Gastric Smooth Muscle Contractility Changes in the Esophageal Atresia Rat Model: An In Vitro Study

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Purpose: The aim of the study was to investigate the gastric smooth muscle reactivity in the Adriamycin-induced esophageal atresia (EA) rat model.

Methods: The fetuses were divided into 3 groups. The control group was exposed to saline. The second group was comprised of fetuses that were exposed to Adriamycin but did not have EA (Adriamycin-no-EA group). The third group was comprised of fetuses that were exposed to Adriamycin and had EA (Adriamycin-EA group). Gastric fundus strips were studied in vitro for their contractile response to receptor activation in the 3 groups.

Results: Contractile responses of gastric smooth muscle to carbachol and KCl were increased in the Adriamycin-EA group compared with the Adriamycin-no-EA group. Also serotonin-induced contractile response in the Adriamycin-EA group decreased compared with the Adriamycin-no-EA group. Relaxation of gastric smooth muscle strips to isoprenaline was comparably unaffected in the Adriamycin-EA and Adriamycin-no-EA groups. Likewise, no change in the response to agonist studies was observed between the control and Adriamycin-no-EA groups. The relaxant response to papaverine was not different in the 3 groups.

Conclusions: This study found changes of receptor-dependent and receptor-independent contraction of the gastric fundus smooth muscle in the fetuses with EA. Therefore, impaired contractile responses may be, at least in part, a contributing factor in the abnormal gastric motility seen in EA.

J Pediatr Surg 38:1366-1370. © 2003 Elsevier Inc. All rights reserved.

INDEX WORDS: Esophageal atresia, Adriamycin, in vitro, gastric fundus, rat.

Materials and Methods

Four-month-old female Sprague-Dawley rats (200 to 250 g) were mated for 12 hours. The rats with vaginal plug on day 0 or that gained more than 14 g on day 8, were determined to be pregnant. The pregnant rats were housed individually in plastic cages in a quiet, temperature- and humidity-controlled room (22 ± 3°C and 62% ± 7%, respectively) in which a 12-12 hour light-dark cycle was maintained (08:00 to 20:00 hours light). Adriamycin, 2 mg/kg (n = 10), or saline (n = 5) was injected into the pregnant rats intraperitoneally on gestational days 8 and 9. The fetuses were collected by cesarean section on gestational day 22. The fetuses were killed by decapitation and opened with thoracoabdominal incision examined under 2.3x magnification (Heine C 2.3 Binocular Loupe; Heine, Herrsching, Germany) to determine proximal esophageal atresia and distal tracheoesophageal fistula. Then, total gastrectomy was performed, and gastric fundus strips were prepared in a manner consistent with the method described by Vanes.

Organ Chamber Experiments

The experiments were performed in the Experimental Medical Research Unit (DETAB, Kocaeli University Medical Faculty, Kocaeli), of the Kocaeli Medical School. The isolated gastric fundus strips were mounted in 20 mL organ chambers for isometric tension measurement. The tissue baths contained Tyrode’s solution composed of (millimoles per liter): NaCl, 136.0; KCl, 2.7; CaCl2, 1.8; MgCl2, 1.05; NaH2PO4·H2O, 0.42; NaHCO3, 11.9; glucose, 5.5. The solution was gassed with 95% O2 and 5% CO2 during the study, and the temperature was maintained at 37°C. Each strip was connected to a force transducer (FDT 10-A, May IOBS 99; COMMAT Iletisim Co. Ankara, Turkey) for the measurement of isometric force, which was displaced continuously and recorded on an online computer via 4-channel transducer data acquisition system (MP30B-CE, BIOPAC Systems Inc, Santa Barbara, CA) using software (BSL PRO v 3.6.7, BIOPAC Systems), which also had the capacity to analyze the data. After mounting, each
GASTRIC CONTRACTILITY IN EA

RESULTS

From the 10 Adriamycin-injected litters, 78 fetuses were obtained, and 45 of them (57.69%) had EA with distal tracheoesophageal fistula. The upper esophageal pouch ended just below the level of the cricoid bone, and the distal end was connected to tracheal bifurcation or to the left of the main bronchus. Associated anomalies in these fetal rats were very similar to the description in the previous studies. Control rat fetuses showed no abnormality. There was no significant difference in the mass of the strips used for the contractility studies. The strips were 4.5 ± 0.3 mg and 4.2 ± 0.1 mg in the Adriamycin-no-EA, Adriamycin-EA groups, respectively. We found similar dose-response curves and statistically nonsignificant E\text{max}, pD\text{2} values between the Adriamycin-no-EA fetuses and saline-exposed fetuses in organ bath studies (data not shown). Therefore, only the Adriamycin-no-EA group was used as a control group.

Cumulative addition of carbachol produced concentration-dependent contractions of the gastric fundus strips and the contractility significantly increased in the Adriamycin-EA group compared with the Adriamycin-no-EA group (Fig 1). There was significant increase in the E\text{max} and pD\text{2} values of gastric fundus strips obtained from Adriamycin-EA group compared with the Adriamycin-no-EA group (Table 1). The contraction elicited by 80 mmol/L KCl was significantly increased in the Adriamycin-EA group compared with the Adriamycin-no-EA group (Fig 2, Table 1). Serotonin produced concentration-dependent contraction in 2 groups. However, significantly decreased contractile responses to serotonin in the Adriamycin-EA group compared with the Adriamycin-no-EA group were observed (Fig 3). There was significant decrease in the E\text{max} and pD\text{2} values of gastric fundus strips obtained from Adriamycin-EA group compared with the Adriamycin-no-EA group (Table 1). The relaxant response to isoproterenol and papaverine were similar with similar E\text{max} and pD\text{2} values in the 2 groups, respectively (Figs 4 and 5).

DISCUSSION

Surgical repair of esophagus restores gastrointestinal continuity; however, a majority of patients present with GER-related symptoms postoperatively. A series of previous investigations showed that congenital or acquired factors might affect esophagus and stomach, which cause GER in patients with EA. Furthermore, these studies on humans are supported by experimental studies that showed structural abnormalities in the rat fetuses with EA. However, exact causes of the GER remained
controversial. These studies suggested that esophageal and gastric dismotility might be partly caused by some kind of congenital structural abnormality. Conversely, it has been claimed that both extensive mobilization, anastomosis under tension, and denervation of the esophageal segments could aggravate reflux and motility disorders. 7,10,12,29

Abnormal gastric motility can be considered an important factor predisposing to symptoms of GER in patients with EA. Some patients with GER complain of vomiting, suggesting the possibility of gastric retention, and most patients complain of bloating, fullness, postprandial satiety, and epigastric discomfort, much of which is explained by delayed gastric emptying. A number of studies using gastric scintigraphy, manometry, and electrogastrography showed that delayed gastric emptying is frequent in long-term follow-up of patients operated on for EA. 16-19 Therefore, delayed gastric emptying may be related to the inadequate gastric muscular contraction in accordance with our results.

In this study, receptor-dependent (Carbachol, Serotonin, Isoproterenol) and receptor-independent agonists (KCl, Papaverine) were used to fully investigate the extent of the maldevelopmental effects of the EA to the gastric smooth muscle. We showed that development of EA affected both receptor-dependent and receptor-independent mechanisms of the gastric smooth muscle contractility in the fetuses with EA, whereas relaxant mechanisms seem well preserved. The mechanism underlying this impaired response is not known yet. Decreased serotonin-induced contractile responses in the study may be in part related to the decreased serotonergic receptors or postreceptor mechanisms. Cox et al 30 showed that in

<table>
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<tr>
<th>Drugs</th>
<th>Adriamycin-no-EA</th>
<th>Adriamycin-EA</th>
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<tr>
<td>pD2</td>
<td></td>
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<tr>
<td>Carbachol</td>
<td>6.81 ± 0.83</td>
<td>5.98 ± 0.10*</td>
</tr>
<tr>
<td>Serotonin</td>
<td>7.22 ± 0.15</td>
<td>6.69 ± 0.12*</td>
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<tr>
<td>Isoproterenol</td>
<td>7.66 ± 0.12</td>
<td>7.30 ± 0.28</td>
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<tr>
<td>Papaverine</td>
<td>4.59 ± 0.44</td>
<td>4.93 ± 0.82</td>
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<tr>
<td>Emax</td>
<td></td>
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<tr>
<td>KCl</td>
<td>73.75 ± 6.89</td>
<td>107.3 ± 7.90*</td>
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<tr>
<td>Carbachol</td>
<td>211.80 ± 10.74</td>
<td>565.92 ± 53.78*</td>
</tr>
<tr>
<td>Serotonin</td>
<td>560.36 ± 14.34</td>
<td>300.53 ± 18.32*</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>93.69 ± 5.59</td>
<td>97.42 ± 5.95</td>
</tr>
<tr>
<td>Papaverine</td>
<td>100 ± 0</td>
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NOTE. Values are arithmetic means ± SEM.
* P < .05, statistically different from the response of strips from Adriamycin-EA and Adriamycin-no-EA groups.

Fig 2. KCl-induced (80 mmol/L) maximal contractile responses of gastric fundus strips. Each point is expressed as milligrams of tension per milligram of tissue and is given as the mean ± SEM. Number in parenthesis indicates the number of preparations used from different animals. * P < .05 compared with the other group.

Fig 3. Serotonin-concentration-response curves in isolated rat gastric fundus strips. All points show the mean ± SEM of responses obtained from individual experiments on different tissues from different animals. Data are expressed as milligrams of tension per milligram of tissue wet weight. * P < .05 compared with the other group.

Fig 4. Isoproterenol-concentration-response curves in isolated rat gastric fundus strips precontracted with carbachol 3.10⁻⁸ mol/L. All points show the mean ± SEM of responses obtained from individual experiments on different tissues from different animals. Data are expressed as a percentage of the contraction induced by carbachol. Number of fetuses in each group is shown in parenthesis.
the rat, serotonin-induced contractility involves intracellular calcium release and activation of protein kinase C without stimulation of phosphorysytide hydrolysis. The same mechanism may be involved in this model, and further investigation is required.

The greater contractile response of the gastric fundus strips to KCl, which leads to contraction through membrane depolarization, and influx of extracellular Ca\(^{2+}\) through voltage-dependent Ca\(^{2+}\) channels, indicates an increased reliance on extracellular Ca\(^{2+}\) by the Adria-mycin-EA group. Also, the contractile response difference to carbachol between the 2 groups may be related to the number of the receptors, receptor sensitivity, or postreceptor mechanisms. According to these results, gastric smooth muscle in the rat fetuses with EA generates more active force than the control group. This developmental change may be the result of increased utilization of extracellular Ca\(^{2+}\) in part through voltage-dependent Ca\(^{2+}\) channels.

Although it is difficult to extrapolate in vitro data to in vivo function, the physiologic changes seen in the fetuses with EA may partly explain the postoperative GER in patients with EA. However, further studies are required for determining which intracellular mechanisms are responsible for signal transduction pathways in EA.

REFERENCES


Fig 5. Papaverine-concentration-response curves in isolated rat gastric fundus strips precontracted with carbachol 3.10^-6 mmol/L. All points show the mean ± SEM of responses obtained in individual experiments on different tissues from different animals. Data are expressed as a percentage of the contraction induced by carbachol.