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Human apolipoprotein A-IV reduces gastric acid secretion and diminishes ulcer formation in transgenic mice

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Abstract We have investigated the involvement of human apolipoprotein A-IV (apoA-IV) in gastric acid secretion and ulcer formation in recently generated apoA-IV transgenic mice. Compared to control littermates, transgenic animals showed a gastric acid secretion decreased by 43–77% whereas only slight variations were observed in the different cell population densities within the gastric mucosa. In addition, no variation in gastrin levels was observed. Transgenics were protected against indomethacin-induced ulcer formation, with lesions diminishing by 45 to 64% compared to controls. These results indicate that endogenous apoA-IV expression can regulate gastric acid secretion and ulcer development.

Key words: Transgenic mouse; Gastric mucosa; Apolipoprotein A-IV; Indomethacin; Ulcer; Gastric acid secretion

1. Introduction

Nutrition processes involve several steps such as appetite/satiety, feeding, digestion, nutriment absorption and distribution to the different tissues of the body. Although it is well known that these mechanisms are associated and co-regulated at different levels, the links between them are still not clearly determined. Apolipoprotein A-IV (apoA-IV) is a plasma protein involved in lipid absorption and lipid transport. Plasma levels of apoA-IV are increased by dietary lipid in postprandial periods [1]. This secretion increase has been proposed to be involved in the regulation of nutrition processes [2]. It have been observed that injection of the protein in the brain induced satiety [3], decreased gastric acid secretion [4,5] and reduced gastric ulceration in rats [6]. All these results suggested that apoA-IV could be involved in feeding behavior and in gastric physiology. However, no feeding behavior alterations were observed in apoA-IV genetically modified mice [7,8].

We have recently created transgenic mice overexpressing human apoA-IV (hapoA-IV) [9]. In the current study, we mainly focused on the role this apolipoprotein might play in gastric acid secretion and ulcer protection. Our results showed that expression of human apoA-IV in transgenic mice decreases gastric acid secretion without alteration of the gastric mucosa and gastrin levels and protects against indomethacin-induced lesions.

2. Materials and methods

2.1. Mice

Two human apoA-IV transgenic mice lines, (lines 8018 and 8021) were analyzed [9]. Mice were housed under temperature- and light/dark (7 am–7 pm) controlled conditions. Animals had free access to mouse chow diet (UAR, France diet, France) and to water. Plasma human apoA-IV concentrations were quantified, in fed or 8 h fasted animals, by immunoelectrophoresis (hydragel SEBIA, France) using specific antibodies.

2.2. Gastric acid secretion

Gastric juices were collected using the pylorus ligation method [10]. Briefly, mice were anaesthetized after 18 h of fasting, the abdominal cavity was opened and the stomach exposed. Then, the pylorus was ligated and the cavity closed. After 4 h, the mice were killed, the gastric content was collected and its volume measured. An aliquot was titrated with NaOH to determine gastric acid production.

2.3. Extraction and radioimmunoassay (RIA) for gastrin

For extraction of tissular gastrin, frozen gastric tissues were thawed, boiled in 1 ml of distilled water for 5 min., homogenized and centrifuged at $5000 \times g$ for 20 min. The supernatants were recovered and used in subsequent RIA. For plasma gastrin, blood samples were collected on ice with heparin. The serum was stored at −20°C until assayed. RIA was performed in duplicate using an anti-gastrin rabbit antibody (Peninsula, Laboratories, Belmont, USA) and ^{125}I-gastrin (Amersham, France) as tracer, respectively. The gastrin antiserum recognizes the large C-terminal gastrin and all forms of gastrin. The separation was performed using the double antibody-PEG precipitation technique. Gastrin concentrations were determined using human gastrin-17 (Sigma, St. Louis MO, USA) and expressed in pg/ml or pg/g tissue.

2.4. Histological analysis

Stomachs were pinned flat. After fixation in Bouin's solution, the secreting surface of the stomach was measured [11]. Two parallel strips were resected from standardized areas in the fundic and antral mucosa. Tissues were cut into 4-μm thick sections, perpendicular to the mucosal surface. Fundic mucosal sections were stained by hemalum-eosin and the mucosal thickness was determined at regular intervals using a calibrated ocular grid 240 μm wide at 400× magnification. Acid-secreting parietal cells were identified by their position along the edge of fundic glands, their round shape, the central location of the large nucleus and their very eosinophilic cytoplasm. Fundic argyrophil endocrine cells, including enterochromaffin-like (ECL) cells, were stained by the Grimelius argyrophilia. Antral gastrin and somatostatin endocrine cells were immuno-histochemically detected with specific antibodies using the avidin-biotin complex Vectastain (Vector Labs, Burlingame, CA, USA) and nuclei counterstained with hemalum. Cell densities were estimated by counting only nucleated cells throughout the fundic or antral mucosal thickness, using the ocular grid at

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Abbreviations: apoA-IV, apolipoprotein A-IV; RIA, radioimmunoassay; NS, not significant; ECL cell, enterochromaffin-like cell

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400× magnification [11]. Results are expressed in number of nucleated cells per millimeter of mucosal length.

2.5. Indomethacin-induced gastric ulcers

Four-month-old mice were fasted 8 h prior to subcutaneous administration of a single dose of indomethacin (Sigma, 200 μg/g of body weight). The mice were killed 18 h later; the stomachs removed, opened and fixed in 10% buffered neutral formalin. Images of the glandular stomach were digitalized and surfaces were calculated in a computer-assisted analysis system. Percentage of ulcerated area was determined by calculating the ratio of ulcerated surface to total mucosal surface.

2.6. Statistical analysis

Non-transgenic littermates were used as control mice in all experiments. All biological data are expressed as mean ± S.E.M. Data were analyzed by Fisher's PLSD multiple comparisons ANOVA tests or, for histological data, by the Student's *t*-test.

3. Results and discussion

3.1. Gastric acid secretion and gastrin levels

We have recently generated apoA-IV transgenic mice [9]. For these studies two transgenic lines were used, a low expressor (line 8021) and a high expressor (line 8022) with respectively 34 ± 6 mg/dl and 140 ± 29 mg/dl of human plasma apoA-IV. We analyzed if plasma levels of the protein were increased in postprandial conditions in these transgenics. Indeed, a 2.3-fold and a 2.0-fold increase were observed in lines 8021 and 8022, respectively in fed condition and fasted levels (Fig. 1). In consequence these mice displayed the up-regulation of apoA-IV plasma levels currently observed in fed conditions. The growing curves of 8021 and 8022 transgenic mice, as well as their feeding behavior, were similar with those observed with the non-transgenic control littermates (data not shown).

Afterwards, gastric acid secretion was analyzed by the pylorus-ligated method. Secretion was reduced by 43% ($P < 0.05$) in the line 8021 and by 64% ($P < 0.01$) in the line 8022 when compared to control mice (Fig. 2A). In order to determine if this hypo-secretion was maintained at older ages, we also analyzed 9-month old mice. Plasma levels of apoA-IV were found to be the same as from those observed at 2 months. A gastric acid secretion reduction of 77% ($P < 0.001$) and of 45% ($P < 0,05$) was observed in lines 8021 and 8022, respectively when compared to control mice (Fig. 2B). Differences of gastric acid output between the two transgenic lines were not significant neither in 2-month ($P = 0.28$) nor in 9-month old mice ($P = 0.16$). This suggests that gastric secretion inhibition is dependent neither of human apoA-IV plasma concentration nor of the age of the animals. It is possible that a maximum effect might be obtained with protein concentrations lower than those present in the transgenic mice. Thus, our results indicate that endogenous expression of human apoA-IV inhibits gastric acid secretion in our mice.

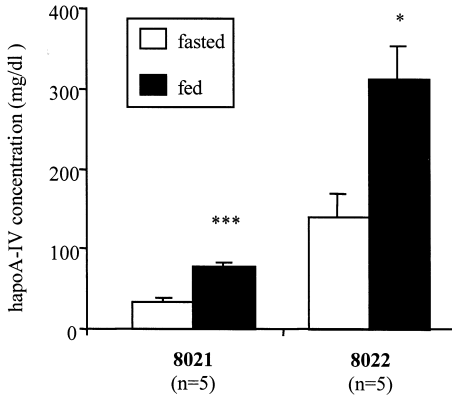

Fig. 1. Human apoA-IV levels in fasted and fed condition. Plasmatic levels of human apoA-IV were measured in 8-h fasted and fed conditions in the two transgenic lines (8021 and 8022). *n* = numbers of animals used for each experiment. *P* values of fasted vs. fed are: * = $P < 0.05$, *** = $P < 0.001$.

Since gastrin is a major secretagogue for gastric acid secretion, we investigated gastrin levels in plasma and in gastric tissue. No significant variations were found. Plasma gastrin levels were 16.6 ± 0.4 pg/ml and 16.0 ± 0.6 pg/ml in control and transgenic mice, respectively, while tissular gastrin levels were 134.7 ± 26.4 pg/g and 128.3 ± 29.0 pg/g in each group of animals. These results showed that gastric acid secretion was decreased in transgenic mice without any change in plasma and tissular gastrin levels, suggesting that other regulatory pathways are involved in secretion regulation by apoA-IV.

3.2. Gastric morphology

Anatomic variations could be induced by the expression of the transgene and might explain the differences observed in gastric acid secretion. To further elucidate the mechanism of action of apoA-IV in gastric acid secretion we analyze the architecture of gastric mucosa in apoA-IV transgenic mice. Histological studies were undertaken in the previously analyzed 2- and 9-month old control and transgenic mice line 8021, where gastric secretion inhibitions were 43% and 77%, respectively. The surface of the secreting stomach and the fundic mucosal thickness showed no differences between transgenic and wild-type mice (Table 1). Densities of cells implicated either in gastric acid secretion or its regulation were relatively unchanged (Fig. 3). In transgenic apoA-IV mice, parietal cells decreased by 10% and by 15% not significant (NS) at 2 and 9 months respectively. Decreases were of the same order at the two states of life for fundic argyrophil and somatostatin cells. Mean gastrin cell density which showed a 16% decrease at 2 months (NS) was strictly normal at 9 months. All these results indicate that the morphological architecture of the gastric mucosa remained roughly normal even after 9 months of life. Other genetically modified animals

Table 1
Morphometric results in the gastric mucosa of wild-type and transgenic mice at two stages of life

Parameters	2 Months		9 Months	
	Wild-type ($n = 6$)	Transgenics ($n = 6$)	Wild-type ($n = 5$)	Transgenics ($n = 6$)
Stomach surface (cm^2)	2.81 ± 0.12	2.89 ± 0.12	3.19 ± 0.04	3.23 ± 0.8
Fundic mucosal thickness (μm)	223 ± 7	206 ± 10	212 ± 13	216 ± 16

Transgenic mice were from line 8021. *n* = number of animals used for each experiment.

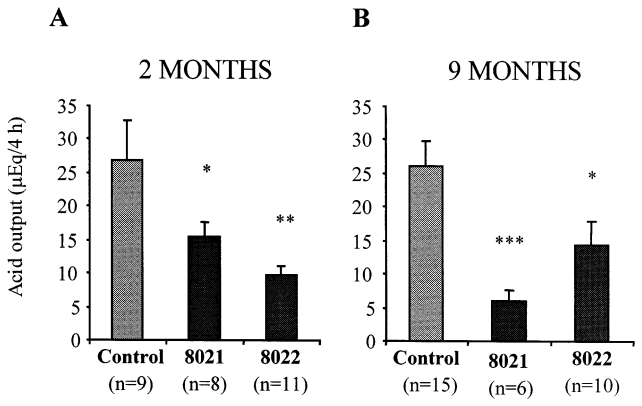

Fig. 2. Gastric acid secretion of 2- and 9-month-old mice. Bars indicate the gastric acid secretion in control mice and in 8021 and 8022 transgenic animals. A and B represent values at 2 and 9 month old mice, respectively. n = numbers of animals used for each experiment. P values of transgenics vs. controls are: * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$. The differences between secretion values of 2- and 9-month-old animals were not significant.

displaying gastric hypo-secretion have showed marked gastric histological changes. In transgenic mice overexpressing transforming growth factor alpha, inhibition of gastric acid secretion was accompanied by severe adenomatous hyperplasia that resulted in striking hypertrophy of the mucosa [12,13]. Knock out mice for gastrin in which basal and stimulated gastric acid secretion were abolished showed a clear-cut decrease in parietal and enterochromaffin-like (ECL) cells numbers (−23 to −46%) [14,15]. In cholecystokinin-B/gastrin receptor-deficient mice, decreased acid production (−70%) was accompanied by an increase in the antral gastrin cell number consistent with the concomitant elevation in circulating gastrin. Moreover parietal and enterochromaffin-like cells (ECL cells) were decreased by 30–50% in these mice, providing a likely explanation for the reduction in acid output [16,17]. In contrast, overexpression of hapoA-IV, and the observed decreased gastric acid secretion, could not be significantly associated with important alterations of the structure of the gastric mucosa and of gastrin levels. Nevertheless, we cannot exclude that the slight variations observed in the number of functional cells in transgenics were related to the overexpression of human apoA-IV.

3.3. Indomethacin induced ulcer formation

To specifically investigate if apoA-IV plays a role in gastric ulcer development, ulcer formation was next assessed in these transgenic mice. Gastric ulcers were induced with indomethacin (200 mg/kg). All the lesions were located in the glandular stomach. In both transgenic lines, the total ulcerated surfaces were significantly smaller than in controls (Fig. 4). Reductions in lesion area were 64% and 45% in 8021 and 8022 mice, respectively. The degree of mucosa ulceration was of the same order in transgenics and in control littermates. This result clearly shows that expression of hapoA-IV is associated with a reduction in indomethacin-induced ulceration. The reduction seems not to be dependent on plasma concentration of human apoA-IV as no significant differences in the ulcer surfaces were observed between the low (8021) and the high (8022) apoA-IV expressor lines. As for gastric acid secretion, it is possible that maximum effect on ulcer protection can be obtained with apoA-IV concentrations lower than those present in these transgenic mice. Even if supplementary data are necessary to understand the mechanism of ulcer protection by apoA-IV, it is tempting to hypothesize that the decrease of lesion area may be related to the reduction of the gastric acid secretion in the transgenic mice.

In conclusion, this paper clearly shows a physiological link between apoA-IV endogenous expression and gastric acid secretion. The animal model presented here can be a useful tool for the study of apoA-IV up-regulation under postprandial

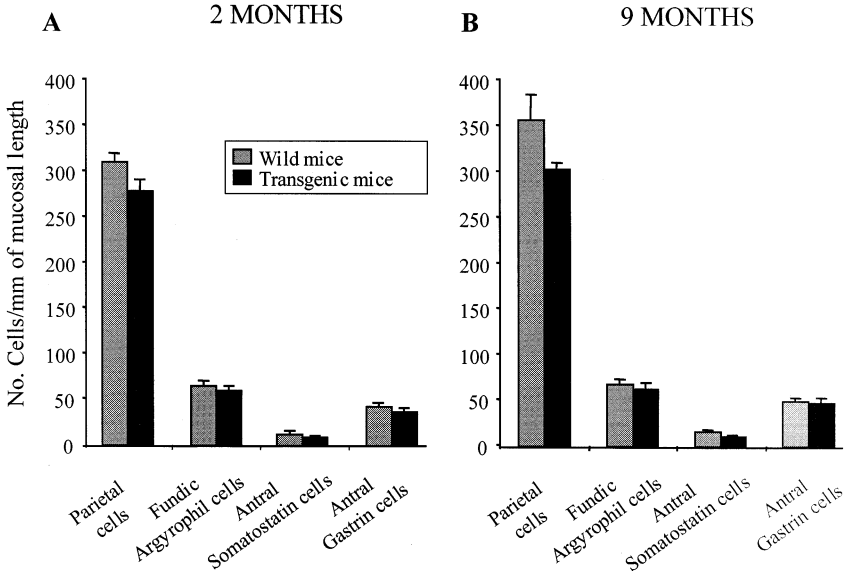

Fig. 3. Numerical densities of nucleated cells implicated in acid secretion in the gastric mucosa of wild-type and transgenic mice. A and B represent values at 2- and 9-month-old mice, respectively. Transgenic mice are from line 8021. Values are mean ± S.E.M. Five or six animals were used for each experiment.

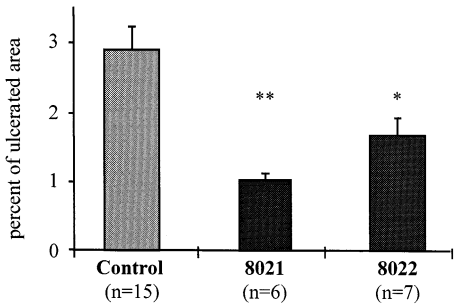

Fig. 4. Indomethacin-induced gastric ulcers. Ulcers were induced by injection of indomethacin (200 μg/g wt). Bars represent the percent of ulcerated areas of each category: control mice or 8021 and 8022 transgenic mice. n = numbers of animals used for each experiment. P values of transgenics vs. controls are: * = $P < 0.05$ and ** = $P < 0.01$.

conditions. Furthermore, our results underline the interest of studying diets or drugs that modify apoA-IV expression in relationship with gastric secretion or ulcer formation.

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