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# Melatonin reduces changes to small intestinal microvasculature during systemic inflammation

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ABSTRACT

*Background:* Systemic inflammation is known to impair the microcirculation in intestine and other organs as a result of multifactorial events. Here, we show that melatonin selectively reduces changes to the small intestinal microvasculature during systemic inflammation.

*Materials and methods:* Lipopolysaccharide (LPS) was infused at a rate of 0.5 mg/kg × h to induce systemic inflammation in male Wistar rats. Melatonin (single dose: 3 mg/kg × 15 min) was intravenously administered before as well as 120 and 240 min after the beginning of the LPS infusion. Systemic parameters were determined in regular intervals. Small intestine, liver, and kidney were histologically (structure of the microvessels, intravascular blood accumulation, and hemorrhages) and immunohistochemically (mast cells, granulocytes, and macrophages) analyzed.

*Results:* Continuous infusion of LPS resulted in dilated microvessels with intravascular blood accumulation (congestion) in liver and small intestine, the latter being particularly pronounced. Blood vessel walls remained intact, there were no hemorrhages. Melatonin significantly reduced these changes to the microvasculature in small intestine, but not in liver. It further reduced mast cell and granulocytes count in small intestine enhanced by LPS. However, except for the systemic blood pressure, melatonin neither improved LPS-dependent changes to systemic parameters nor mortality.

*Conclusions:* Changes to the microvasculature during systemic inflammation are most pronounced in small intestine. Melatonin selectively diminishes these changes to small intestinal microvasculature, probably by reducing the local immune cells recruitment. However, changes to the small intestine are not decisive for the survival. We assume that the therapeutic benefit of melatonin is more likely in local intestinal inflammation.



## Introduction

The intestine plays an important role in situations of critical illness (e.g., trauma, burn, hemorrhage, ischemia–reperfusion, or systemic inflammation), being crucial in the progression of multiple organ failure and, in turn, the outcome of patients.[1-7] Acute injury such as systemic inflammation is known to impair the microcirculation in intestine and other organs as a result of multifactorial events, like changes in systemic blood pressure, increased (micro)

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vascular permeability, decreased erythrocytes deformability, increased immune cell activation, and adherence and development of disseminated intravascular coagulation.[8,9]

The circadian hormone melatonin (MLT) is primarily synthesized and secreted by the pineal gland depending on the daylight–darkness cycle with a peak in the night even in nocturnal animals.[10] But, it is also produced in enteroendocrine cells of the gastrointestinal tract and other extrapineal tissues and cells (e.g., retina, skin, testis, ovaries, bone marrow, leukocytes, and platelets). The extent and rhythm of MLT production in extrapineal tissue occur independently of the daylight–darkness cycle and vary from those in the pineal gland.[11-21] Moreover, the receptor-dependent actions of MLT are mainly mediated by the G-protein–coupled receptors MT1 and MT2.[22,23] These receptors are also expressed in the intestine, and their participation in regulation of (small) intestinal motility is supposed.[24-26] Up to now, protective effects of exogenous MLT on intestinal changes in experimental models of intestinal ischemia–reperfusion (after superior mesenteric artery occlusion = SMAO model)[27-30] or systemic inflammation (after lipopolysaccharide administration = LPS model; and after cecal ligation and puncture = CLP model)[31,32] have primarily been attributed to its anti-oxidative efficacy and intestinal motility-enhancing action. Furthermore, a protective anti-inflammatory role of MLT in different experimental and clinical settings of diseases of the gastrointestinal tract has already been shown.[33-35]

In a preliminary study with male Wistar rats, we already observed that mild systemic inflammation in cause of low-dosed intravenous infusion of LPS led to considerable changes especially to the microvasculature in jejunum and ileum. Here, we could show that the administration of MLT selectively reduces these LPS-related changes to small intestinal microvasculature.

## Materials and methods

### *Chemicals and materials*

LPS (from *Escherichia coli*, serotype 0111:B4), MLT, Tween 20, Naphthol-AS-D-Chloroacetate (Specific Esterase) Kit, and Paraffin wax (Paraplast) were purchased from Sigma–Aldrich (St. Louis, MO), ethanol ≥99.5%, Roti Histokitt II, $H_2O_2$, and formalin solution from Carl Roth (Karlsruhe, Germany), 3′,3′-diaminobenzidine, hematoxylin and eosin from Thermofisher Scientific (Waltham), mouse anti-CD69 antibody and horse anti-mouse IgG antibody from BioLogo (Kronshagen, Germany), object slides superfrost plus from R. Langenbrinck (Emmerdingen, Germany), Portex catheters (0.58 mm i.d./0.96 mm once daily) from Smith Medical International (Grasbrunn, Germany), and 2.0 mL self-filling arterial samplers containing 80 IU electrolyte-balanced heparin (PICO50) from Radiometer Medical (Brønshøj, Denmark). Isoflurane was obtained from AbbVie Deutschland (Ludwigshafen, Germany), ketamine 10% from Ceva (Düsseldorf, Germany), lidocaine (xylocain 1%) from AstraZeneca (Wedel, Germany), 0.9% sodium chloride solution from B. Braun (Melsungen, Germany), medical oxygen from Air Liquide (Düsseldorf), Ringer's solution from Fresenius Kabi (Bad Homburg, Germany), immune peroxidase detection ABC kit from Vector Laboratories (Burlington), protein block from DAKO (Glostrup, Denmark), and toluidine blue from AppliChem (Darmstadt, Germany).

### *Animals*

Male Wistar WU rats (435 ± 12 g) were obtained from the central animal unit of the University Hospital Essen. Rats were kept under standardized conditions of temperature (22 ± 1°C), humidity (55 ± 5%), and 12-h/12-h light–dark cycles (06:00 AM light on/06:00 PM light off, standard time) with free access to food (ssniff-Spezialdiäten, Soest, Germany) and water. All rats received humane care according to the standards of the Federation of European Laboratory Animal Science Association. The experimental protocol has been reviewed and approved by the local Animal Care and Use Committee with a Permit Number Az.: 84-02.04.2012.A205.

### *Anesthesia, analgesia, and surgical procedure*

Rats were anesthetized with isoflurane and received ketamine and lidocaine for analgesia as described before.[36] Afterward, catheters were inserted in femoral artery and vein and perfused with Ringer's solution (arterial) or 0.9% sodium chloride solution (venous) to maintain the functionality. The organ harvesting took place under deep isoflurane anesthesia within the experimental time immediately after death or at the end of the experiment (T = 360 min).

### *Study groups*

Systemic inflammation was induced by an intravenous infusion of LPS (0.5 mg/kg × h) using a syringe pump (Fresenius

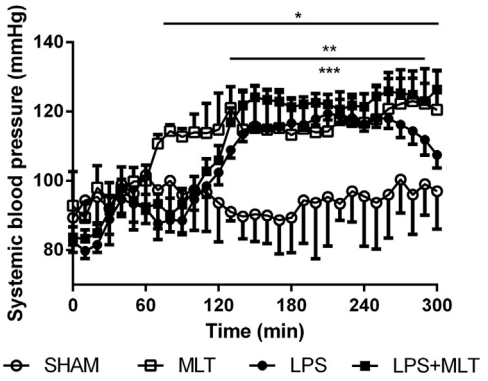


**Fig. 1 – Effect of MLT on systemic blood pressure during systemic inflammation. Systemic blood pressure of the groups SHAM ($n = 4$), MLT ($n = 4$), LPS (A: $n = 8$, B: $n = 8$) and LPS + MLT ($n = 8$) are shown (mean values ± SEM) until T = 300 min, what was the last time point before the first rat of the groups to be compared died. Systemic inflammation was induced by an intravenous infusion of LPS (0.5 mg LPS/kg × h over 360 min). MLT (3 mg MLT/kg × 15 min) was infused 15 min before and 120 min and 240 min after starting the LPS infusion. *P < 0.0001 for MLT *versus* SHAM, **P < 0.0001 for LPS *versus* SHAM, ***P < 0.0001 for LPS + MLT *versus* LPS.**