Segmental Difference in Vasoreactivity of the Human Right Gastroepiploic Artery

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Background: The gastroepiploic artery (GEA) plays an important role in the era of multiple arterial revascularization, but spasm is a major matter of concern. The internal thoracic artery has been shown to have a strong tendency to spasm in its distal bifurcating part, whereas the segmental difference in vasoreactivity of the GEA has never been performed.

Methods and Results: The full length of the GEA obtained from 21 patients undergoing a total gastrectomy was divided into 3 sections: proximal (5 cm from the origin), middle, and distal (5 cm from the end). Concentration-response curves for vasoconstrictors (phenylephrine, prostaglandin F_{2α}, and endothelin-1) and vasodilators (carperitide, nitroglycerin, and nifedipine) were then established using organ baths. All the vasoconstrictors and vasodilators produced concentration-dependent responses in each section. As the concentration of the vasoconstrictors increased, segments at the distal section showed a significantly greater contraction than those at the middle and proximal sections regardless of the type of vasoconstrictor. The effective concentration of drugs that caused 50% of the maximal response for endothelin-1 was significantly greater in the distal section than that in the proximal sections. No significant difference was found in vasodilators-induced relaxation.

Conclusions: The contractility increases toward to the end of the GEA. Clinically, the distal portion of the GEA should be trimmed off and not be used as an anastomotic site wherever possible.

Key Words: Gastroepiploic artery; Internal thoracic artery; Spasm

The optimal grafting strategy for multi-vessel coronary disease remains controversial. In these circumstances, the right gastroepiploic artery (RGEA) plays a certain role in revascularization, especially for the posterior wall of the heart. One prime benefit of the RGEA is that it can be used as an in situ graft. This strategy potentially contributes to reducing the incidence of postoperative stroke by eliminating aortic clamping if in-situ RGEA is teamed with bilateral internal thoracic artery (ITA) grafts in the off-pump surgery.^{1,2} Another feature is that graft patency of the RGEA is equal to or higher than that of the saphenous vein. Recent reports show that the long-term patency rate of the non-skeletonized RGEA ranged from 82% to 86% at 5 years^{3–5} and that of skeletonized RGEA is 94.7% at 5 years and 90.2% at 8 years.⁶ In contrast, one major concern of the RGEA graft is spasm, which is well-recognized and can occur during or immediately after graft harvest.^{7,8} It's been reported that the contractility of ITA grafts is variable along its full length and that its distal section has the highest reactivity, indicating a strong tendency of spasm,^{9–11} whereas the segmental variability of vasoreactivity of the RGEA has never been performed. The present study was designed to examine the segmental variability in vasoreactivity between the proximal, middle,

and distal sections of the human RGEA.

Methods

The Human Ethics Committee of Shiga University of Medical Science approved the use of human blood vessels as well as the experimental protocol used in the present study (Approval no. 25-169). Written informed consent was obtained from each patient.

Vessel Preparation

The full length of the RGEA was obtained from 21 Japanese patients undergoing a total gastrectomy who were shown to have no cardiac disease on preoperative examination. The RGEA was harvested from its origin at the gastroduodenal artery to its terminal anastomosis with the left gastroepiploic branch, immediately placed in a container with Bretschneider's histidine-tryptophan-ketoglutarate solution (Custodiol HTK; Köhler Chemie GmbH, Bensheim, Germany) maintained at 4°C, and transferred to the laboratory as soon as possible. The mean length of the RGEA was 23±4 cm. The adherent connective tissue was carefully dissected and 15-mm-long segments were taken from the proximal, middle, and distal sections of the

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RGEA. The distal section was identified within 5 cm from the end of the RGEA, proximal section within 5 cm from the origin of the RGEA, and the middle as midway between the proximal and distal sections. Each 15-mm-long segment was then evenly divided into 3 segments (5 mm in length) and helically cut into strips with special care being taken to preserve the endothelium, before randomly assigned to different drugs in an isometric tension study.

Isometric Tension Study

Each specimen was vertically fixed between hooks in a muscle bath (10-mL capacity) containing modified Ringer-Locke solution of the following composition: 120 mmol/L NaCl, 5.4 mmol/L KCl, 2.2 mmol/L CaCl₂, 1.0 mmol/L MgCl₂, 25.0 mmol/L NaHCO₃, and 5.6 mmol/L glucose. The bathing media were maintained at a temperature of 37±0.3°C, aerated with a mixture of 95% O₂ and 5% CO₂, and the pH was 7.36–7.43. The hook fixing the upper end of the strips was connected to the lever of a force-displacement transducer (TB-611T; Nihon Kohden Kogyo, Tokyo, Japan) connected to an amplifier (AP-621G; Nihon Kohden Kogyo). Isometric contractions and relaxations were displayed on a pen recorder. Before starting the experiments, all of the preparations were allowed to equilibrate in the bathing medium for 60–90 min, during which time the solution was replaced every 10–15 min. Resting tension is a key determinant of subsequent contractile response. In a preliminary experiment, changes in developed tension at different resting tensions were examined to see where the greatest change in developed tension would occur. We took 3- to 4-cm-long segment from the middle section, then divided it evenly into 6 helical strips, and randomly assigned them to different resting tensions (1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 g). A total of 12 patients provided their RGEA for this experiment. After equilibration for 30 min, active tension induced by 30 mmol/L KCl solution was recorded. The sample was washed for 30 min and repeated twice to confirm reproducibility. The largest increase in active tension was seen in 1.5 g of resting tension (Figure S1). The next largest difference was observed at 1.0 g and 2.0 g. The weakest responses were observed at 3.0 g. Based on this result, the resting tension applied to each preparation was adjusted to 1.5 g in the present study. Contractility was expressed as a relative value of contraction induced by 30 mmol/L KCl.¹² The advantage of this expression is to normalize the contraction by the response to a standard vasoconstrictor and to eliminate the possible differences caused by other factors such as the amount of smooth muscle cells. Integrity of the endothelium was histologically verified by en-face staining of the luminal surface using silver nitrate solution after the isometric tension study (Figure S2).¹³

Measurements

Contraction by Phenylephrine, Prostaglandin F_{2α}, and Endothelin-1 Two types of spasmogens for arterial grafts has been suggested by He et al.¹⁴ Type I includes endothelin, prostanoids, and α₁-adrenoceptor agonists, the most potent vasoconstrictors that induce strong contraction of arterial grafts even when the endothelium is intact. Type II vasoconstrictors (such as 5-hydroxytryptamine) only induce a weak vasoconstriction when the endothelium is intact. We tested 3 type I spasmogens in the experimental protocol (phenylephrine, prostaglandin F_{2α}, and endothelin-1). At the start of the experiment, the tissues were contracted

Figure 1. Concentration-contraction curves for phenylephrine (A), prostaglandin F_{2α} (B), and endothelin-1 (C) in helical strips taken from the proximal, middle, and distal section of the right gastroepiploic artery. Contraction induced by 30 mmol/L KCl was taken as 100%. *Indicates statistical significance at level $P < 0.05$ compared with middle and proximal sections (2-way ANOVA). A post-hoc Bonferroni test was used to detect the individual differences. Values are expressed as mean ± SEM.

twice with 30 mmol/L KCl followed each time by repeated washout with the solution. The second response was taken as an index of the contractile ability. Concentration-response curves for phenylephrine (10^{-8} to 10^{-5} mol/L), prostaglandin F_{2α} (10^{-8} to 10^{-5} mol/L), and endothelin-1 (10^{-10} to 10^{-7} mol/L) were then established in helical strips

Figure 2. The effective concentration of drugs that caused 50% of maximal response (EC_{50}) values for phenylephrine, prostaglandin $F_{2\alpha}$, and endothelin-1 in helical strips taken from the proximal, middle, and distal sections of the right gastroepiploic artery. Mean levels between the 3 groups were compared by using the 1-way ANOVA. A post-hoc Bonferroni test was used to detect the individual differences. Values are expressed as mean $\pm$ SEM. *Indicates statistical significance at level $P < 0.05$. The effective concentration of vasodilators that caused 50% of maximal response was defined as EC_{50} .

prepared from the proximal, middle, and distal sections by adding the drug directly to the bathing media in cumulative concentrations. Only one curve was obtained from each strip. The effective concentration of drugs that caused 50% of maximal response (E_{max}) was defined as EC_{50} . The EC_{50} was determined from each concentration-response curve by a logistic, curve-fitting equation: $E = MA^P / (A^P + K^P)$, where E is response, M is maximal response, A is concentration, K is EC_{50} , and P is the slope parameter.¹⁵

Relaxation by Carperitide, Nitroglycerin, and Nifedipine

Helical strips prepared from the distal, middle, and proximal sections of the RGEA were contracted with phenylephrine. After the contraction reached a plateau, concentration-response curves for carperitide (10^{-11} to 10^{-7} mol/L), nitroglycerin (10^{-11} to 10^{-5} mol/L), and nifedipine (10^{-11} to 10^{-5} mol/L) were obtained by adding the drug directly to the bathing media in cumulative concentrations. Only one concentration-response curve was obtained in each strip. At the end of each experiment, 0.1 mmol/L papaverine was added to induce the maximal relaxation, which was taken as 100% for relaxation induced by the agonist as previously described.¹⁶

Statistical Analysis

Concentration-contraction curves were compared by using 2-way analysis of variance (ANOVA) for repeated measures. Post-hoc Bonferroni test was used to detect the individual differences. The sphericity assumption was confirmed by the Mauchly test in ANOVA analyses. All statistical testing was 2-sided. Results were considered statistically significant at a level of $P < 0.05$. All analyses were performed with the SPSS statistical package version 21.0 (SPSS Inc., Chicago, IL, USA).

Results

Figure 1 shows segmental differences in contraction by phenylephrine, prostaglandin $F_{2\alpha}$, and endothelin-1. Phenylephrine, prostaglandin $F_{2\alpha}$, and endothelin-1 produced concentration-dependent contraction in strips taken from the distal, middle, and proximal sections of the RGEA. As

the concentration of the vasoconstrictors increased, strips at the distal section showed a significantly greater contraction than those at the middle and proximal sections. The E_{max} for phenylephrine was $154 \pm 11\%$ at distal, $92 \pm 13\%$ at middle, $88 \pm 13\%$ at proximal sections (2-way ANOVA, $P < 0.05$). The respective values for prostaglandin $F_{2\alpha}$ and endothelin-1 were $150 \pm 12\%$ at distal, $102 \pm 12\%$ at middle, $98 \pm 13\%$ at proximal sections (2-way ANOVA, $P < 0.05$), and E_{max} : $106 \pm 9\%$ at distal, $72 \pm 11\%$ at middle, $73 \pm 10\%$ at proximal sections (2-way ANOVA, $P < 0.05$). The effective concentration of drugs that caused 50% of maximal response (EC_{50}) of endothelin-1 was significantly lower in the distal section than in the proximal sections (-8.9 ± 0.3 vs. -8.2 ± 0.3 log M, Bonferroni post-hoc test, $P < 0.05$), indicating that the sensitivity to endothelin-1 was 5.0-fold higher in the distal section than in the middle and proximal sections (**Figure 2**). **Figure 3** shows the segmental differences in relaxation by carperitide, nitroglycerin, and nifedipine. All the vasodilators caused a concentration-dependent relaxation response, and no significant segmental difference was found between the proximal, middle, and distal sections.

Discussion

The present study has 3 major findings. First, the contraction induced by phenylephrine, prostaglandin $F_{2\alpha}$, and endothelin-1 was significantly greater at the distal section than at the middle and proximal sections of the RGEA obtained from patients undergoing total gastrectomy. Second, the sensitivity to endothelin-1 was significantly higher at the distal section than at the middle and proximal sections. Third, no significant difference was observed in relaxation response to sodium nitroprusside between the 3 sections.

We often face vasospasm in the distal end of the RGEA with a small lumen diameter rather than in the middle and proximal sections during surgery. The present study may provide scientific backing for our empirical observation. The important point from clinical perspectives is that the distal portion of the RGEA should be trimmed off and not be used as an anastomotic site wherever possible. In order

to do that, it is essential to explore the proximal portion of the RGEA to the level of the pylorus and to harvest an adequate length to prevent excessive tension after grafting. Removal of the surrounding tissue (skeletonization) makes the RGEA longer and larger^{17,18} so that it can be anastomosed at a more proximal position than the non-skeletonized RGEA.

Segmental variability in contractility has been examined in human ITA by He et al.¹¹ They tested the reactivity of ITA grafts taken from patients undergoing coronary artery bypass grafting (CABG) and showed that the contraction force was greater in the distal section than in other sections for norepinephrine and endothelin-1, but no differences were found for potassium, U46619 (a thromboxane A2 receptor agonist), or glyceryl trinitrate. They also demonstrated that the sensitivity for U46619 was 100-fold higher in the distal than in the middle section. These results are almost consistent with the present study results. A histological study on the RGEA obtained at autopsy from a patient who had died of non-cardiac diseases has shown that the media is muscular with rare elastic fibers, the thickness of the media and the luminal diameter become thinner and narrower distally, and the degree of intimal hyperplasia gradually decreases along its course.¹⁹ This histological study does not reveal mechanisms to explain the segmental variability of contractility. The RGEA is considered a visceral artery supplying blood to visceral organs. Physiologically, a higher contractility of visceral arteries compared to somatic arteries such as ITA is considered responsible because blood flow of the splanchnic artery enormously changes under various circumstances to accommodate the function of the stomach.

The study has a number of potential limitations. First, all enrolled subjects were Japanese patients who underwent total gastrectomy and shown to have no cardiac disease on preoperative examination, which limits the generality of the findings, and interpretation of the results should be carefully adopted for patients undergoing CABG. Second, the in vitro design has limitations in the clinical implications, but it allows us to simplify various complex factors that may influence the evaluation of a particular pharmacological agent and focus on the specific effect of the agent on the tissue. Third, we defined each section after the example report by He et al.¹¹ in which the middle section was defined as at least 4 cm from the distal bifurcation or 4 cm from the origin of the ITA from the subclavian artery, and the length of the middle section is $\sim 60\%$ of the total length (19.5 ± 2.4 cm). Arbitrariness, however, cannot be completely denied. Fourth, the segmental difference in endothelial function was not examined. The prevalence of vasospasm in arterial grafts can correlate with their endothelial function. Finally, the effect of perioperative drugs on vascular response was not included in the analysis, but chronic use of oral anti-hypertensive drugs such as calcium antagonists, or intraoperative use of catecholamine or nitroglycerin, might modify the vasoreactivity.

In conclusion, the distal section of the RGEA has greater contractility compared to the middle and proximal sections. This finding indicates that the distal section of the RGEA is the part where spasm easily develops and requires pharmacologic and surgical prevention. The middle and proximal sections are less pharmacologically reactive than the distal section, but these sections are not completely non-reactive passive conduits and also require special care. No significant segmental difference was found in relax-

Figure 3. Concentration-relaxation curves for carperitide (A), nitroglycerin (B), and nifedipine (C) in helical strips taken from the proximal, middle, and distal section of the right gastroepiploic artery. Precontraction was obtained with phenylephrine. Relaxation induced by 10^{-4} mol/L papaverine was taken as 100%. Each point and bar represents the mean $\pm$ SEM.

ation by carperitide, nitroglycerin, and nifedipine. These drugs are favored candidates for prevention and reversal of graft spasm when we have no choice but to use the distal part of the RGEA.

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Conflicts of Interest

All authors have nothing to disclose with regard to commercial support.

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Supplementary Files

Supplementary File 1

Figure S1. Box-and-whisker plots comparing tension induced by 30 mmol/L KCl at different resting tensions.

Figure S2. Typical example of en-face staining of the luminal surface using silver nitrate solution in endothelium-intact (**Left panel**) and -denuded (**Right panel**) gastroepiploic artery strips (bar=50 mm).

Please find supplementary file(s);
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