Risk of Gastric Cancer After Roux-en-Y Gastric Bypass

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Objective: To evaluate the risk of gastric cancer after Roux-en-Y gastric bypass (RYGB).

Design: Rats randomly underwent 1 of the following: RYGB, duodenojejunal bypass (DJB), or a sham operation. Postoperatively, rats underwent a protocol of cancer induction by means of both continuous (200 ppm in tap water for 16 weeks) and intermittent (50-mg/kg intraesophageal injection, once a week, for 12 weeks) administration of \( N\)-methyl-\( N\)-nitrosourea.

Setting: Institut de Recherche Contre les Cancers de l’Appareil Digestif–European Institute of Telesurgery.

Study Animals: Fifty-five Fischer 344 rats.

Main Outcome Measures: Seventeen weeks after the operation, we performed a pathologic examination of the whole stomach in all animals to assess for the presence of cancer and/or premalignant lesions. Bilirubin concentration, gastric bacterial flora, and any other pathologic findings were also recorded.

Results: In rats in the sham and DJB groups, the incidence of gastric cancer was 85% and 75%, respectively (\( P = .63 \)), whereas only 23% of rats in the RYGB group developed gastric cancer (4-fold reduction; \( P = .002 \)). The remnant stomach of rats in the RYGB group also showed a lower bilirubin concentration (\( P < .01 \)) and a lower bacterial count (\( P < .05 \)) compared with both the DJB and sham groups.

Conclusions: This study shows that RYGB reduces the risk of gastric cancer in an experimental model of dietary-induced carcinogenesis. Lack of direct contact with carcinogens, lower bile reflux, and a lower bacteria concentration in the gastric content may be responsible for these observations. These data suggest that RYGB may be a safe option for the treatment of morbid obesity even in areas with high gastric cancer incidence.

Arch Surg. 2007;142(10):947-953

Obesity is a major public health problem worldwide.\(^1\)\(^2\) Currently, surgery is the most effective treatment to reduce excess body weight in severely obese patients, and the number of bariatric procedures performed each year is increasing tremendously.\(^3\) Roux-en-Y gastric bypass (RYGB) is one of the most commonly performed bariatric procedures in the United States; technically, the operation includes the creation of a small proximal gastric pouch and a gastrojejunostomy with Roux-en-Y reconstruction.

The gastric pouch is created by linear stapling. The distal part of the stomach is bypassed and excluded from direct contact with nutrients along with the entire duodenum and the first jejunal loops. Although this is a nonphysiologic condition, large series of RYGB showed low morbidity in the excluded part of the stomach.\(^4\) In the literature, only a few reports\(^5\)\(^8\) have documented cancer in the excluded part of the stomach after RYGB. However, the stomach is an organ that is prone to cancer development, and gastric cancer is the second cause of cancer death in the world, causing 734 000 deaths annually.\(^9\) The incidence of gastric cancer shows geographic variances; for instance, the incidence in Japan is approximately 10-fold that of the United States.\(^9\) Epidemiologic studies\(^10\) of Japanese immigrants to the United States suggest that eating habits represent a risk factor for this disease.

With the current worldwide epidemic of obesity, it is predictable that morbid obesity will soon become more common in geographic areas where the incidence of gastric cancer is high. Reasonably, performance of RYGB in these populations could be a concern because the procedure leaves 95% of the stomach inaccessible to endoscopic exploration.
Whether the condition of an excluded part of the stomach after RYGB carries a greater or lower risk of developing gastric cancer is unknown. In fact, the rarity of the reports about cancer in the remnant part of the stomach after RYGB may simply reflect the fact that only a small number of patients have undergone RYGB among a population with a relatively low incidence of gastric cancer, as in the United States. In contrast, other gastric operations, such as distal gastrectomy, typically expose patients to the same or a higher incidence of cancer in the remnant part of the stomach compared with a matched population.1,12

Anatomical differences between distal gastrectomy and RYGB may be linked to different risk profiles. The carcinogenic factors in the diet reach the stomach directly through the alimentary tract. After RYGB, prevention of direct contact between the gastric mucosa and these factors may possibly reduce cancer incidence in the bypassed part of the stomach. In addition, animal studies have shown that the incidence of cancer after gastrectomy with Billroth II reconstruction is higher than after gastrectomy with Roux-en-Y reconstruction, suggesting that the latter type of reconstruction may reduce the risk of cancer by preventing mucosal injury from bile reflux or other gastrointestinal physiologic changes.

The aim of this study was to investigate the risk of gastric cancer after RYGB in experimental conditions of high gastric cancer incidence (diet-induced rat model of gastric cancer). Furthermore, we aimed to verify whether the Roux-en-Y reconstruction plays a role in determining the risk of gastric cancer after RYGB. This study also evaluated physiologic changes in the bypassed part of the stomach to identify other possible factors that may either predispose patients to or prevent the development of malignant lesions.

STUDY PROTOCOL

Rats were allowed 2 weeks for acclimation before the start of experiments. Ten-week-old rats randomly underwent 1 of 3 surgical procedures: RYGB (n=20), duodenojejunal bypass (DJB; n=20), or a sham operation (n=15). Postoperatively, rats underwent a protocol of cancer induction by means of both continuous and intermittent administration of N-methyl-N-nitrosourea (MNU). At the end of the experimental period, 17 weeks after operation, pathologic examination of the whole stomach was performed in all animals to assess for the presence of cancer and/or premalignant lesions. Bilirubin concentration, gastric bacterial flora, and any other pathologic findings were also recorded.

SURGICAL PROCEDURES

After 12 hours of fasting with free access to tap water, the rats were anesthetized with 2.5% isoflurane anesthetic gas (Aerane; Baxter, Maurepas, France) in oxygen. Abdominal skin was shaved and disinfected with a povidone-iodine solution. A sterilized paper covered the operative field, and the surgeons were sterilized gowns and gloves.

SHAM OPERATION

Laparotomy was performed with a 3.5-cm midline incision. The stomach was mobilized, and a 0.6-cm opening was created on the greater curvature of the forestomach. To minimize bleeding, we performed careful hemostasis at this stage to prevent mixing of blood and gastric juice. Through the opening, gastric juice (0.6 mL) was collected using a sterilized needle to measure the bilirubin concentration (0.5 mL) and bacterial flora (0.1 mL). The opening was closed with 3 stitches of interrupted suture using 6-0 absorbable suture (Maxon; Tyco Healthcare, Plaisir). The laparotomy was closed with a bilayered continuous Polysorb 3-0 suture (Tyco Healthcare, Plaisir).

ROUX-EN-Y GASTRIC BYPASS

Laparotomy and gastric juice collection were performed using the same techniques as described herein. After ligation of the artery crossing the incisional line, gastric division was performed 5 mm downward from the gastroesophageal junction and a gastric pouch was created. The vagus nerves were identified and preserved. The defect of the distal part of the stomach was closed with running sutures (6-0; Maxon).

To perform a Roux-en-Y reconstruction, the small intestine was divided at 10 cm from the Treitz ligament and a gastrojejunostomy was performed using interrupted sutures (6-0; Maxon). Bowel continuity was restored with a jejunojejunostomy at 15 cm from the gastrojejunostomy using resorbable running sutures. The length of the Roux-en-Y loop was calculated as proportional to that of RYGB in humans after measuring the total length of the rat’s small intestine (85-100 cm). The laparotomy was closed as in the sham operation (Figure 1A).

DUODENOJEJUNAL BYPASS

The laparotomy and gastric juice collection were performed by the same techniques as in the sham and RYGB operations. The duodenum was divided from the stomach at the pylorus. The defect of the duodenum was closed with running sutures (6-0; Maxon). As in RYGB, the small intestine was divided at 10 cm from the Treitz ligament and a terminolateral gastrojejunostomy was performed using running sutures (6-0; Maxon). Bowel continuity was restored with a jejunojejunostomy at 15 cm from the terminal ileum using continuous sutures (6-0; Maxon).

ANIMALS

Male, 8-week-old Fischer 344 rats were purchased from Harlan France (Gannat). A total of 55 rats were used. The animals were maintained under conditions of 22°C and 55% humidity with a 12-hour light-dark cycle. They were fed standard solid chow and tap water. The study was approved by the Institutional Animal Care Committee of the Institut de Recherche contre les Cancers de l’Appareil Digestif–European Institute of Telesurgery.
the gastrojejunostomy using resorbable running sutures. The laparotomy was closed in the same way as for the sham operation (Figure 1B).

POSTOPERATIVE FOLLOW-UP

Free access to tap water was permitted just after the operation, and food was supplied 12 hours after surgery. The rats were observed daily, and body weight and food intake were measured twice per week.

PROTOCOL OF CANCER INDUCTION

We used MNU (Sigma, Lyon, France) as a carcinogen. This substance induces carcinogenesis in the forestomach (upper part) by means of intermittent administration and in the glandular part of the stomach (lower part) by means of continuous administration. To induce carcinogenic change in both the forestomach and the glandular part of the stomach, both intermittent and continuous administrations were performed simultaneously.

Starting 1 week postoperatively, the rats were adminis-
tered 200 ppm of MNU in their water supply for 16 weeks. From the fourth postoperative week, additional MNU (50 mg/kg) was administered by oral gavage once per week for 12 weeks. The MNU was dissolved in the deionized water and refreshed daily. The bottles of tap water were covered with aluminum foil to prevent photolysis.

SECOND LAPAROTOMY

At the end of the experimental period, 17 weeks after operation, the rats were anesthetized and a second laparotomy was performed. A small opening was created at the greater curvature of the stomach, and gastric juice was obtained again with sterile techniques to measure the bacterial flora and bile concentration. Then the entire stomach (distal and proximal pouches in the RYGB group) was excised and stored in 10% formalin.

GASTRIC JUICE EXAMINATION

Intragastric Bilirubin Concentration

Gastric juice was aspirated using a 18-gauge needle and 1-mL syringe. Samples with blood contamination were excluded from the study. The samples were stored in ice before centrifugation (14 500g). Total bilirubin concentration was measured with the diazo/caffeine method using the ADVIA system (Bayer Healthcare, Leverkusen, Germany).

Intragastric Bacterial Flora

For quantitative analysis, 100 µL of the specimen and of each dilution (1:100 and 1:10) was spread onto a trypticase soy agar supplemented with 5% sheep blood and then incubated in air and anaerobic conditions. The colony-forming units per milliliter were measured after 48 hours of culture at 37°C. For determination of the bacterial diversity, 1 drop of each specimen was inoculated onto trypticase soy agar supplemented with 5% sheep blood and Drigalsky agar incubated in air, onto chocolate agar incubated in air supplemented by 5% carbon dioxide, and onto hemine agar incubated in anaerobic conditions. All the plates were incubated for 48 hours at 37°C. Bacterial identification was performed on the basis of Gram stain, morphologic analysis of bacteria, and catalase and oxidase reactions.

For enterobacteria and streptococci, complete identification was performed using ID-GNB and ID-GPC cards in the identification system Vitek 2 according to the manufacturer’s recommendations (BioMérieux SA, Marcy L’Etoile, France).

Microscopic Examination

The samples were embedded in paraffin. Serial sections of 3 µm were stained with hematoxylin-eosin. All the slides were analyzed by the same pathologist (V.L.), who was blind to the treatment group. The microscopic evaluation consisted of analysis of carcinogenic changes, intestinal metaplasia, and gastritis.

STATISTICAL ANALYSIS

Data are expressed as mean±SD. Statistical analysis was performed using StatView software for Windows, version 5 (SAS Institute Inc, Cary, North Carolina). Comparisons were made with the Tukey-Kramer multiple comparison test or Pearson χ² test as appropriate. P<.05 was considered statistically significant.

RESULTS

POSTOPERATIVE FOLLOW-UP

During the experimental period, 7 rats in the RYGB group, 8 rats in the DJB group, and 1 rat in the sham group died of leakage or stenosis at the anastomosis. These rats were excluded from the study. A total of 39 rats (13 rats in the RYGB group, 12 rats in the DJB group, and 14 rats in the sham group) completed the 16-week follow-up period.

BODY WEIGHT AND FOOD INTAKE

The rats in the RYGB and DJB groups gained less weight and ate less food compared with the rats in the sham group throughout the 16 weeks of follow-up. No difference was found in body weight gain and food intake between the rats in the RYGB group (with small gastric pouch) and those in the DJB group (with whole stomach) (Figure 2A and B).

GASTRIC JUICE EXAMINATION

Intragastric Bilirubin Concentration

At the first laparotomy, no significant differences were found among the 3 groups. At the second laparotomy, the bile concentration in the RYGB group was significantly lower than that in the sham and DJB groups (Figure 3).

Intragastric Bacterial Flora

At the first laparotomy, no significant differences in bacterial count and species were observed among the 3 groups. At the second laparotomy, the bacterial count of the rats in the sham and DJB groups was significantly higher than in the RYGB group. Gastric juice was sterile in 64% of RYGB-treated rats, an event that occurred sig-
significantly less frequently in the DJB and sham groups (P < .001) (Table). No statistically significant differences were found among groups in the species of bacterial flora isolated from the gastric juice.

**Macroscopic Examination**

No food was found in the remnant part of the stomach of any rat in the RYGB group. The bypassed part of the stomach of the rats in the RYGB group was covered with yellow sediment that was not seen in the stomach of the rats in the sham and DJB groups. No fistula between the gastric pouch and the remnant part of the stomach was observed in the rats in the RYGB group. In the rats in the DJB group, the part of the jejunum just distal from the gastrojejunostomy was edematous, and ulcerations were seen in 3 of 12 rats (25%).

**Microscopic Examination**

The stomach of the rat is clearly separated into 2 parts: the forestomach (proximal part, nonglandular section) and the corpus (distal part, glandular section). The histologic characteristics of the forestomach are similar to those of the esophagus.

No intestinal metaplasia was observed in the remnant part of the stomach after RYGB. The height of glandular mucosa of the remnant part of the stomach was similar to that of the rats in the sham and DJB groups (Figure 4A and B). The yellow sediment macroscopically observed in all bypassed parts of the stomach of the RYGB rats consisted of keratin (Figure 4C).

Carcinoma was found in 85% of animals that had undergone the sham operation, in 75% of DJB-treated rats (P = .63), and in only 23% of the RYGB-treated animals (P = .002; Figure 5). All of the tumors were well-differentiated squamous cell carcinomas in the forestomach (Figure 4C and D). No carcinoma was seen in the glandular section of the stomach. No carcinomas were found in the proximal gastric pouch or esophagus of the RYGB-treated rats.
The results of this study show that in experimental conditions of high-risk gastric cancer, the RYGB determines a 4-fold reduction in the incidence of malignancy compared with sham controls. These findings support the hypothesis that the condition of an excluded part of the stomach, such as after RYGB, causes a lower risk of developing gastric cancer compared with the normal anatomical situation. These data support RYGB as a valuable and safe treatment for morbid obesity even in geographic areas with a high incidence of gastric cancer. Indeed, the potential drawback of leaving 95% of the stomach excluded from standard endoscopic exploration is well balanced by the reduced risk of developing gastric cancer. This knowledge corroborates the impression that gastric cancer is a rare event after RYGB, as suggested by the few clinical observations reported so far despite the tremendous increase in the number of gastric bypass operations performed annually.

This is, to our knowledge, the first study that has attempted to investigate the issue of cancer after RYGB in rats. Others have described surgical techniques in which the proximal gastric pouch is created with a stapler device, without complete division of the stomach, and with lengths of both the biliary limb and alimentary channel that create a significant reduction in absorptive intestinal mucosa. With these variants, the size of the gastric pouch (20% of the stomach) and the length of the bypass are proportionally larger and longer than those of a proximal standard RYGB in humans. Furthermore, the stapled technique is more likely to produce gastric fistulas between the proximal and distal parts of the stomach. We have established a small-pouch RYGB model with complete division of the proximal gastric pouch and intestinal lengths that more closely mimic the standard human RYGB. Our study shows the feasibility of this operation with long-term (16-week) survival, suggesting that this procedure could be a valuable experimental model for investigations aimed at elucidating the physiologic mechanism of action of RYGB.

The DJB model was previously developed by our group to specifically investigate the role of bypassing the duodenum-jejunum only for the control of appetite or satiety and type 2 diabetes mellitus. We showed that the...
duodenal bypass component of RYGB is responsible for some of the most striking effects of RYGB; that is, control of appetite and resolution of type 2 diabetes mellitus. In the present study, we used the DJB model to test the hypothesis that this component of the RYGB could also play a role in reducing the risk of gastric cancer.

Unlike our hypothesis, our results suggest that the Roux-en-Y type of reconstruction is not a primary mediator in the mechanism that decreases the risk of gastric cancer after RYGB. This finding is shown by the fact that DJB-treated rats developed gastric cancer at rates similar to those in sham controls.

Our findings suggest instead that the mechanism that decreases gastric cancer after RYGB lies in the peculiar anatomy of the stomach after this procedure. Risk factors for gastric cancer include consumption of salty food and Helicobacter pylori infection, both of which cannot have contact with the gastric mucosa of the distal part of the stomach after RYGB. Furthermore, nitrites, which are produced by nitrate-reducing bacteria from dietary and salivary nitrates in conditions of low pH, can be further converted to nitrosamines, which, like MNU, induce gastric cancer in animal models and humans. In the present study, administration of MNU was far less effective in inducing gastric cancer in RYGB-treated rats compared with control animals, possibly because gastric exclusion prevented direct contact between nitrate-reducing bacteria and dietary nitrates, resulting in less formation of nitrosamines.

However, cancer incidence in the excluded part of the stomach in the RYGB group was not zero. This finding could be explained by the possibility that an aliquot of the administered carcinogen was absorbed in the small proximal gastric pouch and delivered via the systemic circulation to the entire stomach, including the part excluded from the flow of food. In fact, MNU can induce gastric cancer through both direct and indirect pathways, as shown by reports of successful cancer induction by intraperitoneal injection in rodents.

On the other hand, an intriguing observation in RYGB-treated animals was the absence of any cancer lesion in the proximal gastric pouch, which still has direct contact with MNU. This finding contrasts with the observation that most cancer lesions in both the sham and DJB groups occurred just distal to the gastroesophageal junction and suggests that after RYGB, the entire stomach (not only the distal, excluded part) is at lower risk of developing cancer. A possible explanation for this intriguing finding is that after RYGB a disruption mechanism occurs that counteracts gastric emptying. The gastrojejunostomy, unless stenotic, provides no real obstacles to the rapid passage of intraluminal content to the small bowel, actually disrupting the function of the stomach as a reservoir; hence, it is possible that the ability of the small gastric pouch to store nutrients (and with them carcinogens) is impaired. Consequently, duration of the contact between MNU and the gastric mucosa is shorter than in a normal stomach, thus reducing the risk of carcinogenesis. The higher pH level of the small proximal gastric pouch may also play a role by reducing conversion of salivary nitrates to nitrosamines, a key determinant of proximal gastric cancer.

We observed a low bile concentration in the gastric juice of RYGB-treated rats. In rat models of distal gastrectomy with Billroth II reconstruction, cancer of the gastric remnant occurred even without the use of dietary carcinogens, possibly due to chronic mucosal injury from bile reflux, which is considered one of the mechanisms that promote gastric carcinogenesis. It is possible that the low bile concentration in the stomach after RYGB may have contributed to the reduced cancer incidence observed in these rats.

We also found significantly fewer bacteria in the bypassed part of the stomach after RYGB. The bacterial count in gastric content typically increases after meals because of the swallowing of bacteria from the oral cavity. After 16 weeks of postoperative follow-up in our study, 64% of RYGB-treated rats showed a sterile distal part of the stomach even in the fed condition. This finding is likely because gastric exclusion avoided the passage of oral bacteria into the stomach and eliminated the nutrient supply for local bacteria. Because bacteria are suspected to play a role in gastric carcinogenesis by causing inflammation and damaging the DNA, lower bacterial growth may be another factor to explain the reduction in gastric cancer incidence after RYGB.

An incidental yet interesting finding of our study is the lack of a significant difference in food intake and body weight gain between RYGB-treated rats and DJB-treated rats. This finding suggests that the size of the gastric pouch might not be a primary mediator of appetite control and weight loss after RYGB, as currently believed. Although the present study is not appropriate to evaluate body weight loss and food intake outcomes, since rats were given carcinogens and developed cancer, these data corroborate results from our previous work. We previously showed that DJB alone is sufficient to induce remarkable weight loss and decrease of food intake in an obese rat model, suggesting that changes in the secretion of gastrointestinal hormones secondary to the rearrangement of the small bowel may be more important than gastric restriction in the determination of the antiobesity mechanism of action of RYGB.
Currently, bariatric surgery is uncommon in East Asia, where many countries face a high prevalence of gastric cancer. However, lifestyle and dietary changes are causing a rapid and tremendous increase in obesity rates in these regions, and it is likely that bariatric surgery, including RYGB, will be increasingly performed during the next few years. The lack of access to the distal part of the stomach by means of standard endoscopic exploration may potentially reduce the ability to perform an early diagnosis of gastric cancer after RYGB, which could be seen as a significant drawback of the operation in the setting of a high incidence and prevalence of gastric cancer. Our results suggest that this potential drawback is, at least in part, compensated for by the significant reduction in the tendency to develop gastric cancer after RYGB. These data are reassuring and may help diminish the reluctance in adopting RYGB for effective treatment of morbid obesity in regions of the world where gastric cancer is common.

In conclusion, this study shows that RYGB reduces the risk of cancer in a rodent experimental model of dietary-induced carcinogenesis. Lack of direct contact with carcinogens, lower bile reflux, and fewer bacteria in the gastric content may be responsible for these observations. Our data suggest that RYGB may be a safe option for the treatment of morbid obesity even in areas with high gastric cancer incidence.

Accepted for Publication: June 16, 2007.

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Author Contributions: Dr Inoue had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: H. Inoue, Rubino, and Marescaux. Acquisition of data: H. Inoue, Rubino, Lindner, M. Inoue, and Riegel. Analysis and interpretation of data: H. Inoue, Rubino, Shimada, Lindner, M. Inoue, Riegel, and Marescaux. Drafting of the manuscript: H. Inoue, Lindner, and M. Inoue. Critical revision of the manuscript for important intellectual content: H. Inoue, Rubino, Shimada, Riegel, and Marescaux. Statistical analysis: H. Inoue, Rubino, and Shimada. Administrative, technical, and material support: H. Inoue, Lindner, M. Inoue, and Riegel. Study supervision: Rubino and Marescaux.

Financial Disclosure: None reported.

Additional Contributions: Sophie Bayer, BPharm, and Michele Grima, MD, measured the bilirubin concentration of the gastric juice.

REFERENCES