Effect of endothelin-1 on tumor arteries in patients with colorectal cancer

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ABSTRACT

Endothelin-1 is an endothelium-derived vasoconstrictor peptide whose plasma levels are increased in patients with colorectal cancer, and which may be involved in tumor blood flow regulation. To study whether response to this peptide is altered in tumor arteries, mesenteric arteries supplying blood flow to colorectal tumors, and mesenteric arteries far from said tumors were obtained from 13 patients undergoing colectomy; mesenteric arteries were also obtained from patients with diverticulitis (n = 4) or inflammatory bowel disease (n = 3). Arteries were prepared for isometric tension recording in an organ bath, and in this preparation it was found that endothelin-1 induced contraction in all three types of arteries, but that sensitivity to this peptide was greater in arteries supplying blood flow to the tumor than in arteries far from the tumor or arteries from patients without cancer. These results suggest that endothelin-1 may regulate blood flow to colorectal tumors by inducing a greater contraction in tumor-supplying arteries than in non-tumor arteries.

Key words: Endothelin-1. Colorectal tumors. Vasoconstriction. Endothelium.

INTRODUCTION

The blood vessels that supply tumors exhibit both morphological (1) and functional alterations (2,3), and these tumor vessel characteristics may be relevant to support tumor growth and expansion by altering the incoming blood flow. One of the factors that may be involved in blood flow regulation under both normal and pathological conditions is endothelin-1, a peptide produced by endothelial cells that has a potent vasoconstrictor effect (4). Endothelin-1 may be involved in tumor blood flow regulation since plasma levels are increased in patients with colorectal cancer (5), and the response to this vasoconstrictor peptide is altered in tumor arteries in rats (6) and mice (7).
Tumor blood vessels are a potential therapeutic target in cancer therapy, and understanding the response of these vessels to factors such as vasoactive endothelin-1 may be of interest. At present, as far as we know, no study has been performed on the response to endothelin-1 by human tumor vessels, since existing studies have been conducted in experimental animal tumors (8). Therefore, the objective of this study was to analyze the vasoconstrictor response to endothelin-1 of arteries in human colorectal tumors, compared to normal colon arteries in the same patients, or to arteries from patients without tumor disease.

MATERIALS AND METHODS

Artery collection

In this study, we obtained mesenteric arteries (about 1 mm in outer diameter) from 13 patients with colorectal cancer-related colectomies—arteries directly irrigating the tumor, arteries distant from the tumor, and normal colon-irrigating arteries. Likewise, mesenteric arteries were obtained from patients who had undergone colectomy for non-tumor diseases, such as diverticulitis (4 patients) or inflammatory bowel disease (3 patients). Immediately after removal arteries were dissected and brought into cold isotonicsaline for their transfer from operating theatres at Hospital Universitario 12 de Octubre to the Laboratory at Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma de Madrid.

Mesenteric arteries thus obtained were cleaned of adjacent tissues under a microscope (Fig. 1), and cut into segments 2 mm in length.

Isometric tension registration

For recording isometric tension as developed by the wall in artery segments two tungsten wires 100 µm in diameter were introduced through the vascular lumen. One of the wires was attached to a holder close to the organ container wall, and the other wire, which was mobile, was connected to a transducer for recording isometric tension (Universal Transducing Cell Statham microscale accessory UCL5). A micrometer screw connected to the mobile wire allowed it to be moved vertically, perpendicular to the long axis of the arterial segment, and thus relayed the desired passive tension into the arterial wall. Tension recording was carried out on a computer using a Maclab 8/e (AD Instruments) data acquisition system with the program Chart V 3/4.

Each arterial segment, set up on the system explained above, was placed in an organ bath containing 4 ml of Krebs-Henseleit solution with the following composition (mM): 115 NaCl; KCl 4.6; KH2PO4 1.2; SO4 mg 1.2; CaCl2, 2.5; NaHCO3 25; glucose 11. This solution was balanced with a gas mixture (95% O2 and 5% CO2) to provide pH = 7.3-7.4, which was checked with a Crison laboratory pH-meter. A hot water circuit surrounding the bath kept the Krebs-Henseleit solution together with the vascular segments at a temperature of 37 ± 0.5 °C (Fig. 2).

Optimum tension determination

To determine optimal tension for contraction the serotonin response (10⁻⁴ M) was recorded at various passive tensions. For this determination serotonin was used because it induces a sharp contraction in mesenteric arteries, which was reproducible when the same vascular segment was repeatedly stimulated. After setting up vascular segments in the organ bath vascular walls were stretched by separating the two wires through the micrometer screw until the passive tension measured by the transducer was stable at values of 0.15, 0.3, 0.6, 2, 4, and 8 g. Then serotonin was added to the organ bath at a concent-
tration of $10^{-5}$ M, and smooth muscle contraction was registered. When this contraction reached a plateau serotonin was eliminated from the bath, the Krebs-Henseleit solution was repeatedly renewed, and segments were stretched to the next passive tension. Since the maximum response to serotonin was to a tension of 2-4 g, the later experiments were conducted with a 2-g tension.

**Endothelin-1 response**

After setting up the vascular segments in organ baths passive tensions were set to the value previously determined of 2 g, and maintained for 2-3 hours until balanced. Then the endothelin-1 response was studied by using cumulative concentration-response curves. Endothelin-1 was added to the organ bath in increasing concentrations ($10^{-10}$ - $10^{-7}$ M), and after each addition 10-15 min were left to elapse until wall contraction reached a plateau, before adding the following concentration.

**Data analysis**

Contraction to serotonin or endothelin-1 was recorded as the increment in tension over baseline tension by adding these substances. At each concentration-response to endothelin-1 pD$_2$ was calculated as the negative logarithm of concentration that produced a contraction equal to 50% of peak concentration, obtained by geometric interpolation at the curve. Response to endothelin-1 in the different artery groups was compared using a variance analysis, followed by Bonferroni’s test to determine what differences were statistically significant. The lowest significant probability was 0.05. Results are expressed as mean ± standard error.

**RESULTS**

Serotonin induced a maximum vascular contraction of 1.46 ± 0.34 g, and an optimal response was obtained when vascular segments were stretched to a passive tension of 2-4 g, whereas response was poorer when vascular segments were stretched to lower (0.15, 0.3, 0.6 g) or higher passive tensions (8 g) (Fig. 3).

Endothelin-1 stimulation resulted in concentration-dependent contraction in mesenteric arteries in all cases. Maximum contraction was similar in tumor arteries (4.57 ± 0.49 g), in arteries irrigating a region remote from the tumor in the same patients (4.17 ± 0.52 g), and in arteries of patients without tumors (4.97 ± 0.39 g). However, sensitivity (pD$_2$) was greater in tumor arteries (8.21 ± 0.13) than in arteries of remote regions from the same patients (7.78 ± 0.13, p < 0.05), or in arteries from patients without tumors (7.80 ± 0.08, p < 0.05) (Figs. 4 and 5).
DISCUSSION

The results obtained in this study suggest that endothelin-1 induces a sharp contraction of colorectal tumor arteries, which can be higher than in mesenteric arteries unrelated to tumors.

First, optimal contraction tension in vascular segments was measured by recording contraction to serotonin, which activates vascular smooth muscle. Serotonin contraction was maximal when vascular segments were stretched to a passive tension of 2 to 4 g, and the response diminished for lesser or greater tensions. This dependence of contractile response on initial passive tension is a muscular characteristic (skeletal and smooth muscle), and may be due to an overlap between contractile filaments varying with the length of muscle fibers, with optimal results being obtained with intermediate stretching degrees (9). Muscles length in vivo is close to the optimal value, so in vitro studies try to replicate this physiological condition as far as possible. In this study, while serotonin-induced contraction was slightly higher at 4 g than at 2 g, the difference was not statistically significant, and since a high passive tension for a long time can damage the vascular wall, a passive tension of 2 g was selected for the experiment.

Endothelin-1 induced a very sharp vascular contraction in tumor-irrigating arteries as well as in non-tumor arteries. This contraction in response to endothelin-1 was far greater than the contraction produced by serotonin, despite the fact that serotonin is considered a powerful vascular smooth muscle constrictor. The answer found in our study is similar to that described by Miyauuchi et al. (10) in human mesenteric arteries, as well as to that found in other species such as rats (11) or dogs (12). The objective of this study was to analyze the response to endothelin-1 in tumor arteries, and we found out that the response to this peptide was higher in arteries irrigating colorectal tumors than in normal colon-irrigating arteries from the same patients, or in arteries from patients without tumors.

Although the maximum contraction to high endothelin-1 concentrations (10^-7 M) was similar in all three types of arteries, sensitivity (pD50) was greater in tumor-irrigating arteries than in the other two groups. This difference may have physiological relevance since pD50 suggests a greater response to low concentrations of endothelin-1, and these low concentrations may occur in tissues under physiological conditions. This increased response to endothelin-1 found in our study could be due to local factors released by tumor cells that would affect neighboring blood vessels, since cytokines such as tumor necrosis factor (TNFα) or beta interleukin-1 increase contraction in response to endothelin-1-receptor stimulation in human arteries (13) or in rat arteries (14,15). Although cytokine plasma concentrations may be increased in patients with different tumor types (16), the local concentrations of this cytokine in tumor tissue may be more relevant than its circulating levels (17). In the present study, sensitivity to endothelin-1 was increased in colorectal tumor-irrigating arteries but not in distant arteries from the same patients, as the response to endothelin-1 in these arteries was similar to that in arteries from patients without tumors. This suggests that changes in the response to endothelin-1 may be due to local conditions existing in the tumor area, rather than circulating factors. Subsequent studies will be necessary to analyze whether these local conditions may increase vascular endothelin-1 receptors.

In summary, the results of this study suggest that the arteries that irrigate colorectal tumors are very sensitive to endothelin-1, and this, coupled with the high levels of this peptide found in patients, result in marked vasoconstriction in these arteries. We have proposed therapeutic strategies aimed at increasing tumor blood flow in order to facilitate the arrival of antitumor drugs to tumor cells, and endothelin-1 receptor antagonists may be useful in this regard.

REFERENCES


