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Research Article

J147 Improves Early Recovery after Surgery in Zucker Rats

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Abstract

Older and otherwise vulnerable patients display increased risk for long-term complications after surgery. Slow recovery from surgery may have predictive value for long-term complications. The Zucker rat strain, expressing risk factors for post-surgical complications in patients, could provide a clinically relevant model to study pathophysiology and potential interventions. The anti-dementia drug J147 was shown to improve postoperative recovery in young healthy rats. Aim of the present study was to investigate early postoperative recovery in Zucker rats and effects of J147 treatment. Zucker rats (25-30 weeks of age) were subjected to major abdominal surgery. Non-surgery rats served as control. Surgery rats were randomized into three groups: surgery control, surgery + acute J147 and surgery + chronic J147. While acute J147 treatment was provided by a single intravenous injection at the start of surgery, chronic J147 treatment was provided in the food, from one week before surgery until the end of the protocol. After surgery, rats were placed in individual cages, equipped with an activity tracker, and followed until 6 days after surgery. Body weight loss and time to first activity were obtained as primary outcome parameters for recovery. At 1,6 and 24 hours after surgery, blood samples were collected for analyses of inflammation markers. Surgery induced persistent body weight loss and lower food intake, lower overall activity and higher diurnality (more day active) over the first 5 days after surgery. Whereas body weight loss was not affected, reduced food intake seemed exaggerated by J147. Neither acute nor chronic J147 treatment significantly affected total activity. However, acute, but not chronic J147, reduced the time to first activity after surgery. Accordingly, acute J147 treated rats showed a tendency towards improved diurnality. No significant effects of J147 were observed on Il-1beta responses after surgery, but 24 hours plasma NGAL levels had further increased in acute as well as chronically J147 treated rats.

In conclusion Surgery impacted the Zucker rats by weight loss, reduced food intake, overall lower activity and higher diurnality. Whereas chronic J147 treatment did not affect surgery-induced effects, acute J147 treatment seemed to accelerate recovery by reduction of time to first activity. This effect however could not be attributed to anti-inflammatory actions of acute J147.

Introduction

Anaesthetic and surgical techniques have been improved in the last decades, resulting in more surgical interventions in older or otherwise vulnerable patients. However, these patients also may be prone to develop complications after surgery. Delayed or incomplete recovery may result in more persistent postoperative complications, such as cardiac and pulmonary problems, cognitive decline and psychological problems [1-4], and can lead to prolonged hospital stay and subsequently delayed social activities, reduced quality of life, increased disability, dependency on social services and even death [5-9]. A decade ago, we developed a rat model that closely mimicked the patient's surgical process, to study effects of surgery [10] and potential therapeutic approaches [11-13]. We showed that during the first week after surgery, neuroinflammation was indicated by increased levels of Il-1beta as well as microglia activation in the hippocampus and prefrontal cortex, with concomitant declined hippocampal neurogenesis [10]. Moreover, also weight loss and plasma IL-6 levels after surgery appeared an important predictive factor for postoperative outcome [10,14]. Clinically, the time to regain activity after surgery is mentioned as the most important determinant of postoperative outcome [15-19]. Additionally, a disturbed circadian rhythm has been proposed to determine delayed recovery [20], as circadian rhythm after surgery plays a crucial role in regulating sleep-wake cycles, and hence in surgery-associated sleep disorders [21-23]. Furthermore, circadian rhythm has been shown to regulate the central nervous system and immune cell activity and function and plays a role in cognitive (dys) function, including memory and attention [24,25]. Therefore, a disturbed circadian rhythm after surgery can increase the risk to develop long-term postoperative complications, such as cardiovascular events, immune dysfunction, pain sensitivity, and cognitive dysfunction [26-30]. In addition, recovery of physical activity after surgery is also an important indicator for long-term complications. The faster patients are physical active after surgery, the lower the chance of developing long-term complications [31,15-19]. Moreover, mobilization out of bed within 2 hours after abdominal surgery improved participants' respiratory function [17].

Several risk factors, including pre-operative health, age, types of medication, severity of the surgical procedure and inflammatory responses, can contribute to a delayed recovery after surgery and may increase the risk of long-term postoperative complications [32-34]. These studies showed that clinically defined risk factors for cognitive decline after surgery, such as presurgical conditions and age, were associated with more exaggerated neuroinflammation and more wide-spread cognitive decline [14,33]. Pre-operative health conditions, including hyperglycemia, hypertension, liver disease and renal dysfunction are also identified as major risk factors for delayed recovery and thereby worse outcome after surgery [34]. Rather than the commonly used healthy Wistar rats, in this regard the Zucker rat may provide a clinically relevant increased-risk model, as it combines several risk factors for post-surgical outcome, including hyperglycemia, obesity, hypertension and kidney dysfunction in one rat strain. Moreover, these metabolic syndrome-like rats display low-grade inflammation, neuroinflammation and cognitive dysfunction at a relatively young age (±6 months) [35]. As these factors may have predictive value for impaired recovery and long-term cognitive decline in patients, these Zucker rats may better represent the vulnerable and older patient population undergoing surgery.

J147, originally developed as potential anti-Alzheimer drug has been shown to have general anti-aging [36], anti-dementia [37,38] and anti-diabetic properties [39]. In addition, it is neuroprotective in the most common age-associated neuropathy models [36-41]. More specifically, J147 is effective in reducing inflammation and nerve cell death due to the loss of trophic support, preventing oxidative stress, improving dendritic structure, and cognitive performance [37-39,41]. Moreover, J147 can promote cell survival and reduces changes associated with aging [36,37,42,43] and was shown to exerts cerebral cytoprotective effects in an acute ischemic stroke model [44]. Currently, its clinical effectiveness is investigated in Phase I clinical trials (NCT03838185), expected to be finished soon. In our previous study we have shown that J147 could prevent cognitive impairment after the first week following abdominal surgery in young healthy Wistar rats [12]. However, this effect was not attributable to anti-inflammatory properties. Hence, since J147 combines its anti-inflammatory effects with other neuroprotective properties, it may provide a promising candidate for post-surgical therapeutic intervention. Since we hypothesize that early post-surgical recovery may predict later complications, aim of the present study was to investigate early postoperative recovery in Zucker rats and the effects of J147 treatment, and use postoperative weight loss as seen in our preclinical model, and time to regain activity as indicator in patients, as major outcome parameters.

Material and Methods

Animals and Housing

A total of 69, outbred, 12-weeks old obese male Zucker rats (Crl:ZUC(Orl)-lepri) were obtained from Charles River Laboratories (Wilmington, Massachusetts, USA), and aged in our facility up to 25-30 weeks. Although this age may not optimally reflect the factor age in the development of POCD, mortality in this rat strain increases from this age on [45], which may induce bias towards the most-healthy rats. Until the start of the study, rats were group-housed with 2 or 3 rats per cage on reversed light: dark cycle (12:12) with the lights out at 09:00 am, under controlled climate conditions (temperature of 20+/- 2 degrees and humidity of 50%

+/- 10%). The rats had food (Teklad 2018, Envigo) and water ad libitum. After surgery, rats were housed individually. Approval of all experiments was obtained from the local animal committee of the University of Groningen and the national animal and welfare committee of the Netherlands. An overview of the experimental protocol is given in Figure 1. The present study is part of a larger study on postoperative cognitive dysfunction that will be published separately. Rats were randomly divided over the 4 experimental groups, including: 1. non-surgery; 2. control surgery, 3. surgery + acute J147 and 4. Surgery + chronic J147. Acute J147 treatment was provided by a single injection at the start of surgery, whereas chronic J147 treatment was provided in the food, started one week before surgery and continued until the end of the protocol. A single injection just before the surgery was chosen for the acute J147 treatment to reduce inflammation and activate potential other mechanisms of action during the surgery, but not during the recovery period after surgery. Chronic J147 treatment timing was chosen to create a better physiological state before surgery, with milder inhibition of inflammation and activation of potential other mechanisms during the recovery period after surgery. Half of the control surgery group received a vehicle injection and served as control for acute treatment, while the other half continued on regular food and served as control for chronic treatment. Rats were subjected to abdominal surgery [10], including implantation of a permanent jugular vein catheter, and allowed to wake up. After that rats were housed individually in cages equipped with a locomotion sensor (Circadian Activity Monitor System, CAMS, by H.M. Cooper, J.A. Cooper, INSERM U846, Department of Chronobiology, France). Timed blood samples were collected from the jugular vein catheter. After surgery, rats were weighed daily and food intake was measured, in the first hour of the dark period. Non-surgery control rats were transported to the surgery room, left there for about an hour (approximately the duration of surgery), then placed in individual cages before returning to the housing facility together with the surgery animals.

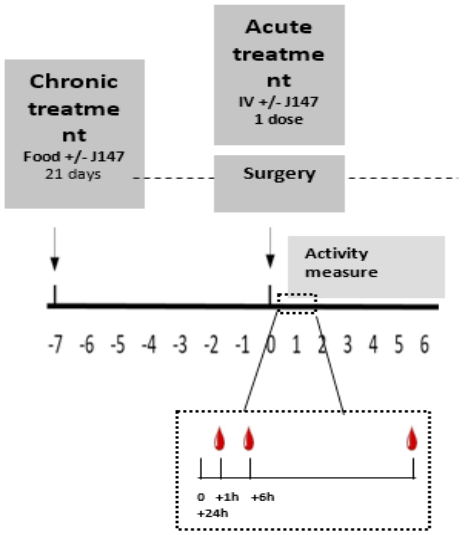

Figure 1: Outline of study: Male Zucker rats (n=69) were randomly divided into four experimental groups; non-surgery (n=14), control surgery (n=16), surgery + acute J147 (intravenous injection, 10mg/ml J147, single dose, n=16) and surgery + chronic J147 (food pallets estimating 30 mg/kg/day, n=16). After surgery, timed blood samples were collected. Postoperative day 0-6, activity measurements were taken.

Acute J147 Treatment

The day of surgery, rats were quickly cannulated in their jugular vein. Acutely treated rats and their vehicle controls received their J147/vehicle by intravenous injection. For acute treatment, a fresh J147 (Abrexa Pharmaceuticals, San Diego USA) solution was prepared each day. For that, crystalline J147 was dissolved in 15% solutol/85% saline (10mg/ml J147) and kept at 37˚C until administration (10mg/kg). Vehicle treated rats received 1ml/kg solvent (15% solutol/85% saline), at 37˚C. Extra blood samples (200 µl) were collected from 3 acutely treated rats at t= 0,1,2,4,6,8,12 and 24 hours after injection, and sent to QPS for measurement of plasma J147 levels.

Chronic J147 Treatment

Chronic J147 treatment started one week before surgery and continued until the end of the experiment. For chronic oral treatment, J147 was added to the food (Teklad 2018, Envigo) at a concentration of 833 mg/kg, aiming at approximately 30 mg/kg/day J147, based on an estimated food consumption of 21 grams/day and body weight of 750g (Zheng et al., 2009). J147 was provided by Abrexa Pharmaceuticals, and Envigo prepared the food. J147 food was stored at -20□C (dark), until use. Control rats received Teklad 2018 food (Envigo). From 3 chronically treated rats, an extra blood sample was collected at the time of surgery to measure steady state plasma levels of J147.

Surgery

Abdominal surgery was performed as described previously [10]. Briefly, rats were anaesthetized using sevoflurane (±2.5% in air/O_2= 2/1) and receive 0.01 mg/kg buprenorphine such as analgesia. A heating blanket was used to preserve body temperature. The animals were equipped with a permanent indwelling jugular vein catheter to mimic insertion of a venous line in patients and allow timed blood sampling [10]. For that, an incision was made at the subclavical region, the jugular vein is freed from surrounding tissue and a catheter is introduced and advanced into the vena cava, just above the right atrium. At this moment acute J147 or vehicle was injected intravenously. The other end of the catheter was guided subcutaneously to the head, filled with PVP dissolved in heparin solution and closed with a plastic plug, and fixated on the skull with dental cement. For the major abdominal surgery, an incision was made along the linea alba and the intestines are exteriorized. The upper mesenteric artery was isolated from surrounding tissue and clamped for a period of 30 minutes during which the intestines and surgical wound were covered with gauze soaked in saline, to prevent dehydration. After removal of the mesenteric artery clamp, the intestines were gently placed back and the abdominal wall and skin were closed separately by sutures. Clamping and reperfusion were visually verified by absence or presence of pulsation in the mesenteric artery distal to the clamp site. From induction to recovery, the procedure took approximately 1 hour. Postoperatively, the animals were allowed to wake up and subsequently were housed individually. Rats were weighed daily. From a clinically point of view, regaining activity was taken as most important factor for recovery from surgery. In addition, from a pre-clinical view point, maximal weight loss within the first 6 days post-surgery was taken as measure of experienced severity of surgery.

Blood Samples

In addition to blood samples collected for measurement of J147 levels, from all surgery rats blood samples were collected from the jugular vein catheter, at 1, 6 and 24h after surgery (200µL). Blood samples were centrifuged for 10 minutes at 2600G, and plasma was collected and stored at -80°C until further analysis.

Plasma Levels of J147

To measure levels of J147, blood samples (200µL) were collected into K_3EDTA coated tubes (Sarstedt). The tubes were inverted several times to facilitate contact with EDTA and subsequently placed on ice until centrifuged. Samples were centrifuged within 30 minutes after collection (4°C, 10 minutes, 2600g) to obtain plasma. Collected plasma was stored at -80°C, until further analysis. At all times vials were wrapped in aluminium foil with reference to light sensitivity of J147. Samples were transported to QPS (Groningen, the Netherlands) on dry ice for measuring plasma J147 concentrations.

Plasma Levels of Inflammatory Markers

Plasma levels of inflammatory markers (Il-1beta, IL6, Il10, TNF-alpha and VEGF) were measured by multiplex (Bio-Techne, Luminex). However, since it seemed not possible to obtain relevant results with this multiplex, only IL-1beta and Neutrophil Gelatinase-Associated Lipocalin (NGAL) levels, were measured by ELISA, as both were shown to be associated with postoperative complications [45,46]. Both plasma IL-1beta (Thermo Fisher Scientific, Invitrogen, US) and NGAL (Bioporto, Denmark) were measured by rat specific ELISA kits, according to manufacturer's instructions.

Activity Data

Within an hour after surgery, rats were placed into individual cages and locomotion sensors were placed above the cages. The sensors remained there until the end of the experiment. From the collected locomotion data, actograms were made, which were further analysed regarding aspects of rhythmicity. Data from the first 5 days after surgery were analysed to assess recovery. Time between the end of the surgery and first activity was considered as measure for regaining activity. Total activity was calculated per 24 hours (12 hours light and 12 hours dark period) for postoperative day 1 to 5 to assess the recovery of total activity. Diurnality was calculated by subtracting the activity during the dark period from the activity during the light period, divided by total activity during 24 hours. Diurnality assesses the shift in activity in the active (dark) period to the light (rest) period; lower values refer to more light activity for the nocturnal rats. All parameters were analysed using ACTOVIEW for Excel 2010, programmed by C. Mulder, University of Groningen (Mulder et al, 2014).

Data Analyses

Effects of vehicle treatment were analysed by comparing the vehicle treated and control food surgery groups by an independent T-test. Since no significant effects of vehicle injection

were observed, rats with vehicle injection and rats on control food were pooled to one control surgery group. This led to 4 experimental groups: non-surgery, control surgery, surgery + acute J147 and surgery + chronic J147. Data are presented as mean ± SEM. Statistical analysis was performed using SPSS (IBM SPSS Statistics, Version 27, and Armonk, NY). Data that exceeded mean ± twice standard deviation of its group were regarded as outliers, and excluded from analyses of comparing means. To assess the effect of surgery, non-surgery and control surgery groups were compared using an independent T-test. Thereafter, effects of J147 were assessed by comparing group averages of the surgery groups by one way ANOVA and if significant effects were present, one-way ANOVA and post hoc Dunnet's analysis, with the control surgery group as control, was used to compare the surgery groups. Time courses of bodyweight and food intake, IL-beta and NGAL curves were analysed using repeated measurements General Linear Model (GLM). Differences were regarded statistically significant when $p \leq 0.05$. Relevant trends are indicated when $p<0.10$.

Results

General

The study was performed as planned. During aging from 12 to 25-30 weeks, 5 rats died spontaneously. All rats that underwent surgery survived; 1 rat had to be sacrificed shortly after surgery, because of internal bleeding; 2 rats died during the study, without any visible cause. In total 61 rats were included in the study, divided over: 1. non-surgery (n=14); 2. Control surgery (n=15), 3. Surgery + acute J147 (n=16) and 4. Surgery + chronic J147 (n=16).

J147 Plasma Levels

Plasma levels of J147 in samples obtained during and after surgery in acutely treated rats are presented in Figure 2. Plasma levels reached almost 450ng/ml at 1 hour after injection, and declined to 50 ng/ml at 24 hours. The level of J147 in food was determined to be 740mg/kg, or 89% of the targeted 833mg/kg. Food intake of Zucker rats was approximately 20g/24h before surgery, as estimated from intake per cage of group-housed rats (≈750 g). This resulted in a calculated steady state dose of approximately 19.7mg/kg/day before surgery. Steady state plasma level of J147 in these chronically treated rats was 11.9±5.1 ng/ml at the time of surgery (n=3). Plasma levels appeared well within the therapeutic range of 3-70 ng/ml, which translates to 10-200 nM [47] (Figure 2).

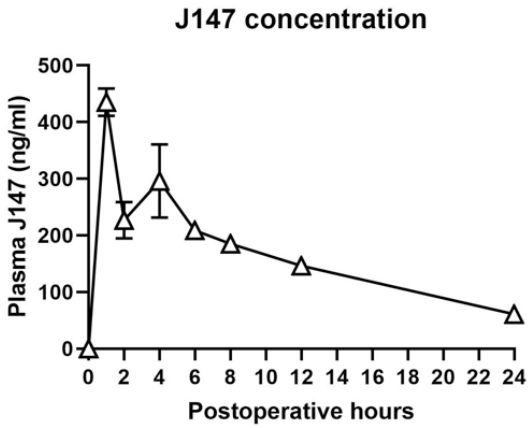

Figure 2: J147 concentrations in plasma at 1,2,4,6,8,12 and 24 hours post-surgery in acutely (intravenous bolus injection) treated rats (mean ± SEM, n=3-4).

Body Weight and Food Intake

At the time of surgery, rats weighed approximately 750g. All groups undergoing surgery significantly lost weight, and were not able to regain their weight within the first week after surgery (Figure 3A). On postoperative day 3, 4 and 5, non-surgery rats weighted significantly more than the control surgery rats (Figure 3A). No significant effects of J147 treatments were observed on the body weight loss during the first 5 days after surgery (Figure 3A and 3B). Rats that underwent surgery ate less than 5 grams the first day after surgery, and after 5 days had not returned to their pre-surgical food intake (Figure 3C). Overall, surgery rats had a significantly lower food intake compared to non-surgery rats at day 1 to 5 (Figure 3C and 3D), and compared to their average food intake before surgery in group housing. J147 treated rats had slight but significantly lower overall food intake compared to the surgery control rats FFigure 3D). Nevertheless, all surgery rats showed similar slopes in retaining their food intake (Figure 3C).

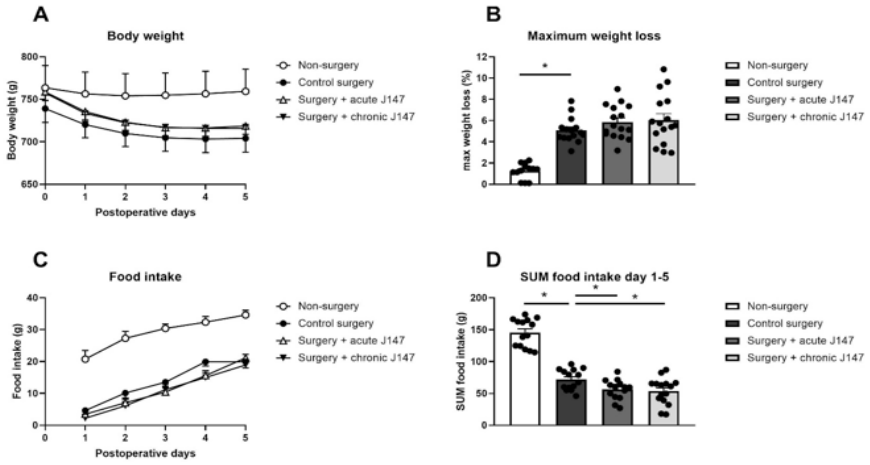

Figure 3: Body weight and food intake for the different experimental groups (mean ± SEM); non-surgery (n=13); control surgery (n=14-15); surgery + acute J147 treatment (n=14-16); surgery + chronic J147 treatment (n=15-16). A) Body weight of the rats on postoperative day 0 to 5, B) Maximum weight loss for postoperative day 0 to 5, C) Food intake on postoperative day 1 to 5 and D) SUM food intake for postoperative day 1 to 5. *: significant difference between indicated groups ($p<0.05$).

Recovery Time

Since time to first activity after surgery was mentioned as a major parameter for recovery in patients, this parameter was obtained from our rats as well. Results of this measurement are presented in Figure 4. Non-surgery rats started moving within a few minutes after being placed in the individual box. Whereas acute J147 significantly reduced time to first activity compared to control surgery rats by about 50%, chronic treatment did not significantly affect this recovery parameter.

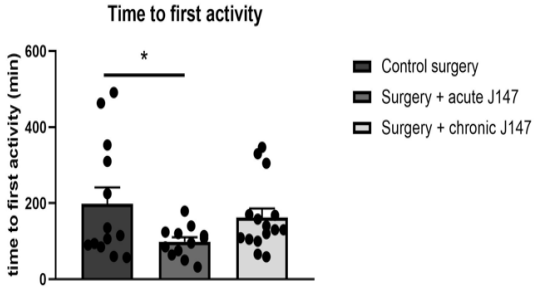

Figure 4: Time to first activity after end of surgery (minutes, mean ± SEM), for the different experimental groups; control surgery (n=13); surgery + acute J147 treatment (n=12); surgery + chronic J147 treatment (n=15).*: significant difference between indicated groups ($p<0.05$).

Activity

Figure 5 presents the time course of the 24 hours activity and diurnality, as well as overall activity and diurnality during the first 5 days after surgery. Surgery rats showed a significant trend towards lower activity ($p=0.085$) during the first 5 days after surgery, which was mostly attributable to the first postoperative day (Figure 5A). The total SUM of activity over the first 5 days after surgery was significantly lower in the control surgery group, compared to the non-surgery group (Figure 5B). All surgery groups quickly regained their activity and although surgery rats maintained lower overall activity (Figure 5B), on postoperative day 3 surgery groups did not differ anymore from non-surgery rats (Figure 5A). Activity over the first 5 days post-surgery was not affected by either J147 treatment (Figure 5A and 5B). Diurnality reflects difference between light and dark activity of the normally night-active rats. Figure 5C shows

that control surgery rats became relatively more active during the light period, reflected in a significantly higher diurnally score at day 1, 3, 4 and 5 (Figure 5C). Both J147 interventions kept the rats at the same level as the non-surgery controls for the first 2 days, while after that they became more day active and stabilized at the level of control surgery rats (Figure 5C). However, the overall diurnality during the first 5 days after surgery showed a trend towards lower diurnality and thereby partially restored night activity in the rats treated with acute J147 (p=0,097) (Figure 5D).

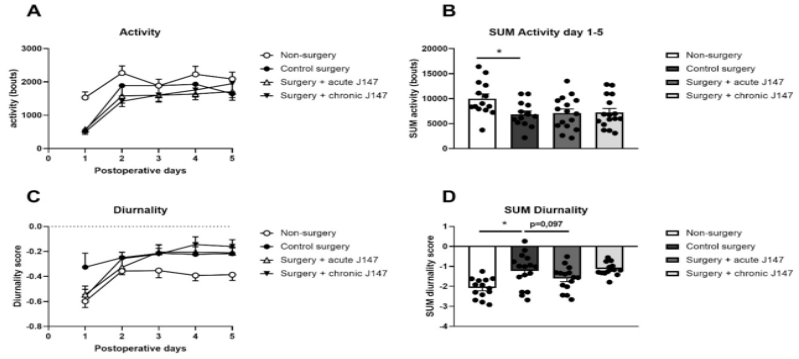

Figure 5: Twenty four hour activity and diurnality after surgery for the different experimental groups (mean ± SEM); non-surgery (n=14); control surgery (n=14-16); surgery + acute J147 treatment (n=15-16); surgery + chronic J147 treatment (n=16). A) Activity of the rats on postoperative day 1 to 5, B) SUM of the activity for postoperative day 1 to 5, C) Diurnality of the rats on postoperative day 1 to 5 and D) SUM of the diurnality for postoperative day 1 to 5. *: significant difference between indicated groups ($p<0.05$).

Inflammation Markers

Plasma IL-1beta and NGAL concentrations after surgery are shown in Figure 6. At 1 hour after surgery the chronically treated rats may show a higher, but not significant, IL-1beta plasma level (Figure 6A). Both the control surgery and acutely treated rats showed an increased level at 6 hours after surgery. However, chronically treated rats showed a decreased IL-1beta level at 6 hours. For all groups the IL-1beta levels were lowest at 24 hours after surgery. At 24 hours chronically treated rats had the lowest IL-1beta plasma levels (Figure 6A). Accordingly, IL-1beta AUC was highest for the control surgery group (2526 ± 285) and lower for both J147 treated groups (acute J147: 2425 ± 377 and chronic J147: 2254 ± 197). NGAL levels after surgery showed an increase for all the groups. Chronically treated animals showed a significant increase in comparison to control surgery animals, while acutely treated animals showed a trend towards an increased NGAL (p=0.056). At 24 h, chronically as well as acutely J147 treated rats showed a significant higher NGAL plasma level than surgery control rats (Figure 6B).

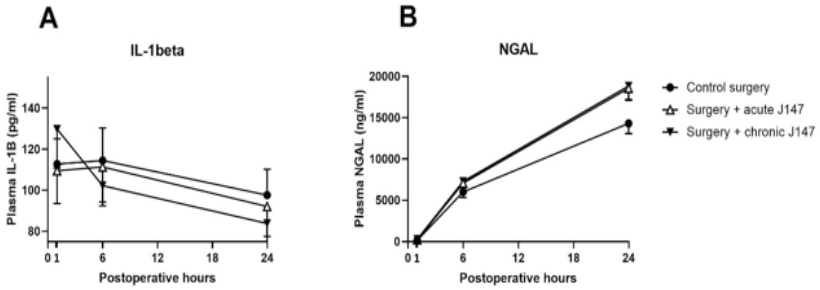

Figure 6: IL-1beta and NGAL plasma concentrations 1, 6 and 24 hours after surgery for the different experimental groups (mean ± SEM); control surgery (n=12-15); surgery + acute J147 treatment (n=15); surgery + chronic J147 treatment (n=15). A) Plasma IL-1beta concentrations at 1, 6 and 24 hours after surgery and B) Plasma NGAL concentrations at 1, 6 and 24 hours after surgery.

Discussion

General

The current study was aimed at evaluating early postoperative recovery in Zucker rats and the effect of treatment with the anti-dementia drug J147. Effects on early recovery were studied by assessing weight loss as main preclinical parameter for outcome, and time to regain activity as major clinical outcome parameter, after major abdominal surgery. In addition, effects on food intake, activity and diurnality and inflammatory factors were evaluated. Results showed that major abdominal surgery induced weight loss, reduced food intake, and was associated with overall lower activity and higher diurnality (more day active). Whereas chronic J147 did not affect these parameters, acute J147 treatment reversed the surgery induced prolonged time to first activity and improved diurnality. Therefore, we conclude that acute J147 treatment may improve early surgical recovery and hence potentially reduces the risk of developing longer-term postoperative complications. However, as neither acute nor chronic J147 treatment reduced plasma IL-1beta and Neutrophil Gelatinase-Associated Lipocalin (NGA) levels after surgery, these results were not attributable to anti-inflammatory actions of J147. The underlying mechanisms therefore would require further investigation.

Surgery in the Zucker Rat Model

Most of our previous work on the effects of surgery was performed in Wistar rats. In young Wistar rats, surgery induced a transient (neuro) inflammatory response, that was associated with temporally increased anxiety and reduced cognitive performance and a persistent reduction in hippocampal neurogenesis [10]. Known risk factors for postoperative complications, such as older age [32] or previous infections [33], lead to more extensive (neuro) inflammation and cognitive decline in these Wistar rats. However, these risk factors were expressed in otherwise healthy rats. Usually, patients undergoing surgery may not be completely healthy, and may present several risk factors. The obese Zucker rats is an outbred strain displaying several known risk factors for post-surgical outcome, including hyperglycemia, obesity, hypertension and renal dysfunction [35]. Moreover, these metabolic syndrome-like rats display low-grade inflammation, neuroinflammation and cognitive dysfunction at a relatively young age (<6 months) [35]. As these factors mimic increased risk factors for complications after surgery in patients, it was hypothesized that Zucker rats would be more vulnerable to complications after surgery, reflected in delayed recovery. Accordingly, surgery in the Zucker rats induced significant and persisting body weight loss, indicating significant impact of surgery on these rats. In rat studies, bodyweight loss seemed to be a good predictor for cognitive dysfunction and neuroinflammation in increased risk conditions as aging and infection history [32,33]. However, maximal weight loss in the Zucker rats appeared even slightly lower than seen in young healthy Wistar rats [10] and substantially lower than in the increased risk conditions in the Wistar rat; older age [32] or previous infection [33]. Nevertheless, Zucker rats did not show recovery of body weight during the first week after surgery, as we found in our previous study in young healthy Wistar rats [12]. This was in line with the persistently reduced food intake after surgery. As anticipated, Zucker rats developed an inflammatory response after surgery. IL-1beta levels were persistently higher within the first 6 hours after surgery, but then gradually declined towards 24 hours. This pattern is similar to the one we observed previously in young healthy rats [11] and is in agreement with other labs [45]. Moreover, NGAL levels steadily increased up to 24 hours after surgery. This is in accordance with previous studies in our lab, where NGAL plasma levels also increased up to 24 hours and decreased after this in healthy Wistar rats but increased even further in Wister rats with a previous infection (data not published).

Furthermore, surgery reduced the spontaneous physical activity after surgery and rats became more day active (higher diurnality score). This effect was most pronounced the first day after surgery for total activity, and persisted for 5 days after surgery for diurnality. Reduced activity and circadian rhythmicity the first few days after surgery were also observed in young healthy Wistar rats [13]. These data are in agreement with the results from clinical studies [48-52], in which surgery leads to temporary disruptions in circadian rhythm, causing sleep disturbances and changes in day-time and night-time activity levels. Moreover, reduced total night sleep, more awakenings during the sleep period, worse sleep structure with decrease of REM sleep have also been observed after surgery [48-52]. The relationship between activity and circadian sleep/wake rhythm is bio-directional, as less activity can also affect the sleep/wake rhythm. These changes in circadian sleep rhythmicity may be an indicator for worse postoperative outcome, such as cognitive problems, poor recovery and cardiovascular events [53,54] although above results support impact of surgery in Zucker rats, the impact was not as high as expected. In the present study, at least one risk factor for surgery-associated complications was made explicit; obesity, as for the other risk factors we relied on literature [35]. However, in this case, obesity in the Zucker rat resulted from eating more of the intentionally healthy rat chow, rather than the common cause of obesity in human patients; eating (too) much of unhealthy food. Recent studies indicated that a short period of high fat diet prior to surgery can lead to more postoperative cognitive problems [55]. Other clinical studies support the idea that obesity per se is not the cause of worse outcome after surgery, but other factors, including inflammation and malnutrition, also play a key role. In contrast, obesity could even have a positive effect in surgery. This contradiction is called the obese paradox [56,57]. The inflammatory state is suggested to be a major player in the difference between healthy and non-healthy obese people,

and fat tissue is a major source for inflammatory mediators [58]. Alternatively, other clinical studies point to the importance of malnutrition in the surgical period. Although patients with obesity may have a high food intake, they can simultaneously have malnutrition, associated with higher rates of complications; prolonged hospital stay, readmissions and mortality in different surgical procedures [59-62].

Effects of J147

J147 is a promising new drug that has been shown to have anti-dementia and anti-aging properties. In addition, our previous study in healthy young Wister rats showed that J147 protected against long-term surgery associated cognitive decline [12]. In the present study, we investigated effects of two routes of administration of J147, acutely at the start of surgery, or chronic in food starting one week before operation. Both administration routes resulted in plasma concentrations, well within the therapeutic range of 3-70 ng/ml [47], at the time of surgery, though obviously with different time courses. Chronically treated rats were supposed to have reached a steady state concentration during recovery, whereas acutely treated rats showed a transient high level that waned off to 24 hours. Besides a positive effect on diurnality on the first day after surgery, results showed that chronic J147 did not seem to have major effects on the surgery-induced changes in Zucker rats. In contrast, acute J147 did have a positive effect on the postoperative recovery. Acutely treated rats seemed to recover significantly faster, which was best reflected in a shorter time to first activity after surgey. These results seem contradictory to our previous study in young healthy rats, showing that chronic, but not acute, J147 treated rats displayed accelerated weight gain after surgery, and restored cognitive impairment [12]. In addition to the use of a different rat strain, and early recovery measures versus later behavioural changes, a major difference between the present and previous study could lay in the route of administration of acute J147. In our previous study acute J147 had been administered by gavage in order to match with the oral administration of chronic treatment. However, we did not measure plasma levels, and hence we cannot exclude a too low J147 plasma level in the previous study. This potential problem was overcome in the present study by given the J147 intravenously. Furthermore, the current study reflects on the early recovery period and the previous study showed results of one to two weeks after surgery. Since we hypothesized that early recovery may predict development of later complications, results from the present study then may predict less late complications after acute J147 treatment. Follow-up studies would elucidate on this aspect with regard to neuroinflammation and cognitive decline. Although J147 was shown to have anti-inflammatory proporties [36,38], the positive effect of acute J147 treatment did not seem attributable to inhibition of inflammation in the current study, as neither IL-1beta or NGAL at 1,6 and 24 hours levels indicated reduced peripheral inflammation. This is in agreement with our previous study where we also did not see an anti-inflammatory effect of J147 [16]. Although no difference was seen in inflammation, the pattern of upregulation of IL-1beta followed by upregulation of NGAL is in accordance with previous research, in which IL-1beta has been reported as an important factor for upregulation of NGAL in vitro [63-66] and in vivo [63].

Based on our previous research, indicating (neuro) inflammation to play a major role in postoperative complications [10], the present study mainly focused on anti-inflammatory effects, as the potential mechanism of J147. However, literature showed that J147 could also affect the mitochondrial Alfa-F1 Subunit of ATP Synthase (ATP5A), resulting in an increase in intracellular calcium, leading to activation of the AMPK/mTOR pathway, a canonical longevity mechanism [37]. Although our previous study on J147 after surgery in Wister rats did not support this theory [12], both our previous study [12] and the present one may point to altered metabolism since body weight remained the same at a lower food intake after J147 treatment. A result that stood out in the present study was the effect of acute J147 on activity patterns. One of the best estimates of recovery in patients is how quick they become active again after surgery [15-19]. In addition, it is well known that immobility after surgery is associated with the development of complications, such as pneumonia [67], deep vein thrombosis [68] and urinary tract infection [69], leading to prolonged hospital stay and increased mortality [70-72]. On the other hand, the immobility in the present study may also reflect general sickness behaviour [73-75]. As acutely J147 treated rats showed shorter time to first activity, this result can be regarded as a clinically relevant parameter for recovery. Furthermore, acute J147 treated rats showed a trend towards lower diurnality and thereby partially restoring the surgery-associated disturbed light-dark rhythm. In the clinical setting, also patients are prone to have a disturbed circadian rhythm after surgery and disturbance of the circadian clock that can cause several pathologies, including obesity, mood disorders, cancer, sleep disorders and cardiovascular diseases [76]. To our knowledge there is no literature on the effect of J147 on the circadian rhythm. However, J147 shows some activities similar to curcumin [77,78]. Curcumin can alter the molecular circadian clock, by inhibiting inflammation through NF-κB and subsequently increasing PPAR-γ activity [79], which can lead to activation of Bmal1, and Sirtuin 1 (SIRT1) regulating circadian rhythms [79-82]. Hence, the circadian rhythm and immune system are tightly intermeshed [83]. Although no anti-inflammatory effects could be observed in our models of postoperative recovery (present study) and complications [12] based on circulating cytokine levels or microglia activity, it cannot be excluded that J147 has acted through other inflammatory pathways, such as the above-mentioned NF-κB, PPAR, Bmal1, and sirtuin 1 pathway, and subsequently on the circadian clock to restore the circadian clock disturbances after surgery.

Limitations

Age is the most prominent risk factor for postoperative complications in patients. In this study, we used 25-30 weeks old outbred Zucker rats, since in older Zucker rats spontaneous mortality steeply increases, potentially biasing our results. Nevertheless, as pre-operative health forms a main risk factor for complications after surgery in patients, the Zucker rat strain, combining several risk factors, may provide a clinically relevant model. Moreover, as we used outbred rats, on the one hand this may better represent the patient population, but on the other hand induces more variation in the data, hampering strong statistically significant outcomes. The latter aspect potentially limiting the visibility of the positive effects of J147. Acute J147 treatment was given at one dose at the start of surgery by intravenous injection. Indeed, at the duration of surgery of approximately 1 hour, plasma levels were well within the therapeutic range, and declined towards 24 hours post-surgery. Although the dose, time and route of administration were chosen carefully, regarding the time course of the (neuro) inflammatory response [10], timing could provide a relevant target for further optimization and may help to better understand the development of postoperative complications. The current study showed that acute J147 treatment may improve recovery by reducing the time to first activity after surgery and partly restoring the circadian rhythm. This result may provide a relevant first step in evaluating the protective potential of J147 in postoperative recovery, but more research is needed in this area. For instance, clinically studies indicate that subjective sleep quality after surgery can be deteriorated, despite increased total sleep duration [22]. This reduced subjective sleep quality may be due to physical recovery from surgery [84], and is linked to worse postoperative recovery [85]. However, subjective sleep quality is hard the measure in preclinical research. Therefore, other parameters to assess sleep quality should be included in further research. Finally, chronically treated rats receive J147 through their food. As rats ate less the week after surgery, they received less J147 in that period. However, as acute J147 appeared effective with a half-life of 1.5 hrs in plasma and 2.5 hrs in brain [38], and have effective plasma concentrations up to 24 hours after surgery, the steady state plasma concentration of chronic J147 still would be sufficient until 24 h after surgery.

Conclusion

In the current study we investigated early recovery after major abdominal surgery and the effects of J147 in Zucker rats. Although Zucker rats may potentially better represent the type of patients that are at risk for post-surgical complications by bringing along co-morbidities, surgery induced only relatively mild weight loss and disturbed activity. Acute, but not chronic, treatment with J147 protected against surgery induced changes in activity and circadian rhythm, which was regarded as improved early recovery. This promising effect could not be attributed to anti-inflammatory effects. We hypothesized that improved early recovery would predict less long-term complications. These long-term effects are subject of our follow-up study.

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