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Full-length Article

# Combined effects of social stress and liver fluke infection in a mouse model

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ABSTRACT

The effects of two influences, social stress and acute opisthorchiasis, were investigated in inbred C57BL/6J male mice. In the model of social stress, mice were repeatedly attacked and defeated by aggressive outbred ICR male mice and were in continuous sensory contact with an aggressive conspecific mouse in their home cage for 20 days. Acute opisthorchiasis was provoked by invasion of *Opisthorchis felineus* (50 larvae per animal) on the fourth day after the social stress was induced. Simultaneous action of both factors caused the hypertrophy of adrenal glands, as well as elevated the activity of cathepsins B and L in the spleen. This effect on the activity of the cysteine proteases in the hippocampus and hypothalamus following *O. felineus* invasion was the predominant result of simultaneous action with social stress. Acute opisthorchiasis, social stress, and their combination caused an increase in the level of blood IL-6 in approximately 30% of the animals. Social stress induced a more pronounced effect on mouse plus-maze behavior than *O. felineus* invasion. Our results suggest a more severe negative effect of the simultaneous influence of both factors on most of the parameters that were investigated.



## 1. Introduction

It is known that different stress factors, in particular, social and psychological, have a negative impact on any human individual and society as a whole. One of the consequences of such an influence is the development of anxiety and depressive disorders in humans (Brown, 1996; Deakin, 1996; Leonard, 2014; Mitchell and Redfern, 2005). It has been established that major depressive disorder (MDD) is associated with high levels of blood interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) (Irwin and Miller, 2007; Prather et al., 2009; Slavich and Irwin, 2014; Stewart et al., 2015; Zunszain et al., 2013). For this reason, the inflammatory hypothesis of depression, or the so-called cytokine theory of depression (Dantzer, 2006; Dantzer et al., 2008; Maes et al., 2009; Miller et al., 2009), has been widely investigated during the last few decades. On the other hand, it is common knowledge that inflammation has an important role in pathological processes, as seen during the development of opisthorchiasis induced by *Opisthorchiidae* family parasites – *Opisthorchis felineus* (*O. felineus*) or *Opisthorchis viverrini* (*O. viverrini*) (Gentile and Gentile, 1994; Mordvinov and Furman, 2010; Ogorodova et al., 2007; Sripa et al., 2007). Bile duct epithelial cell damage, resulting from mechanical irritation caused by a parasite and/or by the secretion of its metabolites, activates the inflammatory immune mechanisms of the host (Poomphakwaen et al., 2009; Sripa et al., 2007). However, the simultaneous action of two factors, social stress and liver fluke invasion of *O. felineus*, has not yet been investigated despite these cases being often observed in human populations. It is of current concern to Western Siberia, where severe conditions (long winters, extreme temperatures, pressure and thermal discontinuities) destabilize the emotional state of a person. It has been determined that Western Siberia comprises the largest endemic region for *O. felineus*, with prevalence ranging from 40% to 95% in the Tyumen and Tomsk districts (Adams, 2006; Mordvinov and Furman, 2010; Mordvinov et al., 2012;

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Ogorodova et al., 2007; Pozio et al., 2013). To a lesser extent than in Western Siberia, *O. felineus* is endemic to Eastern Europe and the whole Russian territory, whereas *O. viverrini* is endemic to Cambodia, Lao PDR, Thailand and Vietnam (Keiser and Utzinger, 2004). However, despite the two species of liver fluke even promoting the development of cholangiocarcinoma (Adams, 2006; Fried et al., 2011; Maksimova et al., 2015; Parkin et al., 1991; Shin et al., 2010; Sripa et al., 2007, 2011), very little is known about the mechanisms underlying the interactions between the parasite and the host.

It is worth noting that infection by liver flukes may not only cause chronic liver inflammation but also damage other organs and systems of the host (Sripa, 2003), including the central nervous system. Liver–brain interactions under liver damage conditions have been discussed previously (Banks et al., 2009; Banks and Erickson, 2010; D'Mello et al., 2009; D'Mello and Swain, 2011). It is believed that inflammatory cytokines, TNF-α, IL-1 and IL-6, which are synthesized by blood monocytes and brain macrophages, mediate this interaction (Kerfoot et al., 2006; D'Mello and Swain, 2011). Cytokines (e.g., IFN-a, IL-1, IL-6 and TNF-a) lead to an increase of the release of corticotrophin releasing hormone (CRH); hypersecretion of CRH has been found in patients with MDD, as manifested by increased cerebrospinal fluid concentrations of CRH and increased CRH mRNA and protein in the hypothalamus (Irwin and Miller, 2007). Cytokines, such as IL-1, IL-2, IL-6, IL-11, IL-12, TNFα and IFNγ, can induce the release of glucocorticoids, which in turn control the production of cytokines (Besedovsky and del Rey, 2000; Mix et al., 2007). First, glucocorticoids act on receptors at the level of the hypothalamus and the pituitary to inhibit the release of CRH and adrenocorticotropic hormone (ACTH), respectively, and second, they act at the level of immune cells to inhibit the release of proinflammatory cytokines, such as TNFα, IL-1β, and IL-6 (D'Mello and Swain, 2011).

Therefore, it may be important to build an experimental model that allows for the investigation of the combined action of two factors: social stress and infection by the liver fluke *O. felineus*. For this reason, the C57BL/6J mouse strain was chosen for use because continuous male-to-male confrontations cause anxiety-depression-like disorder in these mice in which stress-induced anxiety precedes and promotes depression-like states (Avgustinovich et al., 1997; Kudryavtseva and Avgustinovich, 1998). The social defeat stress paradigm is widely used by other investigators, and it has been shown that there is a shift in pro- and anti-inflammatory cytokines in this mouse model (Stewart et al., 2015). Therefore, in the present study, this model was expected to estimate the net result of social stress and infection by the liver fluke *O. felineus* in the C57BL/6J inbred mouse strain, at first, by assessment of blood levels of inflammatory cytokine IL-6 and corticosterone because these two factors reflect the functional state of the brain and liver during inflammation. In addition, this model here was expected to evaluate mouse behavior in a plus-maze test upon the combined action of social stress and *O. felineus* invasion because of behavioral abnormalities that may serve as functional markers of brain inflammation. Studies have shown that inflammatory cytokines induce symptoms of so-called "sickness behavior" in humans and animals (Kelley et al., 2003), which largely overlap with the symptoms of MDD in patients (Dantzer, 2006; Irwin and Miller, 2007).

Moreover, it seems to be important to understand the involvement of lysosomal proteases (cathepsins B and L) because of their mutual interaction with cytokines (Conus and Simon, 2010). Cathepsins B and L are the most studied of the 11 cathepsins (B, C, H, F, K, L, O, S, V, W and X/Z). Aside from the role of cysteine cathepsins in many acute and chronic inflammatory processes, these cathepsins are involved in neurodegenerative diseases (malignant and benign brain tumors, Alzheimer's disease, brain ischemia), tissue destruction and remodeling (limited proteolysis), apoptosis and necrosis (Chwieralski et al., 2006; Conus and Simon, 2010; Felbor et al., 2002; Gan et al., 2004; Guicciardi et al., 2000, 2004; Morinaga et al., 2010; Repnik et al., 2012; Sun et al., 2010; Wang and Tsirka, 2005). In a recent study, experimental data have demonstrated the functional importance of the cathepsin B gene (*Ctsb*) in anxiety-related and depression-like behavior using knockout mice (Czibere et al., 2011). However, the role of lysosomal proteases during fluke invasion has not been established.

Therefore, the aim of this study was to investigate the combined effects of two treatments: social stress and *O. felineus* infection in C57BL/6J inbred mice. These effects were estimated based on blood IL-6 and corticosterone levels; cathepsin B and L activity in the spleen, hypothalamus, and hippocampus; and mouse behavior in the plus-maze test.

## 2. Methods

### 2.1. Animals

In this study, we used C57BL/6J inbred 2.5–3-month-old male mice (20–22 g) and ICR outbred mice (35–40 g). The first were obtained from the animal house of the Institute of Physiology and Basic Medicine, SB RAMS; the latter from the Center for Genetic Resources of Laboratory Animals at the Institute of Cytology and Genetics, SB RAS. During the experiments, all animals were kept under standard conditions: a controlled light regime of 12:12 (light/dark cycle), with an ambient temperature of 24 °C and food and water provided *ad libitum*. The experimental procedures were conducted in accordance with the European Communities Council Directives of 24 November 1986, 86/609/EEC and the decision of the Commission for Bioethics (protocol No 22 of May 30th, 2014).

### 2.2. Experimental design

C57BL/6J mice were divided into four groups: "CON" – control animals that were orally administered with a physiological solution; "OP" – mice that were orally administered with *O. felineus* metacercariae; "SS" – mice subjected to the social male-to-male confrontation stress; "OP + SS" – mice subjected to both treatments – oral administration of *O. felineus* metacercariae and social stress. Mice from the groups "SS" and "OP + SS" were placed into 28 × 14 × 10 cm cages for the duration of the experiment. Mice from the "CON" and "OP" groups were kept in groups of 4–5 animals in 36 × 23 × 12 cm cages. Five days before the behavioral tests, the animals were individually split into experimental cages (28 × 14 × 10 cm) to eliminate the effect of hierarchical encounters within the groups (Avgustinovich et al., 2005). Mice from the "OP" and "OP + SS" groups were orally administered with metacercariae of *O. felineus* (50 larvae per animal) on the fourth day after the social stress was induced. Fourteen days after the liver fluke invasion and seventeen days after male-to-male confrontations, the plus-maze behavior of the four experimental animal groups was analyzed. On the day following the twentieth male-to-male confrontation, the animals were decapitated and biological samples were collected for further microscopic and biochemical analyses. The hypothalamus, hippocampus, and spleen were dissected and kept in a 0.25 M sucrose solution and stored at −70 °C until further measurements of cathepsin B and L activity were conducted. Body weight as well as the relative weights of the adrenal glands and liver were also determined. Whole blood from each animal was centrifuges for 20 min at 800*g* and 4 °C. Blood serum was separated and divided into two parts for corticosterone and IL-6 level measurements and maintained at −70 °C until ELISA.