MINIREVIEW

Does Helicobacter pylori infection per se cause gastric cancer or duodenal ulcer? Inadequate evidence in Mongolian gerbils and inbred mice

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Abstract

A role for Helicobacter pylori infection in the development of gastric cancer in humans is well established; however, evidence for its carcinogenicity in animals remains inadequate. Mongolian gerbils and mice are commonly used to investigate the carcinogenicity of H. pylori, yet it is unclear whether H. pylori infection per se causes gastric cancer or duodenal ulcers in these animal models. Gastric adenocarcinoma in the gerbils was reported over 10 years ago, but this species has proved an unreliable model for studying H. pylori infection-associated gastric cancer. Helicobacter pylori infection alone appears insufficient to induce gastric cancer in these animals; additional carcinogenic insult is required. The development of invasive adenocarcinoma in inbred mice is rare regardless of the mouse or bacterial strain, and many long-term studies have failed to induce gastric cancer in these animals. Helicobacter pylori infection is also an established causative factor for duodenal ulcer in humans. However, few studies have attempted to develop animal models of H. pylori infection-induced duodenal ulcer. We therefore conclude that both Mongolian gerbils and inbred mice may be inadequate models for studying H. pylori infection-associated gastric cancer and that there is no animal model of H. pylori infection-induced duodenal ulcer.

Introduction

Barry Marshall and Robin Warren discovered that Helicobacter pylori bacteria were present in almost all patients with active chronic gastritis, duodenal ulcer and gastric ulcer, suggesting that the infection by these bacteria is an important factor in the etiology of these diseases (Marshall & Warren, 1984). Marshall and Warren were awarded the Nobel Prize in Physiology or Medicine for this work in 2005. The International Agency for Research on Cancer (IARC), a branch of the World Health Organization, published a report in 1994 that, in terms of a link between H. pylori and gastric cancer, there was sufficient evidence for carcinogenicity among humans and inadequate evidence for carcinogenicity in animals, classifying H. pylori overall as a group I definite carcinogen (IARC, 1994). The inadequate evidence in animals reflected the fact that no data on the cancer in experimental animals were available. Thereafter, efforts to establish animal models of H. pylori infection-induced gastric cancer have been repeated in various species, for instance Mongolian gerbils (Hirayama et al., 1996a, b; Watanabe et al., 1998), mice (Rogers et al., 2005), rats (Li et al., 1998), Japanese cotton rats (Mahler et al., 2005), Mastomys (Koga et al., 2002), monkeys (Kodama et al., 2004), cats, guinea pigs, ferrets and pigs (Nedrud, 1999; Lee, 2000). The most commonly used animals are Mongolian gerbils and mice. We recognize that valuable contributions have been made by numerous studies towards understanding the pathogenesis of gastric diseases, including cancer, in relation to H. pylori infection in humans, and many comprehensive reviews addressing the carcinogenicities of H. pylori are available (Ikono et al., 1999; O’Rourke & Lee, 2003; Pritchard & Przemeck, 2004; Rogers & Fox, 2004; Anisimov et al., 2005; Kodama et al., 2005; Matysiak-Budnik & Mégraud, 2006). However, in this mini-review, we will critically address whether H. pylori per se, without...
concomitant administration of potent carcinogens, causes gastric cancer (i.e. adenocarcinoma) or duodenal ulcer in Mongolian gerbils and inbred mice. Helicobacter pylori infection and gastric cancer in Mongolian gerbils

Mongolian gerbils \((\textit{Meriones unguiculatus})\) were found by Milne Edwards in Mongolian and China in 1867. Twenty pairs of the gerbils were brought to Japan for breeding in 1935. After about 10 years, 11 pairs were taken to the United States. During 1963–1972, five females and four males were used as breeding stock in both the US and Europe (Neumann K \textit{et al.}, 2001). Since then, these animals have been used as experimental animals, particularly in the field of neuroscience. In 1996, Hirayama and coworkers successfully infected Mongolian gerbils with \textit{H. pylori} (ATCC 43504 strain) and observed lymph follicles in the gastric submucosa 42 days after the inoculation (Hirayama \textit{et al.}, 1996a), and intestinal metaplasia after about 6 months of colonization (Hirayama \textit{et al.}, 1996b). They later reported the development of a poorly differentiated pyloric adenocarcinoma in one of 16 Mongolian gerbils at 16 months postinfection. When follow-up was prolonged to 18–24 months (14 Mongolian gerbils) or to more than 24 months (26 Mongolian gerbils) no additional adenocarcinomas were detected (Hirayama \textit{et al.}, 1999). Two cases were reported in another study in which five Mongolian gerbils were infected with the same strain (ATCC 43504) for 18 months (Honda \textit{et al.}, 1998) (Table 1). It is of interest to note that the development of carcinoids in the corpus region of the stomach was observed in about 23% (7/30 gerbils) at 12–24 months' follow-up, increasing to about 42% of animals (11/26 gerbils) at 30 months' follow-up (Hirayama \textit{et al.}, 1999).

A high incidence (37%) of well-differentiated adenocarcinoma in the pyloric area was reported by another Japanese group when the animals were infected with \textit{H. pylori} TN2GF4 strain for more than 15 months (Watanabe \textit{et al.}, 1998). Although vascular invasion and metastases were not observed, it was assumed that they might develop with longer periods of observation. These studies have helped to establish Mongolian gerbils as the model of choice when

### Table 1. Summary of Mongolian gerbils and inbred mouse models of gastric adenocarcinoma after \textit{Helicobacter pylori} infection

<table>
<thead>
<tr>
<th>Animals</th>
<th>\textit{Helicobacter pylori} strain</th>
<th>Duration of infection</th>
<th>Gastric adenocarcinoma</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mongolia gerbils</td>
<td>ATCC 43504</td>
<td>42 days</td>
<td>0/40</td>
<td>Hirayama \textit{et al.} (1996a, b)</td>
</tr>
<tr>
<td>Gerbils</td>
<td>ATCC 43504</td>
<td>12 months</td>
<td>0/3</td>
<td>Honda \textit{et al.} (1998)</td>
</tr>
<tr>
<td>Gerbils</td>
<td>ATCC 43504</td>
<td>18 months</td>
<td>2/5</td>
<td>Shirakawa \textit{et al.} (1999)</td>
</tr>
<tr>
<td>Gerbils</td>
<td>ATCC 43504</td>
<td>16 months</td>
<td>1/16</td>
<td>Hirayama \textit{et al.} (1999)</td>
</tr>
<tr>
<td>Gerbils</td>
<td>ATCC 43504</td>
<td>18–24 months</td>
<td>0/14</td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>ATCC 43504</td>
<td>&gt; 24 months</td>
<td>0/26</td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>ATCC 43504</td>
<td>13 months</td>
<td>0/14</td>
<td>Tokieda \textit{et al.} (1999)</td>
</tr>
<tr>
<td>Gerbils</td>
<td>ATCC 43504</td>
<td>12.5 months</td>
<td>0/20</td>
<td>Shimizu \textit{et al.} (1999)</td>
</tr>
<tr>
<td>Gerbils</td>
<td>ATCC 43504</td>
<td>10 months</td>
<td>0/5</td>
<td>Watanabe \textit{et al.} (1998)</td>
</tr>
<tr>
<td>Gerbils</td>
<td>ATCC 43504</td>
<td>13 months</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>ATCC 43504</td>
<td>15.5 months</td>
<td>10/27</td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>TN2GF4</td>
<td>12 months</td>
<td>0/5</td>
<td>Elfvin \textit{et al.} (2005)</td>
</tr>
<tr>
<td>Gerbils</td>
<td>TN2GF4</td>
<td>18 months</td>
<td>0/10</td>
<td>Franco \textit{et al.} (2005)</td>
</tr>
<tr>
<td>Gerbils</td>
<td>7.13</td>
<td>1 month</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>2 months</td>
<td>59%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>4 months</td>
<td>59%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>ATCC 43504</td>
<td>10 months</td>
<td>0/20</td>
<td>Kato \textit{et al.} (2006)</td>
</tr>
<tr>
<td>Inbred mice</td>
<td>SS1</td>
<td>24 months</td>
<td>0/26</td>
<td>Wang \textit{et al.} (2003)</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>119p</td>
<td>10 months</td>
<td>0/20</td>
<td>Wang \textit{et al.} (2003)</td>
</tr>
<tr>
<td>BALB/c</td>
<td>G50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6</td>
<td>SS1</td>
<td>20 months</td>
<td>0/115</td>
<td>Kim \textit{et al.} (2003)</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>SS1</td>
<td>15 months</td>
<td>0/10</td>
<td>Thompson \textit{et al.} (2004)</td>
</tr>
<tr>
<td>BALB/c</td>
<td>SS2000</td>
<td></td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>B6129 (C57BL/6x123S6/SvEv)</td>
<td>SS1</td>
<td>13 months</td>
<td>0/15*</td>
<td>Rogers \textit{et al.} (2005)</td>
</tr>
</tbody>
</table>

\*Increased carcinoma risk: inflammation in corpus is greater than in antrum and metaplastic changes are present. No adenocarcinoma was described in this report.

\#Number of animals in each group was not indicated. Sixty-two mice were divided into four groups and one group with \textit{H. pylori} infection alone was assumed to contain 15 mice.

**Helicobacter pylori** infection and gastric cancer in Mongolian gerbils

Mongolian gerbils (\textit{Meriones unguiculatus}) were found by Milne Edwards in Mongolian and China in 1867. Twenty pairs of the gerbils were brought to Japan for breeding in 1935. After about 10 years, 11 pairs were taken to the United States. During 1963–1972, five females and four males were used as breeding stock in both the US and Europe (Neumann K \textit{et al.}, 2001). Since then, these animals have been used as experimental animals, particularly in the field of neuroscience. In 1996, Hirayama and coworkers successfully infected Mongolian gerbils with \textit{H. pylori} (ATCC 43504 strain) and observed lymph follicles in the gastric submucosa 42 days after the inoculation (Hirayama \textit{et al.}, 1996a), and intestinal metaplasia after about 6 months of colonization (Hirayama \textit{et al.}, 1996b). They later reported the development of a poorly differentiated pyloric adenocarcinoma in one of 16 Mongolian gerbils at 16 months postinfection. When follow-up was prolonged to 18–24 months (14 Mongolian gerbils) or to more than 24 months (26 Mongolian gerbils) no additional adenocarcinomas were detected (Hirayama \textit{et al.}, 1999). Two cases were reported in another study in which five Mongolian gerbils were infected with the same strain (ATCC 43504) for 18 months (Honda \textit{et al.}, 1998) (Table 1). It is of interest to note that the development of carcinoids in the corpus region of the stomach was observed in about 23% (7/30 gerbils) at 12–24 months’ follow-up, increasing to about 42% of animals (11/26 gerbils) at 30 months’ follow-up (Hirayama \textit{et al.}, 1999). A high incidence (37%) of well-differentiated adenocarcinoma in the pyloric area was reported by another Japanese group when the animals were infected with \textit{H. pylori} TN2GF4 strain for more than 15 months (Watanabe \textit{et al.}, 1998). Although vascular invasion and metastases were not observed, it was assumed that they might develop with longer periods of observation. These studies have helped to establish Mongolian gerbils as the model of choice when
studying *H. pylori*-associated cancers, especially gastric cancer. The so-called Koch’s postulate that *H. pylori* can lead to gastric cancer in a susceptible host seemed to be fulfilled (Wang & Fox, 1998). However, an independent group in Sweden has made it their special aim to elucidate whether or not gastric adenocarcinomas develop in *H. pylori*-infected Mongolian gerbils (Elfvin et al., 2005). Notably neither neoplastic, invasive adenocarcinoma nor any other degree of neoplasia was found in 43 Mongolian gerbils at 3, 6, 12, or 18 months postinfection with Sydney strain 1 (SS1) and TN2GF4 strain (Elfvin et al., 2005). It is possible that these various studies differed in their criteria for grading the observed pathology; therefore, several guidelines for the classification of gastric adenocarcinoma development in the gerbil model have been suggested (Elfvin et al., 2005). In brief, it is essential to demonstrate displaced glands and the malignant potential as indicated by the presence of single tumor cells in the frontline of invasion, or at least a lateral expansion of intramucosal tumors, and to exclude regenerative changes of the nuclei (Elfvin et al., 2005). Recently, for the first time outside of Japan, Peek’s group and his coworkers (including a comparative pathologist) working in the US reported the development of gastric adenocarcinomas, characterized by marked cellular pleomorphism, cellular atypia and euchromatic nuclei, in *H. pylori* (strain 7.13)-infected Mongolian gerbils with an incidence of 17%, 59%, and 59% at 1, 2 and 4 months, respectively (Franco et al., 2005). The number of animals in each group was not indicated in that report. The development of adenocarcinomas seemed to be associated with increased nuclear accumulation of β-catenin in gastric epithelium (Franco et al., 2005). The authors suggested that, independent of inflammation, activation of β-catenin by *H. pylori* might be an important early event that precedes malignant transformation. These findings were similar to findings reported for Mongolian gerbils infected with *H. pylori* and treated with carcinogen N-methyl-N-nitrosourea (MNU) (Cao et al., 2004). Moreover, Mongolian gerbils were infected with *H. pylori* (strain B128) and followed-up for 8 months in Germany; gastric adenocarcinoma was not reported but instead an increased carcinoma risk was found (71%: 12/17 gerbils) (Rieder et al., 2005). They defined increased carcinoma risk as inflammation in the corpus greater than in the antrum and the presence of metaplastic changes based on a study in humans (Meining et al., 1998). A recent study by another Japanese group did not find gastric adenocarcinoma in 20 Mongolian gerbils infected with *H. pylori* (ATCC 43504 strain) for 10 months without extra feeding with extra salt (which was named as group G11 in this study) (Kato et al., 2006) (Table 1). To the best of our and others’ (Elfvin et al., 2005) knowledge, no metastases have been reported in any of *H. pylori*-infected gerbils that were diagnosed as adenocarcinoma, and there is no report of a gerbil that died of gastric cancer. It is likely that the incidence of gastric adenocarcinoma in the gerbils is overestimated due to the different criteria used. Considering these data, we agree with Elfvin et al. (2005) that the gastric cancer cannot be induced in Mongolian gerbils by *H. pylori* infection alone; additional carcinogenic insult is required, in agreement with the extensive body of highly reputable literature describing the multifactorial nature of gastric cancer development in the human population (for recent reviews, see Eslick, 2006; Fox & Wang, 2007). Indeed, it has been shown that *H. pylori* infection enhances the carcinogenic effects in the stomach by chemical carcinogens, such as MNU, and N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) (Sugiyama et al., 1998; Shimizu et al., 1999; Tokieda et al., 1999; Cao et al., 2002, 2004).

**Helicobacter pylori infection and gastric cancer in inbred mice**

The mouse (*Mus musculus*) has been considered an ideal model for studying human diseases for many reasons. For instance, 99% of human genes have direct murine orthologues; embryonic development, anatomy, physiology and behaviour are conserved; gene structure, gene expression and genome organization are highly related; and technology is available to generate germline mutations in mice. Genetically manipulated mice (e.g. gastrin transgenic or knockout mice) provide reproducible models to study the carcinogenesis of gastric cancer with or without *H. pylori* infection (Wang et al., 2000; Fox et al., 2003a, b; Houghton et al., 2004; Pritchard & Przemeck, 2004). Conversely, it has been almost impossible to establish the mouse model of gastric cancer by *H. pylori* infection without genetic manipulation of the mouse (i.e. in wild-type inbred mouse strains). Wadström’s group (coauthor in this article) performed a 2-year follow-up of two strains of inbred mice infected with three strains of *H. pylori* (including SS1) and found no adenocarcinoma in the stomach (Wang et al., 2003). This negative observation agreed with observations by another group that studied a large number of mice (*n* = 115) in a 20-month follow-up (Kim et al., 2003) (Table 1). We (Chen’s and Wadström’s groups) repeated Wang’s experiment together and found no gastric adenocarcinoma, but did detect hyperplasia/dysplasia of enterochromaffin-like (ECL) cells (a prelesion of ECLoma or carcinoids) in the corpus region of the stomach in the mice infected with *H. pylori* for 9 months (Zhao et al., 2003). Moreover, Lee’s group performed a 15-month study of *H. pylori* infection with SS1 and Sydney strain 2000 (SS2000) in C57BL/6 and BALB/c inbred mouse strains and found no adenocarcinoma development in the stomach of any mice (Thompson et al., 2004). Interestingly, they found that the SS2000 lacked the entire cag PAI but was still capable of inducing inflammation in
BALB/c mice in excess of SS1, which contains the entire island. Conversely, SS1 induced more inflammation than SS2000 in the C57BL/6 mice, suggesting that the cag PAI effect is host-specific in mice (Thompson et al., 2004). Rogers and coworkers used the mouse strain B6;129s, which was created by intercrossing C57BL/6 x 129S6/SvEv, to study the effect of *H. pylori* infection. For the first time in mice, they found high degree of dysplasia, i.e. gastric intestinal metaplasia/dysplasia (grade 2, carcinoma in situ) at 13 months postinfection with SS1 (Rogers et al., 2005) (Table 1). Nonetheless, gastric adenocarcinoma (dysplasia grade > 3) and invasion of neoplastic glands into the submucosa or lymphatics were not observed in this study. It is possible that the gastric intra-epithelial neoplasia, representing putative neoplastic lesions (Boivin et al., 2003), could have eventually invaded the submucosa if the mice had been followed for a term, e.g. 24 months (Arlin Rogers, pers. commun.). Thus, more long-term studies in susceptible inbred strains are probably needed before the inbred mouse can be excluded or substantiated as a model of invasive carcinoma due to *H. pylori* infection.

**Helicobacter pylori** infection and duodenal ulcer in Mongolian gerbils or inbred mice

Clinically, *H. pylori* infection is associated with decreased gastric acid secretion in gastric cancer patients but with increased acid secretion in patients with duodenal ulcers (Calam et al., 1997). A previous mouse study showed that chronic *H. pylori* infection (9 months) caused achlorhydria, hypergastrinaemia and hyperplasia of ECL cells in wild-type mice but an elevated vagal-evoked acid secretion in gastrin knockout mice (Zhao et al., 2003). We have speculated that the host factor (e.g. gastrin hormone) might influence an individual's risk of acquiring *H. pylori* infection, as well as determining the direction taken by the pathological process. Possible pathophysiological models of *H. pylori* infection-induced gastric and duodenal ulcers in humans have been well acknowledged (Peek & Blaser, 1997), but there are still no reports of gastric ulcer formation (comment by reviewer) or duodenal ulcer development in inbred mice following *H. pylori* infection in the absence of additional ulcer-inducing agents. It should, in fact, be noted that one study reported that *H. pylori* infection induces duodenal ulcer in Mongolian gerbils (Ohkusa et al., 2003); however, the link is tenuous. Indeed, although all of the animals in that study (*n = 26*) developed gastric ulcer within 3 months of *H. pylori* infection (ATCC 43504 or TN2GF4), only two of 26 gerbils seemed to have developed superficial ulcer in the duodenal mucosa, not a statistically significant number even as stated by the authors themselves. Therefore, the title of that report is misleading, and their conclusion that induction of duodenal ulcer in the animal model fulfills the requirements of Koch’s postulates for establishing a role for *H. pylori* as a causative agent is not sound. Interestingly, the two animals with duodenal ulcers were detected in the group that was infected with a highly pathogenic strain (TN2GF4), which when present for long enough might allow the occurrence of gastric metaplasia in the duodenum; this pathology permitted high level colonization at this site, subsequently resulting in superficial ulceration (comment by reviewer). Large numbers of animals are needed to determine its significance (Ohkusa et al., 2003).

**Concluding remarks**

Whether or not *H. pylori* infection alone causes gastric cancer in Mongolian gerbils remains under debate. The gerbils might not be an adequate animal model for studying *H. pylori* infection-associated gastric cancer, probably because these animals lack heat shock protein-induction, an important process in pathways leading to apoptosis, carcinogenesis, and protection from cytotoxic damage (Ota et al., 2006). There are no reports that inbred mice develop gastric cancer due to *H. pylori* infection. Accordingly, we conclude that there still has been no sufficient evidence of the carcinogenicity of *H. pylori* in experimental animals since 1994. In fact, a recent review also indicates that the epidemiological data are conflicting concerning the relationship between *H. pylori* and gastric cancer, and the early experimental animal studies require replication (Eslick, 2006). Further, there is no animal model of *H. pylori* infection-induced duodenal ulcer. Thus, Koch’s postulate has yet to be fulfilled either for gastric cancer or duodenal ulcer. Surely, studies including the controlled addition of candidate carcinogens or ulcer-inducing agents (or stress) in the presence of *H. pylori* infection are straightforward and could contribute to our knowledge of what triggers the oncogenic transformation or duodenal ulceration of *H. pylori* infection in the minority of patients who develop gastric cancer or duodenal ulcer.

**References**


