Release of 4-Nonylphenol from a Silicone Tube Implanted in Mice

Atsushi OKADA, Yoshitsugu NIWA, Takao KATASE, and Osamu KAI

College of Bioresource Sciences, Nihon University, Fujisawa 252-8510, Japan

Abstract:  Silicone tubes are commonly used as a tool for long-term steroid treatment in animals. However, the release rates of steroids from these tubes have not yet been accurately determined. We measured the amounts of 4-nonylphenol (NP) remaining in tubes implanted in mice to obtain accurate release rates for original amounts of 20 or 1,000 µg NP in silicone tube implants. We achieved an accurate figure for the release rate from the tube, calculated using the total recovery from both the contents of the tube and the silicone matrix of the tube. The regression curve for the remaining amount of NP, as a percentage of the original amount (1,000 µg) was calculated as \( y(\%) = 83.033 - 3.4722t + 0.0375t^2 \), where \( t \) is the number of days after implantation. The mean release rate over 56 days was 1.6% of the original amount per day.

Key words: nonylphenol, release rate, silicone tube

Silicone tube implants are used for administering steroid hormones [2, 6] and are designed to release steroids by diffusion over a long period. Release rates of the steroid from silicone implants are obtained from the loss of weight of the tube during implantation in vivo [9] and by the amount of radioactively labeled tracer released into gelatin-phosphate-buffered saline in vitro [1]. Improved measurement of the release rates of steroids from the tube would allow the effects of the materials to be evaluated more precisely. We previously developed a silicone tube covered with a polyethylene tube for long-term administration, and reported on the effects of the tube filled with estradiol-17β (E₂) and bisphenol A (BPA) over 56 days in mice [4, 5]. We estimated the release rates of E₂ and BPA from the tube by calculations based on the remainder levels in the tube using high performance liquid chromatography (HPLC). 4-Nonylphenol (NP), used in the present study, is a non-ionic surfactant that is used extensively in detergents, paints, herbicides, insecticides, and many other synthetic products. NP has been reported to have weak estrogen-like activity, with a potency of about 2,500 times less than E₂ in vitro [3, 8]. It is necessary to have precise knowledge of the amounts of NP released from the implant tube before starting experiments using NP. In our previous study [5], the remaining amounts of E₂ and BPA in the tube were measured and used to estimate the release rate, without measuring the contents in the silicone matrix of the tube itself. Thus, in the present study, we attempted to obtain a more accurate value for the release rate from tubes retrieved from mice. In these experiments, the contents in the tube and contents in the silicone matrix of the tube were measured separately using HPLC.

Male ICR mice were purchased from SLC Japan (Shizuoka, Japan) at 7 weeks of age. The animals were acclimatized to the room to be used for the experiments.

(Received 21 October 2008 / Accepted 19 May 2009)

Address corresponding: O. Kai, College of Bioresource Sciences, Nihon University, Fujisawa 252-8510, Japan
for one week prior to the start of the study. The room was maintained at 22 ± 3.0°C with 14 h of artificial light and 10 h of darkness daily (lights on 05:00 to 19:00) and the animals were provided with food pellets (LABO MR STOCK, Nihon Nosan Kogyo, Yokohama, Japan) and water ad libitum. Eighty-nine animals were used in this study. Five to twelve mice were sacrificed at 3, 7, 14, 21, 28, 42, or 56 days after implantation and the tubes were retrieved for analysis. All experimental procedures were conducted in accordance with the guidelines for animal experiments, College of Bioresource Sciences, Nihon University.

Tubes for implantation in mice were prepared as previously described [5]. Briefly, each tube was a medical silicone tube (inner diameter 2.0 mm, outer diameter 3.0 mm, length 20 mm; KANEKA MEDEX, Osaka, Japan) covered with a polyethylene tube (inner diameter 3.0 mm, outer diameter 4.0 mm, length 8.0 mm; IMAMURA, Tokyo, Japan) at each end. NP (Tokyo Kasei Kogyo, Tokyo, Japan) was dissolved in sesame oil (Sigma-Aldrich Japan, Tokyo, Japan) at a concentration of 0.4 or 20 mg/ml, and 50 µl was loaded into each tube (final amount, 20 or 1,000 µg NP) before the tube was sealed using a special glue for silicone (Shin-Etsu Silicon, Shin-Etsu Chemical, Tokyo, Japan). Each implant was incubated in 6 ml of physiological saline at room temperature for one night before subcutaneous implantation into male mice.

HPLC was performed using an HP 1050 system with a quaternary gradient pump, column oven, variable-wavelength UV detector, auto sampler (Hewlett-Packard, Avondale, PA, USA) and a Capcell Pak C18 column (250 x 4.6 mm i.d., 5 µm; Shiseido, Tokyo, Japan). The column temperature was 40°C. A mobile phase consisting of water-methanol (10:90, v:v) was used at a flow rate of 1.0 ml/min. The UV detection wavelength was 230 nm. The injection volume was 10 µl.

NP was recovered from the tubes collected from the mice 3 to 56 days after implantation (n=5–12) or from the physiological saline (day 0; n=14) in which the tube had been incubated for one night before use, in 1 or 10 ml of absolute methanol, respectively. The tubes which were initially loaded with 1,000 µg NP were halved and immersed in 1.5 ml methanol to retrieve NP from the silicone matrix. The mixture was agitated on a rotator (RT-50, TAITEC, Saitama, Japan) for one day at room temperature. The three kinds of recovered samples (contents in the tube and in the silicone matrix of the tube, and the physiological saline) were then diluted to appropriate concentrations using the solvent for measurement in the HPLC system. Standard solutions of NP (5.0, 10.0, 25.0, or 50.0 µg/ml) were prepared for the HPLC system. The NP standard solution was measured and the resultant peak area was plotted against the corresponding concentrations to obtain a standard curve, using a linear regression, after which the amounts of NP were determined by direct comparison with the standard curve. The data were expressed as mean ± SEM. The significance of the differences between the linear regression lines was determined using analysis of covariance [7].

Figure 1 shows the time course of the two different concentrations of NP remaining in the tubes. The NP contents of the tubes declined steadily throughout the experimental period. NP, as a percentage of the original amount, was closely similar at all time points for the two different concentrations of NP. The linear regression lines used in the statistical analysis showed no significant difference between the two concentrations of NP in the tubes. Figure 2 depicts the time course of NP remaining in the tube as a percentage of the original amount obtained from the NP content in the tube, plus the content of NP in the silicone matrix of the tube. After incubating tubes filled with 1,000 µg NP in sesame oil overnight in physiological saline, the mean recovery rate from the tubes after physiological saline incubation in vitro was 93.6% (contents in the tube: 84.73 ± 1.23%, contents in the silicone matrix: 8.32 ± 0.20%, and contents in physiological saline released from the tube: 0.52 ± 0.04%). The NP remaining in the silicone tube declined more rapidly in the first 3 days after implantation than at any other time in the experimental period. The ratios of the contents in the silicone matrix to the contents of the tubes were very similar at all sampling times from 3 to 56 days. This decline illustrates a progressive release of NP from the tube and it closely fitted with a quadratic regression. The quadratic regression for the percentage of 1,000 µg NP remaining in the tube (contents in the tube and contents in the silicone matrix of the tube) was $y(\%) = 83.033 - 3.4722t + 0.0375t^2$. 
RELEASE OF NP FROM TUBES IMPLANTED IN MICE

(R²=0.9678), where \( t \) is the number of days after implantation.

The results of the present study show that the amounts of NP remaining in the tubes, expressed as a percentage of the original amount, were similar at two different concentrations, as we reported earlier for E₂ [5]. Moreover, in the present study we were able to retrieve NP from the silicone matrix using methanol, and could thus achieve more accurate values for the amounts of NP remaining in the tube, by addition of the recovery of the NP contents in the silicone matrix of the tube to the contents in the tube, than in our previous study. The present study revealed that NP was released at roughly 1.6% of the original amount per day over 56 days, although the release pattern did not follow a linear path.

Fig. 1. Time course of NP remaining in the tube. NP, 20 (■—■) and 1,000 µg (□—□) was dissolved in 50 µl sesame oil and loaded into the tubes. The amounts of NP, as a percentage of the original amount, remaining in the tubes are shown to 56 days after implantation. Each value represents mean ± SEM of n=5–14 samples.

Fig. 2. Time course of NP, as a percentage of the original amount (1,000 µg), remaining in the tubes (■—■), plus the content found in the silicone matrix of the tubes (□—□) to 56 days after implantation. Each value represents mean ± SEM of n=5–14 samples.
Acknowledgment(s)

This study was supported in part by a Grant-in Aid for Effective Promotion of Joint Research with Industry, Academia, and Government, the Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Science, Sports and Culture of Japan.

References


