Impaired Esophageal Reactivity in Adriamycin-Induced Rat Esophageal Atresia: An In Vitro Study

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Purpose: The aim of this study was to investigate the reactivity of lower esophageal smooth muscle 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 comprised fetuses that were exposed to Adriamycin but in whom EA did not develop. The third group comprised of fetuses that were exposed to Adriamycin and EA was observed. The reactivity of distal esophageal strips was studied in organ chambers.

Results: The tension was similar in all groups precontracted with carbachol for the study of relaxation to serotonin. Relaxation of lower esophageal strips to serotonin was comparably unaffected in the control and Adriamycin-no EA groups, whereas it was significantly inhibited in the EA group with decreased E_{max} and pD_2 values. Contractile responses of esophageal smooth muscle to carbachol or 80 mmol/L KCl and relaxant responses to papaverine were similar in all groups. No change in agonist potency was observed among the groups.

Conclusions: Our study showed impairment of serotonin-receptor–mediated relaxation; but not of cholinoceptor-mediated contraction of the lower esophageal smooth muscle in the EA. Thus, impaired relaxant responses may be, at least in part, a contributing factor in the esophageal dismotility seen in EA.
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INDEX WORDS: Esophageal atresia, Adriamycin, carbachol, serotonin, rat.

ESOPHAGEAL MOTILITY is controlled by intrinsic smooth muscle activity, the neuropeptide-rich intrinsic nerve pathways and extrinsic nerves that originate from the central nervous system. Surgical repair of esophageal atresia (EA) restores the continuity of the alimentary tract, but does not ensure normal motor function of the esophagus.[1-5] Whether the motor disorders encountered in these patients are part of the primary syndrome or result from neurologic damage from operative manipulation is subject to ongoing debate.[6] The motor abnormalities include incomplete upper esophageal sphincter (UES) relaxation, incomplete lower esophageal sphincter (LES) relaxation, decreased LES pressure, and abnormalities in the amplitude or coordination of peristaltic waves in the repaired esophageal body.[7] The Adriamycin-induced EA rat model provides a useful model to study esophageal dismotility in animals with EA.[8] A previous study with this model showed abnormality in the course and branching pattern of the vagus nerve and deficient extrinsic nerve fiber plexus in the lower esophagus.[9] Other studies showed significant abnormalities of the intramural nervous components of the esophagus in EA rats involving both the excitatory and inhibitory intramural nerves and abnormal nerve distribution of nerve tissue.[10,11] Previous studies pertaining to EA deal primarily with the morphologic changes occurring within the tissue. In the current study, our aim was to investigate the functional properties of serotonergic as well as muscarinic receptors of esophagus in the Adriamycin-induced EA rat model.[12-14]

MATERIALS AND METHODS

Four-months-old female Sprague-Dawley rats (200 to 250 g) were mated early in the morning for 12 hours. The rats with vaginal plug on day 0 and that gained weight more than 14 g on day 8 were designated as 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 (08:00-20:00 hours light). Adriamycin, 2 mg/kg (n = 13), or saline (n = 5) was injected intraperitoneally on gestational days 8 and 9.[14] The fetuses were collected by cesarean section, weighted, and examined under 2.3 × magnification (Heine C 2.3 Binocular Loupe; Heine, Herrsching, Germany) to detect EA on gestational day 22 immediately before delivery. The fetuses were divided into 3 groups, and lower esophageal segments were harvested for experimentation. The control group comprised fetuses from rats that received only saline, the Adriamycin-no EA group comprised fetuses without esophageal atresia obtained from Adriamycin-injected rats, and the Adriamycin-EA group was made up of fetuses with esophageal atresia obtained from Adriamycin-injected rats.

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Organ Chamber Experiments

Fetuses were killed by decapitation. The distal esophagus was dissected free of surrounding tissues and opened longitudinally from esophagogastric junction to tracheal fistula in fetuses with EA. The esophagus was dissected using the same technique and opened from esophagogastric junction to the level of tracheal bifurcation in the control and Adriamycin-no EA groups. Strips of lower esophagus were studied in 20-mL water-jacketed organ baths for isometric tension recording. Whole-mount preparations were used because the esophageal wall of Adriamycin-treated fetuses with or without EA was too thin to separate into mucosal and muscular layers as reported previously.[10] The strips were tied with silk thread to a force transducer (MAY-COM FDT 10-A; COMMAT Iletisim Co, Ankara, Turkey) at one end and fixed to a glass support at the other end. The tissue baths contained Tyrode's solution composed of (millimoles per liter): NaCl, 136.0; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 1.05; $NaH_2PO_4H_2O$, 0.42; $NaHCO_3$, 11.9; and glucose, 5.5. The solution was gassed with 95% O_2 and 5% CO_2 during the study, and the temperature was maintained at 37°C. After mounting, the strips were allowed to equilibrate under a resting tension 0.5 g for 90 minutes. During this period the bath fluid was changed routinely every 15 minutes. Agonists were added directly to the organ bath. At the completion of each experiment, tissues were blotted lightly and weighted. Isometric tension was recorded on a computer via 4-channel transducer data acquisition system (TDA-94; COMMAT Iletisim Co) using a software (Polywin 95 ver 1.0; COMMAT), which also had the capacity to analyze the data.

In a series of preliminary experiments, lower esophageal strips were stimulated with 80 mmol/L KCl. In examining the contractile response to the muscarinic agonist carbachol (10^{-10} to 3.10^{-4} mol/L), cumulative concentration-response curve was constructed in a stepwise manner after the response to the previous concentration had reached a plateau. After completion of carbachol dose-response curve, tissues were washed for a further 60 minutes and precontracted with a submaximal concentration of carbachol (3.10^{-6} mol/L). After the contractions reached a plateau, concentration-response relationships for serotonin (10^{-10} to 10^{-4} mol/L) was obtained in a cumulative manner.

Analysis of Data

The results were expressed as mean ± SEM. The contractile force was expressed as milligrams of developed tension per milligram of tissue wet weight. The relaxant effects of agonists were expressed as percentage of contraction induced by carbachol. Concentration-response curves were fitted by nonlinear regression with simplex algorithm and E_{max} and pD_2 ($-\log EC_{50}$) were calculated using the software of transducer data acquisition system.

Statistical Analysis

Significance was tested by 1-way analysis of variance with a post-hoc Tukey's-Kramer test. Probabilities of less than 5% ($P < .05$) were considered statistically significant.

Drugs

The following drugs were used: carbachol chloride (Sigma, St Louis, MO), 5-hydroxytriptamine hydrochloride (Sigma), papaverine hydrochloride (Sigma), Adriamycin (Adriblastina, Carlo-Erba, Istanbul, Turkey). In the high K^+ solution, NaCl was exchanged for equimolar amounts of KCl. Drugs were prepared daily in distilled water and kept in ice during the course of experiments. Adriamycin was dissolved in saline.

The Kocaeli University, Medical School Ethics Committee (Kocaeli, Turkey) granted the ethical approval.

RESULTS

Thirteen Adriamycin-injected litters produced 84 fetuses, and 36 of them (42.85%) 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 left main bronchus. Associated anomalies including duodenal atresia, bladder agenesia, bilateral hydroureteronephrosis, and anorectal atresia also were observed as reported.[8,12]

There was no significant difference in the mass of the strips used for the contractility studies. The strips were 6.2 ± 0.2 mg, 6.7 ± 0.8 mg, and 6.5 ± 1.1 mg in the control, Adriamycin-no EA, and Adriamycin-EA groups, respectively. Cumulative addition of carbachol produced concentration-dependent contractions of the lower esophageal strips, and the contractility did not change in esophageal strips obtained from EA compared with control and Adriamycin-no EA groups (Fig 1). There was no significant difference between maximum responses (E_{max}) or pD_2 values of esophageal strips obtained from EA group compared with control and Adriamycin-no EA groups (Tables 1 and 2). The contraction elicited by 80 mmol/L KCl was similar in all groups (Table 1).

Serotonin produced concentration-dependent relaxation in submaximally precontracted (3.10^{-6} mol/L carbachol) esophageal strips in all groups (Fig 2). The tension induced by carbachol was 176.2 ± 39.4 mg, 164.3 ± 97.2 mg, and 165.2 ± 97.2 mg, in the control, Adriamycin-no EA, and Adriamycin-EA groups, respectively. Contractions induced by carbachol for the study of relaxation to serotonin were similar in all groups. The relaxant response to serotonin was significantly inhibited in EA group compared with control and Adriamycin-no EA groups. The concentration-response curve for serotonin shifted to right with significantly lower E_{max} and pD_2 values in the EA group ($P < .005$; Tables 1 and 2). However, responses to serotonin were similar in the control and Adriamycin-no EA groups. The relaxations elicited by papaverine were similar in all groups (data not shown).

DISCUSSION

Abnormal esophageal motility after the repair of EA have been well documented.[15,16] Preoperative esophagography and manometric studies on patients with H type tracheoesophageal fistula showed gastroesophageal reflux and uncoordinated peristalsis below fistula.[17,18] Romeo et al[19] documented motility disorders (ie, incomplete relaxation of the upper esophageal sphincter, reduced LES pressure, and incomplete relaxation of the LES) at the proximal and distal esophageal segments in newborns with EA during the preoperative period. An-

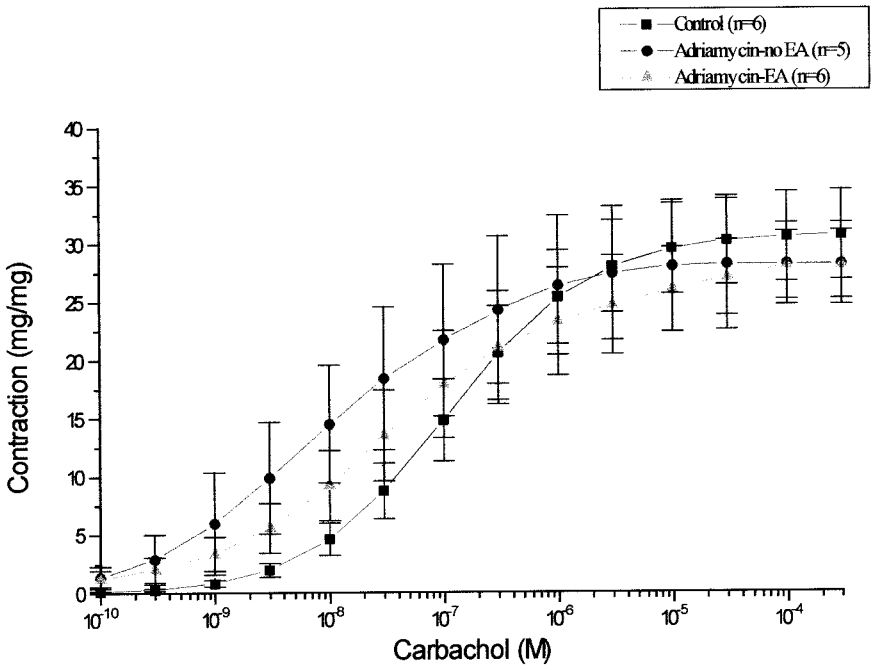


Fig 1. Carbachol concentration response curves in isolated rat lower esophageal strips. All points show the mean ± SEM of responses obtained in 5 to 7 individual experiments on different tissues from different animals. Data are expressed as milligrams of tension per mg of tissue wet weight.

other study showed that the neuronal cells were larger and the interganglionic fibers were looser and thinner than normal in the distal segment of the EA.[20] Using videomanometry, Montgomery et al[21] showed normal propagation of the peristaltic wave from the upper esophageal sphincter to the first 2 cm of the upper esophagus in EA patients, but below this level poor or absent motility was seen in adults who had undergone repair of EA in the newborn period. These findings suggest that esophageal dismotility may be in part caused by some congenital abnormality of the esophagus itself.

Adriamycin-induced EA is a convenient model for studying embryogenesis, histopathology, and associated malformations in rats.[8,12-14,22-24] Thus, the etiology of esophageal dysfunction in EA was investigated in several studies. The abnormalities in the course and branching pattern of the vagus nerves were shown by Qi et al.[9] Another study found significant abnormalities of the intramural nervous components of the esophagus, both the excitatory and inhibitory intramural nerves.[10] Our study showed that EA impairs the relaxant response to serotonin in the lower esophageal smooth muscle, whereas the contractile response to carbachol is completely preserved. KCl-induced contractile responses and papaverine-induced direct relaxant responses were similar in all groups. It is well known that smooth muscle relaxation plays an important role in esophageal motility. Decreased relaxant responses found in this study may lead to diminished peristalsis. The mechanism underlying this impaired relaxation is not known yet. However, EA-induced dysfunction of esophageal tissue does not appear to involve alterations in the level of smooth muscle cells. In the current study, the magnitude of the precontraction was similar in preparations from EA, control, and Adriamycin-no EA groups for carbachol, thus, ensuring that any difference in relaxation between EA or control preparations were not caused by differences in the degree of precontracting. The impaired relaxant responses are not entirely caused by the insult of Adriamycin alone, because the relaxant responses of the lower esophageal strips from EA fetuses did not differ significantly from that of fetuses with normal esophagus receiving Adriamycin. It appears that a concurrent abnormality also may occur in the supporting cells of esophagus with the development of EA.

Recently, it was shown that in the rat, isolated esoph-

Table 1. E_{max} (mg/mg) Values for KCl and Carbachol and E_{max} Values (% of 10^{-6} mol/L carbachol) for Serotonin in Lower Esophageal Strips

	Control	Adriamycin-No EA	Adriamycin-EA
KCl	16.27 ± 6.48	19.18 ± 3.41	21.02 ± 4.31
Carbachol	30.68 ± 0.067	28.47 ± 0.20	27.86 ± 0.26
Serotonin	98.83 ± 8.5	82.33 ± 7.55	37.47 ± 1.74*

NOTE. Values are expressed as mean ± 1 SEM.
*$P < .05$, compared with other 2 groups.

Table 2. pD_2 Values for Carbachol and Serotonin in Lower Esophageal Strips

	Control	Adriamycin-No EA	Adriamycin-EA
Carbachol	6.95 ± 0.32	7.98 ± 0.53	7.43 ± 0.29
Serotonin	7.61 ± 0.32	7.49 ± 0.47	8.34 ± 0.21*

NOTE. Values are expressed as mean ± 1 SEM.
*$P < .05$, compared with other 2 groups.

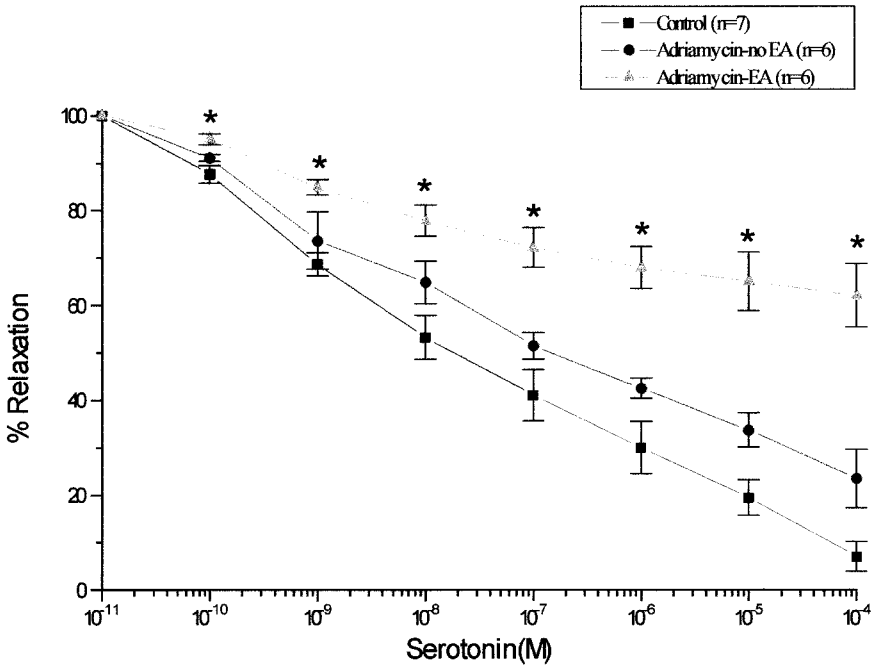


Fig 2. Serotonin concentration response curves in isolated rat lower esophageal strips precontracted with carbachol, 3.10^{-6} mol/L. All points show the mean ± SEM of responses obtained in 5 to 7 individual experiments on different tissues from different animals. Data are expressed as a percentage of the contraction induced by carbachol. *$P < .05$, compared with other 2 groups.

ageal muscularis mucosae serotonin relaxes smooth muscle cells by enhancing cAMP.[25,26] The decreased relaxant responses in this study may be related in part to the decreased serotonergic receptors. It could be assumed that the decreased responsiveness to serotonergic receptor stimulation in esophageal tissues from EA group might be attributable to impaired signal transduction in addition to the decreased number of serotonergic receptors. This observation would suggest a possible common pathophysiologic mechanism of alteration in the adenylate cyclase-cAMP pathway. Because we did not perform experiments with an adenylate cyclase activator such as forskolin, we cannot comment on this issue. The role of changes in G protein, another intermediate indispensable for signal transduction between serotonergic receptors and adenylate cyclase, remains to be investigated. The effect of EA on relaxant responses of esophageal smooth muscle, however, requires further investigation.

Relaxant responses were decreased in the distal esophageal segment of the fetuses with EA. Impaired relaxant responses may be, at least in part, a contributing factor in the esophageal dismotility seen in EA.

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