Unraveling the Role of Neuroinflammation in Surgery-induced Cognitive Impairment

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Abstract

Although inflammatory processes are generally considered to be involved in postoperative cognitive dysfunction (POCD), the mechanism behind this condition is still largely unknown. The aim of our research is to study the role of inflammation in the development of POCD under high and low risk conditions with special focus on the different brain areas and different types of cognitive function involved. To this end we developed a rat model for POCD using standardized surgery and various behavioral tests to assess surgery-induced cognitive dysfunction.

Introduction

Postoperative cognitive dysfunction (POCD) was first recognized as the occurrence of dementia-like symptoms that persisted for weeks to months after surgery [1]. Whereas in younger patients the cognitive impairments are usually transient, in approximately 10 % of the elderly patients POCD persists [2, 3]. Next to advanced age the main risk factors for POCD include surgery severity and duration, the occurrence of peri-operative complications and pre-existing cognitive impairment. POCD has been associated with reduced quality of life, increased disability and dependency on social services and an increased risk of mortality [4].

Consistent evidence is accumulating for the role of surgery-induced inflammation in the development of POCD. It is hypothesized that surgery leads to a systemic increase in pro-inflammatory cytokines, which in turn can influence inflammatory processes in the brain, resulting in microglia activation and neuroinflammation (see Figure 1). Indeed, several clinical and preclinical studies show a co-occurrence of post-operative (neuro)inflammation and cognitive impairment [5, 7–9]. Since this proposed mechanism is expected to occur to some extent in all patients, it remains unclear why some individuals develop persisting POCD and others do not.

Although much research has focused on determining the risk factors and mechanism for POCD, the cognitive domains affected by POCD are an understudied topic [10]. Clinical studies tend to define POCD as a persisting, generalized decline in cognition, without specifying which cognitive functions are impaired. Preclinical studies, mainly focus on surgery-induced hippocampal dysfunction. We reviewed the clinical literature on POCD with the aim to elucidate what cognitive domains deteriorate after surgery and what brain areas might be involved [10]. We concluded that POCD encompasses a wide range of cognitive impairments, suggesting POCD affects a multitude of brain areas. We therefore propose a new approach in (preclinical) research on POCD that encompasses various aspects of cognition and mood related behavior and their associated brain regions. We believe that this new approach will facilitated translation from studied in animal models to clinical practice.

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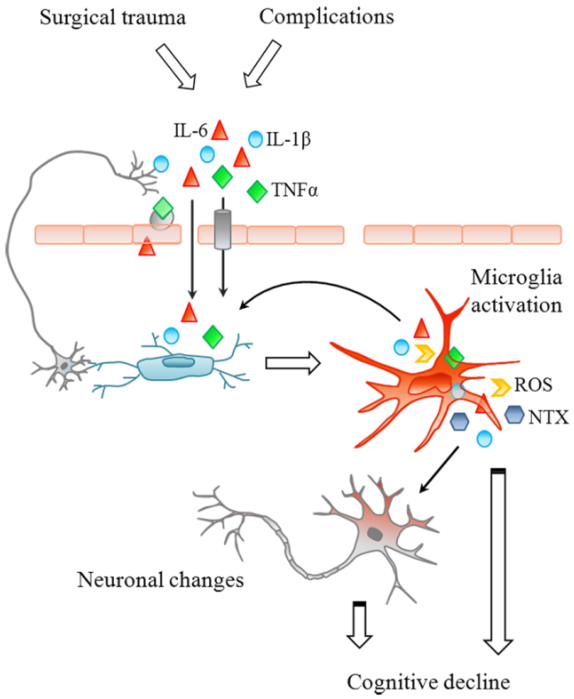

Figure 1. The hypothesized role of inflammation in POCD development. The surgical trauma and complications associated with surgery lead to a systemic increase in pro-inflammatory factors (e.g. IL-1β, IL-6 and TNFα). These can cross the blood brain barrier at permeable regions or by specific transport systems, or they can exert their effects in the CNS through interaction with vagal nerves. In the CNS inflammatory factors activate microglia, which in turn start producing more cytokines, reactive oxygen species and neurotoxins, ultimately leading to changes in neuronal functioning and cognitive decline. (Based on [5–7])

Methods

We aimed to study the role of inflammation in the development of POCD under high and low risk conditions with special focus on the different brain areas and different types of cognitive function involved. To this end we developed an animal model for POCD using standardized abdominal surgery and various behavioral tests to assess surgery-induced cognitive dysfunction. We studied the effects of known risk factors for POCD such as time after surgery, increased age, pre-operative health status and the type of surgery on POCD, (neuro)inflammation and neuronal functioning. In this abstract we describe our outcomes of surgery on behavior and microglia activation.

Animals

To study development of POCD male Wistar rats of 3 months of age (young) were obtained from Harlan (Harlan laboratories, Venray, The Netherlands). To study the effects of aging male Wistar rats of 3, 18 and 25 months (young, aged and elderly respectively) of age were obtained from a breeding colony of the Semmelweis University (Budapest, Hungary). All experiments were approved by the local animal experiment and welfare committee (Dier Experimenten Commissie, Groningen, the Netherlands).

Surgery

Under sevoflurane-O_2 anesthesia and buprenorphine (0.003 mg/ kg s.c.) analgesia the gastrointestinal tract was exteriorized and a temporary hypoperfusion of the upper part of the mesenteric vascular bed was induced by clamping the upper mesenteric artery for 30 minutes [11]. In addition, the rats were equipped with an indwelling jugular vein catheter to allow timed blood sampling with minimal handling.

Behavioral tests

We performed several behavioral tests aimed at measuring different aspects of cognition and mood related behavior. All behavioral tests were performed following standardized protocols as described before [11, 12]. Open field (OF) testing was performed to analyze exploratory behavior in a novel environment as measure of interest and anxiety. The novel object (NO) test was used to determine attention and object memory. Spatial learning and memory were analyzed based on training and probe trials in the Morris water maze (MWM) or Y-maze. Finally, reversal training in the Morris water maze or Y-maze was used to determine cognitive flexibility.

Analysis of micorglia activation in IBA-1 stained brain sections

We developed a novel image-analysis image analysis method that uses morphological parameters of microglia to quantify microglia activation in IBA-1 stained brain sections. We studied the microglia activation in several brain regions that have been associated with aspects of mood and cognition. These areas are: the amygdala, which has been related to anxiety; the prefrontal cortex, which has been associated with object recognition; the hippocampus, which has been related to spatial learning and memory; and the striatum, which has been associated with cognitive flexibility.

Results and Discussion

Figure 2 gives a typical example of behavioral test outcomes and results for microglia activation obtained in our experiments.

Young rats show impaired spatial memory and hippocampal neuroinflammation following surgery

We studied the development of POCD in young healthy rats by analyzing behavior in the first three weeks following major abdominal surgery [11]. Anesthesia alone did not affect cognitive performance at any time point. In young rats spatial learning and memory were temporarily impaired in the first two weeks following surgery, whereas non-spatial cognitive functions seemed unaffected. Since spatial learning and memory depend mainly on hippocampal function, this finding suggests that the hippocampus may be especially vulnerable to surgery-induced impairment.

Microglia activation, indicative of neuroinflammation, was increased in the hippocampus and prefrontal cortex 1 week following surgery, but not thereafter. Comparing this to the behavioral test results, it seems that neuroinflammation is not the sole factor leading to POCD. We hypothesize that for POCD to occur neuroinflammation must affect pathways involved in neuronal functioning.

Factors that are associated with an increased risk of POCD lead to more generalized and prolonged behavioral impairment following surgery

Advanced age and cardiac surgery both increase the risk of POCD. In two separate experiments we compared POCD in young and elderly rats, and compared POCD in young rats after major abdominal and major cardiac surgery (unpublished results). Whereas young rats after abdominal surgery only showed impaired spatial memory, both elderly rats and rats that underwent cardiac surgery showed a more generalized cognitive decline including impairment of object recognition (NO) and cognitive flexibility (MWM). Future studies may indicate whether this

more generalized cognitive impairment is related to a more generalized or prolonged neuroinflammation in the rat brain.

Whereas patients of all age categories can experience transient POCD, persisting POCD is mainly seen in elderly patients. We performed an additional experiment to study long-term postoperative behavioral changes in young and aged rats [12]. In contrast to young rats, aged rats showed a change in exploratory behavior that persists for at least 6 weeks following surgery. This may be indicative of changes in mood. Additionally, only aged rats showed increased microglia activation in the hippocampal CA1 region at six weeks following surgery, which was correlated to the changes in exploratory behavior. Again, these outcomes seem to indicate that the hippocampus is especially vulnerable to surgery-induced impairment. Moreover, a prolonged hippocampal neuroinflammation in aged rats may be associated with post-operative behavioral impairment.

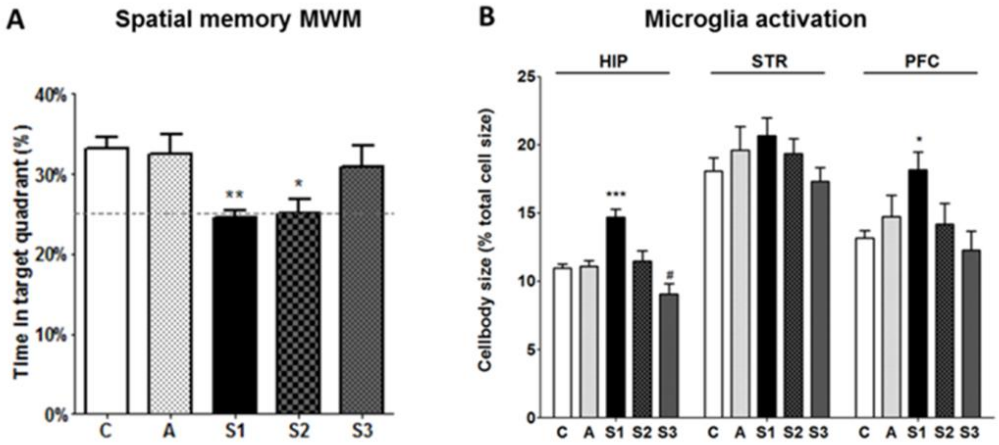

Figure 2. Spatial learning and microglia activation in young rats following abdominal surgery. A) Spatial memory in the Morris water maze (MWM) probe trial was measured by determining the time the rats spent in the target quadrant. Rats showed impaired spatial memory in the first and second week following surgery. Rats that only underwent anesthesia or rats that were tested in the third week following surgery performed similar to control rats. B) Microglia activation in the hippocampus (HIP), striatum (STR) and prefrontal cortex (PFC) was measured by determining the cell body to cell size ratio of IBA-1 stained microglia. Microglia activation was increased in the hippocampus and prefrontal cortex in the first week following surgery. C=naïve control group, A= anesthesia only group, S1, S2 and S3 = experimental groups that underwent behavioral testing 1, 2 and 3 weeks following surgery respectively. *p<0.05, **p<0.01,***p<0.001, #p<0.1. (Based on [11])

Conclusion

Our experiments are the first to study the effects of surgery on an extended range of cognitive functions and brain areas under high and low risk conditions for POCD. In line with previous research, we showed a temporal postoperative impairment of hippocampal dependent cognition and increase in hippocampal neuroinflammation in young healthy rats. Our approach allowed us to compare these outcomes in different cognitive domains. The results may indicate that in young rats hippocampal dependent cognition is most vulnerable to the effects of surgery and that neuroinflammation may not be the sole factor responsible for POCD. Additionally, we observed a more generalized and prolonged behavioral impairment and prolonged hippocampal neuroinflammation after surgery under conditions associated with increased POCD risk. These findings seem to be in line with the clinical findings on POCD and thus provide a promising basis for more extensive studies of the molecular changes in the brain following surgery.

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