Supplemental Digital Content (SDC)

[1] Methods:

Animals

Inbred male Lewis (LEW) rats weighing 180–200 g were obtained from Charles River WIGA GmbH (Sulzfeld, Germany). All experiments were performed in accordance with the federal law regarding the protection of animals. The principles of laboratory animal care (NIH Publication No.8 5-23, revised 1985) were followed. The animals were maintained on a 12-h light/dark cycle and provided with commercially available chow (Altromin, Lage, Germany) and tap water *ad libitum*.

Drugs and solutions

A standard Krebs Ringer buffer solution (KRB) was used as described previously [1]. KRB constituents and bethanechol were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany) and from Merck KGaA (Darmstadt, Germany). Phosphate buffered saline (PBS) was purchased from Lonza (Verviers, Belgium). CPSI-2364 was provided by Cytokine PharmaSciences, Inc. (King of Prussia, PA, USA).

Quantification of cell infiltration

Specimens for histochemical and immunohistochemical analysis were prepared at 3 and 18 hours after reperfusion as whole mounts of the distal jejunum. The tissue was fixed with ethanol (100%) for 10 minutes or paraformaldehyde (4%) for 30 minutes at room temperature [1;2]. Myeloperoxidase (MPO) positive neutrophils were detected by staining with Hanker-Yates reagent (Polysciences Europe GmbH, Eppelheim, Germany) and quantified by counting five randomly chosen areas in each specimen. Infiltrating monocytes and macrophages were quantified in the same manner after staining with primary anti-CD68 antibodies (mouse-anti-rat; ED1; 1:200 at 4°C overnight; Serotec GmbH, Duesseldorf, Germany) and secondary donkey-anti-mouse-Cy3 antibodies (1:200; Dianova GmbH,
Hamburg, Germany). In all staining procedures, secondary antibodies without ED1 preincubation were used in parallel to ensure specificity.

Apoptosis

Apoptotic cells within the smooth muscle layer of the grafts were detected at 3 and 18 hours after reperfusion by detection of DNA double strand breaks using the TdT-mediated dUTP-X nick-end-labeling (TUNEL) method on paraffin-embedded tissue (In Situ Cell Death Detection Kit, Roche Diagnostics GmbH, Mannheim, Germany) and reviewed in a blinded manner.

Functional studies

Mechanical in vitro activity of the mid-jejunum was evaluated at 18 hours after reperfusion using mucosa-free smooth muscle strips of the circular muscularis as described previously [1]. After recording spontaneous contractility for 30 min, dose-response curves were generated using increasing doses of the muscarinic agonist bethanechol (1-300 µmol/l) for 10 min and intervening wash periods (Krebs-Ringer buffer solution/KRB) of 10 min. The contractile response was recorded and analyzed with ADI Chart© software (ADI, Heidelberg, Germany) and calculated as grams per square millimeter per second by conversion of the weight and length of the strip to square millimeters of tissue (g/mm²/s).

Determination of nitric oxide metabolites and cytokines

Nitrite and nitrate at 3 and 18 hours after reperfusion was quantified in the rat serum using Nitrate/Nitrite Colorimetric Assay Kit (Cayman Chemical, Ann Arbor, USA) following the instructions by the manufacturer. Serum levels of Interleukin-6 (IL-6) were measured by an IL-6 ELISA Kit (R&D Systems, Wiesbaden, Germany).
Data analysis

Results are expressed as mean ± standard deviation (SD) or standard error (SEM). Statistical analysis consisted of one-way ANOVA followed by Bonferroni multiple comparison test. \( P \leq 0.05 \) was considered as significant. SPSS Statistics 17.0 (SPSS GmbH Software, Munich, Germany) was used for statistical analysis.

References
