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67	Abstract	<hr/> <p>Small bowel is one of the most sensitive organs to ischemia–reperfusion injury, which is a significant problem during transplantation. Pituitary adenylate cyclase-activating polypeptide (PACAP) has cytoprotective effect in ischemic injuries of various tissues. The aim of our study was to measure changes of PACAP-38 and PACAP-27 immunoreactivities and cytokine levels in intestinal grafts stored in PACAP-38-containing preservation solution. Small bowel autotransplantation was performed on male Wistar rats. Grafts were stored in University of Wisconsin (UW) solution at 4 °C for 1 h (group (GI)), for 3 h (GII), and for 6 h (GIII) and in PACAP-38-containing UW solution for 1 h (GIV), for 3 h (GV), and for 6 h (GVI). After preservation, performing vessel anastomosis reperfusion began, which lasted 3 h in each group. Tissue biopsies were collected after laparotomy (control) and at the end of the reperfusion periods. Intestinal PACAP-38 and PACAP-27 immunoreactivities were measured by radioimmunoassay. To measure cytokine array from tissue homogenates, we used rat cytokine array and Luminex Multiplex Immunoassay. Levels of PACAP-38 and PACAP-27 immunoreactivity decreased after 1 and 3 h preservation compared to control levels. This decrease was significant following 6 h cold storage ($p < 0.05$). Values remained significantly higher in grafts stored in PACAP-38-containing UW. Cytokine array revealed that expression of the soluble intercellular adhesion molecule-1 (CD54) and L-selectin (CD62L/LECAM-1) was increased in GIII. Both 6 h cold storage in PACAP-38-containing UW solution and 3 h reperfusion caused strong reduction in these cytokines activation in GVI. RANTES (CCL5) levels were increased in all groups. Strong activation of the tissue inhibitor of metalloproteinase-1 was in GIII. However, PACAP-38-containing cold storage could decrease its activation in GVI. Furthermore, strong activation of the tissue inhibitor of metalloproteinase-1 was detected in 6 h preserved grafts without PACAP-38 (GIII). PACAP-38-containing cold storage could decrease its activation in GVI. Our present study showed that PACAP-38 and PACAP-27 immunoreactivities decreased in a time-dependent manner during intestinal cold preservation, which could be ameliorated by administration of exogenous PACAP-38 to the preservation solution. Moreover, PACAP-38 could attenuate tissue cold ischemic injury-induced changes in cytokine expression.</p> <hr/>
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4 **Intestinal Autotransplantation Using PACAP-38-Containing**
5 **Preservation Solution**6 **Klara Nedvig · Gyorgy Weber · Jozsef Nemeth ·**
7 **Krisztina Kovacs · Dora Reglodi · Agnes Kemeny ·**
8 **Andrea Ferencz**9 Received: 23 March 2012 / Accepted: 31 July 2012
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12 **Abstract** Small bowel is one of the most sensitive organs to
13 ischemia–reperfusion injury, which is a significant pro-
14 blem during transplantation. Pituitary adenylate cyclase-
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16 in ischemic injuries of various tissues. The aim of our study
17 was to measure changes of PACAP-38 and PACAP-27
18 immunoreactivities and cytokine levels in intestinal grafts
19 stored in PACAP-38-containing preservation solution.
20 Small bowel autotransplantation was performed on male
21 Wistar rats. Grafts were stored in University of Wisconsin
22 (UW) solution at 4 °C for 1 h (group (G)I), for 3 h (G)II), andfor 6 h (G)III) and in PACAP-38-containing UW solution for 23
1 h (G)IV), for 3 h (G)V), and for 6 h (G)VI). After preserva- 24
tion, performing vessel anastomosis reperfusion began, 25
which lasted 3 h in each group. Tissue biopsies were col- 26
lected after laparotomy (control) and at the end of the 27
reperfusion periods. Intestinal PACAP-38 and PACAP-27 28
immunoreactivities were measured by radioimmunoassay. 29
To measure cytokine array from tissue homogenates, we 30
used rat cytokine array and Luminex Multiplex Immunoas- 31
say. Levels of PACAP-38 and PACAP-27 immunoreactivity 32
decreased after 1 and 3 h preservation compared to control 33
levels. This decrease was significant following 6 h cold 34
storage ($p < 0.05$). Values remained significantly higher in 35
grafts stored in PACAP-38-containing UW. Cytokine array 36
revealed that expression of the soluble intercellular adhesion 37
molecule-1 (CD54) and L-selectin (CD62L/LECAM-1) was 38
increased in G)III). Both 6 h cold storage in PACAP-38- 39
containing UW solution and 3 h reperfusion caused strong 40
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tion of the tissue inhibitor of metalloproteinase-1 was in 43
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tion of the tissue inhibitor of metalloproteinase-1 was 46
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which could be ameliorated by administration of exogenous 52
PACAP-38 to the preservation solution. Moreover, PACAP- 53
38 could attenuate tissue cold ischemic injury-induced 54
changes in cytokine expression. 55K. Nedvig · G. Weber
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PACAP-27 · Cytokine 57

58 **Introduction**

59 Small bowel is a highly sensitive tissue to ischemia/reperfusion (I/R) injury in the body. Intestinal I/R injury is caused
 60 by many clinical conditions, including small bowel trans-
 61 plantation. Both clinical and experimental data demonstrate
 62 that transplant I/R injury has deleterious short- and long-
 63 term effects, manifesting as increased episodes of acute
 64 rejection and chronic allograft dysfunction (Ferencz et al.
 65 2002, 2010a, b; Linfert et al. 2009; Yuan et al. 2011). Graft
 66 viability prior to implantation is a key factor in the outcome
 67 after organ transplantation. Along with surgical manipula-
 68 tion, I/R injury and preservation damage are some of the
 69 many essential factors that affect the quality of intestinal
 70 graft and its multiple functions. The current standard in
 71 organ preservation with University of Wisconsin (UW) so-
 72 lution was developed for kidney/liver preservation and it is
 73 suboptimal for the intestinal graft despite good results for
 74 other organs (Maathuis et al. 2007; Roskott et al. 2011). The
 75 benefit of the UW solution for the preservation of other
 76 intraabdominal organs remains unclear and the maximum
 77 storage time for small bowel remains relatively brief
 78 (6–8 h). Thus, no general agreement exists about optimal
 79 preservation solution for intestinal grafts so far (Kokotilo
 80 et al. 2010; Roskott et al. 2011). Recently, there are contin-
 81 uous research efforts to modify the commercially available
 82 solutions (adding more components, high-energy intermedi-
 83 ates, and nutrients) or to develop new preservation solutions
 84 (Inuzuka et al. 2007; Wei et al. 2007; Ferencz et al. 2009;
 85 Yandza et al. 2011).

87 Pituitary adenylate cyclase-activating polypeptide
 88 (PACAP) is a widespread neuropeptide with diverse effects
 89 not only in the nervous system but also in the cardiovascular
 90 system and peripheral organs including endocrine glands,
 91 respiratory organs, and gastrointestinal tract. The polypep-
 92 tide exists in two forms, with 38 and 27 amino acid residues,
 93 named PACAP-38 and PACAP-27 (Vaudry et al. 2009).
 94 Endogenous PACAP-38 and PACAP-27 were demonstrated
 95 in all parts of the gastrointestinal tract with high levels
 96 detected in the jejunum and ileum. PACAP-38 and
 97 PACAP-27-immunoreactivities have been shown in the cell
 98 bodies and nerve fibers throughout the gastrointestinal tract
 99 (Hannibal et al. 1998). PACAP-38 and PACAP-27 act
 100 through the specific PAC1 receptor and VPAC1 and VPAC2
 101 receptors that bind VIP and PACAP with equal affinity
 102 (Koves et al. 1993; Vaudry et al. 2009). All three types of
 103 PACAP receptors have been shown in the intestinal system:
 104 in the mucosa and myenteric neurons, in neuroendocrine
 105 cells, blood vessels, and smooth muscle (Schulz et al. 2004;
 106 Pirone et al. 2011).

107 Endogenous PACAP-38 has been implicated in protec-
 108 tion against harmful stimuli; the peptide has anti-apoptotic,
 109 anti-inflammatory, and anti-oxidant actions in numerous

different in vivo and in vitro models (Ferencz et al. 2010a; 110
 Horvath et al. 2010a; Gasz et al. 2006; Racz et al. 2006, 111
 2010; Reglodi et al. 2011, 2012; Roth et al. 2009). Recently, 112 Q3
 in warm and cold I/R small bowel models, the in vivo 113
 protective effects of PACAP-38 have been shown. These 114
 experiments confirmed an important protective effect of 115
 endogenous PACAP-38 against warm I/R tissue damage. 116
 Moreover, it has been shown that adding exogenous 117
 PACAP-38 to UW solution prevented the oxidative stress 118
 and tissue structure injury in rat small intestine (Ferencz 119
 et al. 2010a, b). Although the exact mechanisms, by which 120
 intestinal I/R and gut injury contribute to the systemic 121
 inflammatory response, are not completely known, cyto- 122
 kines play a key role in these actions. There is also evidence 123
 that intestinal injury resulting from I/R can lead to the bowel 124
 becoming a cytokine-generating organ (Grotz et al. 1999). 125
 The anti-inflammatory actions of PACAP in several inflam- 126
 matory models are partially mediated through its suppress- 127
 ing effect on cytokine/chemokine production (Delgado and 128
 Ganea 2001; Horvath et al. 2010b; Ohtaki et al. 2010). 129
 However, there are no data in the literature about changes 130
 of intestinal PACAP-38 or PACAP-27 and tissue cytokine 131
 levels using PACAP-38-containing preservation solution in 132
 stored and transplanted small bowel grafts. The aim of our 133
 study was to measure changes of PACAP-38 and PACAP- 134
 27 immunoreactivities and cytokine values using PACAP- 135
 38-containing UW preservation solution during intestinal 136
 autotransplantation. 137

138 **Materials and Methods**

139 **Animals**

140 Adult male Wistar rats (250–300 g, $n=35$) were purchased 140
 from the Laboratory Animal Center of University of Pecs, 141
 housed under pathogen-free conditions, and were fasted for 142
 24 h preoperatively but had free access to water. Rats were 143
 anesthetized with intramuscular ketamine hydrochloride 144
 (0.075 mg/g of body weight) and diazepam (0.075 mg/g of 145
 body weight; Richter Gedeon, Budapest, Hungary). All 146
 procedures were performed in accordance with the ethical 147
 guidelines of National Institute of Health and guidelines 148
 approved by the University of Pecs (BA02/2000-9/2008) 149
 to minimize pain and suffering of the animals. 150

151 **Intestinal Autotransplantation Model**

152 Rats were randomly divided into groups (G). In group S, 152
 animals underwent only median laparotomy (sham operated, 153
 $n=5$). In GI–GVI after heparin administration (0.2 U/g), 154
 small bowel was resected to descending colon, and the 155
 lumen was flushed with normal saline. Grafts were perfused 156

157 by the superior mesenteric artery and were preserved in
 158 100 ml 4 °C University of Wisconsin solution (ViaSpan,
 159 DuPont Pharma, Bad Homburg, Germany) for 1 h in GI (*n*=
 160 5), for 3 h in GII (*n*=5), and for 6 h in GIII (*n*=5). Grafts
 161 were preserved in 100 ml UW solution containing 100 µg
 162 PACAP-38 dissolved in 2 ml of physiological saline (Sigma,
 163 Hungary) for 1 h in GIV (*n*=5), for 3 h in GV (*n*=5), and for
 164 6 h in GVI (*n*=5). After preservation, end-to-end anastomo-
 165 sis was performed between the stumps of mesenteric vessels
 166 with microvascular technique. Reperfusion lasted 3 h in
 167 each group. Small bowel biopsies were collected after lap-
 168 arotomy (control, C) and at the end of the reperfusion
 169 periods.

170 Radioimmunoassay

171 Intestinal tissue samples (600 mg) were homogenized in ice-
 172 cold distilled water. After centrifugation at 12,000 rpm/4 °C
 173 for 30 min, the supernate was further processed for RIA
 174 analysis of PACAP-38- and PACAP-27-like immunoreac-
 175 tivity, as previously described (Brubel et al. 2011). Briefly,
 176 the antiserum for PACAP-38 was “88111-3” and for
 177 PACAP-27 was “88123”. The tracer was mono-¹²⁵I-labeled
 178 ovine PACAP 24-38 and mono-¹²⁵I-labeled ovine PACAP-
 179 27 (5,000 cpm/tube). The standard was ovine PACAP-38
 180 and PACAP-27 (0–1,000 fmol/ml). Assays were prepared in
 181 1 ml phosphate buffer (0.05 mol/l, pH 7.4) containing 0.1 M
 182 NaCl, 0.05 % NaN₃, and 0.25 % bovine serum albumin. The
 183 assay procedures include 100 µl antisera (working dilu-
 184 tions—PACAP-38 “88111-3” antiserum, 1:10,000; PACAP-
 185 27 “88123” antiserum, 1:45,000), 100 µl RIA tracers, and
 186 100 µl standards or unknown samples measured into poly-
 187 propylene tubes with assay buffer. After 48–72 h incubation
 188 at 4 °C, the antibody-bound peptides were separated from
 189 the free ones by addition of 100 µl separation solution (10 g
 190 charcoal, 1 g dextran, and 0.2 g commercial fat-free milk
 191 powder in 100 ml distilled water). Following centrifugation
 192 (3,000 rpm for 20 min at 4 °C), the tubes were gently
 193 decanted, and the radioactivity of the precipitates was mea-
 194 sured in a gamma counter. PACAP-38 and PACAP-27 con-
 195 centrations of the unknown samples were read from the
 196 appropriate calibration curves. Results of PACAP-38- and
 197 PACAP-27-like immunoreactivities are given as femto-
 198 moles per milligram tissue.

199 Cytokine Array After Small Bowel Autotransplantation

200 Intestinal tissues from control bowel sample (A), from tissue
 201 exposed to 6 h cold storage in UW (B), from 6 h cold preser-
 202 vation in PACAP-38-containing UW solution (C), and subse-
 203 quent 3 h reperfusion period (D) were measured as previously
 204 described (Horvath et al. 2011). Briefly, cytokine array from
 205 tissue homogenates was performed using rat cytokine array

(Panel A Array kit from R&D Systems, Biomedica Hung., 206
 Budapest, Hungary). Small bowel samples were excised then 207
 homogenized in PBS with protease inhibitors. Triton X-100 208
 was added to the final concentrations of 1 %. The samples were 209
 stored at –80 °C prior to use. After blocking the array mem- 210
 branes for 1 h and adding the reconstituted Detection Antibody 211
 Cocktail for another 1 h at room temperature, the membranes 212
 were incubated with 1 ml of tissue homogenates at 2–8 °C 213
 overnight on a rocking platform. After washing with buffer for 214
 three times and addition of horseradish peroxidase-conjugated 215
 streptavidin to each membrane, we exposed them to a chemi- 216
 luminescent detection reagent (Amersham Biosciences, 217
 Hungary) then side up to an X-ray film cassette. 218

Luminex Multiplex Immunoassay 219

The levels of three host markers (soluble intercellular adhesion 220
 molecule-1 (sICAM1), L-selectin, and metalloproteinase-1 221
 (TIMP-1)) were determined in the selected bowel samples 222
 (see in cytokine array) using customized Flurokine MAP Rat 223
 Base Kit (R&D Systems). This was done according to the 224
 manufacturer's instructions (R&D Systems). Following previ- 225
 ous optimizations, all samples were tested undiluted, in a 226
 blinded manner. All analyte levels in the quality control 227
 reagents of the kits were within the expected ranges. Standard 228
 curve for sICAM-1 is 17–12,500 pg/ml, for L-selectin is 229
 100–73,000 pg/ml, and for TIMP-1 is 55–40,600 pg/ml. Meas- 230
 urements were done with Luminex100 instrument, and Lumi- 231
 nex 100 IS software was used for the analysis of bead median 232
 fluorescence intensity. The R&D Systems Rat Base kit assay 233
 was carried out according to the manufacturer's instructions, 234
 with a few exceptions as stipulated below. Briefly, an eight- 235
 point standard curve was generated by performing serial dilu- 236
 tions of the reconstituted normalized standard (lot # 1279612). 237
 This was done in order to ensure that the matrix used in the 238
 generation of the standard curve resembled that of the samples 239
 as closely as possible as preliminary test showed that this 240
 method was superior to dilution of standards in standard dilu- 241
 ent (data not shown). Bowel samples were homogenized with 242
 RPMI-1640 (GIBCO) containing 1 % protease inhibitor cock- 243
 tail. In order to assess recovery, bowel samples were used in 244
 20 mg/ml concentrations. The assays were run in duplicate, 245
 which produced in total of six concentration replicates. A 246
 50-µl volume of each sample, control, or standard was added 247
 to a 96-well plate (provided with the kit) containing 50 µl of 248
 antibody-coated fluorescent beads. Biotinylated secondary and 249
 streptavidin-PE antibodies were added to the plate with alter- 250
 nate incubation and washing steps. After the last wash step, 251
 100 µl of wash buffer was added to the wells; the plate was 252
 incubated and read on the Luminex100 array reader, using a 253
 four-PL regression curve to plot the standard curve. Data were 254
 subsequently analyzed using the Luminex100 manager 255
 software. 256

257 Statistics

258 Results are expressed as mean values ± SEM. Data were
 259 analyzed with one-way analysis of variance. The level of
 260 significance was set at $p < 0.05$. The MicroCal Origin (ver.
 261 6.0) program (Microcal Software Inc, Northampton, USA)
 262 was used for data evaluation.

263 Results

264 Radioimmunoassay

265 Level of intestinal PACAP-38-like immunoreactivity (LI) was
 266 55.1 ± 2.5 fmol/mg in sham-operated group and it was $57.32 \pm$
 267 3.5 fmol/mg in control samples. After 1 h cold storage, intestinal
 268 PACAP-38-LI was 50.4 ± 3.5 fmol/mg (GI), and after 3 h
 269 preservation it decreased to 40.1 ± 5.5 fmol/mg (GII). These
 270 changes were significant following 6 h cold storage (GIII,
 271 32.6 ± 3.0 fmol/mg; $p < 0.05$) compared to control or sham
 272 values. Levels remained significantly higher in grafts stored
 273 in PACAP-38-containing UW solution (GIV–GVI). In GIV,
 274 levels (65.2 ± 3.4 fmol/mg) increased above the control values,
 275 which was statistically significant. After 3 and 6 h cold storage
 276 in PACAP-38-containing preservation solution, the PACAP-
 277 38-LI levels were 55.6 ± 4.2 fmol/mg (GV) and $48.9 \pm$
 278 3.2 fmol/mg (GVI). These resulted significantly higher compared
 279 to preservation only in UW without PACAP-38 (vs. GII
 280 and GIII; $p < 0.05$) (Fig. 1).

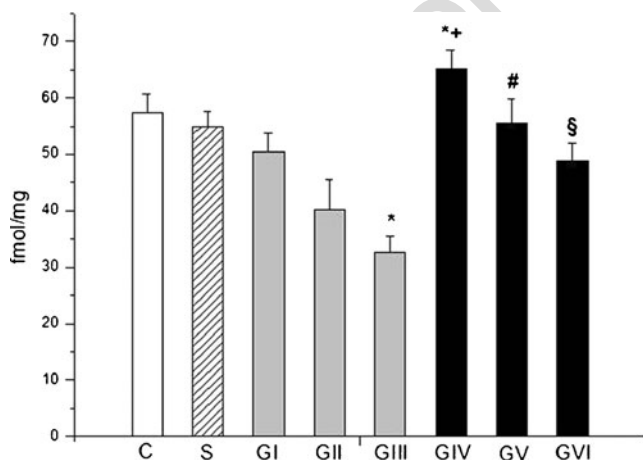


Fig. 1 Level of immunoreactive PACAP-38 in small bowel tissue after intestinal autotransplantation. Small intestinal grafts were perfused and preserved in cold UW solution for 1 h (GI), for 3 h (GII), and for 6 h (GIII) and in UW containing PACAP-38 for 1 h (GIV), for 3 h (GV), and for 6 h (GVI). In sham-operated group (S), animals underwent only median laparotomy. Small bowel biopsies were collected after laparotomy (control, C) and at the end of the reperfusion periods. Final value of PACAP-38 was given as femtomoles per milligram wet weight. Data are presented as mean ± SEM. * $p < 0.05$ vs. control; + $p < 0.05$ vs. GI; # $p < 0.05$ vs. GII; § $p < 0.05$ vs. GIII

Tissue PACAP-27 level also decreased during cold storage
 and autotransplantation procedure compared to the control
 value (4.2 ± 0.2 fmol/mg). This decrease was significant
 in the 1 h (GI, 2 ± 0.2 fmol/mg; $p < 0.05$), 3 h (GII, $1.6 \pm$
 0.3 fmol/mg; $p < 0.05$), and 6 h (GIII, 0.9 ± 0.2 fmol/mg;
 $p < 0.01$) groups. Levels of PACAP-27-LI remained significantly
 higher in grafts stored in PACAP-38-containing UW
 solution (GIV–GVI) at the end of the reperfusion periods. In
 GIV, its concentration was 3.5 ± 0.3 fmol/mg, which was
 significantly higher than in GI. Three and 6 h cold storage
 in PACAP-38-containing preservation solution resulted in
 elevated PACAP-27 levels in GV (3.0 ± 0.2 fmol/mg) and in
 GVI (2.6 ± 0.15 fmol/mg). These values were significantly
 higher compared to tissues preserved in UW without
 PACAP-38 (vs. GII and GIII; $p < 0.05$) (Fig. 2).

Cytokine Measurements

Among several cytokines, according to cytokine array, the
 expression of the sICAM-1 (CD54) (1) and L-selectin
 (CD62L/LECAM-1) (2) regulated upon activation was
 detectable in control bowel samples. The expression did
 not change after 6 h cold preservation in UW and subsequent
 reperfusion period in GIII. Both 6 h cold storage in
 PACAP-38-containing UW solution and 3 h reperfusion
 caused a strong reduction in the activation of these cyto-
 kines in GVI. The RANTES (CCL5) (3) levels were high in
 all groups and did not change, as could be observed in the

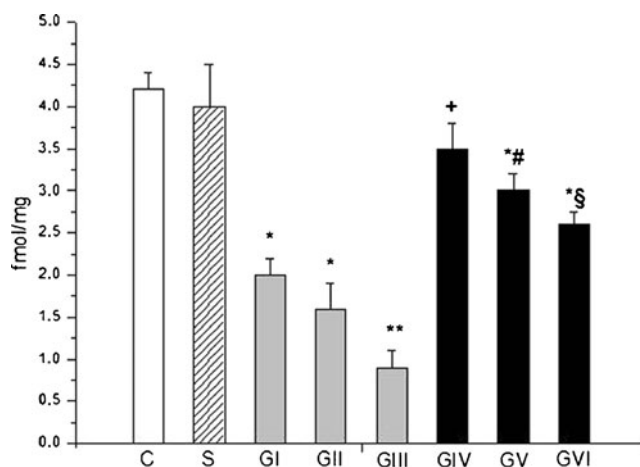


Fig. 2 Level of immunoreactive PACAP-27 in small bowel tissue during autotransplantation. Small intestinal grafts were perfused and preserved in cold UW solution for 1 h (GI), for 3 h (GII), and for 6 h (GIII) and in UW containing PACAP-38 for 1 h (GIV), for 3 h (GV), and for 6 h (GVI). In sham-operated group (S), animals underwent only median laparotomy. Small bowel biopsies were collected after laparotomy (control, C) and at the end of the reperfusion periods. Final value of PACAP-27 was given as femtomoles per milligram wet weight. Data are presented as mean ± SEM. * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control; + $p < 0.05$ vs. GI; # $p < 0.05$ vs. GII; § $p < 0.05$ vs. GIII

307 PACAP-treated groups. We found no activation of the tissue
 308 inhibitor of TIMP-1 (4) in the control samples, but strong
 309 activation was detected in 6 h preserved grafts without
 310 PACAP-38 (GIII). PACAP-38-containing cold storage
 311 could decrease its activation in GVI (Fig. 3).

312 Measurement of cytokines levels by Luminex Immuno-
 313 assay confirmed these results (Fig. 4a-c). sICAM and
 314 L-selectin were expressed at similar levels in the control
 315 (A in Fig. 4a, b) and ischemic groups (B in Fig. 4a, b), while
 316 both were significantly reduced in the PACAP-treated
 317 groups (C and D in Fig. 4a, b). TIMP, on the other hand,
 318 was expressed at detection limit in the control group
 319 (A in Fig. 4c), and it was markedly increased upon ischemia
 320 (B in Fig. 4c). The elevated TIMP levels were significantly
 321 attenuated by PACAP treatment (C and D in Fig. 4c).

322 **Discussion**

323 This study examined the intestinal levels of PACAP-38 and
 324 PACAP-27 and tissue cytokine expression using PACAP-

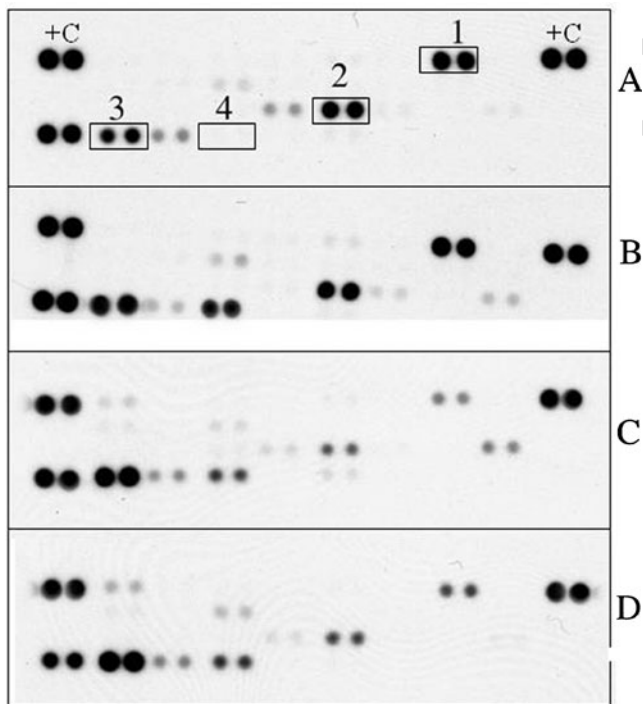


Fig. 3 Cytokine array showing the appearance on various cytokines in control intestine (a) and in small bowel tissue exposed 6 h cold storage in UW (b) or 6 h cold preservation in PACAP-38-containing UW solution (c) and subsequent 3 h reperfusion period (d). Marked changes are observed in sICAM-1 (1), L-selectin (2), and TIMP-1 (4) expressions. +C represents positive control. Other spots, where no changes were observed are (from upper left corner, without numbers): CINC-1, CINC-2alpha/beta, CINC-3, GM-CSF, IFN-gamma, IL-1alpha, IL-1beta, IL-1ra, IL-2, IL-3, IL-4, IL-6, IL-10, IL-13, IL-17, IP-10, LIX, MIG, MIP-1alpha, TNF-alpha, and VEGF

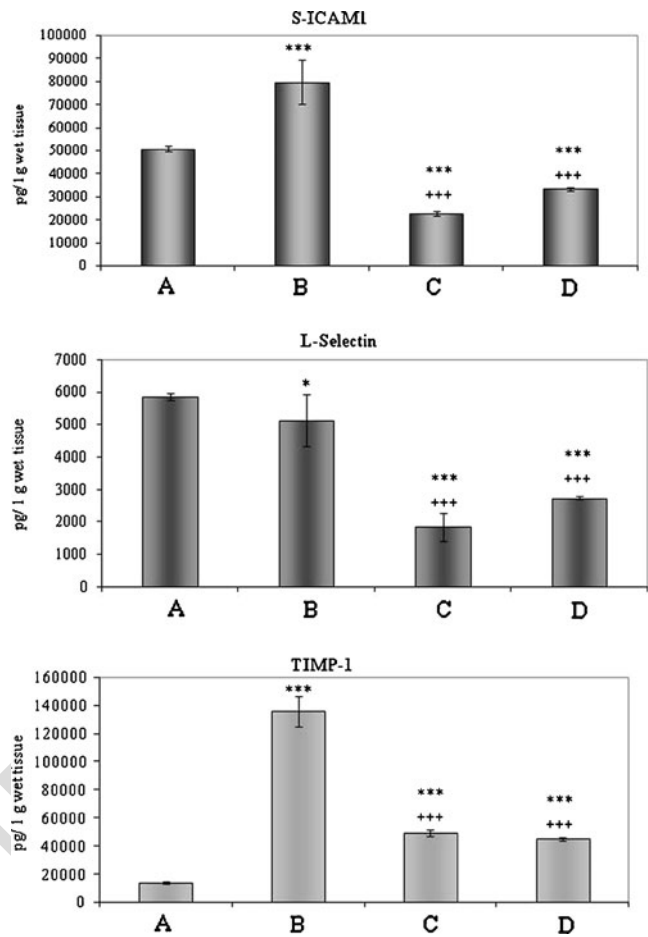


Fig. 4 Cytokine measurement by Luminex immunoassay showing the appearance on various cytokines in control intestine (A) and in small bowel tissue exposed to 6 h cold storage in UW (B) or 6 h cold preservation in PACAP-38-containing UW solution (C) and subsequent 3 h reperfusion period (D). Marked changes are observed in sICAM-1 (a), L-selectin (b), and TIMP-1 (c) expressions. Final values were given as picogram per gram wet tissue weight. Data are presented as mean \pm SEM. * p <0.05 vs. A, *** p <0.001 vs. A, +++ p <0.001 vs. B

38-containing UW preservation solution during small bowel autotransplantation. 325 326

327 Small bowel transplantation is increasingly performed in recent years, yet, clinically, there are still many obstacles to improve patient and graft survival. For most grafts, the preservation solution plays a fundamental role in minimizing the detrimental effects of ischemia during cold storage and subsequent reperfusion periods. The current clinical standard for small bowel consists of a vascular flush with cold UW solution. This solution has many advantages in the preservation of liver and kidney; however, for small bowel storage it still is unclear whether UW is optimal (Kokotilo et al. 2010; Roskott et al. 2011; Salehi et al. 2004). Several research efforts have been directed towards methods to protect against I/R injury, using modified composition of the commercially available solutions or adding new components appropriate for intestinal storage. 331 332 333 334 335 336 337 338 339 340 341

342 In the present study, we demonstrated that intestinal
 343 tissue PACAP-38 and PACAP-27 levels decreased in a
 344 time-dependent manner after 1 and 3 h cold preservation
 345 procedure. These changes were significant following 6 h
 346 cold storage. Our previous study showed similar tendency
 347 in the results of endogenous PACAP-38 concentration
 348 changes in warm I/R intestinal model (Ferencz et al.
 349 2009). The reason for the decreased PACAP-38 levels after
 350 cold ischemia may be due to either excessive uptake by
 351 ischemic cells or decreased synthesis/increased degradation
 352 paralleling tissue degeneration. Similar observations have
 353 been made by others in an experimental ulcer model, where
 354 an acute decrease in PACAP immunoreactivity was
 355 observed (Mei and Sundler 1998). Values remained signif-
 356 icantly higher in grafts stored in PACAP-38-containing UW
 357 solution. Interestingly, PACAP-38 levels increased above
 358 control values following 1 h preservation. Three and 6 h
 359 cold storage in PACAP-38-containing preservation solution
 360 resulted in significantly higher PACAP-38 and PACAP-27
 361 levels in bowel tissue homogenates compared to only in
 362 UW-preserved grafts without PACAP-38. There are no data
 363 indicating the exact mechanism of the elevated values at the
 364 end of the reperfusion periods. It could be due to the
 365 decrease in intracellular cyclic adenosine monophosphate
 366 (cAMP) through the reduction of adenylate cyclase activity
 367 induced by hypoxia in endothelial cells in vitro (Yan et al.
 368 1997). These changes were confirmed in an in vivo small
 369 intestine preservation study. Among these mechanisms, the
 370 cellular cAMP signal may represent a major determinant of
 371 the intestinal integrity after global ischemic preservation
 372 (Minor and Isselhard 1998). Studies confirmed that admin-
 373 istration of PACAP-38 enhancing the cAMP level exer-
 374 ted tissue protection against I/R injury (Riera et al.
 375 2001). Moreover, after extrinsic denervation, which is an
 376 indispensable procedure during intestinal transplantation,
 377 PACAP-38 concentration decreased in the stomach, but
 378 not in the small intestine. These findings suggested a dual
 379 intrinsic and extrinsic origin of the PACAP-containing nerve
 380 fibers in the small intestine (Hannibal et al. 1998). Another
 381 explanation of the present result is that PACAP-38 intake
 382 from the preservation solution and attached to the specific
 383 receptors could result in the anti-oxidant and protective
 384 effect to the bowel structure as described in our previous
 385 studies (Ferencz et al. 2009, 2010a, b).

386 I/R injury is one of the main factors affecting the function
 387 and structure of the small intestine, by generation of pro-
 388 inflammatory mediators including cytokines. The generated
 389 inflammatory cascade may activate leukocytes and endothe-
 390 lial cells, which ultimately lead to tissue inflammation,
 391 multiple organ dysfunction, and death. Following I/R in
 392 small bowel transplantation, the gut turns into a cytokine-
 393 producing organ, threatening graft and patient survival
 394 (Kostopanagiotou et al. 2011).

In the present study, we found that the expression of the
 sICAM-1 (CD54) and L-selectin (CD62L/LECAM-1) regu-
 lated upon activation was detectable in control bowel sam-
 ples, and those after 6 h cold preservation in UW and
 subsequent reperfusion period. In contrast, 6 h cold storage
 in PACAP-38-containing UW solution caused strong reduc-
 tion in the activation of these cytokines. Increased expres-
 sion of sICAM-1 and L-selectin was also observed after
 renal I/R, and it was decreased in PACAP-treated groups
 in renal model (Horvath et al. 2010b). In fact, these adhesion
 molecules, involved in the distinct cellular crosstalk
 between leukocytes, platelets, T cells, and endothelial cells,
 can cause microvascular dysfunction and reperfusion dam-
 age (Vollmar and Menger 2011). The RANTES (CCL5)
 chemokine is not constitutively expressed; it is released
 during inflammation. In our model, the RANTES (CCL5)
 levels were increased in all groups, but slight reduction
 was observed in PACAP-treated groups. During inflammatory
 events, the transcription of matrix metalloproteinase-9 and
 its endogenous inhibitor TIMP-1 is induced by pro-
 inflammatory mediators. In our experiment, TIMP-1
 showed a strong activation in 6 h preserved grafts without
 PACAP-38. PACAP-38-containing cold storage could
 decrease its activation. The anti-inflammatory actions of
 PACAP in several inflammatory models are partially medi-
 ated through its suppressing effect on cytokine/chemokine
 production (Delgado and Ganea 2001; Horvath et al.
 2010b). In summary, our present results support the protec-
 tive role of PACAP-38 in cold UW solution-stored and
 autotransplanted small intestine, which may have clinical
 relevance in bowel transplantation in the future.

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