EXPERIMENTAL PULMONARY EDEMA IN RODENTS FOLLOWING A SINGLE INJECTION OF 4-NITROQUINOLINE 1-OXIDE

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Administration of 4-nitroquinoline 1-oxide has been known to produce pulmonary tumors in rodents (1–3). An electron microscopic examination on the lung of rats shortly after an injection of this compound revealed induction of nucleolar segregation in the alveolar epithelial cells (4). In this experiment, it was further shown that an injection of 4-nitroquinoline 1-oxide could produce pulmonary edema in rats as well as in guinea-pigs and rabbits.

MATERIALS AND METHODS

Pulmonary alterations in rats

Male Sprague-Dawley rats of 4 to 5 week age were used. The test substance, 4-nitroquinoline 1-oxide was suspended in olive oil and subcutaneously injected into rats at interscapular region. The experiments were conducted in two series;

Preliminary experiment: Five groups of 10 rats each received an injection of 4-nitroquinoline 1-oxide in dose levels ranging from 10.0 mg/kg to 24.1 mg/kg. During seven days after injection, all rats were checked for general condition, and the rats which died or became moribund were autopsied.

Pathological examination of the pulmonary alterations: Thirty-six rats were given an injection of 4-nitroquinoline 1-oxide in a dose of 20 mg/kg, and 6 rats each were sacrificed 8, 24, 36, 48 and 56 hours after injection. The lungs were excised fresh, weighed and fixed in 10% formalin or Bouin’s solution for histology. As the control group, additional 8 rats were given a subcutaneous injection of 0.1 ml olive oil, and killed 56 hours after injection.

Species difference

Male ICR mice of 4 week age, male Hartley guinea-pigs weighing around 300 g and male albino rabbits weighing around 2 kg were given a subcutaneous injection of 4-nitroquinoline 1-oxide in dose levels as shown in Table 2. The animals either died or became moribund were autopsied. The lungs were grossly inspected, weighed and fixed in 10% formalin for histology. The animals which survived for 3 days were decapitated under Nembutal anesthesia, and the lungs were examined in the manner mentioned above.
Chemical structure-activity relationship

Four carcinogenic 4-nitroquinoline derivatives; 4-nitroquinoline 1-oxide, 4-nitroquinaldine 1-oxide, 6-methyl-4-nitroquinoline 1-oxide and 6-chloro-4-nitroquinoline 1-oxide, and four non-carcinogenic compounds; 4-nitroquinoline, quinoline 1-oxide, 3-methyl-4-nitroquinoline 1-oxide, and 4-nitropyridine 1-oxide were suspended in olive oil, and subcutaneously injected into rats in dose levels as shown in Table 3. Of these compounds, 3-methyl-4-nitroquinoline 1-oxide, 6-methyl 4-nitroquinoline 1-oxide were shown to be hardly absorbable from the injection site at subcutis, they were therefore dissolved in dimethyl sulfoxide and injected into the tail-vein of rats. Fifty-six hours after injection, all rats were decapitated under Nembutal anesthesia, and the lungs were excised, grossly examined and weighed.

In another series of experiments, 4-hydroxyaminoquinoline 1-oxide, a compound known as a proximate carcinogenic metabolite of 4-nitroquinoline 1-oxide (5) was tested. Guinea-pigs were used in this test; because the pulmonary alterations after 4-nitroquinoline 1-oxide administration were shown to develop much more rapidly in this species than in rats or rabbits. For injection, an aqueous solution of 4-hydroxyaminoquinoline 1-oxide was prepared as follows; 10 mg of 4-hydroxyaminoquinoline 1-oxide hydrochloride was dissolved in 15 ml of 0.02 N HCl and then diluted to a volume of 100 ml with distilled water. This aqueous solution was injected into the cephalic vein of guinea-pig in a dose of 30 mg 4-hydroxyaminoquinoline 1-oxide/kg. The animals were killed at 6 hours or 72 hours after injection. The lungs were excised fresh, grossly inspected and fixed in 10% formalin for histology.

RESULTS

Preliminary experiment

Around 48 hours after injection of 4-nitroquinoline 1-oxide, rats began to manifest a dyspnea with moist rale, decrease of body temperature and frothy nasal discharge. They died in a day after onset of such symptoms. The mortality rate of each dose group is shown in Table 1. At autopsy, the lungs were heavy, appeared dark red, and pinkish frothy fluid oozed from the cut surface.

| Dose mg/kg | Initial number of rats | 1 | 2 | 3 | 4 | 5 | 7 |
|------------|------------------------|---|---|---|---|---|---|
| 10.0       | 8                      | 0/8| 0/8| 1/8| 1/8| 1/8| 1/8|
| 12.5       | 8                      | 0/8| 0/8| 4/8| 5/8| 5/8| 5/8|
| 15.5       | 8                      | 0/8| 0/8| 6/8| 7/8| 7/8| 7/8|
| 19.3       | 8                      | 0/8| 5/8| 6/8| 6/8| 7/8| 7/8|
| 24.1       | 8                      | 0/8| 3/8| 8/8| 8/8| 8/8| 8/8|

Fifty percent lethal dose calculated by Litchfield-Fertig method : 12.6±0.9 mg/kg.
Pathology of the pulmonary alterations in rats

By the 24th hour the animals did not exhibit any gross alteration of the lung other than slight hyperemia. The lung-weight at this stage was comparable to that of the control animals. At the 36th hour, the lungs appeared hyperemic, and focal area with dark red coloration occurred at the juxtahilar region. At the 48th hour, the lungs appeared strongly hyperemic, and more than one third of the total parenchyma became consolidated exhibiting dark red coloration. Pinkish froth appeared at the cut surface of these lungs. At the 56th hour, the froth also occurred in the tracheobronchial lumen. Histological examination of these pulmonary tissues invariably revealed accumulation of eosinophilic material in the alveolar lumina, and fibrin precipitation was occasionally noted there. The alveolar walls were swollen in association with marked dilatation of the capillaries and small arteries. Sequential steps in the development of the pulmonary alterations as expressed by increase of the lung-weight are shown in Fig. 1, which indicates that the pulmonary alterations became aggravated rapidly around 48 hours after injection of 4-nitroquinoline 1-oxide.

**Fig. 1.** Occurrence of pulmonary edema in rats after an injection of 4-nitroquinoline 1-oxide.

**Table 2.** Occurrence of pulmonary edema in rodents after a single subcutaneous injection of 4-nitroquinoline 1-oxide.

| Species   | Dose mg/kg | Onset of dyspnea Hrs. after injection | Froth in tracheobronchial lumina | Lung-weight g% per body-weight |
|-----------|------------|--------------------------------------|----------------------------------|-------------------------------|
| Rat       | 0          | ---                                  | -                               | 0.58 ± 0.02                   |
|           | 30         | 24-36                                | +                               | 1.51 ± 0.13*                  |
| Guinea-pig| 0          | ---                                  | -                               | 0.73 ± 0.02                   |
|           | 30         | 4-6                                  | +                               | 1.93 ± 0.07*                  |
| Rabbit    | 0          | ---                                  | -                               | 0.34 ± 0.03                   |
|           | 30         | 12-24                                | +                               | 0.89 ± 0.12*                  |
|           | 0          | ---                                  | -                               | 0.65 ± 0.03                   |
| Mouse     | 40         | ---                                  | -                               | 0.78 ± 0.12                   |
|           | 60         | ---                                  | -                               | 0.72 ± 0.03                   |
|           | 80         | ---                                  | -                               | 0.70 ± 0.02                   |

*P < 0.01
Species difference

As shown in Table 2, an injection of 4-nitroquinoline 1-oxide caused respiratory distress in guinea-pigs and rabbits as well as in rats. Guinea-pigs exhibited labored breathing with frothy nasal discharge 4 to 6 hours after an injection of 30 mg 4-nitroquinoline 1-oxide/kg. In rabbits, similar symptoms were manifested around at the 24th hour. In all cases, the lungs were heavy and appeared dark red. Histologically, eosinophilic material was shown to fill the alveolar spaces. All five mice treated with 80 mg/kg 4-nitroquinoline 1-oxide died in 24 hours, and 5 of 8 mice treated with 60 mg/kg died in 48 hours. However, edematous changes of the lung as assessed by gross inspection and lung-weight were not shown in any of these mice (Table 2).

Chemical structure-activity relationship

Of the eight compounds tested, 4-nitroquinoline 1-oxide, 4-nitroquinaldine 1-oxide, 6-methyl-4-nitroquinoline 1-oxide and 6-chloro-4-nitroquinoline 1-oxide produced the pulmonary alterations in rats (Table 3). In the groups treated with 3-methyl-4-nitroquinoline 1-oxide, 4-nitroquinoline, quinoline 1-oxide and 4-nitropyridine 1-oxide, the lungs appeared grossly normal and the lung-weight was comparable to that of the control rats.

| Table 3. Occurrence of pulmonary edema in rats by various quinoline derivatives. | Dose mg/kg | Route | Lung-weight per body-weight g% | Froth in tracheobronchial lumina |
|---|---|---|---|---|
| Compound | | | | |
| 4-Nitroquinoline 1-oxide | 20 | s.c. | 1.44±0.09 * | + |
| | 20 | i.v. | 1.57±0.10 * | + |
| 4-Nitroquinoline | 30 | s.c. | 0.63±0.13 | − |
| | 10 | i.v. | 0.69±0.11 | − |
| Quinoline 1-oxide | 60 | s.c. | 0.54±0.09 | − |
| 4-Nitroquinaldine 1-oxide | 30 | s.c. | 0.95±0.09 * | + |
| 3-Methyl-4-nitroquinoline 1-oxide | 60 | s.c. | 0.59±0.03 | − |
| | 20 | i.v. | 0.61±0.03 | − |
| 6-Methyl-4-nitroquinoline 1-oxide | 30 | s.c. | 0.62±0.02 | − |
| | 20 | i.v. | 1.05±0.13 * | + |
| 6-Chloro-4-nitroquinoline 1-oxide | 10 | i.v. | 1.44±0.07 * | + |
| 4-Nitropyridine 1-oxide | 20 | s.c. | 0.58±0.02 | − |
| Control (1) | 0 | s.c. | 0.60±0.01 | − |
| Control (2) | 0 | i.v. | 0.57±0.02 | − |

Control (1) : given a subcutaneous injection of 0.2 ml olive-oil.
Control (2) : given a intravenous injection of 0.1 ml dimethylsulfoxide.

* P<0.01

In Table 4, 4-nitroquinoline 1-oxide and 4-hydroxyaminoquinoline 1-oxide are compared with regard to their activity in causing acute respiratory distress in guinea-pigs. Administration of 4-nitroquinoline 1-oxide either through subcutaneous or intravenous route produced labored breathing in association with edematous changes of the lung, while no respiratory distress appeared in the group treated with 4-hydroxyaminoquinoline 1-oxide.
TABLE 4. Acute pulmonary alterations of guinea-pigs following a single injection of 4-nitroquinoline 1-oxide and 4-hydroxyaminoquinoline 1-oxide.

| Compound                        | No. of animals | Dose mg/kg | Route | Lung-weight g% per body-weight | Froth in tracheobronchial lumina |
|---------------------------------|----------------|------------|-------|-------------------------------|---------------------------------|
| 4-Nitroquinoline 1-oxide        | 7              | 30         | s.c.  | 1.93±0.07*                    | +                               |
|                                 | 5              | 30         | i.v.  | 1.32±0.14*                    | −                               |
| 4-Hydroxyaminoquinoline 1-oxide | 5              | 30         | i.v.  | 0.73±0.03                     | −                               |
| Control                         | 7              | 0          | −      | 0.75±0.02                     | −                               |
|                                 | 5              | 0          | −      | 0.69±0.02                     | −                               |

* P<0.01

Fig. 2. The lungs from rats 56 hours after an injection of 4-nitroquinoline 1-oxide in a dose of 20 mg/kg, showing dark red discoloration and consolidation in various grades.

Fig. 3. The lungs from guinea-pigs 6 hours after an injection of 4-nitroquinoline 1-oxide in a dose of 30 mg/kg, showing marked congestion. Pinkish froth was noted in the tracheal lumen and at a cut-surface of the pulmonary parenchyme.
FIG. 4. An area of the lung from a guinea-pig 6 hours after an injection of 4-nitroquinoline 1-oxide in a dose of 30 mg/kg. The alveolar spaces were filled with eosinophilic material. The blood-vessels were shown to be dilated. Hematoxylin-eosin, 50 x.

DISCUSSION

This experiment indicates that a single injection of 4-nitroquinoline 1-oxide, a potent carcinogen (6), can produce in rats labored breathing with frothy nasal discharge, marked pulmonary congestion, and accumulation of proteinaceous fluid in the alveolar spaces. These alterations, consistent with pulmonary edema, have not previously been reported to be caused in animals by this carcinogen. Similar symptoms were also shown to occur in guinea-pigs and rabbits treated with 4-nitroquinoline 1-oxide, though mice did not exhibit such respiratory distress even after a high dose of this compound. These findings are in contrast with experimental data on the carcinogenicity of 4-nitroquinoline 1-oxide; where mice are known to be highly sensitive to this compound (7, 8), while guinea-pigs have very low sensitivity (9). This inverse relation might suggest that 4-nitroquinoline 1-oxide can produce either pulmonary edema or tumor in animals through different modes of action.

It has been reported that 4-nitroquinoline 1-oxide as well as allied carcinogenic 4-nitroquinoline derivatives, 4-nitroquinaldine 1-oxide, 6-methyl-4-nitroquinoline 1-oxide and 6-chloro-4-nitroquinoline 1-oxide, undergo two types of chemical reaction; 1) Reduction to hydroxyaminoquinoline derivatives (5, 10) and 2) Substitution reaction with thiol-groups (11-13). Since 4-hydroxyaminoquinoline 1-oxide itself was shown to be a potent carcinogen (14, 15), experimental data have been published to support the view (5, 10, 15) that 4-nitroquinoline 1-oxide elicits its carcinogenic reaction after conversion into 4-hydroxyaminoquinoline 1-oxide. In contrast, this experiment indicated that 4-hydroxyaminoquinoline 1-oxide was incapable of producing pulmonary edema. In this experiment it was further shown that pulmonary edema was not produced either by such compounds as 4-nitroquinoline, quinoline 1-oxide, 3-methyl-4-nitroquinoline 1-oxide and 4-
nitropyridine 1-oxide, which are known to possess low reactivity with thiol-groups (11, 13). On the basis of these findings, it might be assumed that the substitution reaction with a thiol-group is an essential biochemical step through which 4-nitroquinoline 1-oxide can cause pulmonary edema.

Pulmonary edema can be induced in animals by many chemicals including α-naphthylthiourea (17), epinephrine (18, 19) and phosgen (20). Born (21) and Aviado et al. (22) have reported that edematous changes can be produced in the excised lung by perfusing it with a solution containing SH-inhibitors such as alloxan, parachloromercuribenzoate, iodoacetic acid and N-ethylmaleimide. However, administration of these compounds into living animals is not associated with a consistent production of pulmonary edema; because the injurious effect of these compounds is not specific to the lung. The present experiment which resulted in high incidence of pulmonary edema in living animals therefore might reflect a strong affinity of 4-nitroquinoline 1-oxide with the pulmonary tissue.

SUMMARY

A single administration of 4-nitroquinoline 1-oxide, a potent carcinogen, produced pulmonary edema in rats, guinea-pigs, and rabbits. Mice appeared not to be affected in this way by 4-nitroquinoline 1-oxide. Other carcinogenic quinolines, 4-nitroquinaldine 1-oxide, 6-methyl-4-nitroquinoline 1-oxide and 6-chloro-4-nitroquinoline 1-oxide were as active as 4-nitroquinoline 1-oxide; though 4-hydroxyaminoquinoline 1-oxide, a compound known as a proximate carcinogenic metabolite of 4-nitroquinoline 1-oxide, was inactive in producing pulmonary edema. Quinoline derivatives having weak reactivity with thiol-groups did not produce pulmonary edema.

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