JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2011, 62, 6, 669-675
www.jpp.krakow.pl

S. WROTEK, T. JEDRZEJEWSKI, E. POTERA-KRAM, W. KOZAK

ANTIPYRETIC ACTIVITY OF N-ACETYLCYSTEINE

Department of Immunology, Institute of General and Molecular Biology, Nicolaus Copernicus University, Torun, Poland

N-acetylcysteine (NAC) has been used primarily as a mucolytic agent for the treatment of respiratory diseases. It has been recently suggested that NAC also possesses some anti-inflammatory properties. The aim of the present study is to investigate the effects of NAC on fever provoked either by bacterial lipopolysaccharide (LPS) or a turpentine-induced aseptic abscess in the rats. The body temperature (Tb) and the motor activity of the Wistar rats were measured using biotelemetry system. NAC (200 mg/kg) was injected intraperitoneally (i.p.) One hour prior to the injection of LPS (50 μg/kg; i.p.) or turpentine (100 μl/rat; subcutaneously) into separate groups of rats. The injection of NAC into normal non-febrile rats did not alter the animal circadian rhythm in Tb and activity. Pretreatment of rats with NAC resulted in the reduction of both infectious and aseptic fevers. Fever in rats was associated with inhibition of the motor activity and loss of body weight. Treatment with NAC diminished the decrease of motor activity and had no effect on the reduction of body weight in rats injected with LPS. It did, however, attenuate the drop of body mass in rats challenged with turpentine oil. Based on these data one may conclude that NAC, in addition to its mucolytic, antioxidant and anti-inflammatory properties, may be considered as a therapeutic fever-modulating agent under certain clinical circumstances.

Key words: *N-acetylcysteine, lipopolysaccharide, turpentine oil, biotelemetry, body temperature, fever, motor activity, body mass*

INTRODUCTION

N-acetylcysteine (NAC) is a widely used thiol-containing antioxidant, which has been in clinical practice since the 1960s (1). It is a safe, well-tolerated drug with no clinically significant adverse effects. NAC is chemically similar to cysteine, but in comparison with the cysteine, is less toxic, less susceptible to oxidation and it is far better soluble in water (2). Both *in vitro* and *in vivo* studies have documented that NAC acts as a donor of cysteine and precursor of glutathione (GSH) synthesis (3, 4).

NAC as a drug has been administrated orally and intravenously. The oral administration of NAC is not known to cause clinically significant adverse reactions (5), and it has been proved to be beneficial in settings where deficiency of GSH occurs, for example upon HIV infection (5-7), Alzheimer disease (8), diabetes (9), cardiac dysfunction (10) and colon cancer (11). NAC is also used commonly as a mucolytic agent for the treatment of respiratory disorders including chronic bronchitis (12), cystic fibrosis (13) and influenza-like syndromes (14). The clinical situations may often dictate the need for intravenous administration of the NAC, *e.g.*, to reduce hepatic injury during the treatment with acetaminophen, which is a well known antipyretic and anti-inflammatory specimen (15, 16). The acetaminophen dosage may cause a dramatic liver GSH depletion and permanent liver damage. NAC administration supplies the cysteine required for *de novo* synthesis of hepatic GSH, which is considered as one of the important biochemical features involved in the hepatic detoxification processes. Among others, they also include detoxification of numerous exogenous toxins and microbial products that have the potency to initiate fever.

Fever is a common response to infection, inflammation, injury and trauma (17). There are considerable data indicating that moderate fevers are beneficial to the infected host. It was demonstrated that the elevation of body temperature (Tb) from normal to a fever-like level augments number of key mechanisms of the defense against infections, such as T and B cells proliferation and differentiation, production of antibodies, phagocytic activity, secretion of interferon, and the migration of macrophages and neutrophils (18-20). On the other hand, the rise in Tb above the typical febrile levels (overpyrexia) may often be harmful to the patient (21). There are certain clinical situations in which fever is rather undesirable and can be associated with a poor medical prognosis. For instance, upon the acute stroke, fever onset during the early post-stroke period is often associated with significantly worse outcomes (22), and the usage of a liver-challenging extensive standard antipyretic medication in such cases have shown rather poor results in the reduction of body temperature to normal (23-26). Thus, laboratories worldwide currently search for additional, efficient and safer fever modulating therapeutics, which could be used in life-threatening conditions.

The aim of the present study is to investigate the effect of NAC on fever provoked either by bacterial lipopolysaccharide

(LPS), the standard exogenous pyrogen used extensively in the laboratory settings to study the mechanisms of an infectious-like fever or by a turpentine abscess which models fever due to the non-infectious (aseptic) tissue necrosis in the laboratory rats. Data presented indicate that NAC can reduce both types of fever. Since NAC has been proved safe and well tolerated by the patients during more than a half-century of its usage in clinics, one may assume that it could be considered as a drug of choice in controlling fever under certain medical circumstances, especially when the liver protection could play a critical role.

MATERIALS AND METHODS

The specific pathogen-free male Wistar rats weighing 250-300 g were used throughout the experimentation. The rats were obtained from Mossakowski Medical Research Centre Polish Academy of Sciences (Warsaw, Poland) and were housed in individual plastic cages and maintained in a temperature/humidity/light-controlled chamber set at 25±1°C, 12:12 h light:dark cycle, with light on at 07:00 a.m. The rodent laboratory food and drinking water were provided *ad libitum*. A week after the shipment, the rats were implanted under sterile conditions with biotelemetry devices to monitor the physiologic and behavioural measures. The experiments were started after 10 days of the post-surgery recovery. The body weight was monitored daily at 09:00 a.m. by weighing on a top-loading balance accurate to ±0.1 g (Radwag, Poland). Only rats showing a regular and stable 24-h body mass gaining were taken to the experiments. All experimental procedures were approved by the Local Bioethical Committee for Animal Care.

Body temperature and motor activity measurement

The deep body temperature (Tb) of the rats was measured using battery-operated telemetry transmitters (model TA-F40, Data Sciences International, U.S.A.) implanted intra-abdominally under sterile conditions according to producer's instruction. Before the implantation, the rats were anaesthetized with a mixture of ketamine (Biowet)/xylazine (ScanVet) (87 mg/kg and 13 mg/kg, respectively) injected intramuscularly. Then, following shaving and sterilization of a small abdomen surgical area, an incision was made in the skin and muscles of the abdomen, and a miniature temperature-sensitive telemetry device was placed into the peritoneal cavity. The muscle level of the abdomen and the skin were separately sutured closed. All the experiments started 10 days after the recovery from the surgical procedures. The motor activity of the rats was detected by changes in the position of the implanted temperature-sensitive transmitter over the receiver board, resulting in a change of the signal strength that was detected by the external receiver antenna and recorded as a "pulse" or "count" of the activity.

Induction of lipopolysaccharide fever in the telemetry implanted rats

Lipopolysaccharide (LPS) extracted from *Escherichia coli* (0111:B4, Sigma Chemicals) was dissolved in sterile 0.9% sodium chloride (saline). Before injection, the stock solution of LPS (2 mg/ml) was warmed to 37°C, diluted in a warm sterile saline to the desired concentration, and injected intraperitoneally (ip) at a dose of 50 μg/kg, as described previously (27). The control rats were injected i.p. with an equivalent volume of pyrogen-free saline. The rats were briefly restrained and not anesthetized during the LPS and/or saline i.p. injections. Immediately after the injections, the rats were placed in their home cages.

Induction of turpentine fever in the implanted rats

Commercial-grade steam-distilled undiluted turpentine oil (Turp) (Elissa) was injected subcutaneously (s.c.) into the left hindlimb at a volume of 100 μl/rat (28). The injections of sterile saline at a volume of 100 μl (s.c.; left hindlimb) into the separate groups of rats were used as reference. All rats were briefly anesthetized with inhaled isoflurane shortly before the injection procedure, and returned to their home cages afterwards.

N-acetylcysteine preparation and administration

N-acetylcysteine (NAC) (Sigma Chemicals) was dissolved in a 0.9% pyrogen-free sodium chloride and adjusted to pH 7.4 by the addition of 0.1 M NaOH. NAC at a dose of 200 mg/kg was injected i.p. (29) 1 h prior to the LPS and/or turpentine challenge. Solvent without NAC at an equivalent volume was used as control injection. The effects of NAC on the normal circadian rhythm in Tb and motor activity of the rats was also evaluated and compared to the non-treated free-running rats.

Data analysis

The temperature and activity data are expressed as means ±S.E. The injections were performed at time as indicated in figures. The data were recorded and computed at 5-min intervals using Data Acquisition Programme (Data Sciences International, U.S.A). For the data analysis, the excel plotting and the presentation, the temperature and the activity recordings were pooled into 30-min and 12-h averages, respectively. A Student *t*-test with a minimum level of significance set at $p<0.05$ were used for post hoc comparisons.

RESULTS

Circadian rhythm of body temperature and motor activity of rats following injection of N-acetylcysteine

Rats are nocturnal animals revealing low daytime and high nighttime Tb and motor activity. Injection of N-acetylcysteine (200 mg/kg) alone, and/or saline as control, did not affect the rats physiologic measures either. *Fig. 1* depicts changes of Tb of rats injected at 09:00 AM with NAC plotted against normal circadian rhythm of Tb in rats. The activity data followed the temperature changes, with low daytime (1.98±0.72 counts; 12-h average,) and high nighttime (5.4±0.68 counts; 12-h averages) values (data not shown).

Effect of N-acetylcysteine on lipopolysaccharides-induced fever in rats

Intraperitoneal bolus injection of the saline suspension of *E. coli* LPS at a dose of 50 μg/kg of body mass induced biphasic fever in the rat *(Fig. 2)*. Fever started within 2 hours post-injection, and the first peak of the Tb rise was reached and maintained during the next 1.5 h (Tb=37.87°C ±0.09 in LPS-treated rats versus 37.36°C ±0.04 of the NAC/NaCl-treated animals). The second peak of fever was reached within 5 h post-injection and maintained for the following 2.5 h (average Tb for the LPS-treated animals was 38.38°C ±0.15 and for NAC/NaCl-treated animals was 37.11°C ±0.02 during this time). Then, a 4 h-lasting gradual decrease of the rats Tb towards normal was observed.

Pre-treatment of the rats with NAC resulted in a significant alterations of the post-LPS Tb, that can be regarded as a reduction of the time-course and the level of fever response to

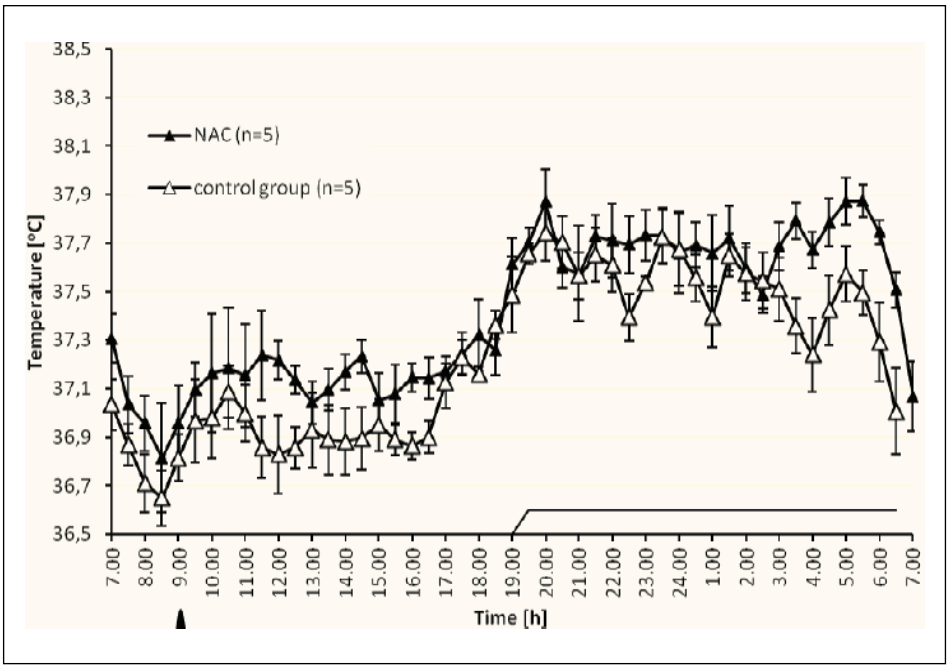

Fig. 1. Changes of body temperature over time of rats treated i.p. at 09:00 a.m. with NAC (200 mg/kg; arrowhead) and non-treated animals (control circadian rhythm). Values are means ±S.E.M. at 30-min averages. Letter n indicates sample size in each group. Black horizontal line represents lights-off in a 12:12-h light-dark cycle.

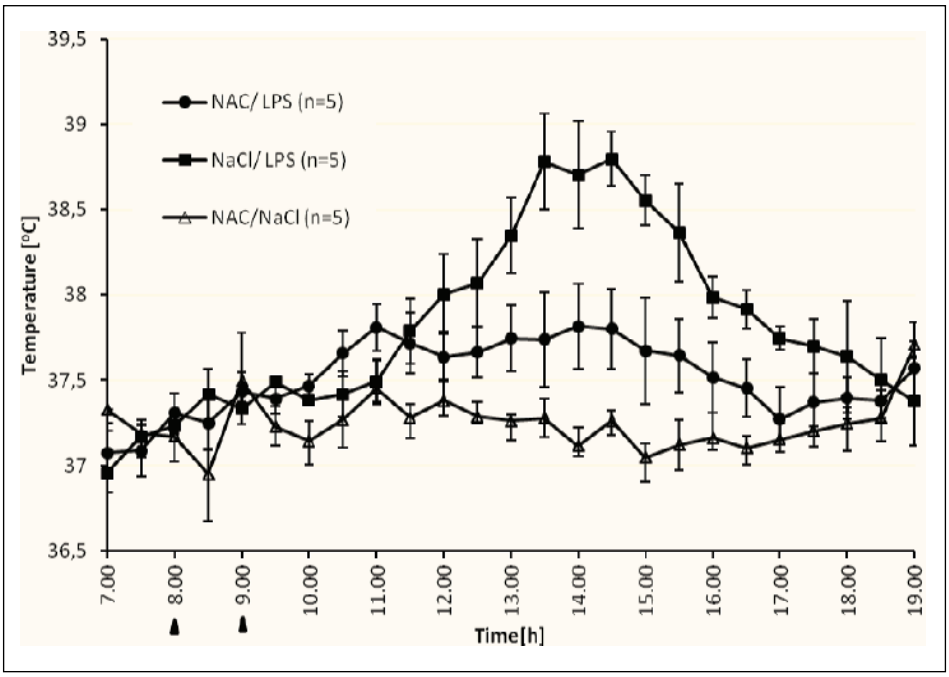

Fig. 2. Changes of body temperature of rats treated i.p. with saline and/or NAC (200 mg/kg; arrowhead at 08:00 a.m.) and then injected with saline and/or LPS (50 µg/kg; arrowhead at 09:00 a.m.). Values are means ±S.E.M. at 30-min averages. Letter n indicates sample size in a respective groups.

the administration of endotoxin. As it can be seen in *Fig. 2*, the rise of Tb in the NAC/LPS-treated rats started approximately 30 min earlier than that of the rats treated with saline/LPS, and fever sooner disappeared. Above all, however, the LPS-induced elevation of Tb in rats pre-treated with NAC never reached the level of fever seen for the saline/LPS-injected rats during the entire observation time (the average Tb for the NAC/LPS-treated animals between 2 h and 5 h post LPS-injection was 37.75°C ±0.06 *vs*. 38.31°C ±0.20 in the saline/LPS treated rats during this time).

NAC also affected the post-LPS lethargy of rats. The daytime 12-h averages of the motor activity of rats treated with NAC followed by LPS was higher by about 0,6 counts than those of saline/LPS-treated rats (data not shown).

Effect of N-acetylcysteine on fever in response to a turpentine-induced abscess

The subcutaneous administration of turpentine oil (100 µl/rat) into the left hindlimb provoked fever in rats with 6-h latency (*Fig. 3*). Within the next 4 h the animals pre-injected ip with saline prior to turpentine (NaCl/Turp group) increased Tb to 39.40°C ±0.16, while the rats pre-treated for 1 h with NAC before the sc administration of turpentine (NAC/Turp group) reached 38.40°C ±0.12 of Tb. Then, after achieving these treatment-dependent maximum levels of Tb, fever in both turpentine-injected groups lasted 6 h. It can be seen that NAC affected the upper limit of the turpentine-induced fever but it did not influence the time-course of this fever.

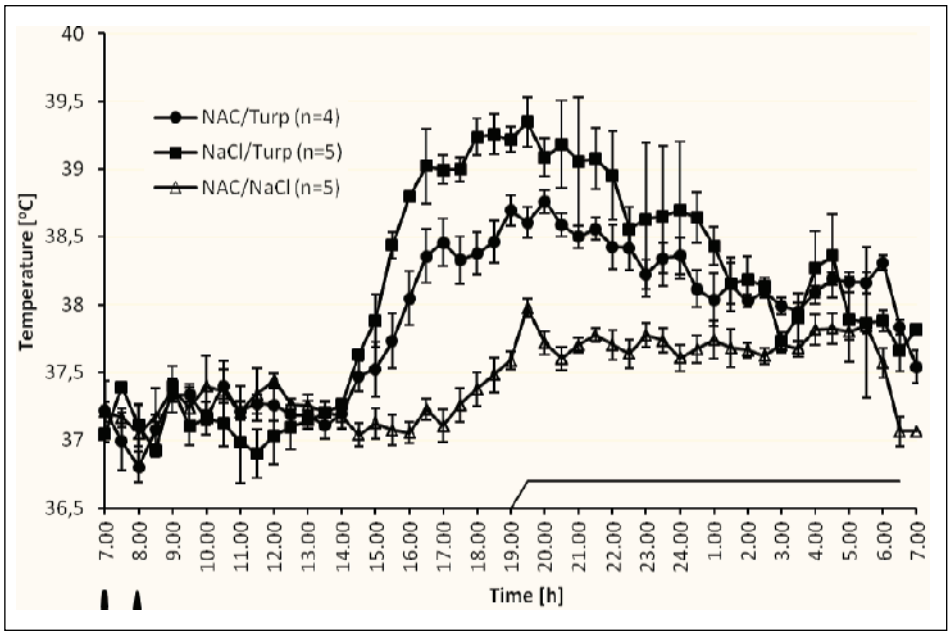

Fig. 3. Changes of body temperature of rats treated i.p. with NAC at a dose of 200 mg/kg or saline at 07:00 a.m. and then at 08:00 a.m. injected with turpentine (Turp) at a volume of 100 µl/rat. Values are means ±S.E. at 30-min averages. Letter n indicates sample size in a respective groups. Black horizontal line represents lights-off period in a 12:12-h light-dark cycle.

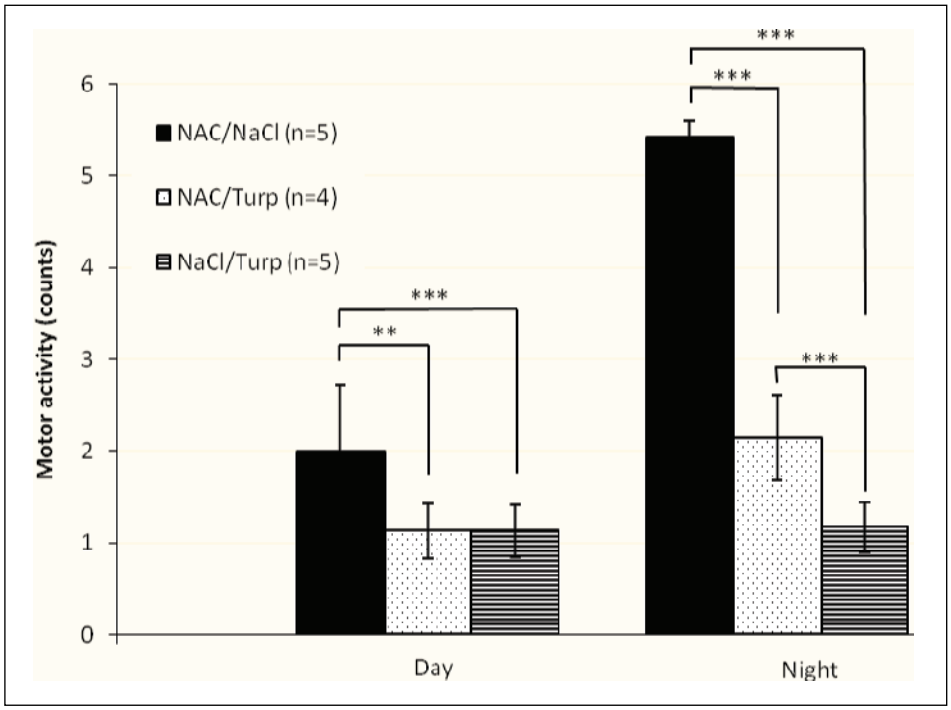

Fig. 4. 12-h averages of daytime and nighttime motor activity of rats treated i.p. with NAC and/or saline and then injected sc with turpentine (Turp) as shown in *Fig. 3.* Letter n indicates sample size in a respective groups. Asterisk indicates significant difference (**p<0.01, ***p<0.001).

NAC had no effect on post-turpentine motor activity at a daytime (lights on) of the injection (12-h averages activity in the groups of rats pre-treated with NAC and NaCl were 1.13±0.3 and 1.12±0.28, respectively, *vs*. 1.99±0.72 of NAC/saline treated rats *Fig. 4*). It diminished, however, the post-turpentine lethargy seen during the nighttime: 12-h averages of motor activity during the post-turpentine night were 2.13±0.47 counts for NAC/Turp and 1.17±0.27 counts for saline/Turp *vs*. 5.4±0.18 for NAC/saline treated animals.

Effect of N-acetylcysteine on changes of body weight in response to lipopolysaccharide and turpentine

NAC did not affect the normal body mass in rats (see *Fig. 5*). As it can be seen in *Fig. 5*, an experimental fever in the rats challenged either with LPS or turpentine was associated with significant drop of body weight measured 24-h post-treatment. NAC did not affect the decrease of body weight in the rats treated with LPS. It did, however, attenuate the drop registered in response to the turpentine-induced abscess.

DISCUSSION

These studies demonstrate for the first time that N-acetylcysteine, a specimen used primarily as a mucolytic agent for the treatment of respiratory disorders, possesses also antypiretic activity. NAC significantly attenuated fever induced by the injection of LPS, a standard pyrogenic factor in the laboratory settings which, since its initial discovery and

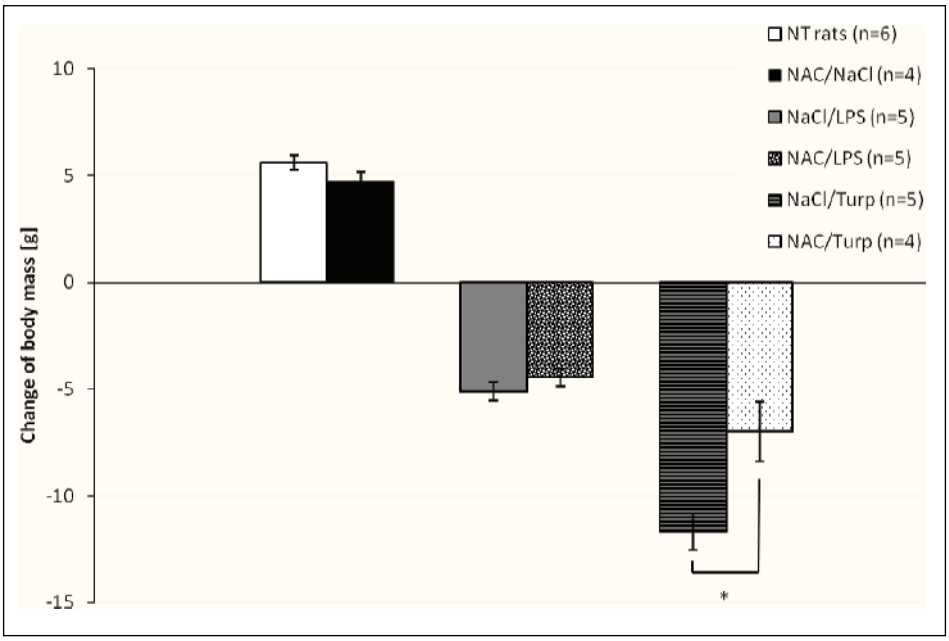

Fig. 5. 24-h changes of the body mass of rats treated as indicated. Initial body weight of the rats was taken at the injection (treatment) time and then 24 h later. NT - indicates change of weight of the control non-treated animals. Letter n indicates sample size in a respective groups. Asterisk indicates significant difference (*$p<0.05$).

extraction, has been considered the most important exogenous pyrogen acting upon bacterial Gram-negative infections (30). It also attenuated fever in response to pure turpentine oil, a tissue irritant used in the laboratories worldwide to induce fever as a result of an aseptic abscess and sterile tissue necrosis (28, 29). These two kinds of febrile responses, although of different etiology, have similar molecular mechanism; they are cytokine and prostaglandin PGE_2-dependent phenomena (31-33). Recent data indicate that injection of LPS into rats increases the production not only PGE_2 but also that of PGD_2 in the periphery (34). The molecular basis of the antypiretic action of NAC so far is a matter of speculation due to the insufficient experimental data. Relevant to this, however, are data demonstrating that NAC can inhibit the nuclear transcription factors (35, 36). In this respect, we hypothesize that antifebrile activity of NAC is due to a modulation of intracellular transcription factors including nuclear factor-κB (NF-κB) - one of the most important transcription factor which can induce proinflammatory genes expression. This conclusion may also be extended to the mechanism of an anti-inflammatory property of NAC documented recently (36). In support of the above hypothesis, it has been shown by an earlier pharmacological studies by Lee and co-workers on rabbits (37), as well as in our more recent studies using genetically engineered mice (38), that the febrile response to LPS is related to the intracellular signaling pathway focusing on the dimerisation of nuclear factors and the activation of the NF-κB. Moreover, those studies have clearly shown that NF-κB was essential for the induction of fever, since the lack of signaling through NF-κB in mice resulted in the absence of fever to the LPS challenge and the blockade of the proinflammatory interleukin-6 (IL-6) synthesis (38), a cytokine regarded as one of the most important endogenous mediators of fever in mammals (32).

Recently, numerous reports were published on the effects of NAC on inflammation. Palacio *et al.* (39) demonstrated the essential role of NAC in the regulation of proinflammatory cytokine expression. In their experiment, NAC significantly inhibited TNF-α, IL-1β and IL-6 at the protein and mRNA level, in the LPS-activated macrophage cell line THP-1 under mild oxidative conditions. In *in vivo* studies using mice Lima Trajano *et al.* (40) have shown that NAC downregulated the transcription of IL-6 and TNF-α induced by LPS administration.

A putative factor involved in the regulation of fever by the NAC is nitric oxide (NO). Kozak *et al.* (41) have reported that nNOS (neuronal NO synthase) and iNOS (inducible NO synthase) are engaged in the generation of fever in mice treated with LPS, whereas none of these NOSs participate in triggering fever induced by turpentine. Recently Pechanova *et al.* (42) demonstrated that NAC increased the brain nNOS activity in normotensive and hypertensive rats. These data suggest that NOS may be involved in antipyretic action of NAC during systemic inflammation. Taking together, one may assume that NAC provided antipyresis reflects the action of the drug on the NO syntheses and may be a consequence of its anti-oxidant/anti-inflammatory action.

Interestingly, pre-treatment with NAC did not influence the body weight loss in the rats responding to LPS in our experiments. NAC did attenuate, however, a reduction of body mass of rats challenged with turpentine oil. It indicates that cachexia upon inflammation is not a function of fever itself, and is probably mediated by a concomitant independent mechanism. On the other hand, NAC attenuated the decrease of motor activity in both types of inflammatory responses which may further suggest that aspects of the sickness behaviour such as fever, lethargy and cachexia may be regulated independently. However, the underlying mechanism needs to be tested.

In spite of its fever-lowering activity, NAC in a dosage used did not affect normal circadian rhythm of the body temperature and motor activity in rats. This effect of NAC supports the notion that the drug is relatively safe for the consumption in its therapeutic dosage ranges (1). There is a considerable number of steroidal and non-steroidal antipyretic drugs often prescribed by physicians. Some of these drugs, as we mentioned earlier, are not well tolerated by patients and are not efficient and even harmful under certain medical circumstances. Therefore, there is a need to search for substitutes which could better fit into the health status and the condition of the patient, a therapeutic application of NAC during response to various pyrogenic insults merits further investigation.

Acknowledgements: This study was supported by the Nicolaus Copernicus Intramural Grant 475-B to W. Kozak and 558-B to S. Wrotek.

Conflict of interests: None declared.

REFERENCES

1. De Vries N, De Flora S. N-Acetyl-L-cysteine. *J Cell Biochem* 1993; 17: 270-278.
2. Bonanomi L, Gazzaniga A. Toxicological, pharmacokinetic and metabolic studies on acetylcysteine. *Eur J Respir Dis Suppl* 1980; 111: 45-51.
3. Ziment I. Acetylcysteine: a drug that is much more than a mucokinetic. *Biomed Pharmacother* 1998; 42: 513-520.
4. Zafarullah M, Li WQ, Sylvester J, Ahmad M. Molecular mechanisms of N-acetylcysteine actions. *Cell Mol Life Sci* 2003; 60: 6-20.
5. De Rosa SC, Zaretsky MD, Dubs JG, *et al.* N-acetylcysteine replenishes glutathione in HIV infection. *Eur J Clin Invest* 2000; 30: 915-929.
6. Breitkreutz R, Pittack N, Nebe CT, *et al.* Improvement of immune functions in HIV infection by sulfur supplementation: two randomized trials. *J Mol Med* 2000; 78: 55-62.
7. Spada C, Treitinger A, Reis M, *et al.* The effect of N-acetylcysteine supplementation upon viral load, CD4, CD8, total lymphocyte count and hematocrit in individuals undergoing antiretroviral treatment. *Clin Chem Lab Med* 2002; 40: 452-455.
8. Adair JC, Knoefel JE, Morgan N. Controlled trial of N-acetylcysteine for patients with probable Alzheimer's disease. *Neurology* 2001; 57: 1515-1517.
9. De Mattia G, Bravi MC, Laurenti O, *et al.* Reduction of oxidative stress by oral N-acetyl-L-cysteine treatment decreases plasma soluble vascular cell adhesion molecule-1 concentrations in non-obese, non-dyslipidaemic, normotensive, patients with non-insulin-dependent diabetes. *Diabetologia* 1998; 41: 1392-1396.
10. Svendsen JH, Klarlund K, Aldershvile J, Waldorff S. N-Acetylcysteine modifies the acute effects of isosorbide-5-mononitrate in angina pectoris patients evaluated by exercise testing. *J Cardiovasc Pharmacol* 1989; 13: 320-323.
11. Estensen RD, Levy M, Klopp SJ, *et al.* N-Acetylcysteine suppression of the proliferative index in the colon of patients with previous adenomatous colonic polyps. *Cancer Lett* 1999; 147: 109-114.
12. Parr GD, Huitson A. Oral fabrol (oral N-acetyl-cysteine) in chronic bronchitis. *Br J Dis Chest* 1987; 81: 341-348.
13. Ratjen F, Wonne R, Posselt HG, Stover B, Hofmann D, Bender SW. A double-blind placebo controlled trial with oral ambroxol and N-acetylcysteine for mucolytic treatment in cystic fibrosis. *Eur J Pediatr* 1985; 144: 374-378.
14. Gillissen A, Nowak D. Characterization of N-acetylcysteine and ambroxol in anti-oxidant therapy. *Respir Med* 1998; 92: 609-623.
15. Prescott LF, Park J, Ballantyne A, Adriaenssens P, Proudfoot AT. Treatment of paracetamol (acetaminophen) poisoning with N-acetylcysteine. *Lancet* 1977; 2: 432-434.
16. Prescott L. Oral or intravenous N-acetylcysteine for acetaminophen poisoning? *Ann Emerg Med* 2005; 45: 409-413.
17. Kozak W, Kluger MJ, Tesfaigzi J, *et al.* Molecular mechanisms of fever and endogenous antipyresis. *Ann NY Acad Sci* 2000; 917: 121-134.
18. Kluger MJ, Kozak W, Conn CA, Leon LR, Soszynski D. The adaptive value of fever. *Infec Dis Clin North Am* 1996; 10: 1-21.
19. Roberts NJ. Impact of temperature elevation of immunologic defense. *Rev Infect Dis* 1991; 13: 462-472.
20. Roberts NJ. The immunological consequences of fever. In: Fever: Basic Mechanisms and Management. Mackowiak P.A. (ed). Raven Press, New York 1991; p.125.
21. Kluger MJ, Ringler DH, Anver MR. Fever and survival. *Science* 1975; 188: 166-168.
22. Wrotek SE, Kozak WE, Hess DC, Fagan SC. Treatment of fever after stroke: conflicting evidence. *Pharmacotherapy* 2011; 31: 1085-1091.
23. Sulter G, Elting JW, Maurits N, Luijckx GJ, De Keyser J. Acetylsalicylic acid and acetaminophen to combat elevated body temperature in acute ischemic stroke. *Cerebrovasc Dis* 2004; 17: 118-122.
24. Dippel DW, van Breda EJ, van der Worp HB, *et al.* Effect of paracetamol (acetaminophen) and ibuprofen on body temperature in acute ischemic stroke PISA, a phase II double-blind, randomized, placebo-controlled trial [ISRCTN98608690]. PJ; PISA-Investigators. *BMC Cardiovasc Disord* 2003; 3: 2.
25. Kasner SE, Wein T, Piriyawat P, *et al.* Acetaminophen for altering body temperature in acute stroke: a randomized clinical trial. *Stroke* 2002; 33: 130-134.
26. Koennecke HC, Leistner S. Prophylactic antipyretic treatment with acetaminophen in acute ischemic stroke: a pilot study. *Neurology* 2001; 57: 2301-2303.
27. Kozak W, Klir JJ, Conn CA, Kluger MJ. Attenuation of lipopolysaccharide fever in rats by protein kinase C inhibitors. *Am J Physiol* 1997; 273: R873-R879.
28. Lim CL, Wilson G, Brown L, Coombes JS, Mackinnon LT. Pre-existing inflammatory state compromises heat tolerance in rats exposed to heat stress. *Am J Physiol Regul Integr Comp Physiol* 2007; 292: R186-R194.
29. Bijarnia RK, Kaur T, Singla SK, Tandon C. Oxalate-mediated oxidant-antioxidant imbalance in erythrocytes: role of N-acetylcysteine. *Hum Exp Toxicol* 2009; 28: 245-251.
30. Bennett IL Jr, Petersdorf RG, Keene WR. Pathogenesis of fever: evidence for direct cerebral action of bacterial endotoxins. *Trans Assoc Am Physicians* 1957; 70: 64-73.
31. Cooper AL, Brouwer S, Turnbull AV, *et al.* Tumor necrosis factor-alpha and fever after peripheral inflammation in the rat. *Am J Physiol* 1994; 267: 1431-1436.
32. Kozak W, Kluger MJ, Soszynski D, Conn CA, *et al.* IL-6 and IL-1 beta in fever. Studies using cytokine-deficient (knockout) mice. *Ann N Y Acad Sci* 1998; 856: 33-47.
33. Kozak W, Soszynski D, Rudolph K, Conn CA, Kluger MJ. Dietary n-3 fatty acids differentially affect sickness behavior in mice during local and systemic inflammation. *Am J Physiol* 1997; 272: 1298-1307.
34. Gao W, Schmidtko A, Wobst I, Lu R, Angioni C, Geisslinger G. Prostaglandin D_2 produced by hematopoietic prostaglandin D synthase contributes to LPS-induced fever. *J Physiol Pharmacol* 2009; 60: 145-150.
35. Schubert SY, Neeman I, Resnick N. A novel mechanism for the inhibition of NFκB activation in vascular endothelial cells by natural antioxidants. *FASEB J* 2002; 16: 1931-1933.
36. Paintlia MK, Paintlia AS, Singh AK, Singh I. Attenuation of lipopolysaccharide-induced inflammatory response and phospholipids metabolism at the feto-maternal interface by N-acetyl cysteine. *Pediatr Res* 2008; 64: 334-339.
37. Lee JJ, Huang WT, Shao DZ, Liao JF, Lin MT. Blocking NF-κB activation may be an effective strategy in the fever therapy. *Jpn J Physiol* 2003; 53: 367-375.
38. Kozak W, Wrotek S, Kozak A. Pyrogenicity of CpG-DNA in mice: role of interleukin-6, cyclooxygenases and nuclear factor-κB. *Am J Physiol* 2006; 290: R871-R880.

39. Palacio JR, Markert UR, Martinez P. Anti-inflammatory properties of N-acetylcysteine on lipopolysaccharide-activated macrophages. *Inflamm Res* 2011; 60: 695-704.
40. Lima Trajano ET, Sternberg C, Caetano M, *et al*. Endotoxin-induced acute lung injury is dependent upon oxidative response. *Inhal Toxicol* 2011; 23: 918-926.
41. Kozak W, Kozak A. Differential role of nitric oxide synthase isoforms in fever of different etiologies: studies using Nos gene-deficient mice. *J Appl Physiol* 2003; 94: 2534-2544.
42. Pechanova O, Kunes J, Dobesova Z, Vrankova S, Zicha J. Contribution of neuronal nitric oxide (NO) synthase to N-acetylcysteine-induced increase of NO synthase activity in the brain of normotensive and hypertensive rats. *J Physiol Pharmacol* 2009; 60(4): 21-5.

Received: March 21, 2011
Accepted: November 23, 2011

Author's address: Prof. Wieslaw Kozak, Department of Immunology, Nicolaus Copernicus University, 9 Gagarina Street, 87-100 Torun, Poland; Phone: +48 56 611 2025; E-mail: wkozak@umk.pl