in vivo *22*: 721-724 (2008)

The Frequency of Histological Features Mimicking Reflux Esophagitis: A Study in Non-human Primates

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Abstract. *Background: The frequency of histological changes mimicking those described for reflux esophagitis in humans was assessed in a cohort of non-human primates (NHP). Materials and Methods: A total of 121 consecutive esophagi (from 103 baboons and 18 macaques) were classified according to Ismail-Beiji for reflux esophagitis in humans into grade 1, grade 2 and grade 3 esophagitis. Results: Histological features compatible with reflux esophagitis were found in 28.2% of the baboons and in 22.2% of the macaques. Esophagitis grade 1 was more common in baboons (24%) than in macaques (6%), while esophagitis grade 2 was more common in macaques (17%) than in baboons (2%). Conclusion: Although the prevalence of reflux esophagitis in man is at least 2%, only a fraction of patients demonstrate histological features consistent with grades 1, 2 or 3 esophagitis. Hence, the finding that 27% of a cohort of consecutive, unselected NHP had grades 1, 2 or 3 esophagitis at histology is remarkable. The possible causes for the difference between species, such as the oblique position often adopted by NHP during the gastric phase of digestion, the diet, regurgitation and subsequent re-ingestion, as well as the stress of NHP when kept in captivity, are reviewed.*

A multilayer of non-keratinized stratified squamous cells covers the esophagus. This multilayer protects the underlying tissues from noxious agents and irritants contained in passing solids and fluids (1). Gastric juices of low pH refluxed into its lumen provide a major insult to the esophageal epithelium. The disease resulting from this gastroesophageal reflux (GER) is called gastroesophageal reflux disease [GERD (2)]. Spechler (3) define GERD as any symptomatic condition (*e.g.* heartburn), anatomic alteration (*e.g.* esophagitis), or both that result from the reflux of noxious material from the stomach into the esophagus. The symptoms in GERD are due to mucosal inflammation of the distal esophagus and regurgitation and not to hiatus hernia, as most patients with hiatus hernia are asymptomatic (4).

In Western countries it has been estimated that up to 40% of adults experience heartburn occasionally, episodically or long-lastingly (3). It should be stressed that inflammatory changes in the esophagus may also be induced by several external factors such as bacteria, viruses, fungi (*Candida albicans*), radiation, caustic substances, corticoid or antibiotic therapy or by diseases such as diabetes (5, 6), but acid reflux from the stomach is the leading cause of reflux esophagitis.

GERD is usually diagnosed by barium examination, endoscopic observtion, pH monitoring and esophageal biopsies. Endoscopical screening has revealed that the prevalence of reflux esophagitis in the general population is at least 2% (3).

A persistent reflux alters the homeostasis of the esophageal microenvironment leading to mucosal inflammation followed, in severe cases, by ulceration and the replacement of deeper layers by fibrotic tissue (7). Ismail-Beiji described the histological spectrum of reflux esophagitis in humans (8). Recently, similar histological changes as those described in reflux esophagitis in humans were reported in 8 baboons (9).

The purpose of the present work was to assess the frequency of histological changes analogous to those described for reflux esophagitis in humans in a cohort of non-human primates (NHP) housed at our facility.

Materials and Methods

A total of 121 consecutive esophagi (sampled at autopsy) were collected in NHP: 103 were from olive or olive/yellow hybrid baboons (*Papio hamadryas anubis*, *P.h. cynocephalus*) and the remaining 18 from macaques (*Macaca fasciculata*).

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Key Words: Esophagitis, reflux, non-human primates indent.

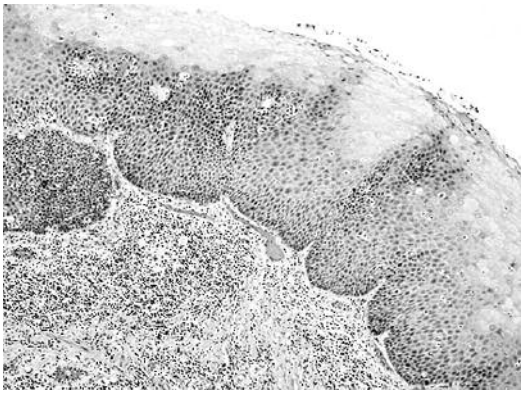

Figure 1. *Reflux esophagitis grade 1 in a baboon. Note tall papillae and basal cell proliferation and few intraepithelial lymphocytes. Chronic inflammation in the lamina propria is also seen (H&E, original magnification ×10).*

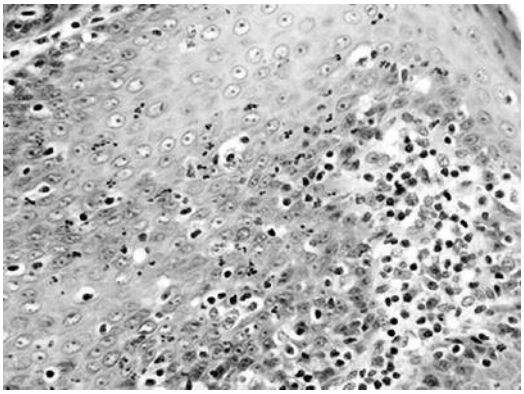

Figure 2. *Reflux esophagitis grade 2 in a baboon. Note intraepithelial granulocytic infiltration as well as chronic inflammation in the lamina propria (H&E, original magnification ×20).*

The NHPs were members of colonies at the Southwest National Primate Research Center, Southwest Foundation for Biomedical Research. The conditions of animal housing have been reported elsewhere (9). Briefly, the NHPs were housed in metal and concrete indoor-outdoor cages and were fed commercial monkey diets, occasionally supplemented with a variety of fruit and vegetables. Water was available *ad libitum*.

The animals were euthanized with a commercial barbiturate agent due to non gastrointestinal (GI) diseases or natural causes. All procedures were carried out in accordance with the Institutional Animal Care and Use Committee guidelines.

At necropsy, longitudinal tissue samples from the esophagus were fixed in 10% neutral buffered formalin, processed conventionally, embedded in paraffin, cut at 5 μm, stained with hematoxylin and eosin (H&E) and evaluated by light microscopy.

Histological changes. Following the histological classification of Ismail-Beiji for reflux esophagitis in humans (8), esophagitis in NHPs were also divided into 3 distinct subgroups: grade 1 esophagitis, characterized by basal cell hyperplasia and abnormally tall (*i.e.* deep) papillae; grade 2 esophagitis, showing intraepithelial infiltration of polymorphonuclear leucocytes; and grade 3 esophagitis having eroded or ulcerated epithelium due to inflammation.

Statistical analysis. The nonparametric test of Wilcoxon were used (Stat-View Version 4.5 software; Abacus Concepts, Berkley, CA, USA). Statistical significance was defined as $p<0.05$.

Results

Of the 121 consecutive esophagi examined, 28.2% (29/103) of the baboons and 27.8% (5/18) of the macaques showed normal squamous epithelium (N.S.). In 27.3% (33/121), the histological features mimicked those of reflux esophagitis in humans. These changes were found in 28.2% (29/103) of the baboons and in 22.2% (4/18) of the macaques (N.S.).

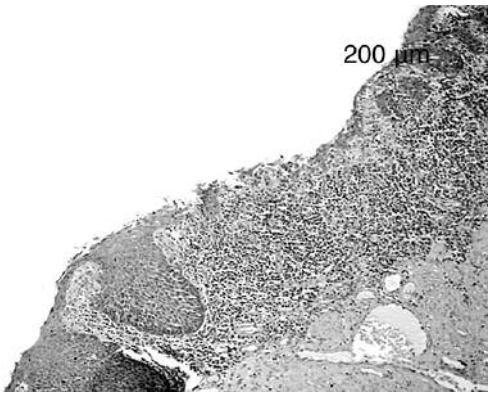

Figure 3. *Reflux esophagitis grade 3 in a baboon, showing mucosal ulceration and severe chronic inflammation in the lamina propria (H&E, original magnification ×20).*

In the baboons, changes compatible with grade 1 esophagitis (Figure 1) were recorded in 24.3% (25/103), with grade 2 esophagitis (Figure 2) in 1.9% (2/103) and grade 3 (Figure 3) in 1.9% (2/103).

In the macaques, grade 1 esophagitis was found in 5.6% (1/18) and grade 2 in 16.7% (3/18); none of the macaques had grade 3 esophagitis.

The difference between grade 1 and grade 2 esophagitis in baboons and in macaques was significant ($p<0.05$).

Discussion

This work showed that histological changes similar to those described by Ismail-Beiji for reflux esophagitis in man (8) had evolved in 27% of the 121 consecutive esophagi in NHPs. Esophagitis grade 1 was more common in baboons

(24%) than in macaques (6%), while esophagitis grade 2 was more common in macaques (17%) than in baboons (2%). The cause of this difference remains unclear.

In humans, the diet (such as alcohol, acidic foods and foods with a high fat content) may cause heartburn; the abuse of these items may result in GERD (10). It was once thought that there was a connection between obesity and GERD (11), but now it is believed that the connection is not with weight but rather with a high fat intake (12).

Large meals often lead to regurgitation with rumination in NHP, thus encouraging acid reflux symptoms (13-15). Higher-ranking NHPs housed in a group setting would have a greater access to food than lower-ranking NHPs (9). This behaviour has been noted in numerous captive species of NHPs (14-16). Before it was thought that regurgitation was a behavioural psychopathology and was frequently compared to human disorders such as bulimia and rumination syndromes (17, 18). While a causal relationship between regurgitation and GERD has not yet been determined, a correlation between this behaviour and gastrointestinal disorders seems to exist (19, 20).

Stress has long been known to both cause and increase the severity of symptoms in the GI tract (21). Corticotropin-releasing factor (CRF) is the prime mediator of the stress response. One response of CRF receptors to stress is slowed gastric emptying (21). An impaired or delayed emptying of stomach contents may contribute to heartburn and regurgitation, thus affecting the development of GERD (16). Recent research on the relationship of stress and GERD symptoms, most notably heartburn, suggest that a subset of individuals with GERD may be psychologically distressed (21). Stress exposure (22-24) can also lead to changes in esophageal motility and in lower esophageal sphincter (LES) function.

Although the prevalence of reflux esophagitis in man is at least 2% (3), only a fraction of patients demonstrate histological features consistent with grades 1, 2 or 3 esophagitis (8). Hence the finding that 27% of a cohort of consecutive, unselected NHPs had grades 1, 2 or 3 esophagitis at histology is remarkable.

The apparent high frequency of histological changes in NHP mimicking those seen in reflux esophagitis in man may partly be dictated by differences in food processing and by the unique postural position during the gastric phase of digestion. Whereas humans usually have an upright (orthostatic) position during the gastric phase of digestion, NHPs, by nature, usually adopt an oblique position (when not sitting or lying).

From the present work, several questions remain to be elucidated: i) Does the oblique position often adopted by NHPs during the gastric phase of digestion play a role in the development of reflux esophagitis in these animals? ii) Does a fatty diet, regurgitation and the subsequent re-ingestion trigger the initiation and maintenance of the disease in NHPs? iii) Does the stress in lower-ranking NHP individuals kept in captivity encourage the development of reflux esophagitis?

One possibility to explore the effect of one or several of the aforementioned environmental factors in NHPs would be to assess the frequency of histological changes compatible with reflux esophagitis at other facilities engaged in primate research. The use of different food regimens at other facilities would identify whether the diet has any bearing in the triggering of the histological spectrum of esophagitis.

In closing, it should be mentioned that the histological changes described here differ from those recorded in lymphocytic esophagitis, a newly described subset of esophagitis in patients without GER (25). Interestingly, lymphocytic esophagitis was recently recorded in NHPs (26).

Acknowledgements

Thanks are due to the staff of the Histology Laboratory and to Priscilla Williams, Data Management, Biostatistics and Scientific Computing, at the Southwest Foundation for Biomedical Research, San Antonio, Texas, for their invaluable help.

This study was supported by a grant from the Karolinska Institute, Stockholm, Sweden.

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Received April 23, 2008
Revised June 26, 2008
Accepted August 6, 2008