation on the care and use of laboratory animals at Shiga University of Medical Science, Japan.

Animal model

Eight-week-old male Wistar rats (250 g; CLEA, Tokyo, Japan), and housed 2 per cage in our animal centre under the following controlled condition: 23 ± 2°C room temperature, 50 ± 10% humidity, 21 times per hour air change and a 12 h light/dark cycle. Standard solid chow (CE-2; CLEA) and tap water were available *ad libitum*. The rats were fasted for 24 h, and a midline laparotomy incision was made under diethyl ether anaesthesia. The gastro-duodenal reflux was induced according to a previously reported procedure (Chen *et al*, 2007a,b). Briefly, the oesophago-gastric junction was transected, the distal cut-end was closed and the proximal cut-end was anastomosed end-to-side to the upper jejunum, approximately 2 cm anal to the origin. After the oesophago-jejunostomy, a serosal suture (interrupted 7-0 nylon) was inserted between the oesophagus and jejunum to support the afferent loop side of the anastomosis. As a result, the serosal suture allowed ingested food to easily enter the efferent loop and also prevented food from entering the afferent loop. The gastro-duodenal contents flowed through the oesophago-jejunal stoma and back into the oesophagus (Chen *et al*, 2007a,b) (Figure 1). Sham-operated rats were used as a control, because we did not need the minimal association between diethyl ether anaesthesia or laparotomy and dental erosion. These rats underwent a laparotomy with blunt manipulation of the abdominal contents under diethyl ether anaesthesia. All of the animals were allowed access to water 12 h after the operation and to food 36 h later. About 35% of rats died of complications of reflux oesophagitis, such as malnutrition, pneumonia, bleeding and ileus or of surgery. The survived animals in the reflux and control groups were painlessly killed with an overdose of diethyl ether at postoperative week 15 ($n = 7$) or 30 ($n = 7$). Subsequently, the entire oesophagus, stomach and mandible with the teeth were resected. The crown height and the severity of dental erosion were measured from the resected samples.

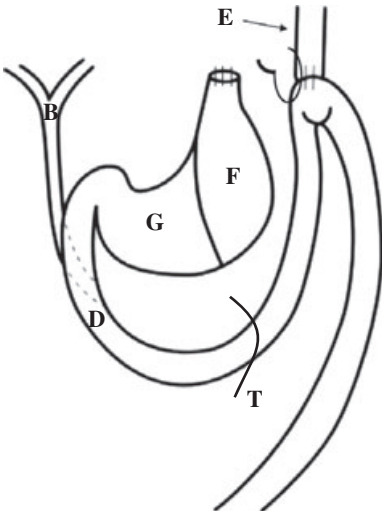

Figure 1 Gastro-duodenal content reflux model. F, fore-stomach; G, glandular stomach; E, oesophagus; D, duodenum; B, bile duct; T, treitz ligament. Gastro-duodenal content reflux was induced by oesophago-jejunostomy without gastrectomy

Analysis of crown height

Using a loupe (AS ONE, Osaka, Japan), first we measured the distance from the enamel–cement junction to the mesial and distal cusp tips on the lingual side. We then calculated the average of these measured values as crown height of the tooth (bilateral first to third molars, six teeth total).

Grading of dental erosion

Most indices of dental erosion used to grade the severity of tooth wear are based on the degree and extent of the structural loss of the human teeth, and there are no similar indices for the rat. This study evaluated dental erosion in the rats based on a previously published index (Eccles, 1979; Hattab and Yassin, 2000) (Table 1).

Analysis of the pH of the oesophageal and gastric contents in the duodenal reflux model

Estimation of pH was employed in the oesophageal and gastric contents following killing (Compact pH meter; Horiba, Kyoto, Japan).

Statistical analysis

Crown height and the oesophageal- and gastric-content pH were expressed as mean ± s.e. The Student's *t*-test or Welch's *t*-test were used for the comparisons. $P < 0.05$ was considered statistically significant.

Histological examination

The resected samples were fixed overnight in 10% formalin in phosphate-buffered saline solution. The mandibular bones were decalcified for 7 days in a decalcification solution (K-CX; FALMA, Tokyo, Japan) at 4°C. Tissue material was embedded in paraffin (Kawashima *et al*, 2005), and 3-μm serial sections were cut and stained with haematoxylin and eosin.

Results

Crown height

After 15 and 30 weeks, the lingual crown height of the first and third molars, and the second molar after

Table 1 Index for grading the severity of dental erosion in rats

Grade 0	No visible enamel changes
Grade 1	Smooth enamel; developmental structures of the enamel have partially vanished
Grade 2	Loss of cusp; developmental structures of the enamel have totally vanished or dentin exposed surface $\leqq 1/3$
Grade 3	Dentin surface exposed $>1/3$ or pulp visible through the dentin

Table 2 The crown height on the lingual side

	1st Molar	*2nd Molar*	*3rd Molar*
30 week ope. group	0.60 ± 0.04*	0.55 ± 0.05	0.56 ± 0.03*
30 week cont. group	0.71 ± 0.03	0.68 ± 0.04	0.70 ± 0.03
I5 week ope. group	0.60 ± 0.01*	0.59 ± 0.03*	0.64 ± 0.03*
15 week cont. group	0.86 ± 0.02	0.82 ± 0.02	0.75 ± 0.03

The values are given as mean ± s.e. (mm).
*Significant difference from the control, $P < .05$ by Student's *t*-test.

Table 3 Oesophageal and gastric-content pH

	Oesophagus	*Gastric*
30 week ope. group	6.93 ± 0.15	3.7 ± 0.39
30 week cont. group	n.d.	3.64 ± 0.63
15 week ope. group	6.77 ± 0.09	3.81 ± 0.49
15 wk cont. group	n.d.	3.36 ± 0.21

n.d., not determined; too little fluid in the oesophagus of control animals.

15 weeks, were significantly shorter in the reflux rats than in the control rats (Student's *t*-test, $P < 0.05$) (Table 2).

The pH of the oesophageal and gastric contents
The pH of the gastric contents in the reflux rats was 3.81 ± 0.49 after 15 weeks and 3.7 ± 0.39 after 30 weeks (Table 3). The pH of the gastric contents in the control rats was 3.36 ± 0.21 after 15 weeks and 3.64 ± 0.63 after 30 weeks. And, the pH of the oesophageal contents in the reflux rats was 6.77 ± 0.09 after 15 weeks and 6.93 ± 0.15 after 30 weeks. Because of the small volume of oesophageal contents in the control rats, we could not measure the pH after 15 or 30 weeks.

Grade of dental erosion
Dental erosion was characterized by more severe in the first molar than in the second and third molars (Table 4, Figure 2). After 30 weeks, Grade 1 dental erosion was detected in 6 of the 14 teeth in the control rats; however, it was difficult to distinguish these lesions from those that could have been caused by abrasion. After 15 and 30 weeks, the dental erosion indices were much higher in the reflux rats than in the control rats. After 30 weeks, the lesions in the reflux rats were much worse than those after 15 weeks. In three of the seven reflux rats (five of the 14 teeth), the dentin was exposed (Grade 3) in the first molar (Table 4, Figure 2c,f).

Table 4 Indices of dental erosion

	1st Molar				*2nd Molar*				*3rd Molar*			
Grade	*0*	*1*	*2*	*3*	*0*	*1*	*2*	*3*	*0*	*1*	*2*	*3*
30 week ope. group	0	0	9	5	0	8	5	1	1	11	2	0
30 week cont. group	8	6	0	0	14	0	0	0	14	0	0	0
15 week ope. group	0	5	9	0	0	9	5	0	3	11	0	0
15 wk cont. group	14	0	0	0	14	0	0	0	14	0	0	0

The numbers represent tooth counts (bilateral teeth in 7 cases) in each grade.

Pathological findings
Figure 3 shows micrographs of a representative tooth from a control rat and a severely eroded tooth from a reflux rat after 30 weeks of exposure to refluxate, respectively. No dental erosion was found in the tooth of the control rat (Figure 3a), whereas the crown of the reflux rat was severely destroyed (Figure 3b). The dental pulp and the alveolar bone were almost destroyed. Food debris was found in the loosened portion of the dental pulp and the alveolar bone. An abscess and granulation tissue were noted within the defected alveolar bone (Figure 3c). In higher magnification of Figure 3c, completely necrosis of dental pulp, destruction of tooth root and dense proliferation of neutrophils are found (Figure 3d). Granulation tissue with marked neovasculization and infiltration of lymphocytes and plasma cells are detected (Figure 3e).

Discussion

Acid is the usual cause of dental erosion (Eccles, 1979). Dental erosion may be caused by sources outside of the mouth (extrinsic) or by sources within the mouth (intrinsic). The two types of extrinsic erosion are industrial, as a result of the exposure of the teeth to atmospheric acids, and dietary, because of demineralizing foods such as citrus fruits and acid beverages (Strafne and Lovestedt, 1947; Fuller and Johnson, 1977). In contrast, intrinsic dental erosion results from the habitual regurgitation of gastric contents and may be a symptom of anorexia nervosa, hiatal hernia or recurrent vomiting caused by some abnormality of the gastrointestinal tract (Hellström, 1977; White *et al*, 1978). GORD has recently been reported as a risk factor for intrinsic dental erosion. Samples of teeth from GORD patients had dental erosion ranging from 5% to 47.5% (median, 32.5%) (Järvinen *et al*, 1988; Meurman *et al*, 1994; Schroeder *et al*, 1995; Loffeld, 1996; Muñoz *et al*, 2003; Moazzez *et al*, 2004; Oginni *et al*, 2005); however, the true impact of GORD in the genesis of dental lesions remains unclear (Pace *et al*, 2008). The aims of this study were to explore the association between dental erosion and GORD, and to establish an animal model suitable for mechanistic studies of human dental erosion.

We studied teeth from rats that were surgically manipulated to reflux gastro-duodenal contents through the oesophagus using a modified index of dental erosion for rats. In human beings, transient relaxation of the lower oesophageal sphincter is the major mechanism of gastro-oesophageal reflux in normal subjects and patients with GORD without hiatal hernia (Mittal *et al*, 1995; Mittal and Balaban, 1997). On the other hand, the reflux continuously occurred in the present model because of the oesophago-jejunostomy and the absence of a lower oesophageal sphincter after the operation.

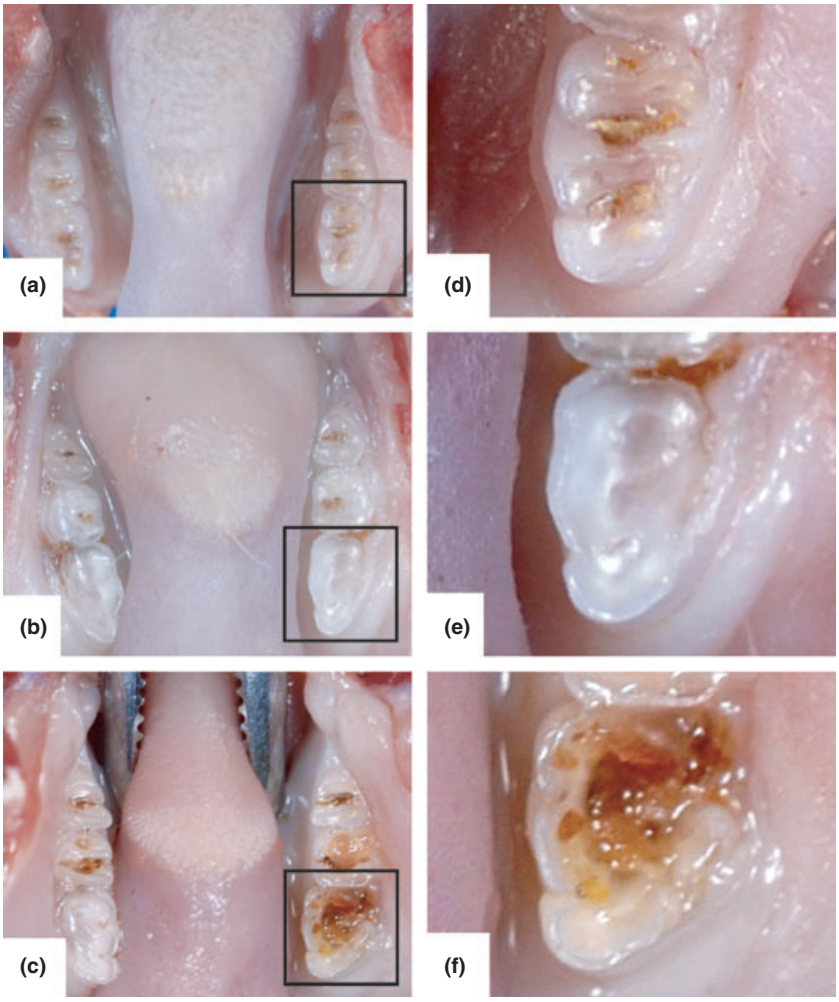

Figure 2 Macroscopic findings of the teeth in the reflux rats 30 weeks following oesophagojejunostomy. (**a**, **d**) Grade 0 control. Food debris is noted in the tooth fissure. (**b**, **e**) Grade 2 dental erosion. No food debris is found, and the development structures are absent. (**c**, **f**) Grade 3 dental erosion. Dentin surface is exposed in more than one-third of the pulp area, and pulp is visible through the dentin

However, postprandial oesophageal refluxate with gastric acidity and bile secretion may significantly increase because of the preservation of gastric function in the present model, indicating that severe reflux may occur after feeding, as in humans. In addition, the refluxate in the present model was mixed acid and duodenal contents, which frequently occurs in patients with GORD. Thus, the present reflux model is useful to analyse the mechanism of dental erosion caused by GORD. In this study, severe dental erosion was only detected in the reflux rats and was unlikely to have been caused by abrasion. Furthermore, dentin exposure was detected in three of the seven cases after 30 weeks. The height of first molar was significantly shorter in the reflux rats than in the controls. As rats are ordinarily prone, the acidic regurgitation probably occurred heavily in the first molar. Moreover, the severity of the dental erosion was much greater in these animals after 30 weeks than after 15 weeks. These findings suggest that acid reflux increased the risk for dental erosion and that the risk increased with a longer exposure to the refluxate.

It has been reported that acid reflux with a pH of less than 5.5 is required for the development of dental erosion (Lussi *et al*, 1993). These findings suggest that the acid reflux in the oral cavities of our reflux rats should have been less than 5.5; however, we found the pH to range between 6.8 and 6.9 during the study period. As we measured the pH of the oesophageal contents following the killing of rats, the contents were mixed and included gastric acid juice, duodenal contents and saliva. *In vivo*, the gastric contents would probably reflux to the oral cavity before being neutralized by the duodenal contents and saliva. In addition, the oesophageal refluxate may have not only been an acidic liquid but also acidic vapour generated from the gastric contents. This is a reasonable assumption as dental erosion can occur from excessive exposure to acid from extrinsic agents in the work environment (Fuller and Johnson, 1977). Thus, the development of dental erosion, especially loss of surface enamel, was probably associated with the regurgitation of not only an acidic liquid but also of gas or vapour generated from the refluxed gastric contents (mixed reflux) before neutralization. Further study is required to confirm this hypothesis. However, a recent interesting report showed that reflux episodes that extend proximally 15 cm above the lower oesophageal sphincter contained a mixed (liquid–gas) composition and are significantly associated with GORD symptoms irrespective of whether the pH

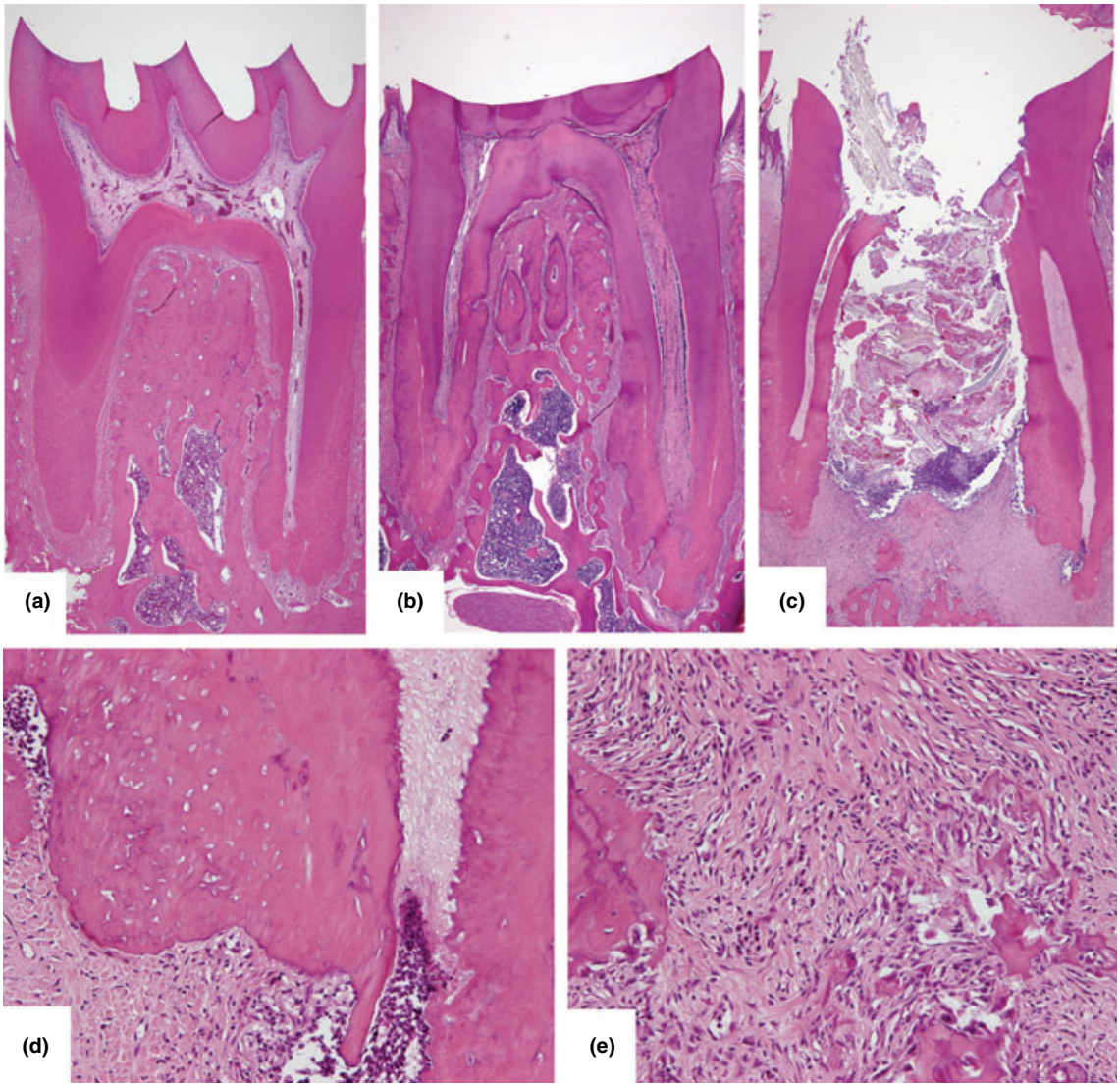

Figure 3 Microscopic findings of the teeth in the reflux rats 30 weeks following oesophago-jejunostomy. (**a**) Control animal. (**b**) Grade 3 dental erosion in reflux animal. (**c**) Grade 3 dental erosion with osteomyelitis. (**d**, **e**) Higher magnification of (**c**) and (**d**)

was acidic (< 4) or non-acidic (≥4) (Tutuian *et al*, 2008). These findings suggest that it may be easier for gaseous reflux, rather than the liquid-only reflux, to reach the proximal end of the mouth. So, intrinsic gas from the stomach is not disregarded to the decalcification in the crown enamel.

In this study, dentinal pulp as well as the alveolar bone had dissolved in rats with severe dental erosion. Alveolar bone destruction and osteomyelitis may have been caused by duodenal contents including bile juice and many bacteria rather than only gastric acid. Thus, dental erosions caused by GORD occur by mixed reflux of acid and duodenal contents. Although pH ranges of those mixed juices materials are neutral to little acidic, decalcification activity to dental hard tissues as well as alveolar bones could be strong.

In summary, the present manuscript is the first report to establish an animal rat model of human dental erosion. We confirmed the relationship between dental erosion and GORD. The gastro-duodenal reflux model was originally used in studies of Barrett's oesophagus. However, the results show that the gastro-duodenal reflux animal model is a good tool for studying the development of dental erosion caused by GORD. Compared with developing dental decay in dental caries, dental erosion caused by GORD involves the acute dissolution of dental hard tissue. A possible mechanism for the development of dental erosion caused by GORD includes the reflux of not only an acidic liquid but also acidic vapour or gas in the first step. Moreover, mixed juice with gastric and duodenal contents plays a role on developing further destruction of dental hard tissues and tooth supporting structure.

Author contributions

Dr. Mukaisho designed the study, analyzed the data and contributed to the writing of the paper. Dr. Higo performed all experiment, analyzed the data and drafted the paper. Dr. Ling, Dr. Oue and Dr. Chen helped experiment. Dr. Araki,

Dr. Sugihara Dr. Yamamto and Dr. Hattori advised the first author on data analysis, interpretation and paper writing. All authors contributed to the editing of the paper and presentation of the data.

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