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Altered expression profiles of clock genes *hPer1* and *hPer2* in peripheral blood mononuclear cells of cancer patients undergoing surgery

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Abstract

Patients undergoing surgery often develop symptoms of circadian rhythm disorders such as insomnia or delirium. However, the effect of surgery on the biological clock remains unknown. The present study examines the expression of clock genes in peripheral blood mononuclear cells (PBMCs) and measures plasma hormone concentrations in patients with esophageal cancer and early gastric cancer who underwent surgery. Six blood samples per day were collected from 9 patients with esophageal cancer before and after esophagectomy and from 9 patients with early gastric cancer before and after laparoscopy-assisted distal gastrectomy (LADG). The expression profiles of *hPer1* and *hPer2* mRNAs in PBMCs were determined by real-time RT-PCR. Plasma melatonin and cortisol concentrations were measured by radioimmunoassay. Plasma melatonin levels decreased in both groups throughout the day and plasma cortisol levels changed after surgery. The acrophase of clock gene expression was altered after surgery as follows: *hPer1*, from 6:19 ± 1:50 to 13:59 ± 0:59 ($p = 0.0003$) and from 7:47 ± 1:27 to 12:33 ± 1:30 ($p = 0.0043$) and *hPer2*, from 5:01 ± 2:59 to 19:30 ± 2:15 ($p < 0.0001$) and from 6:49 ± 1:59 to 13:39 ± 3:06 ($p = 0.0171$) in patients with esophageal and early gastric cancer, respectively. The post-operative phase change of *hPer2* was more prominent after esophagectomy than after LADG. Our results suggest that surgical stress affects the peripheral clock as well as endogenous hormones in humans.

Keywords: Circadian rhythm; Biological clock; Surgical stress; Clock genes; Surgery; *hPer1*; *hPer2*

Introduction

Circadian rhythm is seen in various behavioral, physiological and biochemical processes in living organisms from cyanobacteria to mammals. These include the sleep–wake cycle, temperature, heart rate, hormone secretion and enzyme activity.

Abbreviations: LADG, Laparoscopy-assisted distal gastrectomy; PBGD, Porphobilinogen deaminase; PBMCs, Peripheral blood mononuclear cells; SCN, Suprachiasmatic nucleus.

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The biological clock that controls circadian rhythms in mammals is located in the suprachiasmatic nucleus (SCN) of the hypothalamus (Inouye and Shibata, 1994). The biological clock does not only generate circadian rhythmicity but also maintains homeostasis by resetting the phases of biological clocks in response to environmental changes.

Patients who undergo surgery are post-operatively exposed to remarkable external and internal environmental changes that require considerable adjustment to maintain homeostasis. These include recovery rooms without adequate natural light, limited movement, artificial ventilation, continuous infusion, blood loss during surgery, pain, activation of neuroendocrine and cytokine systems and post-operative complications. Several recent studies have suggested that some human diseases are associated with circadian rhythm disorders (Toh et al., 2001; Tomoda et al., 2003). Post-surgical patients also develop symptoms suggesting circadian rhythm disorders, such as insomnia or delirium. The circadian rhythm of plasma melatonin levels is abolished during the immediate post-operative period (Cronin et al., 2001; Guo et al., 2002). The loss of the melatonin rhythm seems to be a critical causative factor of post-operative sleep disturbances (Miyazaki et al., 2003), because melatonin plays an important role in regulation of the sleep–wake cycle. Although post-operative hormonal rhythms have been thoroughly studied, the status of clock gene expression in post-operative patients has not been investigated.

Recent progress in circadian rhythm physiology at the molecular level has revealed that transcriptional feedback loops of clock genes are essential for rhythm generation in both mammals and flies.

The positive regulators in mammals are *Clock* and *BMAL1*, whereas *Per1*, *Per2*, *Per3*, *Cry1* and *Cry2* are involved in negative feedback in the circadian clock (Dunlap, 1999). Circadian clock genes are expressed in most peripheral tissues as well as in the SCN (Sakamoto et al., 1998). Our previous studies showed that *Per2* mRNA is expressed in a circadian manner in rat peripheral blood mononuclear cells (PBMCs) (Oishi et al., 1998), suggesting that clock gene expression profiles in PBMCs could be a useful marker for circadian rhythm in humans. Several clock genes are also expressed in the oral mucosa and skin of humans (Bjarnason et al., 2001). As in rodents, *hPer1*, *hPer2*, *hPer3* and *hDec1* are expressed in a circadian manner in human PBMCs under controlled conditions (Boivin et al., 2003).

Esophagectomy is one of the most invasive types of surgery (Sakamoto et al., 1994; Tashiro et al., 1999) and sleep disorders and delirium often develop after curative procedures (Miyazaki et al., 2003). On the other hand, laparoscopy-assisted distal gastrectomy (LADG) is less invasive and patients usually recover without complications (Adachi et al., 1999, 2000; Kitano et al., 2002). We postulated that surgical stress generally affects the circadian expression of clock genes in humans. We test this hypothesis in the present study by measuring the circadian expression profiles of *hPer1* and *hPer2* mRNA in PBMCs together with plasma melatonin and cortisol levels in perioperative patients with esophageal cancer and with early gastric cancer.

Patients and methods

The Human Ethics Review Committee of Osaka University approved the study protocol and all patients provided written, informed consent to participate in all procedures associated with the study.

Patients

Nine patients with esophageal cancer and nine with early gastric cancer participated in this study. Exclusion criteria were exogenous hormone therapy and known endocrine, renal, hepatic, or central nervous system dysfunction. Surgically resected specimens, including the primary tumor and the lymph nodes, were histopathologically evaluated based on the TNM/UICC classification (Sobin and Wittekind, 2002).

Anesthesia and surgery

All patients received 0.5 mg of atropine sulfate and 1.0–2.5 mg of midazolam intramuscularly 1 h before surgery and an epidural catheter was inserted to control pain. General anesthesia was induced in patients with esophageal cancer using propofol and then maintained with propofol and fentanyl. Esophagectomy with lymphadenectomy proceeded through right thoracotomy and laparotomy. After surgery, all patients remained intubated and were transported to the intensive care unit (ICU), where artificial ventilation was maintained. During this period, the patients were sedated *via* propofol infusion (50 to 150 mg/h). All patients were extubated on post-operative day 1 upon achieving stable respiratory status. Vecuronium was administered to patients with early gastric cancer and then general anesthesia was induced *via* thiamylal and tracheal intubation. Anesthesia was maintained with sevoflurane, nitrous oxide and fentanyl. These patients underwent LADG after pneumoperitoneum with carbon dioxide. All of the patients with early gastric cancer were extubated in the operation room and then returned to the recovery room in the surgical ward.

Post-operative management

Pain was controlled with infused epidural morphine (0.2 mg/h). Fluorescent lighting was switched on between 07:00 and 22:00 h, and most of the nighttime lighting was reserved for the nursing areas.

Sampling

Blood samples were collected at 20:00, 0:00, 04:00, 08:00, 12:00 and 16:00 h through an intravenous catheter a few days before and immediately after surgery. The procedures for all patients with esophageal cancer started at about 10:00 h and ended at between 17:30 to 20:00 h ($n=6$), between 20:00 to 21:00 h ($n=2$) and at about 23:00 h ($n=1$). Surgery for all patients with early gastric cancer started at about 09:30 h and finished at about 13:00 h. Blood was sampled from both groups of patients from 20:00 h on the day of surgery.