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 diseases 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 infective-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 intraperitoneally 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. A week after the implantation, 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.

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 anaesthetized 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 into the separate groups of rats were used as reference. All rats were briefly anaesthetized 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. I 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...
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.
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 antipyretic 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
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 PGE2-dependent phenomena (31-33). Recent data indicate that injection of LPS into rats increases the production not only PGE2 but also that of PGD2 in the periphery (34). The molecular basis of the antipyretic 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 infection 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 stereoidal and non-stereoidal 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.

![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).](image-url)
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