JOURNAL OF AEROSOL MEDICINE AND PULMONARY DRUG DELIVERY
Volume 22, Number 1, 2009

Pp. 000–000
DOI: 10.1089/jamp.2008.00721

Analgesic Effect from Ibuprofen Nanoparticles Inhaled by Male Mice

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Abstract

Aerosol lung administration is a convenient way to deliver water-insoluble or poorly soluble drugs, provided that small-sized particles are generated. Here, for the outbred male mice we show that the pulmonary administration of ibuprofen nanoparticles requires a dose that is three to five orders of magnitude less than that for the orally delivered particles at the same analgesic effect. The aerosol evaporation–condensation generator consisted of a horizontal cylindrical quartz tube with an outer heater. Argon flow was supplied to the inlet, and aerosol was formed at the outlet. The particle mean diameter and number concentration varied from 10 to 100 nm and 10^3–10^7 cm^{-3}, respectively. The analgesic action and side pulmonary effects caused by the inhalation of ibuprofen nanoparticles were investigated. The chemical composition of aerosol particles was shown to be identical with the maternal drug. Using the nose-only exposure chambers, the mice lung deposition efficiency was evaluated as a function of the particle diameter. The dose-dependent analgesic effect of aerosolized ibuprofen was studied in comparison with the oral treatment. It was found that the dose for aerosol treatment is three to five orders of magnitude less than that required for oral treatment at the same analgesic effect. Accompanying effects were moderate venous hyperemia and some emphysematous signs.

Key words: ibuprofen, nanoparticles, aerosol drug administration, particle lung deposition, mice, analgesic effect

Introduction

The aerosol administration represents a valuable means by which a therapeutic agent may be delivered to a patient. This way of drug delivery is used now for the treatment of both respiratory and systemic diseases.(1) In the case of systemic targeting the advantages of the aerosol delivery with respect to the peroral treatment include the possibility to avoid the losses in the gastrointestinal tract as well as metabolic destruction in the liver. In contrast to the injection therapy, the inhalation therapy is noninvasive, so it is a more convenient and safe route, leading to an improved treatment outcome. On the other hand, the aerosol treatment has no limitations for the use of water-insoluble drugs giving evident advantage with respect to the injection therapy. The representatives of the drugs with poor water solubility are indomethacin and ibuprofen. The previous paper(2) of the present authors was devoted to the anti-inflammatory effect from lung delivered indomethacin nanoparticles. It was found that the aerosol administration was much more effective than the peroral treatment. The aerosol route required a therapeutic dose six orders of magnitude less than that for peroral administration.

The present paper studies the analgesic effect from the aerosolized ibuprofen nanoparticles. Ibuprofen is a nonsteroidal, chiral, anti-inflammatory drug that inhibits the enzyme cyclooxygenase and thus acts as an analgesic.(3) It is most often prescribed to treat rheumatoid arthritis and pain. Like other

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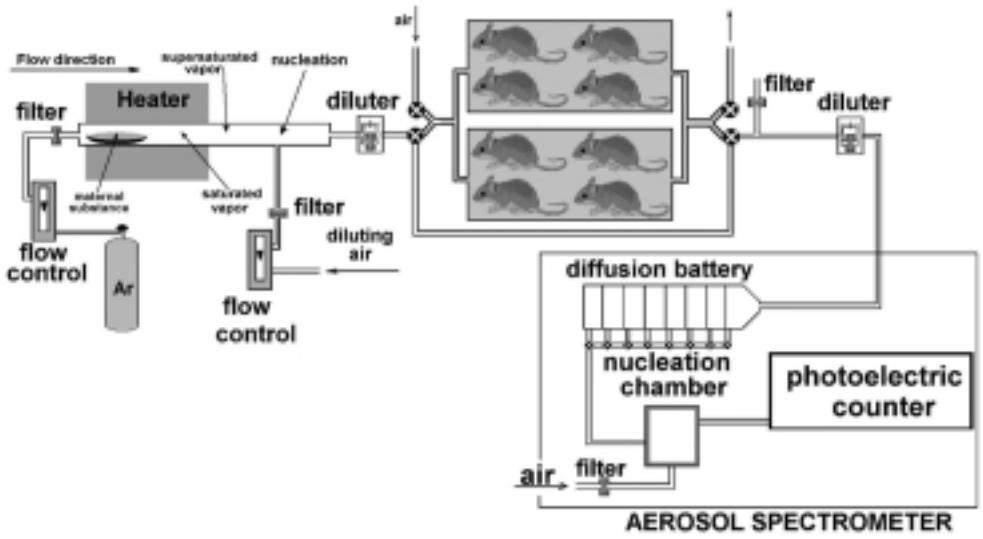

FIG. 1. Scheme of the experimental setup for inhalation experiments.

nonsteroid anti-inflammatory drugs (such as indomethacin or aspirin) its peroral treatment may result in serious disorders such as bleeding and perforation of the gastrointestinal tract, depression, drowsiness, mental disorder, increased blood pressure, congestive heart failure, etc. A possible way to diminish side effects may be a decrease of the dose delivered to the patient. The data presented here show that the lung-delivered dose of ibuprofen is a few orders of magnitude less than the peroral dose at the same analgesic effect.

Materials and Methods

The inhalation scheme includes a flow aerosol generator, plastic boxes for mice, filters, diluters, flow control equipment, and aerosol spectrometer (Fig. 1).

The horizontal evaporation–condensation aerosol generator is made of a molybdenum glass tube (with an inner diameter of 0.8 cm) with an outer heater. The generator temperature profile is shown in Figure 2. Argon flow is supplied to the inlet of the generator through the Petrianov's high efficiency aerosol filter[4] at the rate of 8 cm^3/sec (at standard temperature and pressure). The original substance (racemic ibuprofen from Ratiopharm, Germany) is put to the hot zone inside the tube. The saturated vapor is formed inside the generator. The temperature drops down at the outlet of the heated zone resulting in vapor supersaturation. The flow of argon + supersaturated vapor of ibuprofen is mixed with the air flow (with the ratio 1:13, respectively) downstream resulting to the homogeneous nucleation. Then the aerosol is diluted up to the necessary concentration using an aerosol diluter (based on flow splitting, filtering one of the subflows and mixing the subflows again). The final aerosol is admitted into the plastic whole-body (WB) exposure chambers (each of 1500 cm^3 volume). Two chambers were used in parallel; thus, the aerosol flow rate was about 55 cm^3/sec in each chamber. The temperature in the chambers was 295 K, which

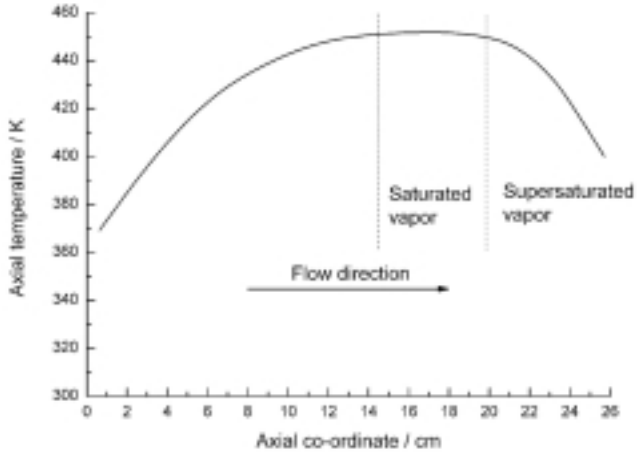

FIG. 2. Axial temperature profile in aerosol generator.

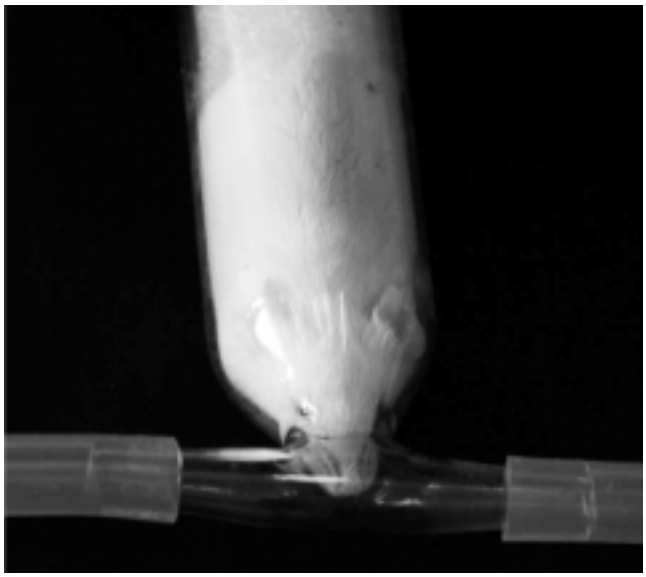

FIG. 3. Nose-only exposure chamber.

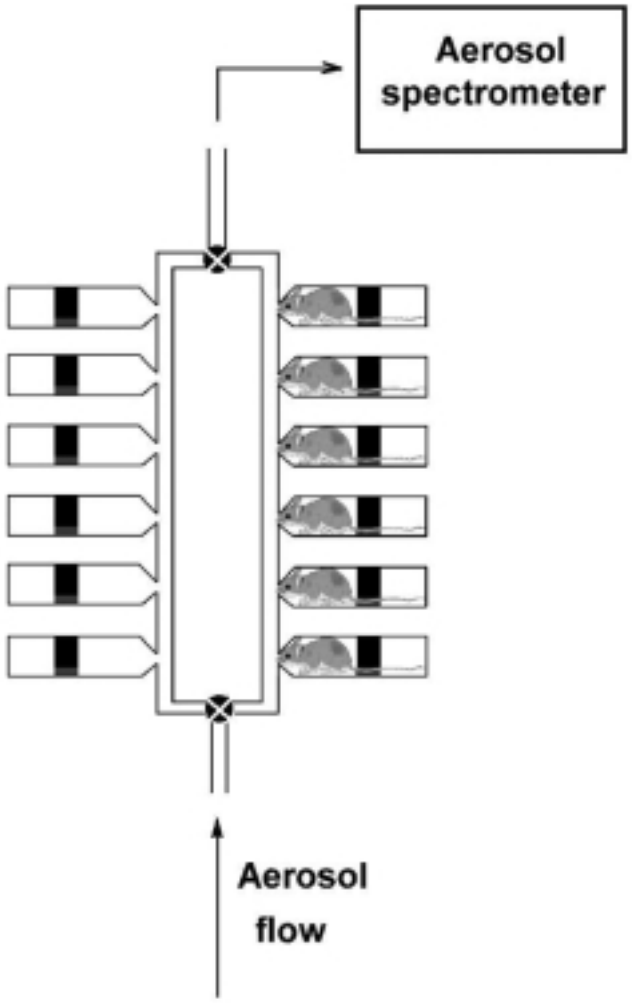

FIG. 4. Scheme of the experimental setup for the lung-deposited dose measurements.

corresponded to the room temperature, and the relative humidity was in the range of 50–70%. Each chamber contained four mice during the experiment. The outbred laboratory male mice were used. Their age and weight were 12 weeks and 25 to 30 g, respectively. The inhalation time in all the experiments was 20 min. The mice were free to move along the chamber during the aerosol exposure. The experiments with mice were provided in the Pharmacology Research Laboratory (Institute of Organic Chemistry, Novosibirsk) which was accredited as satisfying to the international standards ISO/IEC 17025-2000, approval code ROSS RU.0001.514430; No. 000269. All studies were carried out in accordance with the Guideline for the Care and Use of Laboratory Animals (Geneva Convention for the Protection of Animals, 1986).

The aerosol concentration and size distribution were measured with the aerosol spectrometer designed and built at the Institute of Chemical Kinetics and Combustion, Novosibirsk, Russia.[5] This aerosol spectrometer consists of an automatic diffusion battery, condensation chamber, and photoelectric counter. The spectrometer measured the aerosol number concentration and particle size distribution at the chamber inlet and outlet during the exposure.

The UV absorption spectra of ibuprofen solution in ethanol were recorded (with Shimadzu UV-2401PC spectrophotometer, within wavelength region of 190–900 nm) both for the nanoparticles formed by evaporation–condensation and for the original ibuprofen substance. To analyze the nanoparticles, the aerosol was passed through the glass fiber filters (Shleicher & Schuell, GF 6). Then the deposit was dissolved in ethanol.

The chemical composition of ibuprofen nanoparticles was analyzed by means of high-performance liquid chromatography with Milikhrom-1 coupled to a computer through a 14-bit analog-to-digital converter. The eluent was acetonitrile : water = 1:3; sample volume 40 μL; UV detection at the wavelength of 264 nm; column: KAKh-2 filled with reverse-phase sorbent Nucleosil C18 (5 μm); elution rate: 100 μL/min.

The crystal phase analysis of nanoparticles was carried out using X-ray diffractometer system Bruker-AXS D8 Discover with a GADDS Area Detector.

Special experiments were carried out to determine the lung-deposited dose. Nose-only exposure (NOE) glass chambers were used to minimize the skin or fur effect. The laboratory animals were confined so that only nose was exposed to the aerosol (Fig. 3). The aerosol flow was switching between two parallel lines (Fig 4). One line was loaded with

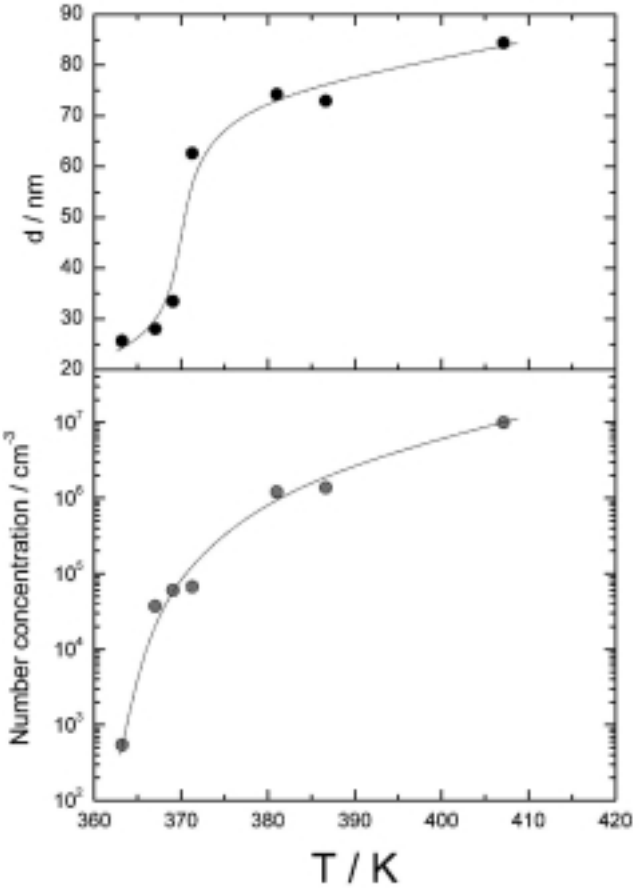

FIG. 5. Mean particle diameter and number concentration versus temperature in the saturated vapor zone (as measured by the aerosol spectrometer).

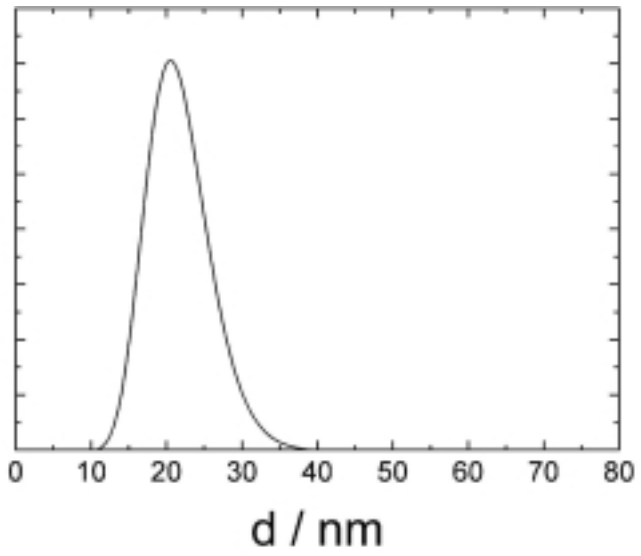

FIG. 6. Typical diameter distribution for ibuprofen nanoparticles (as measured by the aerosol spectrometer).

the animals; the other one had empty chambers. Each line contained six chambers in tandem. The aerosol depletion due to mice breathing was determined by comparing the ibuprofen particle concentration at the outlets of loaded and unloaded lines as measured by the aerosol spectrometer.

The analgesic effect of ibuprofen was estimated in "acetic acid writhing" test.[6] Each animal was used only once in the inhalation procedure and sacrificed at the end of the experiment. The mice were separated into three groups. The animals of the first group (untreated) were not exposed to ibuprofen (neither orally nor by aerosol inhalation); the animals of the second group (oral) were treated orally with the water–Tween suspension of ibuprofen with the dose varied from 8×10^{-3} to 170 mg per kg bodyweight (bw); the animals from the third group (aerosol group) were subjected to the aerosol inhalation. The mice from all the three groups were put to the WB chambers for 20 min. The mice from the first and the second groups were exposed to pure air; the mice of the third group were exposed to the aerosol (the average lung-deposited mass was determined from the NOE chamber experimental data using the ibuprofen density of 1.1 g/cm^3).[7] One hour after the chamber exposure, 0.1 mL of 0.75% acetic acid solution in water was injected intraperitoneally to all animals. Five minutes after the injection, the number of writhes (i.e., abdominal constriction followed by dorsiflexion and stretching of hind limbs) occurring during a 3-min period was measured. Each animal was observed by one observer. The observations were performed blindly with respect to the treatment regime.

A histologic analysis was performed to observe the ibuprofen aerosol effect on the mice lungs morphology. The mice were killed 6.3 h after the exposure. Lungs were fixed in 4% paraformaldehyde in the phosphate buffer (pH 7.2–7.4). The fixed tissues were treated in a standard way using histological equipment "MICROM" (Carl Zeiss, Germany) and then embedded to paraffin. Sections of 3–4 μm-thick were stained with hematoxylin and eosin. Slides were examined under the light microscope Axioskop 40 (Carl Zeiss).

Results and Discussion

Aerosol size, concentration, and composition

The mean particle size and number concentration (as measured at the WB chamber inlet) are shown in Figure 5 as a function of generator temperature. As seen in the plot, the range of the particle diameter is 20–100 nm under the standard operating conditions. Changing the vapor to air mixing conditions, it was possible to decrease the mean particle diameter to 10 nm. The particle size distribution was well described by the lognormal function. For the whole range of the particle diameters, the geometric standard deviation was 1.4. The typical particle size distribution is shown in Figure 6. Figure 7 compares the UV spectra from nanoparticles with that from the original substance. One can see that the spectra from the particles and the maternal substance are identical.

The chromatographic analysis showed that the chromatogram from nanoparticles was identical to that from the original ibuprofen sample (Fig. 8).

The powder X-ray diffraction patterns of nanoparticles and original powder are compared in Figure 9. Both XRD patterns from nanoparticles and original substance correspond to racemic ibuprofen,[8–10] that is, the nanoparticles form the same crystal phase as the original substance. The small difference in the peak relative intensities between the curves a and b in the range of $17 < 2\theta < 21$ is probably related to the difference in distribution of crystallographic orientations in the microsized original powder and nanoparticles.

Lung-deposited dose

To determine the lung-deposited dose, we measured the fraction α of particles consumed per chamber due to mouse breathing:

$$\alpha = 1 - \left(\frac{n_{out}}{n_{out}^{(0)}} \right)^{1/N} \tag{1}$$

where n_{out} and $n_{\text{out}}^{(0)}$ are aerosol number concentrations at the outlets of the loaded and unloaded NOE lines, respectively,

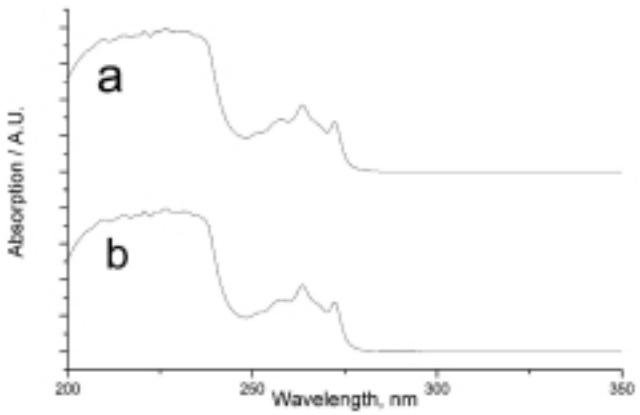

FIG. 7. UV absorption spectra of ibuprofen solution in ethanol: (**a**) from nanoparticles (mean particle diameter $d = 85$ nm) sampled to a filter and then dissolved; (**b**) from the original substance.

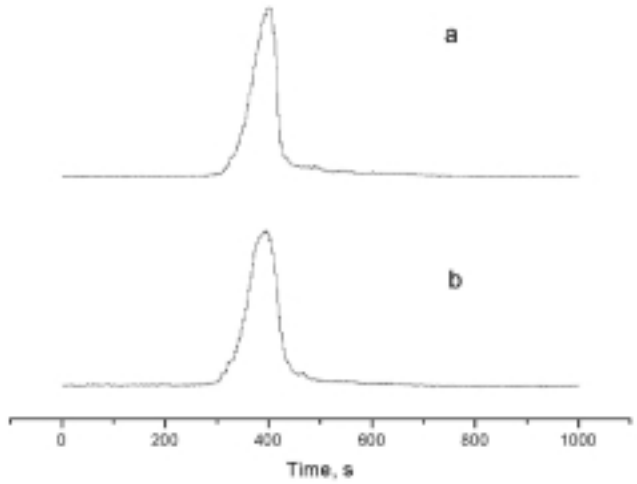

FIG. 8. Comparison between the chromatograms of original ibuprofen powder and nanoparticles (mean particle diameter d = 85 nm) formed by evaporation-nucleation.

N is number of chambers in the tandem (see Fig. 4). The rate D [$\sec^{-1}$] of particle lung deposition per mouse can be written as:

$$D \approx F\alpha n, \tag{2}$$

where F (cm^3/sec) is flow rate through the NOE chambers tandem and n is the arithmetic mean between the loaded NOE line inlet and outlet particle concentrations.

One can use also the relative deposition rate

$$D_0 = \frac{D}{n} = F\alpha. \tag{3}$$

Equation (3) of this paper differs from equivalent equation of our previous paper. In the previous paper[2] we used $n_{\text{out}}^{(0)}$ (the particle concentration at the outlet of the unloaded NOE line) and in the present manuscript we use n (the average between the inlet and outlet concentrations for the loaded NOE line). These two magnitudes are very close to each other. In the previous paper we used $n_{\text{out}}^{(0)}$ to make the logic simpler. Now we incline to n to make the formula more realistic.

Figure 10 shows the relative deposition rate D_0 as a function of the mean particle diameter. The logistic regression analysis applied to the relative deposition rate data showed that there was a statistically significant correlation between the particle diameter and the relative deposition rate (R^2 = 0.96). The fitted curve is shown as a solid line. Using the function $D_0(d)$ we determined the lung-deposited dose (weight of the deposited particles) for the WB chambers inhalation experiments:

$$\text{Dose} = D_{0n}{}_{\text{av}}^{\text{WB}} mt \tag{4}$$

where $n_{\text{av}}^{\text{WB}}$ is the arithmetic mean between the WB chamber inlet and outlet particle concentrations, m is the mean particle mass, t is the inhalation time. Note that the lung-deposited dose is a function of the product fV_T (where f *and* V_T are the average mouse breathing frequency and tidal volume, respectively) which for the WB inhalation experiments can differ from that of the NOE chamber by 20%.[11–14] Therefore, we assume that the accuracy of Equation (4) is also about 20%.

To demonstrate the validity of our measurements of the lung-deposited dose, we evaluated the particle deposition efficiency which follows from Equation (5):

$$D_0 \approx fV_T\varepsilon \tag{5}$$

where ε is the lung deposition efficiency, that is, the ratio of the difference between the numbers of inhaled and exhaled particles to the number of inhaled particles. From Equations (3) and (5) we get:

$$\varepsilon \approx \frac{F}{fV_T}\left[1 - \left(\frac{n_{\text{out}}}{n_{\text{out}}^{(0)}}\right)^{1/N}\right]. \tag{6}$$

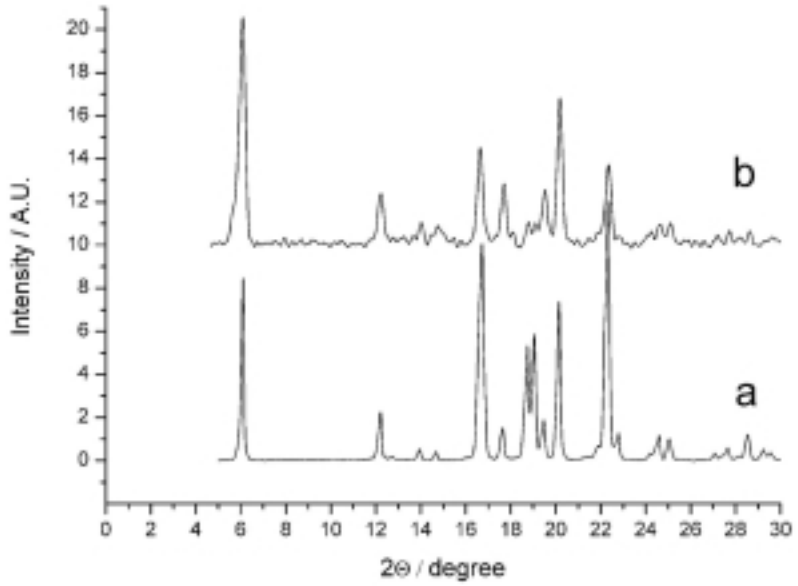

FIG. 9. X-ray diffraction patterns of (**a**) original ibuprofen powder, (**b**) ibuprofen nanoparticles formed via evaporation–nucleation route (mean particle diameter d = 85 nm).

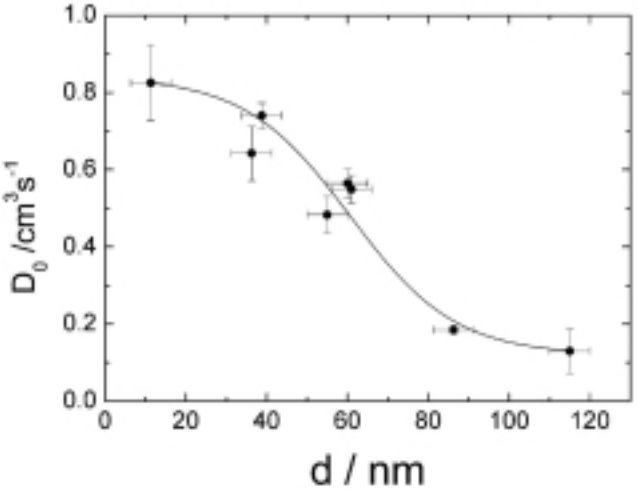

FIG. 10. Relative deposition rate per mouse versus mean particle diameter. Bars indicate standard error. Line is logistic regression analysis result.

We used the mouse breathing frequency and the mice tidal volume as equal to $f = 5.0\ \text{sec}^{-1}$ and $V_T = 0.16\ \text{cm}^3$.[2,15] The particle deposition efficiency as a function of the mean particle diameter is shown in Figure 11. One can see that ε tends to unity at small-diameter values, which is in good agreement with numerical simulations for the particle lung deposition.[16] We added also in Figure 11 the lung deposition efficiency data, which were determined in our previous paper on the indomethacin aerosol inhalation. These two sets of data are in agreement at $d < 15$ and $d > 80$ nm. Within the intermediate range of mean particle diameter, there is some discrepancy between the deposition efficiencies measured for indomethacin and ibuprofen particles. One of the possible reasons for this discrepancy may arise from different hygroscopic properties of indomethacin and ibuprofen nanoparticles.

The aerosol depletion in both the empty and mice-occupied WB chambers was measured in special experiments and found to be independent of the mean particle diameter within the range $35 < d < 120$ nm. To this end aerosol concentration outlet ($n^{(0)}_{\text{out}}$) to inlet ($n^{(0)}_{\text{in}}$) ratio was measured with the aerosol spectrometer for the empty chamber to be

$$\frac{n^{(0)}_{\text{out}}}{n^{(0)}_{\text{in}}} = 0.9\,4 \pm 0.01.$$

To measure the aerosol depletion in the occupied chambers due to the fur deposition, two chambers were put in parallel, the same way as it was done in the inhalation experiments. One chamber was empty and the other one was occupied by four mice. The ratio of the particle concentration at the outlet of the occupied chamber (n_{out}) to that of the empty one ($n^{(0)}_{\text{out}}$) were measured to be

$$\frac{n_{\text{out}}}{n^{(0)}_{\text{out}}} = 0.91 \pm 0.02.$$

Thus, the total aerosol depletion in the occupied chamber was about 15%. When evaluating the lung deposited dose the average between inlet and outlet concentrations was used.

Analgesic effect of ibuprofen nanoparticles

It is convenient to use the relative analgesic index (RAI), that is, the ratio between the mean number of writhes for the aerosolized group (or oral group) and that for the untreated group. During 1 day, one to three WB exposure runs were provided for aerosolized or oral groups and one run for the animals from group 1 (eight animals per one run). Thus, during 1 day for all the runs of group 2 or 3, one and the same run of group 1 was considered as a match. Figure 12 shows the RAI for the aerosolized animals as well as RAI for the orally treated animals versus the lung-deposited dose. The RAI data for both the aerosolized and oral groups were analyzed for the dose–response relationship. The fitted curves are shown as the solid lines. Both aerosol and oral treatments give no analgesic effect (RAI is about unity) at small lung/orally deposited doses (less than 10^{-6} and 1 mg per kg bw for the aerosol and oral treatments, respectively). The regression analysis applied to the RAI results showed that there was a statistically significant dose response ($R^2 = 0.91$ and 0.96 for aerosolized and oral groups, respectively). Note that the lung-deposited dose was varied by changing the heating temperature in the generator of nanoparticles. As seen from Figure 5, an increase in temperature results in an increase of both the mean particle diameter and number concentration. Therefore, the RAI points for different dose magnitudes correspond to different mean particle diameters. One can also see from Figure 12 that the aerosol treatment is more effective (gives the same RAI for a less dose) than the oral administration. The body-delivered doses for aerosol and oral treatments differ by three and five orders of magnitude at the lung deposited dose 10^{-1} and 10^{-4} mg per kg bw, respectively. One of the reasons for this difference is probably the high level of metabolism of ibuprofen being administered orally.[17]

Histology of the lungs

A histologic analysis was performed to observe possible hemodynamic abnormalities and pulmonary edema after

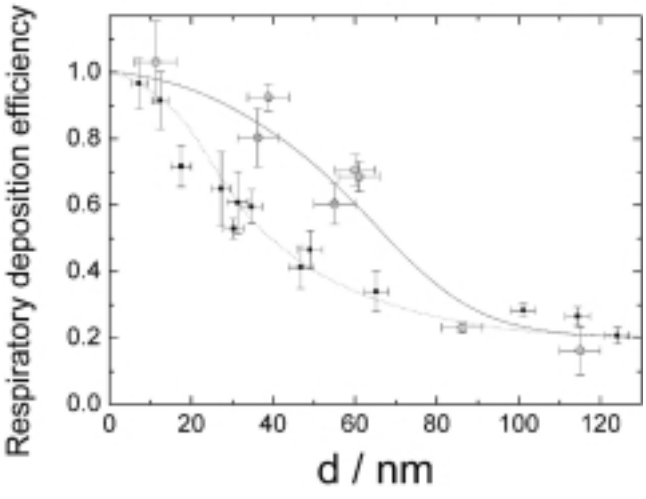

FIG. 11. Mouse respiratory efficiency (ε) versus mean particle diameter; circles—the data for ibuprofen (present work); squares—the data from the indomethacin nanoparticle inhalation experiments[2] which are given for comparison. Bars indicate standard error. Lines are given as eye guides.

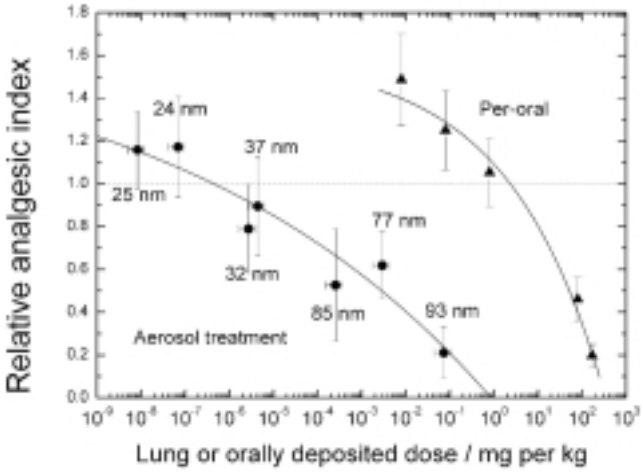

FIG. 12. Relative analgesic index (RAI) (the ratio between the mean number of writhes for the aerosolized group and that for untreated group) versus the lung deposited dose (circles). Triangles are RAI values for peroral treatment. Bars indicate standard error. The fitted dose–response curves are shown as solid lines. Mean particle diameter is indicated for each inhalation point.

the aerosol treatment. The animals were exposed to nanoparticles of $d = 100$ nm, with the lung deposited dose of 5.5×10^{-3} mg per kg bw (group 3.1, 16 animals), and $d = 75$ nm, with the lung deposited dose of 2.9×10^{-3} mg per kg bw (group 3.2, 16 animals). The group 1 (eight animals) included again untreated mice being exposed to the pure air in the chambers. The lungs of animals from the group 1 have a normal structure without any destructive and hemodynamic pathologic changes (Fig. 13). A moderate venous hyperemia was observed for seven animals from group 3.1 and for all the animals from group 3.2. (Fig. 14). The other nine animals of group 3.1 have demonstrated more pronounced venous and arterial hyperemia (Fig. 15). A homogeneous venous deposition (presumably fibrin) was observed. Typical emphysematous signs occurred in the lungs of those animals, that is, the dilatation of bronchioli and alveolar channels, alveolar wall thinning, and partial capillary bed reduction.

Conclusions

The analgesic action and side pulmonary effects caused by the inhalation of ibuprofen nanoparticles 10–100 nm in diameter were investigated. The nanoparticles were formed by the evaporation–condensation route. The chromatographic and UV analysis showed that the aerosol particles were chemically identical to the maternal substance (i.e., there was no thermal decomposition or oxidation during evaporation). The X-ray diffraction analysis showed that the nanoparticle crystal phase (racemic ibuprofen) was identical to that of the original ibuprofen powder.

Using the NOE chambers, the mice lung deposition efficiency was evaluated as a function of the particle diameter changing from about unity at $d = 10$ nm to about 0.2 at $d = 100$ nm.

The dose-dependent effect of aerosolized ibuprofen was studied in comparison with the oral treatment. It was found that aerosol administration is much more effective than the oral delivery; thus, the aerosol treatment needs the dose a

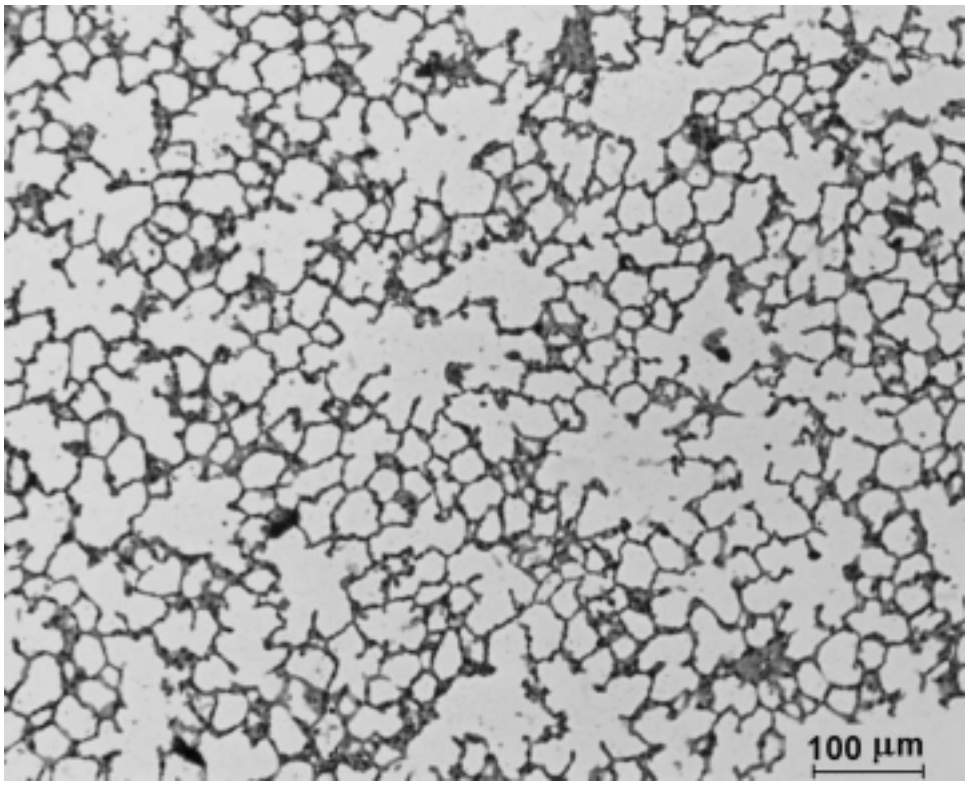

FIG. 13. Representative sections from the lungs of untreated animal (group 1).

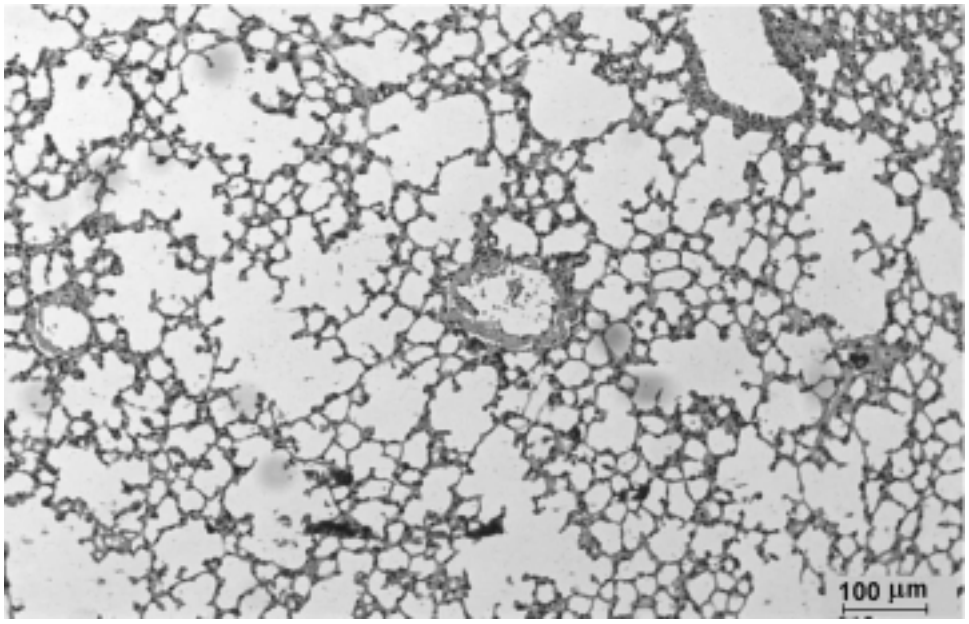

FIG. 14. A cross section of the lung from a mouse treated with 100-nm nanoparticles showing enlarged airspaces.

three to five orders of magnitude less than the oral one at the same analgesic effect.

The lung histology analysis for the mice treated with particles ($d = 75$ and 100 nm) at dose about 2.9×10^{-5} and 5.5×10^{-5} mg per kg bw, respectively, was carried out. The majority of mice demonstrated moderate venous hyperemia; 9 of 32 animals revealed emphysematous signs like the dilatation of bronchioles and alveolar channels, alveolar wall thinning, and partial capillary bed reduction still remaining 6 h after the inhalation.

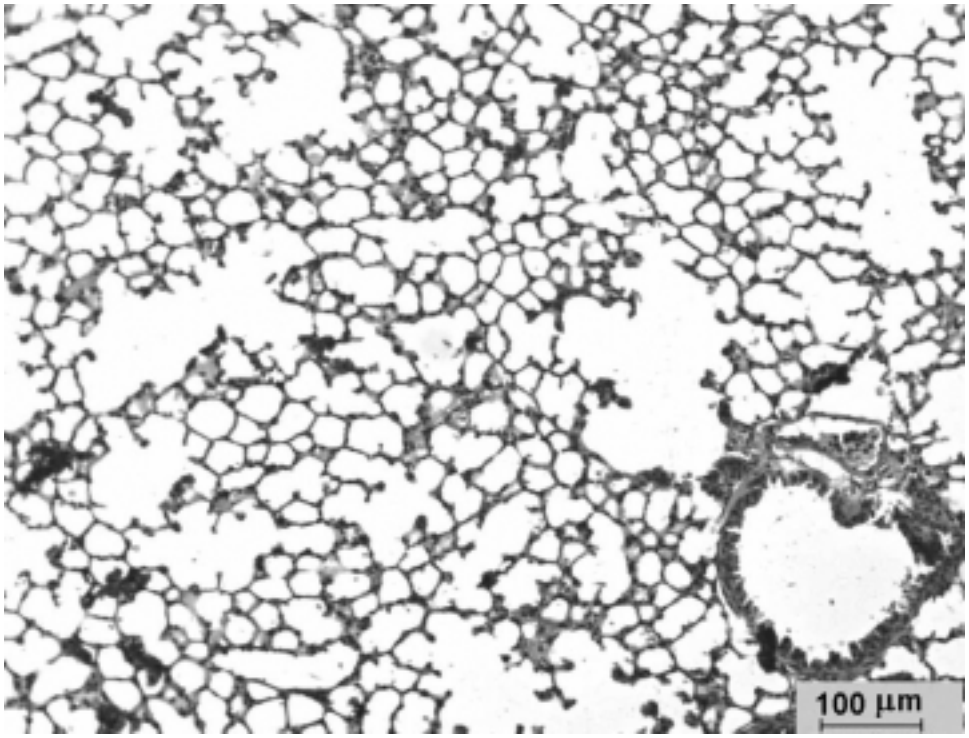

FIG. 15. A cross-section of the lung from a mouse treated with 100-nm nanoparticles showing a pronounced venous and arterial hyperemia as well as dilatation of bronchioli and alveolar channels, alveolar wall thinning, and partial capillary bed reduction.

Acknowledgments

Financial support for this work was provided by the Siberian Branch of Russian Academy of Sciences (Interdisciplinary Integration Project), the Russian Foundation for Basic Research (RFBR) (project nos 07-03-00643a, 08-04-92003-HHC_a). The authors are grateful to S. V. Tsybulya for carrying out the XRD analysis of samples

Author Disclosure Statement

No conflicts of interest exist.

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Received on September 4, 2008
In final form, December 26, 2008

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