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4-Thiouridine induces dose-dependent reduction of oedema, leucocyte influx and tumour necrosis factor in lung inflammation

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Summary

Recent reports demonstrate a role for nucleotides as inflammatory modulators. Uridine, for example, reduces oedema formation and leucocyte infiltration in a Sephadex-induced lung inflammation model. Tumour necrosis factor (TNF) concentration was also reduced. Previous *in vivo* observations indicated that 4-thiouridine might have similar effects on leucocyte infiltration and TNF release. The aim of this study was thus to investigate the effects of 4-thiouridine in greater detail. We used a Sephadex-induced acute lung inflammation model in Sprague–Dawley rats. The dextran beads were instilled intratracheally into the lungs, which were excised and examined after 24 h. Sephadex alone led to massive oedema formation and infiltration of macrophages, neutrophils and eosinophils. Microgranulomas with giant cell formations were clearly visible around the partially degraded beads. A significant increase in bronchoalveolar lavage fluid (BALF) content of TNF and leukotrienes was also seen. 4-Thiouridine co-administration affected all variables investigated in this model, i.e. oedema, microscopic and macroscopic appearance of lung tissue, total leucocyte and differential leucocyte counts in BALF, TNF and leukotrienes C_4 (LTC_4), LTD_4 and LTE_4 in BALF, indicating a reproducible anti-inflammatory effect. In conclusion, we have demonstrated that 4-thiouridine has anti-inflammatory effects similar to those of uridine. To our knowledge, this is the first demonstration of pharmacological 4-thiouridine effects *in vivo*. The results suggest nucleoside/nucleotide involvement in inflammatory processes, warranting further studies on nucleoside analogues as attractive new alternatives in the treatment of inflammatory diseases.

Keywords: 4-thiouridine, inflammation, leukotriene, TNF, uridine

Introduction

Nucleobases and their derivatives are known to play multiple roles in cells, e.g. as building blocks of RNA or as chemical energy carriers. Evidence is also accumulating regarding nucleotides as inflammatory modulators. Nucleotides are released excessively during inflammation, both from degranulating platelets and leucocytes. They act subsequently through the binding to and activation of P2 receptors; in the case of uridine nucleotides, so-called P2Y receptors. These are G-protein-coupled receptors, mainly of the $G_{i/q}$ type, activation of which lead to either adenylyl cyclase inhibition ($G_{i\alpha}$) or phospholipase C beta (PLC-β) activation and intracellular Ca^{2+} flux ($G_{q\alpha}$) [1]. Mammalian cells express at least eight different P2Y receptor subtypes, of which $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{14}$ are expressed in lung tissue [2]. The former three are activated by uridine di- or triphosphate (UDP, UTP), the latter by UDP-glucose.

Uridine nucleotides, especially UTP, have received increasing attention in the treatment of cystic fibrosis, asthma and chronic obstructive pulmonary disease [3], due greatly to their effects on airway mucociliary clearance. Exogenous uridine reduced inflammatory parameters such as oedema and leucocyte infiltration in a Sephadex-induced lung inflammation model, effects which were attributed to the nucleotides resulting from uridine metabolism [4]. Reduced concentrations of tumour necrosis factor (TNF), a central inflammatory mediator in many diseases, were also observed in our model.

The Sephadex model has been used in parallel with allergen-induced lung inflammation models by many groups during the last decades. Although the Sephadex model can be classified as an acute inflammation model, in several respects it shows similarity in inflammatory profile to clinical asthma [5,6]. An eosinophil-dominated lung inflammation arises within 24 h of intratracheal Sephadex instillation without the use of adjuvant or prior sensitization to allergens. Apart from the marked eosinophil component of the cellular infiltrate, prominent features are rapid development of oedema, granuloma formation with multi-nucleated giant cells and expression of different cytokines, e.g. TNF. Some groups also report strain-related airway hyperreactivity in response to Sephadex [7], but this was not investigated in the current setting.

4-Thiouridine differs from uridine only by the substitution of the 4′ oxygen with a sulphur atom, and is thus structurally almost identical. While 4-thiouridine is present in bacterial tRNA, it is non-endogenous to mammalian cells. Depending upon the capacity of mammalian cells to handle the analogue, it could be hypothesized that the effects might differ from those of uridine. A preliminary *in vivo* study indicated that 4-thiouridine might have even stronger effects on inflammatory cell infiltration and TNF release [8].

In addition to the inflammatory variables described above, we investigated whether cysteinyl leukotrienes (cysLTs) were affected. Parallel reports support a connection between nucleotide and cysLT pathways, in particular papers from Abbracchio and colleagues [3,9–15], who have identified the orphan receptor GPR17 as a dual uracil nucleotide/cysLT receptor and described possible feedback mechanisms between cysLT receptor and P2Y receptor signalling.

Materials and methods

Study design

The study design, materials and methods follow the procedures described in Evaldsson *et al.* [4]. Rats were provoked with Sephadex intratracheally to induce an eosinophil-dominated lung inflammation without prior sensitization. Lungs were excised after 24 h for analysis and lavage. Bronchoalveolar lavage fluid (BALF) was used for cell count, differential counts and TNF and leukotriene LTC_4, LTD_4, LTE_4 enzyme-linked immunosorbent assay (ELISA) analysis. In addition to these variables, tissue appearance, oedema formation, blood smears and thymus weight were also studied.

Animals

Male Sprague–Dawley rats (175–200 g, 5–7 weeks of age) from Scanbur BK AB (Sollentuna, Sweden), were housed at the Linköping University animal department. Animals were acclimatized for at least a week before inclusion. Food and water were supplied *ad libitum*. The Swedish National Board for Laboratory Animals (CFN) guidelines were followed and experimental procedures were approved by the local ethics committee in Linköping (D.no. 25-04).

Lung inflammation model

A Sephadex (G-200 superfine; Pharmacia, Sweden) suspension was prepared with pharmacological grade sterile 0·9% NaCl the day before instillation. For co-administration, 4-thiouridine (Sigma-Aldrich Chemical Co., St Louis, MO, USA) was diluted separately at twice the final concentration and mixed with an equal part of double-concentration Sephadex just prior to instillation. Sephadex and 4-thiouridine were, according to the providers, both produced and delivered in such a way as to ensure that they were free from endotoxin.

Day 1. Rats were weighed, then anaesthetized by Isoflurane (Forene; Omnia Mutantur, Helsingborg, Sweden) inhalation for a few minutes. Under anaesthesia, animals were instilled intratracheally with 1 ml/kg of saline, Sephadex (5 mg/ml), 4-thiouridine (50 mg/ml) or a combination of Sephadex and 4-thiouridine (50, 25, 12·5 or 6·25 mg 4-thiouridine/ml). Animals were supervised until fully awake and active.

Day 2. Rats were weighed again and euthanized with 250 mg/kg of sodium pentobarbital.

Blood smears were made using whole blood from heart puncture. Lungs and thymus were excised, rinsed and weighed separately. A bronchoalveolar lavage was performed on lungs from all animals. Lungs were washed once by intratracheal instillation of lavage fluid, which was withdrawn after 60 s using a syringe. In the initial experiments, lungs were lavaged with 5 ml of NaCl/Xylocain [3·33 mg Xylocain® (AstraZeneca, Södertälje, Sweden)/ml 0·9% NaCl solution]. In later experiments, lavages were performed using the same amount of phosphate-buffered saline instead, with comparable results. As the volume of lavage fluid obtained varied between the animals, all data based on counts or detection in BALF has been given per ml BALF. BALF was kept on ice until further use. Lungs were preserved in formalin.

The BALF was centrifuged at 400 ***g*** and 4°C for 5 min before total leucocyte counts (TLCs) and cell smears (Cytospin®) for differential counts were made. Because cells from Sephadex-treated rats appeared more fragile, the Cytospin method was preferred above a manual smearing technique. BALF supernatants were frozen (−20°C) until further use. Blood and lavage cell smears were fixed in methanol for 15–20 min.

Cytospin smears were stained in May–Grünewald solution (Merck, Stockholm, Sweden) for 1 min, rinsed in water and then dyed in Giemsa stain (Merck) for about 25 min.

Excised formalin-fixed lung tissue was sent to the Swedish National Veterinary Institute in Uppsala for analysis. The sections were stained with haematoxylin and eosin, Giemsa or W. Starry, the latter for detection of micro-organisms.

Lavage-fluid samples were analysed in triplicates in a TNF ELISA procedure, following the manufacturer's protocol (Quantikine rat TNF immunoassay; R&D Systems, Minneapolis, USA). LTC_4, LTD_4 and LTE_4 were detected collectively (in triplicates) with a competitive ELISA procedure, following the manufacturer's instructions (Biotrak Assay, Amersham/GE Healthcare, Uppsala, Sweden).

Statistical analysis

Experimental series 1 and 2 were performed at different time-points. Otherwise, all animals were treated strictly according to protocol. Treatment groups were compared one by one against the Sephadex control group using the non-parametric Mann–Whitney *U*-test. The exact significance was calculated with the SPSS exact tests module (SPSS version 15.0; SPSS Inc., Chicago, IL, USA) and the two-tailed value is given. Figures show box-plots where the median is represented by a line. The box, limited by the first and third quartiles, shows the interquartile range (IQR). Whiskers indicate the highest and lowest observations that are not considered outliers. Any data point more than 1·5 IQR away from the first or third quartiles is considered an outlier (O). If the distance is more than 3 (IQR), the data point is considered extreme (*).

Results

No systemic inflammation in the Sephadex model

The differential leucocyte counts in blood smears did not differ between the groups (neutrophils 8–12%, eosinophils 0–1%, monocytic cells 2–3%, lymphocytes 84–88%), indicating that the inflammation was local. Thymus weights were also compared as it is known that some drugs, especially glucocorticoids, can induce T cell apoptosis. The thymus weights did not differ (median value 0·6 g in all groups), showing that 4-thiouridine does not cause involution of the thymus and/or that it is efficiently metabolized locally. Nor did body weight differ significantly between groups or in individual rats between days 1 and 2. All groups increased or lost their weight by less than 1% between days 1 and 2, and the weight range for all groups at the time of experiment was 230–260 g.

Oedema formation reduced by 4-thiouridine

Clearly visible signs of lung oedema and inflammation were seen macroscopically in Sephadex-instilled lungs. While lungs from the vehicle control group had a smooth, light pink surface, the Sephadex-treated lungs were darker in colour, appeared swollen and displayed multiple inflammatory plaques and small spotty bleedings on the surface. The median lung wet weight in experimental series 1 increased from 1·23 g in the NaCl group to 1·65 g in the Sephadex group. Corresponding median values in the second series also increased from 1·38 g and 1·96 g. The fluid influx caused by Sephadex thus corresponded to a 30–40% increase in lung wet weight.

Co-treatment of Sephadex and 4-thiouridine reduced both the number and size of plaques, especially at higher 4-thiouridine doses; 50 mg/kg led to a significant reduction of the oedema (series 1, median lung weight 1·43 g, $P < 0{\cdot}001$), and the effect appeared to diminish dose-dependently (Fig. 1).

Histopathology

To identify the cells of the inflammatory plaques and examine the tissue distribution of different cell types, microscopy analysis was performed on lung sections from the different controls and treatment groups.

Some lung sections from the NaCl (vehicle) control group displayed only small increases in inflammatory cells. These

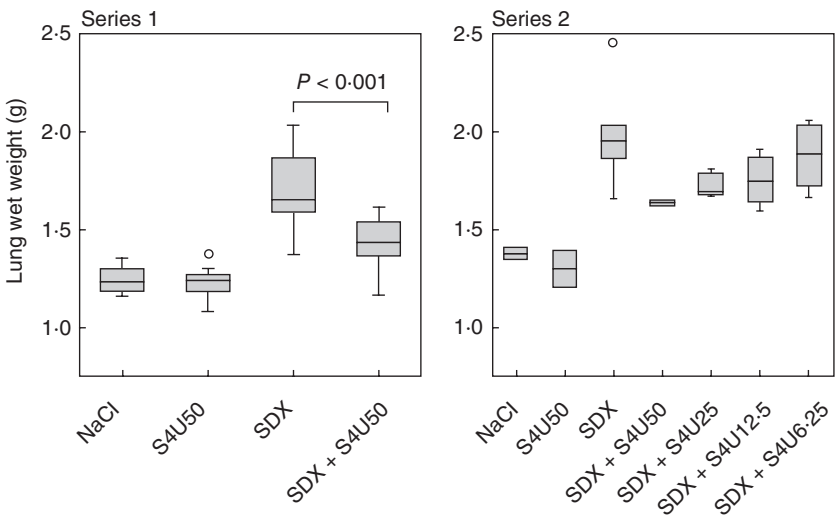

Fig. 1. Lung weight changes (median values) in response to Sephadex and 4-thiouridine. Lungs were excised 24 h after instillation and cleared of all tissues except lung lobes and trachea before weighing (wet weights). All statistical comparisons are made against the Sephadex control group with the non-parametric Mann–Whitney *U*-test. S4U = 4-thiouridine, SDX = Sephadex (5 mg/kg); 50, 25, 12·5 and 6·25 are the administered doses of 4-thiouridine, in mg/kg. Number (*n*) of animals in series 1 (from left to right) are 12, 11, 15 and 10 respectively. In series 2, group *n* equals 2, 2, 5, 2, 5, 4 and 4 respectively. The box-plot denotes median values (line) and outliers are represented with an O.

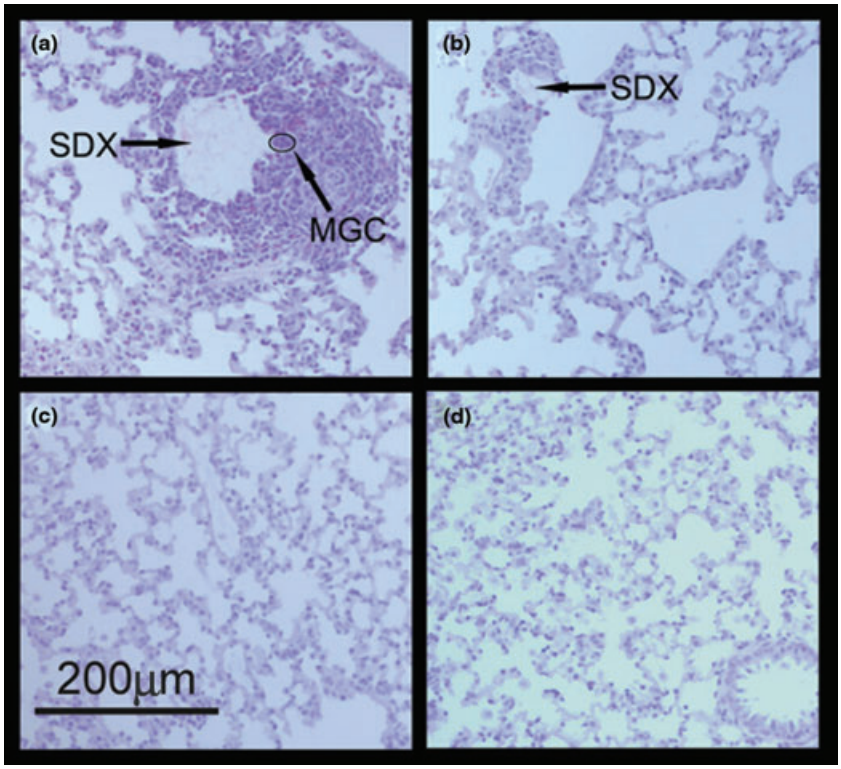

Fig. 2. Haematoxylin-stained lung sections from Sprague–Dawley rats. Animals had been instilled intratracheally with (a) Sephadex (SDX, 5 mg/kg), (b) SDX (5 mg/kg) and 4-thiouridine (25 mg/kg), (c) NaCl (vehicle control) and (d) 4-thiouridine only (50 mg/kg). Sections (a) and (b) show the typical granulomas that were seen associated with partially degraded Sephadex beads, indicated with arrows marked SDX. In addition to macrophages, monocytes, eosinophils and occasional lymphocytes, the Sephadex-induced lesions often displayed multi-nucleated giant cells (macrophage polykaryons) with peripheral nuclei. One example is indicated with the arrow marked MGC.

were concentrated to the trachea, thus probably the result of minor damage from the instillation procedure.

Administered Sephadex beads were interspersed throughout the lungs and concentrated to alveoli and adjacent tissue, as could be expected according to the administration route. Microgranuloma formations seen around the majority of the beads were most pronounced in the Sephadex control group. The granulomas were rich in macrophages, monocytes and eosinophils with the addition of multi-nucleated giant cells (Fig. 2).

4-Thiouridine co-treatment was generally associated with smaller granulomas and a more degraded appearance of the Sephadex beads.

Bacteria were not detected in any section, which confirms Sephadex instillation as the cause of inflammatory changes.

Leucocyte profile

The cell infiltration seen microscopically was in accordance with TLCs in the BALF samples. Sephadex led to increased infiltration of leucocytes, as observed in the first series, where median values increased from $0{\cdot}10 \times 10^6$ in the NaCl, to $0{\cdot}31 \times 10^6$ per ml BALF in the Sephadex control group. Sephadex instillation thus nearly tripled the TLC compared with vehicle. Co-treatment with 4-thiouridine led to a significant reduction of this increase at 50 mg/kg (series 1, $P = 0{\cdot}005$), an effect that diminished dose-dependently (Fig. 3).

The Sephadex-induced TLC increase was explained mainly by a massive influx of eosinophils and neutrophils. Both eosinophils and neutrophils were nearly absent in the vehicle and 4-thiouridine control groups (Fig. 4) and must therefore have been attracted by Sephadex instillation. 4-Thiouridine appeared to affect the specific leucocyte infiltrations dose-dependently. Eosinophils, which were the cell type affected most clearly by Sephadex instillation, were also the cell type most affected by 4-thiouridine co-treatment. The eosinophil reduction was significant at 50 mg/kg (series 1, $P = 0{\cdot}002$) and this effect seemed stable down to 12·5 mg/kg. Neutrophil counts were affected by 4-thiouridine, apparently dose-dependently, but the effect was less pronounced, especially for counts in the second series. A dose–response effect was also noted for monocytic cells (macrophages included).

Lymphocytes were not detected in BALF from any group, which is expected as lymphocytes usually take more than 24 h to recruit.

The TNF

The TNF in BALF was one of the variables that differed most between the two experimental series. Vehicle and 4-thiouridine control animals from both series had no or negligible amounts of TNF in their lavage fluid, while Sephadex lead to a significant increase. The Sephadex group median was 25 and 138 pg/ml BALF in series 1 and 2 respectively. The corresponding range was 142 and

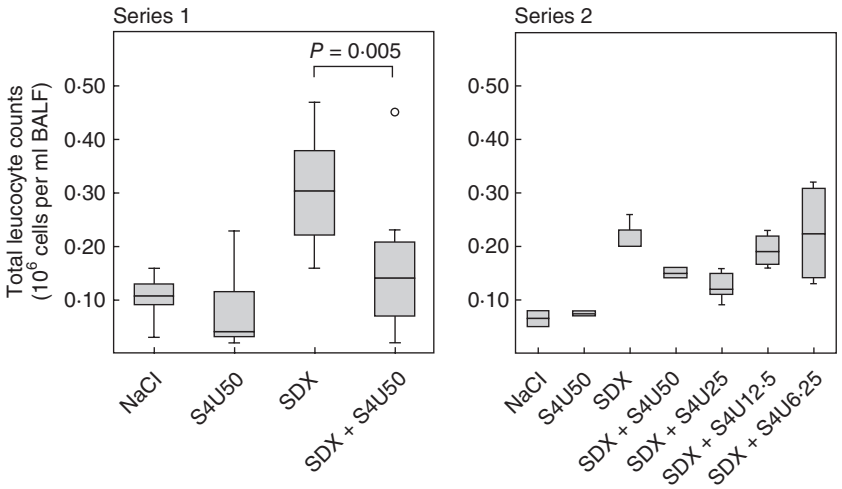

Fig. 3. Total leucocyte counts in bronchoalveolar lavage fluid. The lavage was performed in excised lungs with 5 ml of phosphate-buffered saline or a mixture of saline and xylocain. For differential counts, see Fig. 4. All comparisons are made using the non-parametric Mann–Whitney *U*-test against the Sephadex group. Median values are shown by a line within the box, O represents outliers. S4U = 4-thiouridine, SDX = Sephadex (5 mg/kg); 50, 25, 12·5 and 6·25 are the administered doses of 4-thiouridine in mg/kg. Numbers (*n*) of animals in the groups of the first series (from left to right) are 13, 11, 14, and 10 respectively. In series 2 *n* equals 2, 2, 5, 2, 5, 4 and 4 respectively.

546 pg/ml respectively, which shows that the Sephadex effect on TNF production varies considerably. Although the TNF levels are much lower than those seen in the preliminary experiments [8], 4-thiouridine co-treatment reduced TNF significantly even in the first series, where the increase was relatively small (50 mg/kg, $P = 0{\cdot}004$). At the highest 4-thiouridine dose, the median was reduced to 5 and 45 pg/ml in series 1 and 2, which corresponds roughly to a 70–80% reduction of TNF. The effect was dose-dependent (Fig. 5).

LTC_4, LTD_4 and LTE_4

LTC_4, LTD_4 and LTE_4 were measured collectively in an ELISA using an antibody binding to all three leukotrienes. Sephadex treatment led to a large increase in BALF LTC_4, LTD_4 and LTE_4. The Sephadex group median value in series 1 was 402 pg/ml BALF. Co-treatment with 4-thiouridine led to a significant decrease ($P < 0{\cdot}0001$, Fig. 5). It could also be noted that 4-thiouridine alone seems to somewhat increase the LTC_4, LTD_4 and LTE_4 levels, which is unique to this variable.

The results from series 2 were inconclusive (data not shown).

Discussion

Our previous study [4] showed that uridine, an endogenous nucleoside, could alter the inflammatory response when the levels of uracil nucleotides were increased by exogenous addition of uridine. Preliminary *in vivo* studies with 4-thiouridine [8] indicated that this non-endogenous uridine analogue might have similar effects. In the experiments described here, we have investigated the effects of 4-thiouridine on Sephadex-induced lung inflammation in greater detail.

4-Thiouridine occurs naturally in bacterial tRNA at position 8, where an oxygen in uridine is replaced post-transcriptionally with a sulphur atom [16]. The compound is not normally found within mammalian cells, but has been used experimentally for different purposes; for example, in the synthesis of artificial DNA and RNA to achieve greater thermal stability or resistance to enzymatic degradation [17] and in the studies of protein–nucleic acid interactions [17–19]. To our knowledge, this study is the first to describe pharmacological effects of 4-thiouridine *in vivo*.

Nucleosides such as uridine and 4-thiouridine are small hydrophilic molecules with low spontaneous diffusion across cell membranes. Transporter proteins ensure the cell's supply of nucleosides through the salvage pathway. In Sprague–Dawley rat lungs the concentrative nucleoside transporter (CNT) 3 and the equilibrative nucleoside transporter (ENT) 1 appear to dominate, while CNT2 and ENT2 are expressed in low amounts [20]. CNT3 and ENT1 transport both purines and pyrimidines. In the ENT permeant–transporter interaction, the sugar moiety play a major role [21,22], and a minor structural difference such as the 4′ thio base substitution is unlikely to affect transporter efficiency.

Intracellular uridine is converted subsequently by kinases into uridine-5′-mono-, di- and triphosphates (UMP, UDP, UTP), which can be used directly within the cell or exported in several ways, for example by granule release. At least two of the uridine kinases involved, UCK1 and UCK2, are capable of phosphorylating 4-thiouridine, the latter to 80% the extent of uridine [19].

Of the four P2Y receptors known to be present in lungs ($P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{14}$), $P2Y_2$ has been shown to respond to 4′-thioUTP activation in an inositol triphosphate second-messenger assay within an order of magnitude of activation by UTP [23]. From these data, and assuming that extracellular ecto-nucleotidases can cleave the 4′-thionucleotides to some extent, additional activation of $P2Y_4$, $P2Y_6$ and $P2Y_{14}$ is not inconceivable. $P2Y_2$ activation in lung epithelial cells leads generally to PLC activation via G_q, intracellular Ca^{2+} increase, increased ciliary beat frequency, increased Cl^- efflux and increased mucin release.

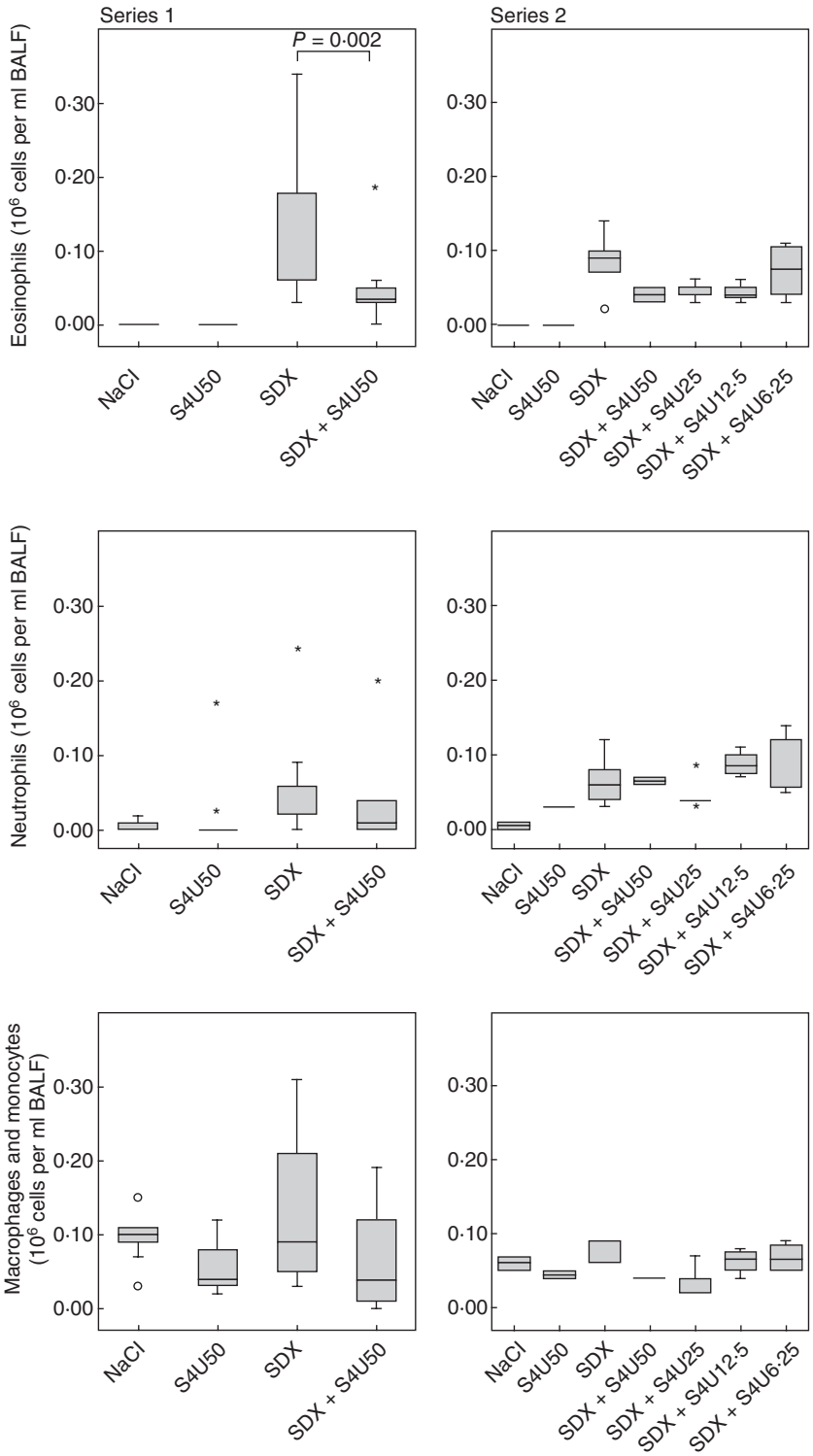

Fig. 4. Differential cell counts in bronchoalveolar lavage fluid. The counts were made on May–Grünewald-stained Cytospin smears. All comparisons are against the Sephadex control group, as calculated with the non-parametric Mann–Whitney *U*-test. S4U = 4-thiouridine, SDX = Sephadex (5 mg/kg); 50, 25, 12·5 and 6·25 are the administered doses of uridine, in mg/kg. Number (*n*) of animals in the groups of series 1 (from left to right) are 13, 11, 14 and 10 respectively. In the second series *n* equals 2, 2, 5, 2, 5, 4 and 4 respectively. The line within the box shows the median value, O represents outlier, * represents extreme value.

The results from this study clearly show anti-inflammatory effects of 4-thiouridine. The Sephadex-induced lung oedema was reduced dose-dependently, the tissue appeared less inflamed when assessed macroscopically and the Sephadex beads were generally surrounded by smaller granulomas when animals were co-instilled with 4-thiouridine. Leucocyte infiltration was also reduced by 4-thiouridine with the most visible effect on eosinophils, a cell type associated strongly with, for example, asthma and some parasite reactions. These variables are important measures of tissue inflammation as they represent the net outcome of several individual and contributing inflamma-

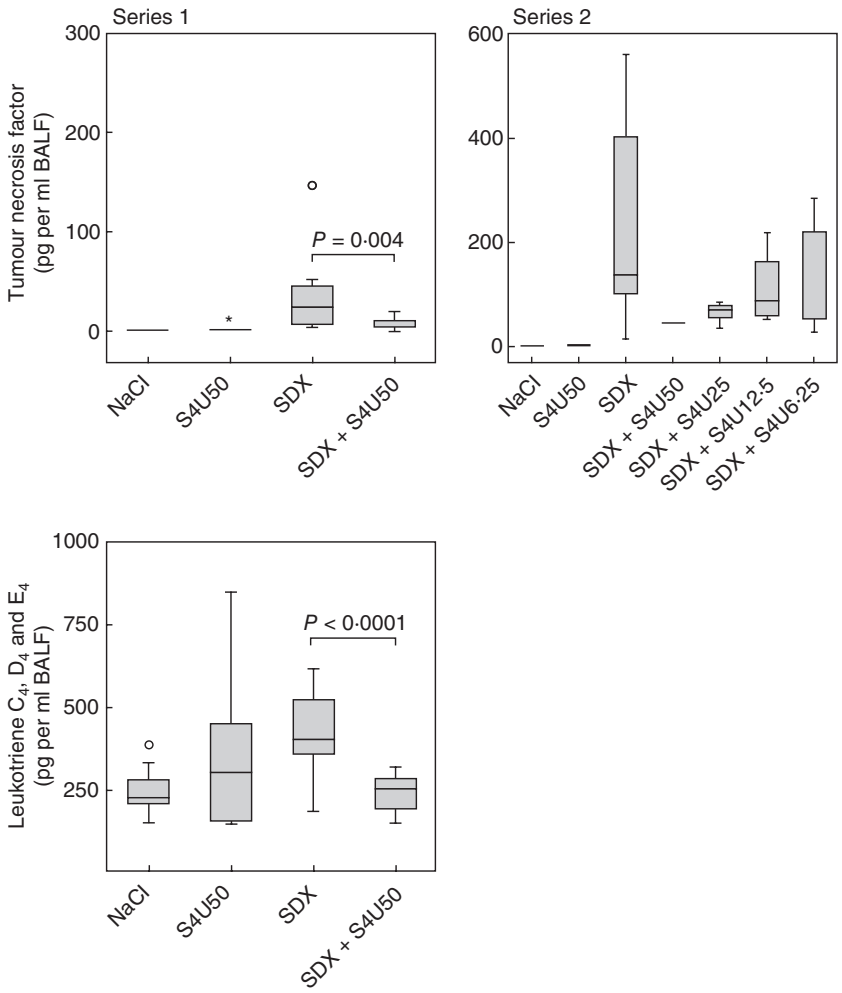

Fig. 5. Tumour necrosis factor and leukotriene (LT) release into bronchoalveolar lavage fluid, detected by enzyme-linked immunosorbent assay. Statistical comparisons were made against the Sephadex (SDX) group with the non-parametric Mann–Whitney *U*-test. With LTC_4, LTD_4 and LTE_4, the only 4-thiouridine (S4U) dose that had any effect on the SDX-induced increase was 50 mg/kg. Number (*n*) of animals in series 1 (from left to right) are 13, 11, 14 and 10 respectively. In series 2 *n* equals 2, 2, 5, 2, 5, 4, 4 and 2 respectively. The line within the box shows the median, O represents outlier, * represents extreme value.

tory mechanisms. The fact that 4-thiouridine reduces them, and generally in a slightly more pronounced manner than seen in our previous uridine experiments, is therefore very interesting. The dose–response trends are also more apparent in this study.

We chose to look at the BALF content of one cytokine (TNF) and three cysLTs (LTC_4 LTD_4 and LTE_4) on the basis that (a) a consensus has been reached regarding their importance in general inflammation and asthma and (b) they are targeted therapeutically in clinical practice.

Co-administration of 4-thiouridine (50 mg/kg) reduced the TNF release by nearly 70–80%, an effect that seems to be dose-dependent. TNF, which is a well-known inflammatory cytokine, is present for example at elevated levels both in the lavage and lung tissue of asthmatics [24]. This makes such potent inhibition highly interesting. Anti-TNF regimens have been tested by others in the Sephadex model [25,26], which reportedly led to decreases in total cellular infiltration or of individual cell types more specifically. TNF is also known to be an important eosinophil chemoattractant [27]. Our own observations of reduced total leucocyte infiltration by 4-thiouridine, and eosinophil/macrophage infiltration in particular, might thus be explained partly by the reduction in TNF.

A note could be made about the amount of TNF in the lavage samples. Compared with the BALF levels in the preliminary *in vivo* study [8] and reported lung tissue levels [28], the TNF content in BALF samples from the current study was low. They correspond well, however, with observations made by other groups using both Sprague–Dawley or Wistar rats, and where the administration routes were either intratracheal or intravenous [29–32]. The differences cannot be attributed to the ELISA method, as the same ELISA protocol was used in both the preliminary and current study. The rat strain is the same, but the animals are purchased from another supplier and the laboratory is another, which may account for a difference in environmental factors. In addition, the technique for lavage sampling differs although the sampling solutions were the same.

The roles of leukotrienes and cysLTs, in particular, are also well established in asthma. CysLTs released from several cell types including eosinophils and mast cells potently constrict the bronchi, affect oedema and aggravate mucociliary clearance [33,34]. Several recent papers [3,9–14] suggest a

connection between leukotriene and pyrimidine pathways, hence an effect of 4-thiouridine would not be unlikely. Our results showed that the highest dose of 4-thiouridine (50 mg/kg) did indeed decrease cysLTs. It is possible that the lowering of oedema could, in part, be attributed to a cysLT-lowering effect, but the results from the second experimental series were inconclusive, making it difficult to estimate what influence possible cysLT effects had on our overall results. More experiments need to be made in order to verify the effects of 4-thiouridine on cysLTs in this model.

The effects we demonstrate with 4-thiouridine generally parallels those seen in our previous uridine study [4], with significant reduction of oedema and leucocyte infiltration and an even more apparent dose-dependent reduction of TNF. This strengthens both the validity of our results with uridine and the notion of anti-inflammatory effects of uridine and uridine analogues.

In conclusion, we have shown that 4-thiouridine have anti-inflammatory effects in a rat model of Sephadex-induced lung inflammation. Oedema, leucocyte infiltration and TNF levels are reduced, which corroborates both our previous results with uridine in a similar setting [4] and our preliminary *in vivo* study performed with 4-thiouridine [8]. A coupling to leukotriene synthesis is also suggested by the results, although the effect was seen only at the highest 4-thiouridine dose. This is in line with recent reports of cysLT and pyrimidine signalling pathway interactions. The mechanisms are not investigated specifically in this study, but are likely to include those of uridine which, according to the literature, involve several different modes of action. For example, both nucleosides can be incorporated into RNA (with the thiolation possibly having an RNA stabilizing effect) or be used in energy transfer when phosphorylated, in addition to signalling through specific pyrimidine-sensitive receptors. This work adds further weight to the observation that nucleosides/nucleotides have an important role in inflammatory processes.

Acknowledgements

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