ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1992, p. 473–476
0066-4804/92/020473-04$02.00/0

Vol. 36, No. 2

Utilization of Pulse Oximetry for the Study of the Inhibitory Effects of Antiviral Agents on Influenza Virus in Mice

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Received 9 July 1991/Accepted 22 November 1991

Pulmonary disease in mice induced by influenza virus was monitored by measurement of oxygen saturation (SaO_2) in blood with a pulse oximeter. The SaO_2 declined in inverse proportion to the viral inoculum. The known antiviral agent ribavirin inhibited the SaO_2 decline, prevented death, lowered lung consolidation, and reduced the level of recoverable virus. Pulse oximetry is an effective means of monitoring murine influenzal disease and can be used in the study of potential antiviral drugs.

It has become standard practice in hospitals that care for patients in potential respiratory distress to continuously monitor their respiratory condition by attaching a pulse oximeter probe to a finger, toe, foot, or earlobe. The oxygen saturation (SaO_2) of the arterial hemoglobin is measured and is based on the pulsatile absorbance of light (1).

There has existed a need for improved means of monitoring the respiratory condition of mice infected with agents such as influenza virus without having to sacrifice the animal at various stages of the disease. Inhibition of rales (5) and weight loss (4, 7) have been used infrequently in attempts to satisfy this need. In this report we describe the use of pulse oximetry for the study of murine influenzal disease and the application of this methodology to supplement the commonly used parameters of death, lung consolidation, and virus titers in lungs to evaluate the inhibitory effects of an antiviral compound, ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) (6, 9), on influenza virus disease.

Young adult male and female BALB/c mice and C57BL/6 mice (Simonsen Laboratories, Gilroy, Calif.), which were maintained on drinking water containing 0.006% oxytetracycline (Pfizer, New York, N.Y.) to control possible secondary bacterial infections, were used in this study. The mice were lightly anesthetized with ether, and in designated studies they were infected intranasally (i.n.) with one of the following viruses: influenza A/NWS/33 (H1N1), obtained from K. W. Cochran (University of Michigan, Ann Arbor); influenza A/Japan/305/57 (H2N2), provided by F. M. Schabel, Jr. (Southern Research Institute, Birmingham, Ala.); and influenza A/Port Chalmers/1/73 (H3N2) and B/Hong Kong/5/72, both obtained from the American Type Culture Collection (Rockville, Md.). All viruses were passaged multiple times through specific-pathogen-free mice to develop lethality for these animals. Each was passaged separately with no other viruses in the area to avoid recombination. Virus pools were prepared and titrated in confluent monolayers of Madin-Darby canine kidney (MDCK) cells.

The viral infection was monitored by using a Biox 3740 pulse oximeter (Ohmeda, Louisville, Ohio). Both the finger and ear probes accompanying the instrument were used in separate studies. The numeric-dominant instrument display was used with the instrument in the "slow" mode. Initial experiments established that the SaO_2 values determined with a pulse oximeter were essentially duplicated by direct measurements of SaO_2 in heparinized blood performed with a OSM-3 hemoximeter and an ABL-2 blood gas analyzer (Radiometer, Inc., Copenhagen, Denmark).

Comparison of methods for determining pulse oximeter readings in mice. The following two methods were found to be useful in obtaining SaO_2 readings by using the pulse oximeter: (i) inserting the entire mouse, tail first, into the finger probe in a manner that the light-emitting diodes and the photodiode were opposite each other across the midsection of the animal, and (ii) placing the ear probe on the inner thigh muscle of the animal so that the light-emitting diodes and the photodiode were on opposite sides of the thigh muscle mass. The finger probe method yielded somewhat lower (2 to 4%) readings; however, the healthy animals tended to become overexcited when they were placed in the finger probe, and more strenuous measures were required to hold them in the probe for a sufficient time to obtain an accurate SaO_2 measurement. Intraperitoneal injection of a 1:80 dilution in sterile saline of 94 mg of Avertin (1.5 g of tribromethanol in 1.0 ml of isoamyl alcohol; Aldrich Chemical Co., Milwaukee, Wis.) per kg of body weight administered 5 min prior to SaO_2 determination successfully eliminated the animals' struggles without affecting the SaO_2 values that were determined.

Such tranquilization was not necessary when the measurements were taken by using the ear probe on the thigh. It was found, however, that care had to be taken in placing the ear probe approximately an equal distance between the hip and the knee. Determinations taken near the hip often varied by ±10%, apparently because of obstruction by the pelvis bone of the light transmission through the vascular bed. When the probe was moved too near the knee, the vascular bed was apparently too small, and external light occasionally interfered with the determinations, again resulting in significant variations.

To determine whether holding the animals too tightly may tend to constrict the chest and hinder appropriate respiration, five mice were held by the scruff of the neck very tightly, with SaO_2 measurements taken on the thigh five separate times for each animal. They were then held very loosely and the measurements were repeated. The mean percent SaO_2 for the animals held tightly was 86.3 ± 1.0; for animals held loosely it was 86.5 ± 1.1. These data indicate that the chest was not being constricted sufficiently to affect the readings.

Literature accompanying the Ohmeda instrument used in these experiments recommended massaging the skin region on which the probe is to be attached with an isopropyl alcohol

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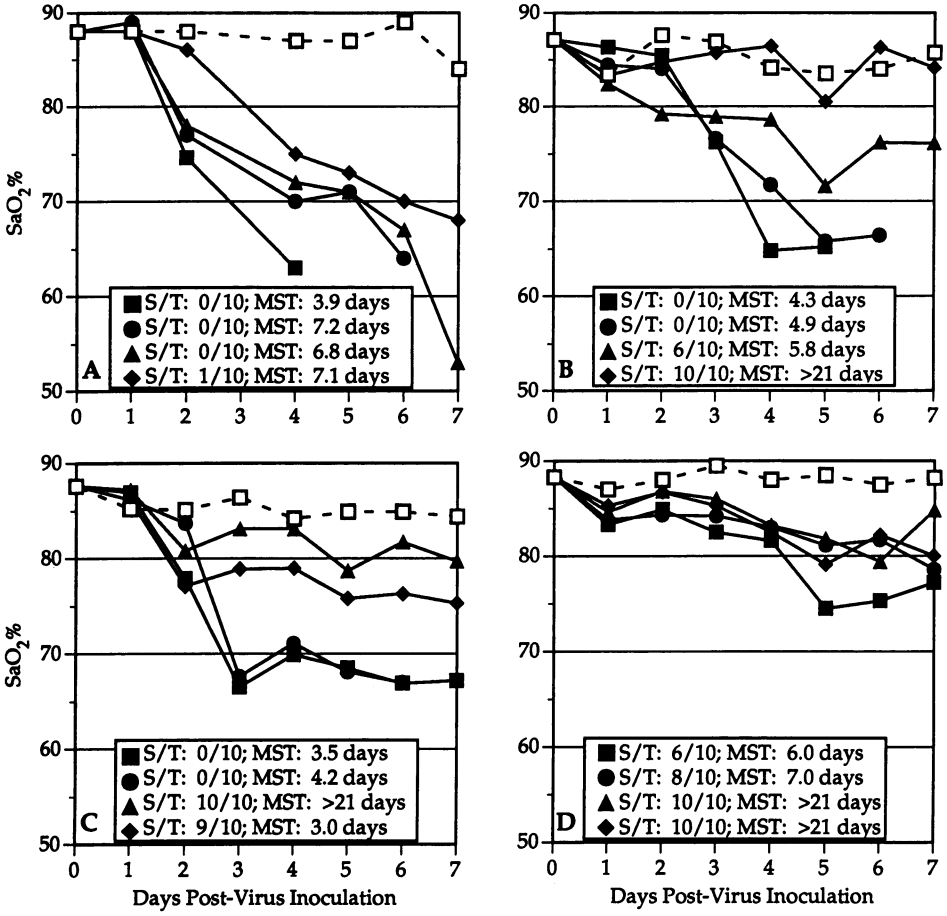

FIG. 1. Effect of pulmonary influenza virus infections on murine arterial SaO_2 determined by pulse oximetry. The titers of each undiluted virus, expressed as 50% tissue culture infectious doses per milliliter, were as follows: influenza virus type A (H1N1), $10^{7.5}$ (A); influenza virus type A (H2N2), $10^{8.5}$ (B); influenza virus type A (H3N2), $10^{7.7}$ (C); influenza virus type B, $10^{8.5}$ (D). ■, 10^{-1} virus dilution; ●, $10^{-1.5}$ virus dilution; ▲, 10^{-2} virus dilution; ◆, $10^{-2.5}$ virus dilution; □, normal controls. S/T, Number of survivors by day 21/total number of mice tested. MST, mean survival time of mice dying on or before day 21.

(70%) pad or with a rubefacient cream for 20 to 30 s to cause local vasodilation and, hence, to increase perfusion. Several experiments with mice in which the skin was initially dampened with isopropyl alcohol indicated an increase of approximately 4% in SaO_2 readings compared with the readings in the same animals not exposed to alcohol. We felt that relative readings were most important in antiviral experiments, so the alcohol pretreatment was not done in these studies.

SaO_2 determinations in influenza virus-infected mice. Young adult BALB/c mice were infected i.n. with various 0.5 $\log_{10}$ dilutions of each influenza virus. The arterial oxygen saturation was determined in these animals daily for 7 to 10 days by using the ear probe placed on the thigh; deaths were also recorded daily. To prevent skewing of data, mice that died of obvious respiratory distress were arbitrarily assigned an SaO_2 value of 65% until all animals died, at which time no further values were recorded. As shown in Fig. 1, the three influenza A viruses at higher inocula were lethal to the mice. In contrast, only 40% of the influenza virus type B-exposed mice died, and these deaths occurred somewhat late (mean survival time, 6 to 7 days). In mice with lethal infections, the SaO_2 values declined in a dose-response fashion. In the case of the influenza virus type B infection, in which the viral inoculum did not cause death in the majority of the animals, the SaO_2

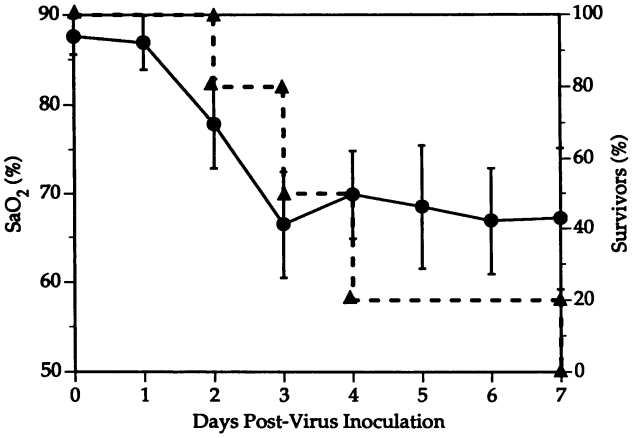

FIG. 2. Comparison of occurrence of death and SaO_2 decline in influenza (H3N2) virus-infected BALB/c mice. ▲, occurrence of death; ●, SaO_2. The bars indicate standard errors.

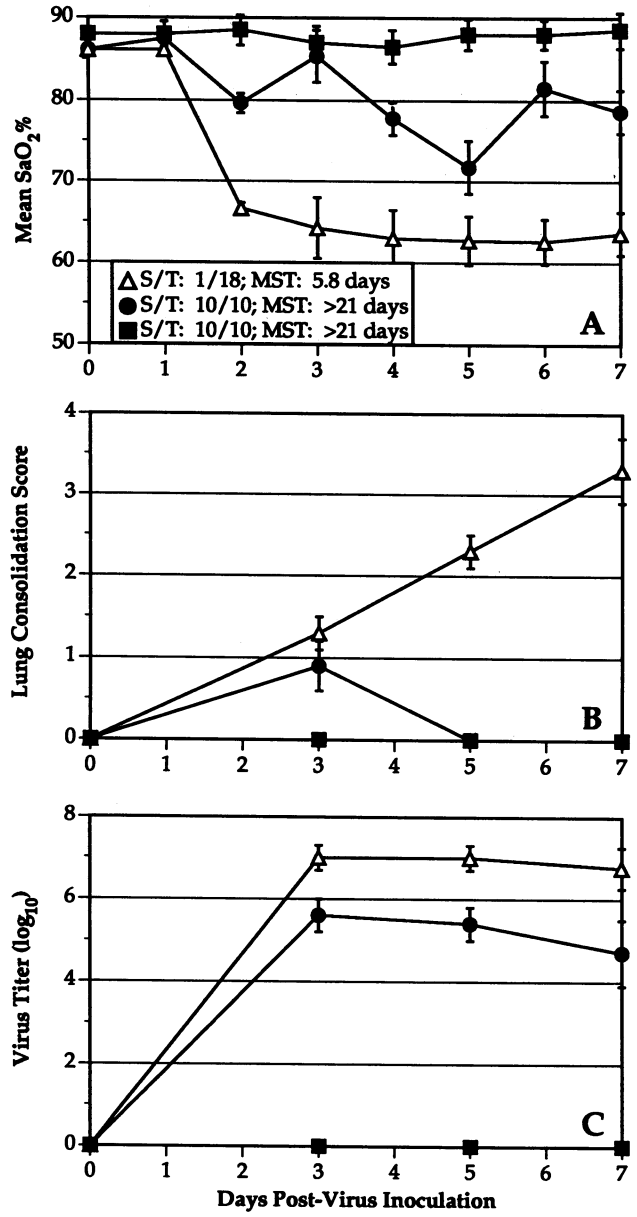

FIG. 3. Effect of intraperitoneal ribavirin therapy on influenza virus type A (H1N1) infections in BALB/c mice. ■, normal controls; △, infected, saline-treated mice; ●, infected, ribavirin-treated mice. (A) Arterial SaO_2; (B) lung consolidation; (C) lung virus titer. The bars indicate standard errors.

decline was less pronounced. As shown in Fig. 2, the majority of the deaths occurred when the SaO_2 values dropped below 75%. The linear correlation coefficient (r) for these data was 0.908, indicating a strong correlation between SaO_2 and death in the animals.

Anti-influenza virus effects of ribavirin. BALB/c mice were infected i.n. with an approximately 90% lethal dose of influenza virus type A (H1N1) (10^5 50% cell culture infectious doses per ml). Ribavirin (ICN Pharmaceuticals, Inc., Costa Mesa, Calif.), at a dose of 75 mg/kg/day (n = 30), or saline (n = 40) was administered intraperitoneally twice daily for 5 days beginning 4 h after virus exposure. Ten infected, ribavirin-treated mice and 18 virus-infected placebo-treated control animals were observed daily for 21 days for death; pulse oximeter readings were determined on these mice daily for 7 days by using the ear probe, as described above. From pools of the remaining infected, treated mice, five mice were randomly selected and killed on days 3, 5, and 7 after virus exposure. Visual evidence of lung consolidation was scored on a blind basis, with 0 being normal and 4 being indicative of 100% consolidation. The lungs were then homogenized and assayed for virus titer in MDCK cells by testing various logarithmic dilutions in triplicate, as described previously (8). As shown in Fig. 3, the ribavirin treatment prevented death, reduced the depression of SaO_2, inhibited lung consolidation, and to a lesser extent, reduced virus titers in the lungs. Visible lung consolidation had a moderate correlation (r = 0.792) with SaO_2 values; the SaO_2 values declined more rapidly than consolidation increased.

This difference between the time of lowering of SaO_2 values and development of visible lung consolidation suggests that the pulmonary tissues were experiencing sufficient damage to lower their gas transference capacity prior to a significant change in lung color. Lung consolidation in influenza virus infections in animals is a result of a combination of events. The virus infection damages the alveolar cells and causes necrosis of the capillary walls, leading to hemorrhage in the lungs. The alveolar exudate usually consists of neutrophils and mononuclear cells (2). The increasing color seen in the lungs later in the infection is probably a manifestation of vascular phenomena resulting from a later immune response to the infection (3). The data from the antiviral experiment, which showed an earlier indication of serious lung damage by SaO_2 measurement, illustrate an additional value of the use of the pulse oximeter for studying murine influenza virus infections.

These data indicate a definite application for pulse oximetry in laboratory animals, that is, as a means of monitoring moderate to severe respiratory disease. The method appears to be of particular value as an additional parameter for studying drugs that are potentially inhibitory to influenza virus.

This study was supported by contract NO1-AI-15097, Antiviral Research Branch, Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

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