Helicobacter pylori and gastric carcinoma – from the view point of animal model

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Abstract. Many epidemiological studies have shown a strong association between chronic *Helicobacter pylori* infection and subsequent development of gastric carcinoma in humans. To confirm this link more clearly, it is necessary to use this bacterium in experimental studies to develop gastric carcinoma in suitable experimental animals. Persistent *H. pylori* infection has recently been achieved in the Japanese Monkeys and Mongolian gerbil models, with results demonstrating that the sequential histopathological changes in the gastric mucosa are closely mimic the gastric mucosal changes caused by *H. pylori* infection in humans. Gastric mucosa infected with *H. pylori* exhibited significantly higher gastritis score, reduction in glandular height, increase in the number of Ki-67 positive cells and over expression of p53 protein and p53 gene mutation in the Japanese Monkey Model. In the Mongolian gerbil model, *H. pylori* infection enhances gastric carcinogenesis in combination with known carcinogens such as MNU and MNNG, and also demonstrated that *H. pylori* infection alone can result in the development of gastric carcinoma. However, diagnostic criteria of gastric carcinoma in animal models remain in the great discussion. These important results provide a starting point for further studies to clarify the mechanism of gastric carcinogenesis as a result of *H. pylori* infection and assist the planning of eradication therapy to prevent gastric carcinoma.

Key words: *Helicobacter pylori*, gastric carcinoma, animal model, Japanese Monkeys, Mongolian gerbils

Introduction

In June 1994, the International Agency for Research on Cancer (IARC), a branch of the World Health Organization (WHO), convened experts from 11 countries to examine the evidence linking a number of infectious agents with human cancer. Although there is inadequate evidence in experimental animals for carcinogenicity of infection with *H. pylori*, it was designated as a definite carcinogen (Group I) to the human stomach based on three prospective case-control studies¹⁻³ reported in 1991.

However, the mechanisms by which *H. pylori* infection increases the risk of gastric carcinoma are currently unknown and the IARC emphasizes that there is inadequate evidence in experimental animals for carcinogenicity of infection with *H. pylori*.⁴ In further studies attempting to reveal the possible mechanisms by which *H. pylori* contributes to the development of gastric carcinoma, many researchers worldwide have developed animal models of infection using *Helicobacter* species.

In our department, persistent *H. pylori* infection has

recently been achieved in the Japanese Monkey and Mongolian gerbil model, with results demonstrating that the sequential histopathological changes in the gastric mucosa closely mimic the gastric mucosal changes caused by *H. pylori* infection in humans.⁵

Development of the Japanese Monkey Model

The use of monkey model provide us with the possibilities of long-term gastrofiberscopic follow up and repeated histological examinations of gastric mucosa using endoscopic biopsy specimens.

In our initial animal experiments, we endoscopically inoculated the *H. pylori* isolates from humans. The macroscopic gastritis induced by *H. pylori* was most prominent in the antrum and was milder from the body to the fornix of the stomach. Histologically, inflammatory cells were most intense in the lamina propria of the antrum.

Using these Japanese Monkey model, long term sequence of gastric mucosal changes such as gastritis scores, reduction of glandular height and an increase in

the number of immuno-positive cells for Ki-67⁶ and p53 proteins⁷ compared with the gastric mucosa of uninfected subjects.

In our recent study, we concluded that the appearance of p53 gene mutation⁸ may be closely associated with the degree of gastric mucosal atrophy, which depends on the duration of *H. pylori* infection.

However, we have not confirmed the occurrence of gastric carcinoma in the stomach of Japanese Monkeys.

Development of the Mongolian Gerbil Model

In 1996, the first study in Japan by Hirayama *et al.*⁹ reported that persistent *H. pylori* infection had been found in the glandular stomach of Mongolian gerbils (MGS/Sea; Seac Yoshitomi, Fukuoka, Japan), which had progressed to gastritis, gastric ulcer, and then to intestinal metaplasia. Honda *et al.*¹⁰ and Ikeno *et al.*¹¹ subsequently confirmed similar sequences which demonstrated that these changes in the gastric mucosa were closely similar to those found in human gastric mucosa infected with *H. pylori*.

On the other hand, in 1998, Tatematsu *et al.*¹² demonstrated that glandular stomach carcinomas developed in Mongolian gerbils to which nitroso compounds alone had been given. Specifically, it was confirmed that oral administration of the known carcinogen *N*-methyl-*N*-nitrosourea (MNU) and *N*-methyl-*N*-nitro-*N'*-nitrosoguanidine (MNNG) resulted in the development of adenocarcinoma in the glandular stomach of *H. pylori*-sensitive Mongolian gerbils.

As a result of the successful development of gerbil models with *H. pylori* infection, several studies were reported from Japan, which agreed on a causal link between gastric carcinoma and *H. pylori* infection.

Carcinogenicity of *H. pylori* in Combination with Carcinogens

Fox *et al.*¹³ reported an experimental study suggesting a possible carcinogenic role for *Helicobacter* species in the gastric mucosa after oral administration of MNNG in ferrets infected with *Helicobacter mustelae*. Ten ferrets were orally dosed with 50 mg/kg MNNG in olive oil. Nine of the ten ferrets developed adenocarcinoma. This was the first experimental study using carcinogens combined with infection with a *Helicobacter* species. However, a major problem with this study was that no uninfected ferrets were included as controls. Although spontaneous gastric adenocarcinomas have been reported¹⁴ in aged ferrets with *H. mustelae* even in the absence of carcinogen exposure, an important additional problem is that the bacterium used was not *H. pylori* but *H. mustelae*.

The results of three experimental studies recently conducted in Japan have confirmed that administration of MNNG or MNU to Mongolian gerbils with chronic *H. pylori* infection enhanced the development of different histopathological types of gastric carcinoma (Table 1).¹⁵⁻¹⁷

Sugiyama *et al.*¹⁵ reported the development of carcinoma in the gastric mucosa of Mongolian gerbils eval-

Table 1 Gastric Carcinogenesis in *Helicobacter pylori*-infected Mongolian Gerbils

Author	Year	Strain	Study design (p.p.m)	Incidence of cancer (%)	Duration of experiment (weeks)
Sugiyama <i>et al.</i> ¹⁵	1998	ATCC43504	HP → MNU(10)	7/19(36.8)	40
			HP alone	0/20(0)	40
			MNU(30) → HP	6/18(33.3)	40
			MNU alone	0/74(0)	40
Tokieda <i>et al.</i> ¹⁶	1999	ATCC43504	HP → MNNG(50)	5/17(29.4)	52
			persistant HP positive	5/8(62.5)	52
			HP eradicated	0/9(0)	52
			Br → MNNG(50)	3/22(13.6)	52
Shimizu <i>et al.</i> ¹⁷	1999	ATCC43504	MNNG(300) → HP	12/27(44.4)	50
			MNNG(300) → Br	1/19(5.3)	50
			MNNG(60) → HP	6/25(24.0)	50
			MNNG(60) → Br	0/20(0)	50
			HP → MNNG(100)	4/27(14.8)	50
			Br → MNNG(100)	3/18(16.7)	50
			HP → MNNG(20)	12/25(60)	50
			Br → MNNG(20)	1/20(5)	50
			HP alone	0/20(0)	50

HP: *Helicobacter pylori*, MNU: *N*-Methy-*N*-nitrosourea, MNNG: *N*-methy-*N'*-nitro-*N*-nitrosoguanidine, Br: Brucella broth.

Table 2 Gastric Carcinogenesis in *Helicobacter pylori*-infected Mongolian Gerbils

Author	Year	Strain	<i>cagA</i> gene	Vacuolating cytotoxin	Incidence of cancer(%)	Duration of experiment	Histological type of carcinoma
Watanabe <i>et al.</i>	1998	TN2GF4*	+	+	10/27(37)	62 weeks	well differentiated adenocarcinoma
Honda <i>et al.</i>	1998	ATCC43504 [†]	+	+	2/5(40)	72 weeks	well differentiated adenocarcinoma

* *H. pylori* isolated from patient with gastric ulcer. [†] Type strain.

uated at 40 weeks after an experiment in which 7-week old animals were inoculated with *H. pylori* (ATCC43504) and given a range of doses of MNU before or after inoculation. In this report, the most interesting finding is that only the groups of the animals infected with *H. pylori* together with administration of MNU developed gastric cancers; more specifically, they developed two different types of adenocarcinoma: intestinal and diffuse types, at significantly higher frequencies than did animals given single treatment of either *H. pylori* or the carcinogen. These interesting experimental results support the results so far obtained in large-scale epidemiological investigations.¹⁸

The group inoculated with *H. pylori* after being given MNU showed a distinctive initiation-promotion effect, whereas the group to which MNU was given after inoculation with *H. pylori* appeared to demonstrate the simultaneous action of these two factors, with *H. pylori* acting as a co-initiator. No gastric carcinoma was found within 40 weeks of *H. pylori* infection alone.

In 1999, two successful experiments on carcinogenicity were reported in which *H. pylori* (ATCC43504) was studied in combination with MNNG. Tokieda *et al.*¹⁶ conducted a study in which 5-week-old Mongolian gerbils were inoculated with *H. pylori* and orally given MNNG at 50 g/ml for 20 weeks for comparison against animals administered with MNNG alone. At 52 weeks after initiation of the study, the stomachs were excised for histopathological analysis. The group treated with MNNG and *H. pylori* developed gastric carcinoma at a significantly higher frequency than the group treated with MNNG alone. In addition, when cell proliferation of the pyloric mucosa was evaluated using a labeling index of 5-bromo-2'-deoxyuridine (BrdU), as a labeling index, cell proliferation was revealed to have markedly accelerated in those animals infected with *H. pylori*. This result suggests the possibility of explaining the link between *H. pylori* infection and early events in gastric carcinogenesis. Of special interest in this study is the discovery that administration of MNNG reduced the infection rate of *H. pylori* with the lapse of time, due to the likelihood of MNNG showing low-level (200 µg/ml)

antibacterial activity against *H. pylori*.¹⁶ It is also of interest that *H. pylori*-free animals did not develop gastric carcinoma. This is an important finding implicating *H. pylori* infection as one of the major factors in the development of gastric carcinoma. Again in this experiment, no carcinoma was found within 52 weeks in those animals with *H. pylori* infection alone.

Shimizu *et al.*¹⁷ reported similar results, in which *H. pylori* infection was confirmed to enhance gastric carcinogenesis in Mongolian gerbils treated with MNNG. Furthermore, giving MNNG to infected gerbils decreased the infection rate for *H. pylori* with concomitant reduction in the promotion effect.

Although it has been reported by Sugiyama¹⁵ that *H. pylori* can persistently colonize the stomach of MNU-treated Mongolian gerbils, it is interesting that the two studies by Tokieda¹⁶ and Shimizu¹⁷ report that MNNG administration eradicates *H. pylori* infection, resulting in a reduction of its carcinogenic effects on the glandular stomach.

Carcinogenic Experiments with *H. pylori* Infection Alone

Two experimental studies attempted to confirm prior epidemiological studies that have demonstrated an association between *H. pylori* infection and gastric carcinogenesis in humans using Mongolian gerbils chronically infected with this bacterium (Table 2).^{19,20} Both studies confirmed gastric carcinogenesis resulting from *H. pylori* infection alone, and were the first papers to fulfil Koch's postulates concerning *H. pylori* infection and gastric carcinoma.

Watanabe *et al.*¹⁹ used *H. pylori* isolated from patients with gastric ulcer (TN2GF4), and Honda *et al.*²⁰ used ATCC43504 (type strain), both of which were inoculated into 5-week old SPF Mongolian gerbils. The results showed that 37% (10 out of 27) of the animals in the former study developed well-differentiated adenocarcinoma at 62 weeks after inoculation, whereas 40% (2 out of 5) of the animals in the latter study developed well-differentiated adenocarcinoma at 72 weeks after

inoculation. Both of these strains contained cytotoxin-associated gene (*cagA*) and produced vacuolating cytotoxins. Presence and *H. pylori* infection was confirmed throughout the experiments.

These two studies provided similar findings on sequential histopathological changes. Chronic active gastritis developed initially in the lesser curvature of the antrum, accompanied by infiltration of polymorphonuclear and mononuclear cells, extending eventually to the fundic regions. In our study,²⁰ developments including gastric ulcer, atrophic gastritis, and focal intestinal metaplasia began to emerge about six months after *H. pylori* inoculation. These findings also extended to the fundic regions and greater curvatures in association with inflammatory cell infiltration. Intestinal metaplasia then became severe and associated with dysplasia at 12 and 18 months after *H. pylori* inoculation. At 18 months after the sequential histopathological changes in the gastric mucosa, well-differentiated gastric adenocarcinoma was found in the pyloric regions of the Mongolian gerbil.

Watanabe *et al.*¹⁹ also reported that all gastric cancers developed in the pyloric regions and consisted of well-differentiated intestinal-type epithelium, corresponding to intestinal-type adenocarcinoma in humans. All the gastric cancers reported in the two experimental studies developed in the pyloric regions and were of a well-differentiated type. Furthermore, sequential histopathological changes leading to carcinogenesis of the gastric mucosa were found to be common to the two studies, and very closely resembled the histopathological changes in human gastric mucosa caused by *H. pylori* infection.

The most important study yet to be carried out will be to extrapolate the sequential histopathological changes found in Mongolian gerbils to lesions in the human gastric mucosa, and to address the possible mechanism through which *H. pylori* infection may result in a predisposition to gastric carcinoma. However, a slight discrepancy was found between these studies regarding intestinal metaplasia and location of gastric carcinoma, and further studies are required to clarify the association between gastric carcinogenesis and intestinal metaplasia. Until now, there have been no reports of animal studies which confirm the development of diffuse type gastric adeno-carcinoma as a result of *H. pylori* infection alone, thus necessitating further studies.

Table 3 shows our tentative opinions on p53 and *H. pylori* infection in human, Japanese monkeys and Mongolian gerbils.²¹ In humans and Japanese monkeys both p53 immunostaining and point mutations were observed in those with *H. pylori* infection.

It is quite difficult to explain why gastric carcinoma occurs so easily in Mongolian gerbils when there is so

Table 3 Relation Between p53 and *H. pylori* and Gastric Cancer (tentative opinion)

Condition	Human	Japanese monkey	Mongolian gerbil
<i>H. pylori</i> and p53 overexpression (histology)	○	○	△ ^a
<i>H. pylori</i> and p53 point mutation (exons 5-8)	○	○	×
<i>H. pylori</i> and gastric cancer	△ ^b	×	○

^a The p53 overexpression was observed only in gastric cancer. ^b Not proven by interventional study. Circles: strong evidence, triangles: weak evidence, crosses: no evidence.

little evidence of p53 mutations in *H. pylori* infected gastric mucosa. It possibly suggest that the other tumor-related genes are involved in the carcinogenesis in this animal.

Conclusions

Experiments developed in Japan using Japanese Monkeys and Mongolian gerbils have demonstrated that *H. pylori* infection is clearly responsible for the progression of atrophic gastritis and gastric carcinogenesis, and provide important confirmation of the statements issued by IARC/WHO. It will be of critical importance to extrapolate the sequential histopathological changes found in the Japanese monkey and Mongolian gerbil to lesions in the human gastric mucosa, since this model is proven to provide important pointers for the study of the mechanism of gastric carcinogenesis as a result of *H. pylori* infection. The most important study yet to be performed is extrapolation of p53 mutations and histopathological changes in animal model with lesions common to the human gastric mucosa.

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