Comparative Analysis of Mixed Saliva Flow Rate, pH, Buffer Capacity and Biochemistry among Miniature Pigs, Rats and Humans

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Objective: *To analyse comparatively flow rate, pH, buffer capacity and biochemistry of the mixed saliva in miniature pigs (minipigs), Sprague-Dawley (SD) rats and humans.*
Materials and Methods: *Twelve minipigs, 10 rats, and 16 human subjects were selected for tests of stimulated mixed saliva flow rate, pH, buffer capacity, and biochemistry.*
Results: *Mixed saliva flow rate of minipigs was 1.401 ± 0.387 ml/min, similar to those of humans (1.183 ± 0.869 ml/min, $p > 0.05$). The pH and buffer capacity were higher in the minipigs' mixed saliva than in the humans' mixed saliva. There were some differences in the mixed saliva biochemistry indices among minipigs, rats, and humans.*
Conclusions: *The minipig is considered a good animal candidate for saliva research. The characteristics of mixed saliva of minipigs and rats can be used in the selection of animal models for dental research.*
Key words: *biochemistry, buffer capacity, miniature pig, pH, rat, saliva flow rate*

A saliva test is a simple way to monitor body metabolism, drugs and toxicity etc[1-3]. In some cases, saliva tests are used to diagnose oral diseases and the general condition of body, as in Sjögren's syndrome[4]. Although routine saliva examination is not widely used in the clinic, the advantages of the saliva test still make it a good clinical approach.

Animals are often used in saliva analyses and salivary diseases research. Miniature pigs (minipigs) and rats are commonly used for investigations of oral diseases, but there are few comparative studies of the saliva of these two animals. The present study provides data on the mixed saliva flow rate, pH, buffer capacity, and biochemistry of saliva from minipigs, rats, and humans.

Materials and Methods

Twelve healthy minipigs were obtained from the Institute of Animal Science of the Chinese Agricultural University. The animals were 10–12 months old and

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weighed 25–35 kg. They were kept under conventional conditions with free access to water and food. Ten Sprague-Dawley (SD) rats were obtained from Beijing Animal Company. The animals were 3 months old and weighed 240–260 g. All animals were fed with clean full-nutrition feedings and had free access to drinking water during the experiment.

Sixteen human subjects were students from the Capital Medical University, seven males and nine females, aged from 21 to 23 years. Human subjects had no caries, periodontitis or systemic diseases. No antibiotics or agents affecting salivary secretion were used during the experiments.

Mixed saliva flow rate measurement

All animals were fasted for at least 12 hours before saliva flow rate measurement. The minipigs were anaesthetised by intramuscular injection at the posterior ear with a combination of ketamine chloride (6 mg/kg) and xylazine (0.6 mg/kg) (Institute of Military and Veterinary Science, Changchun, China). The mixed saliva was collected after 0.5 mg/kg pilocarpine administration (i.m.). The head of the animal was held down and the drooling saliva was collected into a 50 ml sterile tube. The rats were anaesthetised intraperitoneally with 10% chloral hydrate (Tiantan Hospital, Beijing, China). During saliva collection, the rats were placed in a restrained position on a table inclined at the angle of 10º. Their heads were positioned over plastic vessels in a way that prevented contamination by nasal secretions[5]. The secretion of saliva was stimulated with a subcutaneous injection of pilocarpine 2.5 mg/kg (i.m.). Mixed saliva was collected for 10 min from the beginning of first drop of saliva and the secretion rate was calculated gravimetrically.

All students fasted overnight without toothbrushing for at least 8 hours before examination[6]. They were required to spit the saliva into a graduated cylinder after chewing 5 g of medical paraffin for 6 min. The saliva produced during this period was measured volumetrically.

Mixed saliva pH value and buffer capacity measurement

The pH of saliva was measured with pH strips (MN, Germany). Fresh mixed saliva was applied to a pH strip with a clean straw and the pH value was read[7].

The buffer capacities of mixed saliva were measured with CRT Buffer Strips (Ivoclar Vivadent, Liechtenstein). Fresh mixed saliva was applied to the yellow area of a buffer strip with a clean straw. The change in the strip's colour was observed after 5 min. The colour of the strip was compared with the colour card to determine the buffer capacity.

Mixed saliva electrolyte ion and enzyme measurement

Fresh mixed saliva (5 ml) was placed in a 10 ml Eppendorf tube, centrifuged for 5 min at 2,000 rpm at room temperature and analysed using an automatic biochemistry analyser (7060 type; Hitachi Company, Japan). Concentrations of ions and enzymes were measured, including calcium (Ca^{2+}), phosphorus (P), sodium (Na^{+}), chloride (Cl^{-}), potassium (K^{+}), salivary amylase (AMY), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP). Data were analysed by SPSS (11.5) statistical software, and $p < 0.05$ was considered significant.

Results

Mixed saliva flow rate, pH and buffer capacity

The mixed saliva flow rates of minipigs, rats and humans were 1.401 ± 0.387 ml/min, 0.029 ± 0.040 ml/min and 1.183 ± 0.869 ml/min respectively. No significant difference was found between minipigs and human beings ($p > 0.05$). However, the rats' mixed saliva flow rate was significantly lower than that of humans ($p < 0.05$). The mixed saliva pH values of the minipigs, rats and humans were 7.77 ± 0.18, 8.80 ± 0.19 and 7.32 ± 0.17 respectively. The pH values of minipig and rat mixed saliva were significantly higher than that of humans ($p < 0.05$). The mixed saliva buffer capacity of rats was the highest, followed by minipigs and then humans.

Mixed saliva electrolyte ions

There were significant differences in electrolyte ion concentrations among minipigs, rats and humans (Table 1).

The Ca^{2+} and K^{+} concentrations in minipig saliva were higher than those in human saliva ($p < 0.01$), while the P, Na^{+}, and Cl^{-} concentrations in minipig saliva were lower than those in human saliva ($p < 0.01$). The P concentration in rat saliva was lower than that in human saliva ($p < 0.01$), and the K^{+} concentration was higher in rat than in human saliva ($p < 0.01$).

Mixed saliva enzymes

AMY concentrations in minipig saliva were higher than those of the humans ($p < 0.01$; Table 2), while ALT and LDH concentrations in minipig saliva were lower than in humans ($p < 0.01$). The concentrations of AST and ALP were lower in minipigs than in humans ($p < 0.05$),

Table 1 Electrolyte ion concentrations in mixed saliva (mean ± SD)

Source	Ca^{2+} (mmol/l)	P (mmol/l)	Na^{+} (mmol/l)	K^{+} (mmol/l)	Cl^{-} (mmol/l)
Minipig (n = 12)	2.32 ± 1.18*	0.19 ± 0.10*	17.63 ± 9.01*	21.50 ± 5.09*	16.63 ± 4.95*
Rat (n =10)	1.16 ± 0.29	0.94 ± 0.55*	29.88 ± 12.33	37.26 ± 11.59*	32.25 ± 6.05
Human (n =16)	1.10 ± 0.40	3.51 ± 1.15	31.25 ± 9.90	14.36 ± 3.23	29.13 ± 7.18

* Significant difference ($p < 0.01$)

Table 2 Enzyme concentrations in mixed saliva (mean ± SD)

Source	AMY(IU/L)	ALT(IU/L)	AST(IU/L)	LDH(IU/L)	ALP(IU/L)
Minipig (n = 12)	1125.73± 22.73*	5.18 ± 3.71*	21.91 ± 14.51#	42.27 ± 15.85*	4.27 ± 1.19#
Rat (n = 10)	2.25 ± 0.89	0.75 ± 0.46*	5.25 ± 1.83*	31.88 ± 4.52*	3.75 ± 1.04*
Human (n = 16)	1.64 ± 1.00	15.21 ± 7.41	35.93 ± 15.18	215.64 ± 79.41	9.00 ± 4.10

Significant difference ($p < 0.05$)
* Significant difference ($p < 0.01$)

while salivary ALT, AST, LDH, and ALP concentrations were lower in rats than in humans ($p < 0.05$).

Discussion

Rats and mice are the most frequently used animal models for biomedical studies of salivary glands[8-12]. The advantages of rodent models are that they are easily affordable and easy to manage. Hence the biology of the salivary glands of rodent animals has been well described. However, the disadvantages of rodent models are also obvious: their gross anatomy, morphology and physiology are quite different from humans in many organs; the smaller size of their oral maxillofacial region makes it difficult to perform dental operations, and they have small blood volumes that make it difficult to evaluate general systemic responses by following serum chemistry over long follow-up periods. Previous works from our laboratory have demonstrated the similarities in morphology and volume between minipig parotid and submandibular glands and those of humans[13,14]. Subsequently, we have used the minipig parotid gland as a suitable animal model for the study of gene transfer to salivary glands[15,16]. Accordingly, we believe that parameters for the minipig's mixed saliva flow rate, pH, buffer capacity and biochemistry might be useful in the study of human oral diseases.

In the present study we report a general characterisation of minipig saliva compared with that of rats and humans. The minipigs' stimulated mixed salivary flow rate was approximately similar to that of humans, which was significantly greater than that of rats. The saliva collection methods for stimulated mixed saliva flow rate of minipigs, rats and humans used in the present study are well-established methods in salivary research[5,6,15,16]. Although the methods used here are not the same due to the technical reasons, the measured data is stimulated saliva flow rate, and can be used to evaluate saliva secretion. Salivary pH value was higher in minipigs and rats than in humans, and salivary buffer capacity was stronger in minipigs and rats than in humans. These observations may explain the lower incidence of caries in minipigs and rats, so this feature should be considered when these animals are used for caries research. Salivary electrolyte ion and enzyme concentrations in minipigs and rats were different from human saliva. Ca^{2+} concentration of minipigs' saliva was markedly higher than that of humans and rats, which may relate to high incidence of dental calculi in minipigs. The concentration of amylase in minipigs' saliva was extremely high, which may indicate active functions of parotid gland in this animal model.

In summary, the present study provides basic information about minipig saliva and suggests that features of minipig saliva may make it useful as a large animal model for further biomedical studies of oral diseases.

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