

Polysaccharide peptide induces a tumor necrosis factor- α -dependent drop of body temperature in rats

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Abstract

1 Polysaccharide peptide (PSP) extracted from the *Coriolus versicolor* mushroom is frequently
2 suggested as an adjunct to the chemo- or radiotherapy in cancer patients. It improves quality
3 of the patients' life by decreasing pain, fatigue, loss of appetite, nausea, and vomiting.
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5 However, the effect of PSP on body temperature has not thus far been studied, although it is
6 well known that treatment with other polysaccharide adjuvants, such as lipopolysaccharides,
7 may induce fever. The aim of the present study, therefore, was to investigate the influence of
8 PSP on temperature regulation in rats. We report that intraperitoneal injection of PSP
9 provoked a dose-dependent decrease of temperature in male Wistar rats equipped with
10 biotelemetry devices to monitor deep body temperature (Tb). The response was rapid (i.e.,
11 with latency of 15-20 minutes), transient (lasting up to 5 hours post-injection), and
12 accompanied by a significant elevation of the blood tumor necrosis factor- α (TNF- α) level.
13 Pretreatment of the rats with anti-TNF- α antibody prevented the PSP-induced drop in Tb.
14 Based on these data, we conclude that rats may develop an anapyrexia-like response to the
15 injection of peptidopolysaccharide rather than fever, and the response was TNF- α -dependent.
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Key words: rats, body temperature, biotelemetry, polysaccharide peptide, anapyrexia, tumor necrosis factor- α .

1. Introduction

1 Polysaccharide peptide of the *Coriolus versicolor* mushroom (Cui and Chisti, 2003) has been
2 recommended as an adjuvant in the treatment of cancer (Zaidman et al., 2005) and several
3 other disorders, such as chronic hepatitis and infections of the upper respiratory, urinary, and
4 digestive tracts (Eliza et al., 2012). The doses usually used range from 1 to 3.6 grams per day
5 and the duration of therapy is from 1 to 36 months, depending on the patient's condition (Kid,
6 2000). Experimental and clinical studies demonstrate that PSP extracts show non-toxic effects
7 (Cheng and Leung, 2008), inhibit the growth of cancer cells in *in vitro* and *in vivo* settings
8 (Tsang et al., 2003; Lau et al., 2004), and decrease cancer treatment-related symptoms such as
9 fatigue, loss of appetite, nausea, vomiting, and pain (Chan and Yeung, 2006). It is suggested
10 that the compound exerts its activity primarily via immunomodulation (Dong et al., 1996),
11 therefore has been classified as a 'biological response modifier' (Ng, 1998). Indeed, data
12 demonstrate that PSP induces synthesis of pro-inflammatory cytokines, eicosanoids,
13 histamine, reactive oxygen and nitrogen intermediates (Chan and Yeung, 2006; Schepetkin
14 and Quinn, 2006), activates complement-3, and stimulates the NK activities and T-cell
15 proliferation (Sekhon et al., 2013) among others.

16 Immunomodulatory activities of the PSP, especially those data that show *in vitro* synthesis of
17 interleukin-1 β (IL-1 β) and TNF- α by macrophages co-cultured with PSP (Schepetkin and
18 Quinn, 2006) implicate that the use of the compound may exert a profound influence on the
19 regulation of body temperature in men and in laboratory animals. These cytokines are
20 particularly involved in the mechanism of fever induced by a large variety of the pathogen-
21 associated molecular patterns also called 'exogenous pyrogens', including another
22 polysaccharide 'biological response modifier', i.e., lipopolysaccharides (LPS) extracted from
23 the Gram-negative bacteria (Kluger, 1991; Kozak et al., 2000; Kozak et al., 2006a). However,
24 to the best of our knowledge, the effect of PSP on body temperature regulation has not been
25 studied. Based on experimental data reported from other laboratories using PSP we have put
26 forward a hypothesis that polysaccharide peptide, similar to the action of LPS on body
27 temperature, induces fever and the compound can be classified to the family of exogenous
28 pyrogens. We report that instead of fever, PSP provokes a significant decrease of body
29 temperature (T_b) in the rat. Since the *in vitro* studies showed that PSP stimulates the synthesis
30 of TNF- α by macrophages (Schepetkin and Quinn, 2006), and *in vivo* research demonstrated
31 that this cytokine can exert an anapyretic effect in rats (Evans et al., 1994; Klir et al., 1995)
32 and mice (Kozak et al., 1995), our present studies are focused on the role of TNF- α in

1 decreasing of body temperature of the rats treated with PSP. We demonstrate that PSP-
2 provoked drop of Tb was prevented by treatment of rats with anti-TNF- α antibody.
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5 **2. Materials and Methods**

7 *2.1. Experimental animals and body temperature measurement*

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9 Male Wistar rats weighing from 200 g to 250 g were obtained from the Mossakowski Medical
10 Research Centre Polish Academy of Sciences (Warsaw, Poland). Animals were housed in
11 individual plastic cages and maintained in a temperature/humidity/light-controlled chamber
12 set at $23 \pm 1^\circ\text{C}$, 12:12 h light:dark cycle, with light on at 07:00 a.m. Rodent laboratory food
13 and drinking water were provided *ad libitum*. A week after the shipment, the rats were
14 implanted under sterile conditions with battery-operated miniature biotelemeters (PhysioTel[®]
15 model TA10TA-F40, Data Sciences International, USA) to monitor deep body temperature
16 (Tb) with accuracy $\pm 0.1^\circ\text{C}$ as described previously (Wrotek et al., 2011). Described
17 experiments were started 10 days after surgery. All procedures were approved by the Local
18 Bioethical Committee for Animal Care in Bydgoszcz (Poland; permission no. 17/2013).
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29 *2.2. Treatment of the animals*

30 Polysaccharide peptide (PSP; extract from the Cov 1 strain of *Corioliolus versicolor*;
31 MycoMedica, Czech Republic) was dissolved in sterile 0.9% sodium chloride (saline) and
32 injected intraperitoneally (i.p.) at a dose of 50, 100 and 200 mg kg⁻¹. Control group of rats
33 was treated i.p. with an equivalent volume of a pyrogen-free saline.
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59 *2.3. TNF- α assay*

60 Blood samples were collected via cardiac puncture onto the solution of ethylenediamine
61 tetraacetic acid disodium salt (Na₂EDTA, Sigma-Aldrich; cat. no. E 5134) two hours post-
62 injection of PSP and/or pyrogen-free saline from rats anesthetized with a mixture of
63 ketamine/xylazine (87 mg kg⁻¹ and 13 mg kg⁻¹, respectively, intramuscular injection). After
64 centrifugation (20 min, 1500 x g), the resulting plasma was stored at -20°C until assay.
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1 The levels of TNF- α were determined by a standard sandwich ELISA kit from R&D Systems
2 (cat. no. RTA00; sensitivity of assay less than 5 pg ml⁻¹) according to the manufacturer's
3 instructions. Colorimetric changes in the assay were detected using Synergy HT Multi-Mode
4 Microplate Reader (BioTek Instruments, USA).
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8 9 2.4. Statistical analysis

10 All values are reported as means \pm standard error mean (S.E.M.) and were analyzed by
11 analysis of variance (ANOVA) followed by the Student's *t*-test with the level of significance
12 set at $P < 0.05$. ANOVA was used to compare the mean values in the all tested groups, whereas
13 the Student's *t*-test was used to calculate the difference only between the two selected groups.
14 For the conclusions of our study we used the Student's *t*-test. For the Tb measures, the data
15 were recorded and computed at 5-min intervals using Data Acquisition Programme (Data
16 Sciences International, USA). For data presentation, these 5-min temperature recordings were
17 pooled into 20-min averages.
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26 27 3. Results

28 29 3.1. Injection of PSP provokes a decrease in body temperature and elevation of blood TNF- α 30 of rats 31

32 Effect of PSP on changes of Tb in male Wistar rats is illustrated in *Fig. 1*. Three doses of the
33 PSP (50, 100 and 200 mg kg⁻¹) were injected intraperitoneally into the separate groups of rats.
34 In the case of all doses of PSP, the response was rapid with latency of 15-20 min and transient
35 (lasting up to 5 hours post-injection). Moreover, the maximum drop in Tb of rats was
36 maintained from 45 min to 2 h after PSP administration. However, the decrease in Tb was
37 dose-dependent. PSP at a dose of 50 mg/kg induced a decrease in Tb to $36.3 \pm 0.2^{\circ}\text{C}$
38 (measured at 90 min post-injection), whereas the doses of 100 mg kg⁻¹ and 200 mg kg⁻¹
39 provoked a similar greater drop in Tb to $35.8 \pm 0.2^{\circ}\text{C}$ and $35.6 \pm 0.1^{\circ}\text{C}$, respectively at the
40 same time ($P < 0.05$). Dose of 100 mg kg⁻¹ of PSP was selected for further experiments.
41 Injection i.p. of sterile saline (solvent for PSP) did not induce any alterations in Tb of rats
42 (data not shown).
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52 53 (Insert Figure 1 here)

54 Rats injected i.p. with saline (control vehicle) as well as non-treated animals did not
55 show any significant elevation of TNF- α at 2 h post-injection (*Fig. 2*). Plasma concentration
56 of the cytokine in these two groups of rats was below the lowest standard of ELISA kit
57 (respectively $3.07 \pm 0,2$ pg ml⁻¹ and $2.13 \pm 0,2$ pg ml⁻¹; sensitivity of assay less than 5 pg ml⁻¹
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¹). In contrast, however, the levels of TNF- α in plasma of the rats treated with PSP at a dose of 100 mg kg⁻¹ 2h post-injection were significantly higher (225 \pm 4 pg ml⁻¹). Blood for analyses was collected at the time of the greatest decrease in body temperature of rats.

(Insert Figure 2 here)

3.2. Administration of TNF- α antibody inhibited a decrease in body temperature of rats injected with PSP

To determinate whether or not the TNF- α is involved in a PSP-induced decrease of Tb, in separate experiments rats were treated i.p. with an anti-TNF- α immunoglobulin G (IgG) at a dose of 50 μ g/rat 1 h prior to the injection of PSP at a dose 100 mg kg⁻¹. As can be seen in *Fig. 3*, administration of TNF- α antibody inhibited the decrease of Tb provoked by the injection of PSP. The Tb of rats treated i.p. with TNF- α antibody and then injected with PSP was similar to that observed in the control rats injected i.p. with rabbit IgG 1 h prior to the administration of 0.9% saline (37.1 \pm 0.1 $^{\circ}$ C and 37.2 \pm 0.1 $^{\circ}$ C, respectively; P=0.38; counting from 0 min to 240 min post-injection). On the other hand, the Tb of rats injected i.p. with rabbit IgG at a dose of 50 μ g kg⁻¹ 1 h prior to the injection of PSP (100 mg kg⁻¹) was significantly lower in this period of time (36.1 \pm 0.4 $^{\circ}$ C; P<0.001). Moreover, the rats injected with IgG and then with PSP responded with a similar drop in Tb as the group of animals treated i.p. only with PSP. In both groups of animals, the response was similarly rapid (with latency of 15-25 min), transient (lasting up to 5 hours post-injection) and with the same maximum drop in Tb (35.6 \pm 0.1 $^{\circ}$ C and 35.8 \pm 0.2 $^{\circ}$ C, respectively; measured at 90 min post-injection).

(Insert Figure 3 here)

4. Discussion

In the present report we demonstrate for the first time that polysaccharide peptide, a bioactive component derived from the mushroom *Coriolus versicolor*, which exhibits antitumor and immunomodulatory properties, provoked also a dose-dependent decrease in body temperature of rats (*Fig. 1*). Moreover, we have shown that the drop in Tb was accompanied by a significant elevation of the blood TNF- α level of rats 2h post-injection (*Fig. 2*), and was prevented by an i.p. injection of anti-TNF- α antibody 1h prior to the PSP administration (*Fig. 3*).

Nowadays, PSP is considered as an useful adjuvant especially combined with chemotherapy in clinical treatment of cancer patients (Qian et al., 1997). The *in vivo* studies

1 revealed that PSP is able to restore weakened immune response observed in patients with
2 cancer or during chemotherapy (Chu et al., 2002). Moreover, PSP stimulates an element of
3 the innate immune system, which markedly influences the host's ability to respond to various
4 external stimuli, including pathogenic microorganisms. Phagocytic cells such as neutrophils
5 and macrophages are a key component of the innate immune system. They represent the first
6 line of the defense and are involved in all phases of the immune response (phagocytosis,
7 antigen presentation, cytokine secretion) (Dempsey et al., 2003; Wynn et al., 2013). Data
8 indicate that PSP is capable to restore and enhance the phagocytic function of macrophages
9 and it stimulates these cells to the release of pro-inflammatory cytokines (IL-6, TNF- α , IL-1)
10 and prostaglandin E₂ (PGE₂) (Chan and Yeung, 2006). It is well-known that all these
11 activities are temperature-dependent. Based on these data one may conclude that
12 understanding of the effect of PSP on body temperature is very important, especially when
13 considering PSP as an useful adjuvant in clinical treatment of cancer patients or during the
14 severe infections.

25 PSP is used as an anticancer and immunomodulatory agent. Although the mechanism
26 of its antitumor action is still not completely clear, this polysaccharide is suggested to enhance
27 cell-mediated immune response *in vivo* and *in vitro* and act as biological response modifiers.
28 PSP is considered as multi-cytokine inducer that is able to induce gene expression of various
29 immunomodulatory cytokines, such as IL-1 β , TNF- α , IFN- γ (Ooi and Liu, 2000; Lee et al.,
30 2006). Moreover, PSP regulates gene expression and cytokine secretion related to Toll-like
31 receptors (TLRs) signaling pathway. In human peripheral blood mononuclear cells, TRAM-
32 TRIF-TRAF6 subsignaling pathway of TLR seems to be one of the key associated signaling
33 pathways in the immunomodulation of PSP (Li et al., 2010). Thus, polysaccharide peptide
34 may constitute the pathogen-associated molecular patterns (PAMPs) recognized by TLRs. It
35 is well-known that many PAMPs activate a common downstream pathway operating in the
36 signal transduction via TLRs, that leads to the upregulation of the nuclear factor- κ B (NF- κ B)
37 - one of the most important transcription factors which can induce cytokine genes expression,
38 including TNF- α (Kozak et al., 2006a; Kaisho and Akira, 2001; Baeuerle and Henkel, 1994).
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50 TNF- α is a circulating 17-kDa protein that interacts with two distinct transmembrane
51 signaling receptors, termed the type I (p55) and the type II (p75), to induce its biological
52 effects (Schall et al., 1990). Injection of exogenous TNF- α is capable of causing fever in
53 healthy animals of several species (Netea et al., 2000). The pyrogenic properties of the
54 cytokine are induced by binding TNF- α with the type II receptor (Leon et al., 1997; Kozak et
55 al., 1995). On the other hand, there are data indicating that TNF- α can also have a cryogenic
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1 or antipyretic effect when this cytokine is bound with type I receptor (Evans et al., 1994; Klir
2 et al., 1995). Anapyrexia is defined as a regulated decrease in core temperature distinct from
3 hypothermia in that thermoregulatory responses indicate a defense of the anapyretic level of
4 core temperature (Glossary of Terms for Thermal Physiology, 2001). There are conditions
5 under which anapyrexia appears to be adaptive rather than pathologic (Kozak, 1997). For
6 instance, upon hypoxic circumstances (i.e., during anemia, ischemia, cardiac arrhythmias,
7 hypotension) anapyrexia protects tissues from the lack of oxygen by decreasing its
8 consumption and by slowing the rate of cellular damage that occurs from the formation of free
9 radicals, chemical metabolites and tissue edema (Steiner and Branco, 2002; Kozak et al.,
10 2006b). It's assumed that the process may be mediated or initiated by a "cryogen", defined as
11 an endogenous or exogenous substance that lowers the set point of deep body temperature
12 (Kluger, 1991). Our studies demonstrate that PSP may induce an anapyrexia-like effect,
13 which may be mediated by TNF- α . However, in order to fully investigate this phenomenon it
14 is necessary to measure the behavior and autonomic responses to determine the regulated
15 nature of the hypothermia following treatment with PSP. Because of the fact that anti-tumor
16 properties of PSP are postulated, we hypothesized that this phenomenon is associated with the
17 rise in plasma TNF- α .

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Figures legend

Figure 1. Changes of body temperature ($^{\circ}\text{C}$) over time (min) of rats injected intraperitoneally (i.p.) at 0 time (arrowhead) with PSP at a dose 50 mg kg^{-1} (open triangles), 100 mg kg^{-1} (close diamonds) and 200 mg kg^{-1} (open circles) in comparison to non-treated animals (close circles). Values are means \pm S.E.M. at 20-min averages (n indicates sample size in a respective group).

Figure 2. Plasma levels of $\text{TNF-}\alpha$ (pg ml^{-1}) at 2 h post-injection of PSP at a dose of 100 mg kg^{-1} and control injection of saline in comparison to non-treated animals (control). Values are expressed as means \pm S.E.M; n depicts sample sizes for each group. Asterisk indicates significant difference compared to the control and saline i.p. (** $P < 0.001$; * $P < 0.05$, respectively).

Figure 3. Changes of body temperature ($^{\circ}\text{C}$) over time (min) of rats injected i.p. at -60 time (black arrowhead at 08:00 a.m.) with anti-rat $\text{TNF-}\alpha$ IgG ($50\text{ }\mu\text{g/rat}$) or IgG ($50\text{ }\mu\text{g/rat}$) and then treated i.p. at 0 time (white arrowhead at 09:00 a.m.) with PSP (100 mg kg^{-1}) or with 0.9% saline in comparison to rats injected i.p. with PSP (100 mg kg^{-1}) at 0 time. Values are means \pm S.E.M. at 20-min averages. Letter n indicates sample size in a respective groups.

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Highlights

- we examine the effect of polysaccharide peptide (PSP) on rat body temperature (Tb)
- injection of PSP provokes a dose-dependent decrease of body temperature in rats
- decrease of temperature is accompanied by an elevation of the blood TNF- α level
- rats pretreatment with anti-TNF- α antibody prevents the PSP-induced drop in Tb
- rats developed an anapyrexia-like response to the injection of PSP

Figure 1
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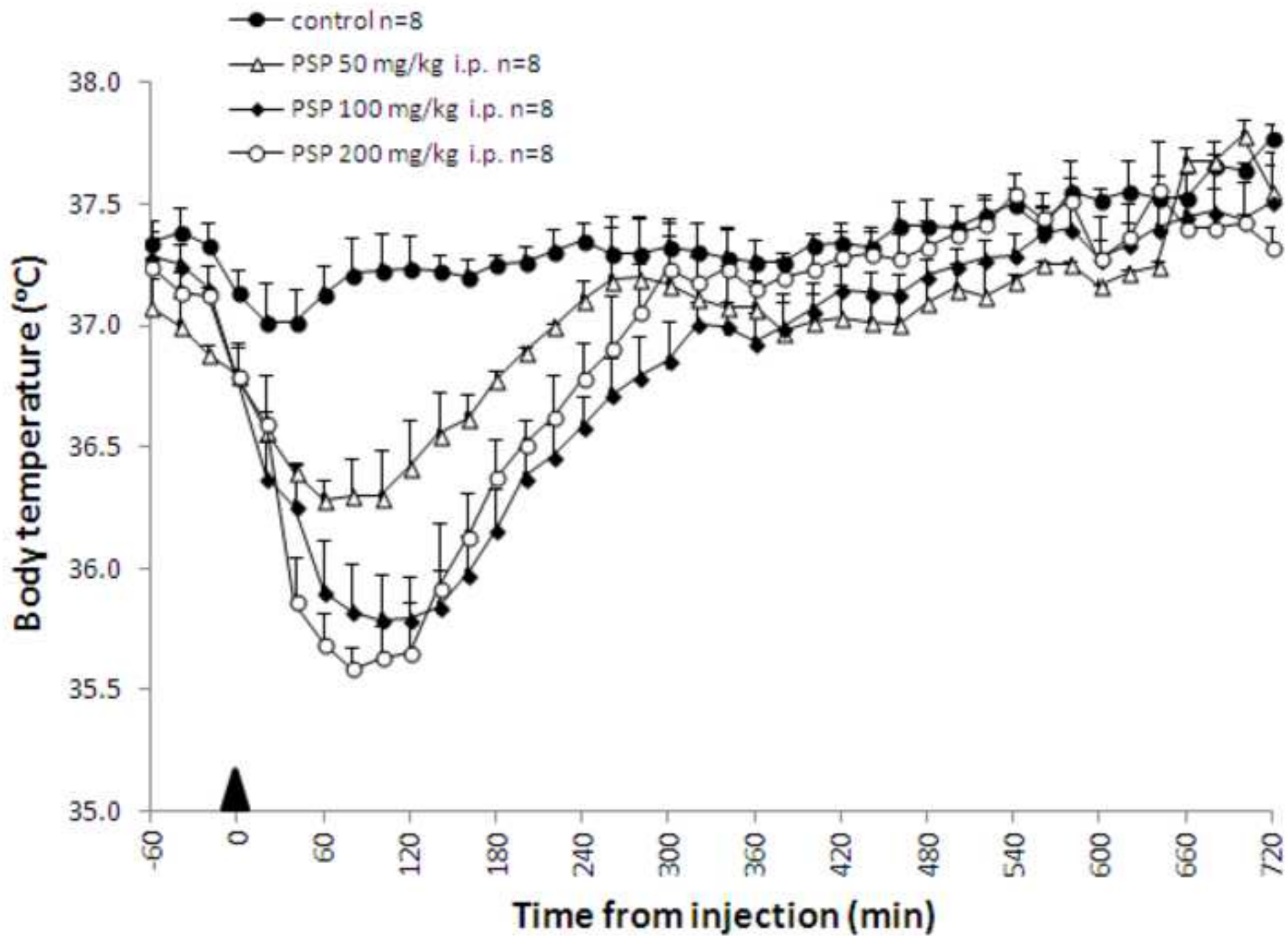


Figure 2
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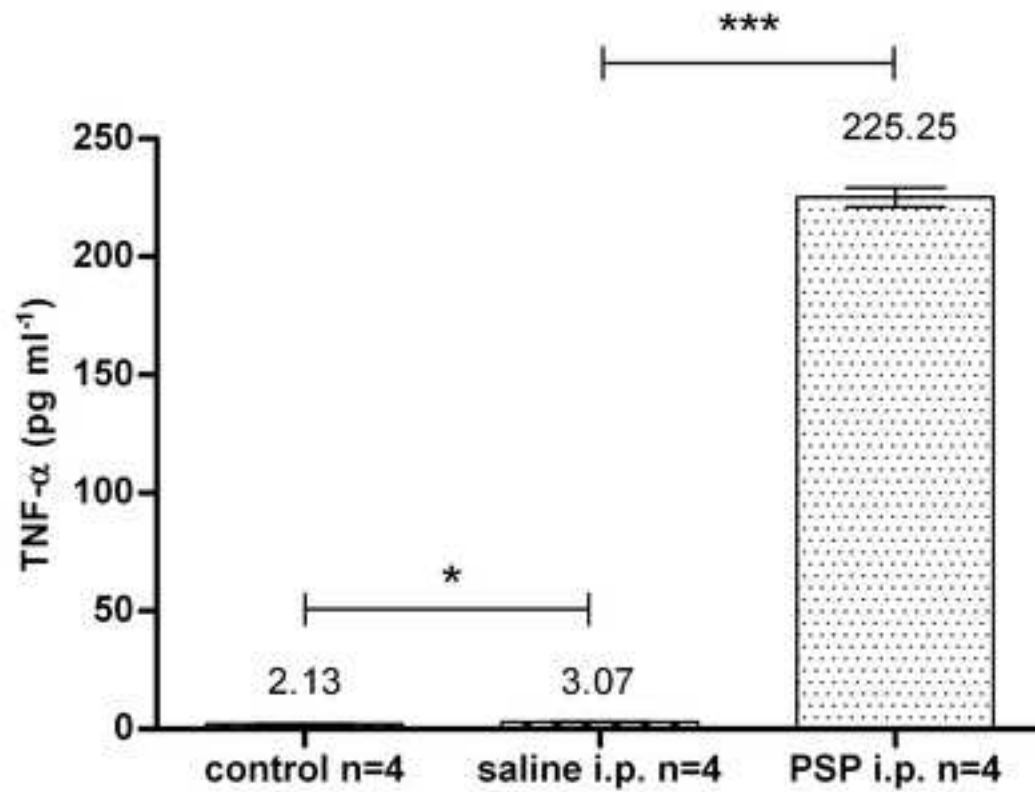


Figure 3
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