Effects of morphine on murine peritonitis – interstrain differences

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Abstract

We have shown previously that supplementation of inflammation-inducing factor with a high dose of morphine inhibits intraperitoneal accumulation of exudatory cells in representatives of fish but not in amphibian species, and in some but not all strains of mice. In a case of mice, males were ip injected with zymosan (40 mg/kg, Z group) or with zymosan supplemented with morphine (20 mg/kg, ZM groups). At 4th hour of peritonitis, the total numbers of intraperitoneal leukocytes (PTLs), and numbers of polymorphonuclear leukocytes (PMNs) were significantly lower in ZM animals than in their Z counterparts in Swiss, C57C3H, Balb/c, and C57BL/6 strains but not in CBA mice. The aim of the present investigations was to monitor the time course of peritonitis (up to 24 hours) in Z and ZM groups of males of four strains. The results fully confirmed the lack of anti-inflammatory action of morphine in CBA strain. In Balb/c, C57BL/6, and Swiss mice, the number of intraperitoneal PMNs was lower in ZM groups during the whole investigated period, being statistically different during the first 12 or 8 hours. In a sharp contrast, the time course of intraperitoneal PMNs accumulation was very similar in Z and ZM groups of CBA mice.

Key words: *zymosan, peritonitis, time course, morphine, mice.*

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Introduction

Peritonitis is a convenient model for comparative studies of an acute inflammation as in vertebrate species it is easy to retrieve peritoneal exudate for qualitative and quantitative analysis of inflammation-related cells and soluble factors. The course of inflammation is species- and strain-specific and varies according to the kind and dose of stimulant used [1-3]. In the case of ectothermic animals, kinetics of inflammation is also season-dependent and affected by the ambient temperature [1]. The typical time course of inflammation may be modified by factors connected with the experimental procedure. For example, peritoneal inflammation in mice is significantly inhibited by daily injections of physiological saline, handling of experimental animals [4] or housing conditions, i.g. isolation or grouping of individuals [5].

Peritoneal inflammation is also convenient for investigations of a modulatory action of various pharmacological factors including morphine. We have shown that supplementation of stimulant with this exogenous opioid affects inflammation in naltrexone-reversed manner, that is through the binding to opioid receptors [6, 7]. Morphine co-injection significantly reduced numbers of exudatory leukocytes and levels of chemotactic factors in several but not all strains of laboratory mice [6-9] and in two investigated fish species [6, 10, 11]. Such effects of morphine were absent in three investigated species of anuran amphibians [12, 13]. Morphine-induced inhibition of peritonitis in Swiss mice and goldfish corresponded with inhibition of *in vitro* migration of Swiss mice and fish (but not frogs) leukocytes pre-incubated with morphine to chemoattractants present in zymosan-activated serum [12].

We have shown that the pain symptoms (characteristic body writhes) of zymosan-injected mice are completely absent in individuals of all investigated strains co-injected with morphine already at a low dose (5 mg/kg b.w.) [7, 8], while only the high dose of morphine (20 mg/kg b.w.) additionally inhibits intraperitoneal influx of leukocytes in Balb/c, C57BL/6 and Swiss mice, but not in CBA strain [7]. These results indicate that local administration of

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morphine might offer both antinociceptive and anti-inflammatory profits, thus it is important to reveal why anti-inflammatory effects are not universal even among various strains of the same species. However, it is important to notice that these inter-strain comparisons were performed only in a one-time point, e.g. at 4 hours after zymosan or zymosan with morphine injection [7]. Therefore one could argue that anti-inflammatory effects of morphine might be revealed also in CBA mice investigated at other time points. In order to verify such an assumption, the aim of present investigation was to compare the time course of peritonitis induced by zymosan or zymosan with morphine injection in four murine strains: Balb/c, C57BL/6, Swiss, and CBA. The results fully confirmed that anti-inflammatory effects of morphine are lacking in CBA strain.

Materials and Methods

Animals

Adult male mice of Balb/c, C57BL/6, Swiss and CBA (4-6 week-old, 23-25 g), purchased from the Unit of Laboratory Animals (Collegium Medicum, Jagiellonian University, Kraków, Poland) were used in the present experiments. All mice were housed 5 per cage in cages 20×13×18 cm under strictly controlled conditions (free access to food (mouse chow) and water, 12-hr dark-light cycle (19:00-7:00 lights off), temperature 22°C. The ethical guidelines of the local committee on animal care (license no. 23/OP/2005) were followed throughout the experiments.

In vivo experimental procedures

After one-week adaptation to the laboratory conditions, the animals were divided into experimental groups and at 9 a.m. ip injected with 0.5 ml/25 g b.w. of freshly prepared zymosan A (Z, 40 mg/kg b.w.) (Sigma, London, UK) or that supplemented with morphine hydrochloride (20 mg/kg b.w.) (Polfa, Kutno, Poland) (ZM). One group of animals was left untreated (intact, INT group).

At time 0 (controls) and at the selected time points after injections (4, 6, 8, 12 and 24 hours) the animals were sacrificed by cervical dislocation and their peritoneal cavities were lavaged with 1 ml of PBS. Peritoneal exudate (fluids and leukocytes) were retrieved and centrifuged at 1500 rpm for 15 minutes. Peritoneal leukocytes (PTL), among them, polymorphonuclear cells (PMN) and mononuclear cells (MN), stained with Türk solution, were counted in haemocytometer. The retrieved fluids were stored at –20°C. Experiments were repeated at least three times with at least 3 animals per each group.

Statistical analysis

The results were statistically analysed by 2-way ANOVA (to check if the time course of ZM groups differs significantly from that of Z groups) with post hoc Tukey's test (indicating those time points with means significantly different). The differences were considered statistically significant at $P<0.05$.

Results and discussion

In all investigated strains of mice the number of resident PTLs was low in intact animals (time 0), while the total number of PTLs increased sharply till 4th hour after zymosan injection (Z), reached peak at 6-12 hours after injection and than slowly declined being still far above the control level at 24 hours after injection (Fig. 1a, solid lines and circles). Among the total pool of PTLs, the polymorphonuclear leukocytes (PMNs) were apparently absent in intact animals and appeared quickly at the early stages of peritonitis with a peak at 4-6 hours after injection. 24 hours after zymosan injection the PMN number was still above respective controls (time 0) in Balb/c, C57BL/6 and Swiss, but was close to zero in CBA mice (Fig. 1b, solid lines and circles). In the present experiments, the number of mononucler cells (MNs), consisting off monocytes/macrophages and lymphocytes decreased slightly soon after zymosan injection in Balb/c, C57BL/6 and Swiss, while it was unaffected in CBA mice. According to Ajuebor et al. [14] an irritant injection causes an initial disappearance of the resident peritoneal macrophages from peritoneal fluid (lasting for the first 2 hours of the inflammatory process) possibly due to an increased adherence to the internal mucosal layers. Following this initial drop, the number of MNs increased gradually with a peak after that of PMNs.

Intraperitoneal influx of PTLs (Fig. 1a, broken lines, open circles), mainly PMNs (Fig. 1b, broken lines, open circles) was inhibited and delayed in the Balb/c, C57BL/6 and Swiss mice injected with zymosan supplemented with morphine, with differences between Z and ZM groups being statistically significant at 4-12 hours in Balb/c and C57BL/6 and 4-8 hours in Swiss mice. In sharp contrast, time courses of peritonitis in Z and ZM groups were very similar in the CBA strain.

The results of present experiments clearly indicate that anti-inflammatory effects of morphine are absent in CBA strains, while they are evident and long lasting in the three remaining murine strains. Experiments aimed in elucidation of the reasons of discrepancy between CBA versus other strains are in progress. So far, we know that CBA possess the highest number of mast cells easily degranulated upon morphine treatment [2, 9], thus morphine-induced inflammatory processes might counterbalance its anti-inflammatory effects. Moreover, we discovered that exudatory leukocytes of Swiss and C57BL/6 strain possess a lot of opioid receptors [in preparation] which might be responsible for inhibition of intraperitoneal influx of leukocyte due to heterologous desensitisation of chemokine receptors [12, 15, 16] and might be involved in the enhanced apoptotic death of leukocytes in focus of inflammation [in preparation]. The lack or scarcity of opioid receptors in CBA

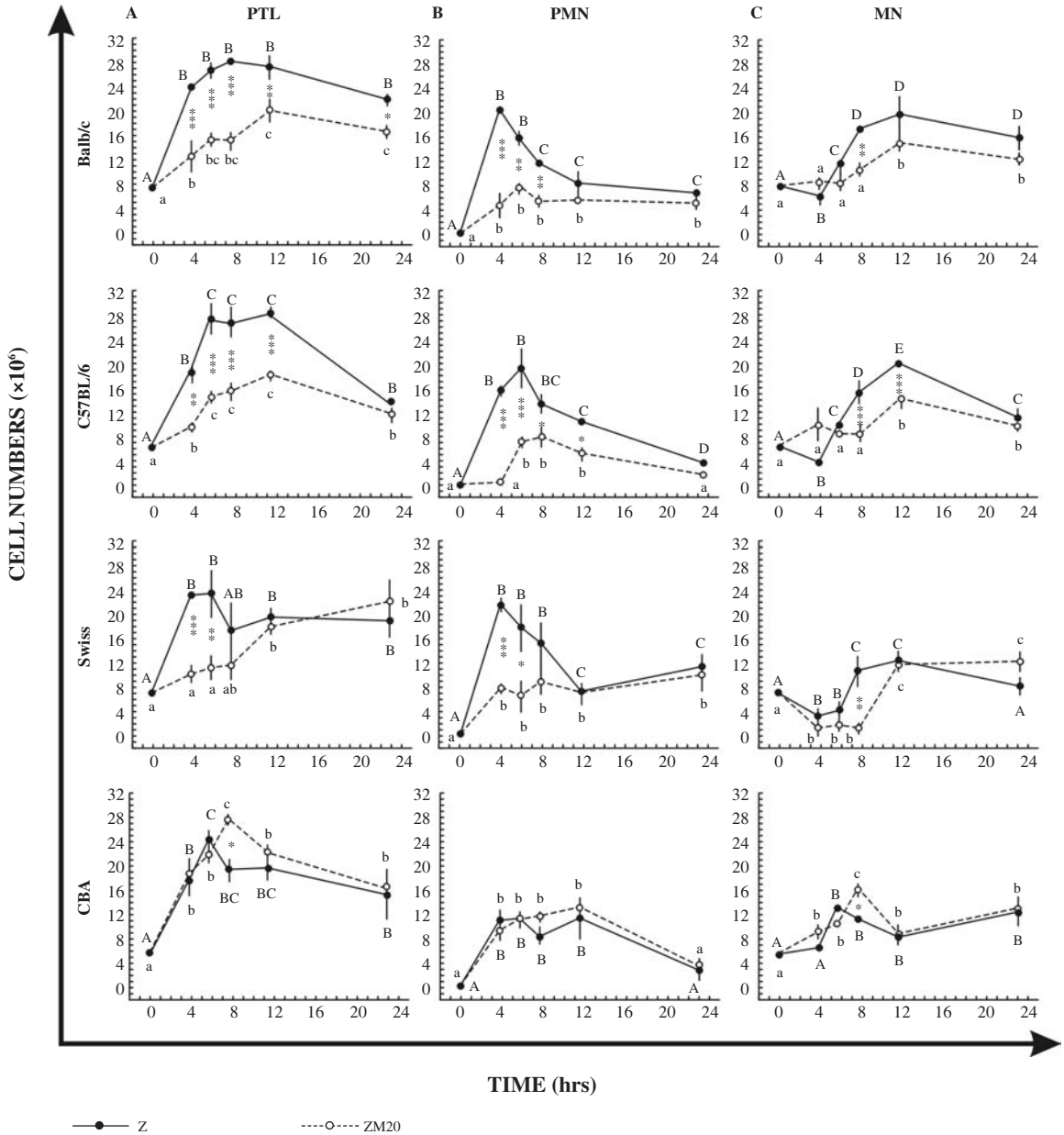

Fig. 1. Time course of peritonitis in males of Balb/c, C57BL/6, Swiss and CBA strains of mice either intact (time 0) or *i.p.* injected with zymosan (Z, solid lines and circles) or zymosan with morphine (ZM, broken lines and open circles). Peritoneal cell numbers: (A) total peritoneal leukocytes (PTL); (B) polymorphonuclear leukocytes (PMN); (C) mononuclear leukocytes (MN). X±SE, n=8-12. Values with different letters (A vs. B; a vs. b) vary significantly within Z or ZM groups. Asterisks between statisticaly different means of Z and ZM groups, according to Tukey's test, with P<0.05 (*), P<0.01 (**) or P<0.001(***)

mice might result in their undisturbed intraperitoneal accumulation and prolonged viability [in preparation].

In conclusion, anti-inflammatory action of morphine supplied directly to the focus of inflammation is absent not only in amphibians (toads and frogs) [13], but also in some representatives of mammals, exemplified here by CBA strain of mice.

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