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Effects of Pretreatment with Anti-Inflammatory Drugs on Ozone-Induced Lung Damage in Rats (39130)

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(Introduced by S. A. Peoples)

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Ozone (O_3), a major constituent of photochemical smog, is a strongly reactive oxidant of atmospheric origin (1). The primary targets of its action in the body are the lung and respiratory tract, although extrapulmonary effects have also been noted (2).

The toxic signs associated with exposure to O_3 at 4–20 ppm include massive pulmonary edema, capillary hemorrhage, and inflammation of the tracheobronchial tree (1, 3). A number of theories have been advanced to explain the mechanism for O_3-induced toxicity (4–7). These include oxidation of intracellular components such as unsaturated fatty acids, sulfhydryl groups, amino acids, flavins, and pyridine nucleotides. The oxidation of intracellular components presumably leads to inactivation of enzymes and alteration of membrane structure and function.

In addition to the above mechanisms, histamine, serotonin, and bradykinin have also been suggested as the possible mediators of O_3-induced inflammatory reactions (8, 9). Therefore, attempts have been made to modify the vascular reaction following O_3 exposure by prior treatment with antihistaminic and antibradykinin drugs (10).

The direct and/or indirect involvement of prostaglandins (PGs) in tissue inflammatory reactions (11) as well as in lung diseases (12) has been well substantiated. Their role, however, in O_3-induced toxicity has not yet been evaluated. This prompted us to investigate the effects of pretreatment with certain compounds which inhibit the synthesis of PGs on O_3-induced pulmonary edema. We have examined the effects of O_3 exposure following pretreatment with four compounds, indomethacin, acetylsalicylic acid (aspirin), hydrocortisone, and heparin. The first two compounds were used because of their well-documented ability to inhibit lung-PG synthesis while hydrocortisone has a feeble effect (13). Heparin was used because of its favorable clinical effects reported in a variety of inflammatory conditions (14).

Methods and materials. Male Sprague–Dawley rats weighing 350 to 450 g, unless otherwise specified, were purchased from Simonsen Lab Incorporated, Gilroy, California, and chronic respiratory disease-free rats from Hilltop Lab, Inc., Pennsylvania. They were fed standard Purina rat chow and water *ad libitum*. Hydrocortisone and heparin were purchased from Sigma Chemical Company and aspirin from Merck and Company, Incorporated. Indomethacin was a generous gift from Merck.

Hydrocortisone and indomethacin were suspended in phosphate buffer (0.155 *M*, pH 7.4) by triturating in a motor-driven glass homogenizer (Tri-R). Aspirin and sodium salt of heparin were easily soluble in phosphate buffer at the concentrations employed in the study. Regardless of the dose, the injectable volume for each drug was kept constant, 0.8 ml/100 g body wt. The anti-inflammatory drugs were injected ip 60 to 90 min prior to exposure to 4 ppm O_3 for 4 hr. Rats in the control group were treated similarly with an equivalent volume of phosphate buffer before exposure to ambient air for the same length of time. The rats were placed in fiberglass chambers with a volume of 21 cubic feet, and received either room air (control), or air mixed with O_3 (experimentals) at a flow rate of 5 ft^3/min for 4 hr. Ozone level during exposure was continuously monitored with a microcoulomb ozone senser. Ozone concentration was also determined chemically by the potassium iodide method (15).

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Following exposure, rats were lightly etherized, the abdominal cavity opened and the descending aorta severed to allow free bleeding. For quantitation of pulmonary edema, the trachea was exposed and ligated immediately. The thoracic cavity was then opened to remove the lungs and heart *en bloc*. The lungs were separated from the heart, blotted gently, and weighed. The dry weight of lung tissue of each rat was determined by heating in an oven at 60°C until a constant weight was attained (72 hr). Both wet lung weight (WLW) and dry lung weight (DLW) per kilogram body weight were used to compare the degrees of pulmonary edema between the control and experimental groups (16).

All data are reported as the mean ± standard error (SE). The data were analyzed throughout using Student's t test, with $P < 0.05$ as the level of significance.

Results. The effects of O_3 exposure (4 ppm for 4 hr) on rats pretreated with phosphate buffer or anti-inflammatory drugs were investigated in two experiments. In the first, rats were treated with a high dose of aspirin, hydrocortisone, indomethacin, or heparin prior to O_3-exposure; in the second series of experiments only aspirin and indomethacin were investigated, using a much smaller dose than the one employed in the first series. The results of the first experiments are summarized in Table I, and the second group is summarized in Table II.

Regardless of the type of pretreatment, the exposure to O_3 was always associated with the occurrence of pulmonary edema. This was indicated by the findings in both series that rats exposed to O_3 had significantly higher wet and dry lung weights than those exposed to ambient air. The increase in dry lung wt in O_3-exposed rats was attributed to the leakage of plasma proteins and blood cells in the alveoli.

The interesting finding of the present study was that the treatment with the anti-inflammatory drugs using high doses prior to O_3 exposure altered the magnitude of lung damage normally encountered in rats exposed to O_3. As summarized in Table I, the anti-inflammatory drugs affected the O_3-induced pulmonary edema in the following ways. An enhanced dergee of pulmonary edema was associated with the aspirin pretreatment, as indicated by increased WLW over rats exposed to O_3 with no drug pretreatment. There was no significant difference, however, in the DLW between these two groups. The aspirin pretreatment by itself did not produce any lung damage as noted in five rats treated with the same amount of aspirin (125 mg/kg) prior to exposure to ambient air. For example, the WLW/kg body wt in these rats averaged 3.76 ± 0.20 (SE) as compared to 3.54 ± 0.12 (SE) in 12 rats pretreated with phosphate buffer and exposed to ambient air. Treatment with a low dose (10 mg/kg) of aspirin prior to O_3-exposure had no effect on O_3-induced lung damage, as no significant difference in WLW or DLW was noted between this group and the one exposed to O_3 without low dose aspirin pretreatment (Table II).

The hydrocortisone pretreatment did not alter the injurious effects of O_3 on the pulmonary tissue. The degree of pulmonary edema in these rats, as reflected by the WLW and DLW, was not statistically different from rats exposed to O_3 without hydrocortisone pretreatment. A substantial reduction in the pulmonary edema was observed when rats were treated either with a high dose (20 mg/kg) of indomethacin or heparin prior to O_3 exposure. This was substantiated by a highly significant decrease in both WLW and DLW in these rats as compared to rats exposed to O_3 without these drug pretreatments (Table I). The protective effect of indomethacin against O_3-induced lung damage was not seen when rats were treated with a smaller dose (2.5 mg/kg) of this drug (Table II) prior to O_3-exposure.

Discussion. It has been well established that anti-inflammatory drugs inhibit PG-synthesis by inhibiting the activity of PG-synthetase, and this has consequently led to the popular belief that the PGs are involved in certain stages of tissue inflammation. Tan and Privett (17) have recently reported that hydroperoxide, which produces lung damage in a manner similar to O_3, impaired the biosynthetic ability of the lung tissue to convert arachidonic acid to PG.

TABLE I. EFFECTS OF O_3 EXPOSURE ON WET LUNG WEIGHT AND DRY LUNG WEIGHT IN RATS PRETREATED WITH PHOSPHATE BUFFER, INDOMETHACIN, ASPIRIN, HYDROCORTISONE, AND HEPARIN[a]

Group	Pretreatment[b]	Exposure	WLW/kg	DLW/kg	*P*-values between groups: Groups	WLW	DLW
1	PO_4 buffer	Ambient air	3.54 ± 0.12 (12)[c]	0.81 ± 0.02 (12)	1, 2	<0.001	<0.001
2	PO_4 buffer	Ozone	7.68 ± 0.41 (10)	1.16 ± 0.02 (10)	1, 3	<0.001	<0.001
3	Indomethacin (20 mg/kg ip)	Ozone	6.14 ± 0.32 (9)	1.06 ± 0.03 (9)	1, 4	<0.001	<0.001
4	Aspirin (125 mg/kg ip)	Ozone	9.40 ± 0.50 (7)	1.20 ± 0.02 (7)	1, 5	<0.001	<0.001
					1, 6	<0.001	<0.001
5	Hydrocortisone (50 mg/kg ip)	Ozone	7.44 ± 0.67 (8)	1.15 ± 0.05 (8)	2, 3	<0.02	<0.05
					2, 4	<0.05	NS
6	Heparin (1,000 units/kg ip)	Ozone	5.77 ± 0.32 (7)	1.02 ± 0.04 (7)	2, 5	NS[d]	NS
					2, 6	<0.01	<0.01

[a] Weights are expressed in g/kg body wt (mean ± SE). Exposure was 4 ppm for 4 hr.
[b] Rats were injected 60–90 min prior to O_3 exposure.
[c] Numbers in parentheses are the numbers of animals used.
[d] NS = $P > 0.05$.

TABLE II. EFFECTS OF O_3 EXPOSURE ON WET LUNG WEIGHT AND DRY LUNG WEIGHT IN RATS[a] PRETREATED WITH PHOSPHATE BUFFER, SMALL DOSES OF ASPIRIN, OR INDOMETHACIN[b]

Group	Pretreatment[c]	Exposure	WLW/kg	DLW/kg	*P*-values between groups: Groups	WLW	DLW
1	PO_4 buffer	Ambient air	3.02 ± 0.08	0.74 ± 0.03	1, 2	<0.01	<0.05
2	PO_4 buffer	Ozone	5.50 ± 0.55	0.86 ± 0.03	1, 3	<0.001	<0.05
					1, 4	<0.01	<0.01
3	Indomethacin (2.5 mg/kg ip)	Ozone	6.80 ± 0.22	1.01 ± 0.04	2, 3	NS[d]	NS
4	Aspirin (10 mg/kg ip)	Ozone	6.55 ± 0.70	0.97 ± 0.06	2, 4	NS	NS

[a] Rats weighing 475 to 680 g were used.
[b] Weights are expressed in g/kg body weight. Each value is the mean ± SE of four animals. Exposure was 4 ppm for 4 hr.
[c] Rats were injected 60–90 min prior to O_3 exposure.
[d] NS = $P > 0.05$.

The level of PG, however, remained paradoxically unchanged with hydroperoxide treatment. This also suggested an impairment in the ability of the lung to biotransform PG. Direct evidence for the involvement of PG-inactivating enzyme of the lung in O_2-induced lung damage was recently reported by Eling and Parkes (18). According to these authors, the exposure of guinea pigs to 100% O_2 decreased the ability of the lung to biotransform PG without any effect on its synthesis. Whether or not the injurious effect of O_3 on the lung tissue is mediated by the presence of an unphysiological amount of PG, resulting from either an increased biosynthesis or decreased biotransformation, is not known.

In the present study, treatment of animals with anti-inflammatory drugs known to inhibit PG-synthesis prior to O_3 exposure produced unexpectedly inconsistent results. Aspirin at a high dose (125 mg/kg) intensi-

fied the O_3-induced lung damage in rats, but had no effect at the low (10 mg/kg) dose. This was in direct contrast to the finding of Dixon and Mountain (19) that aspirin given 8 mg/kg systematically just before and again 2 hr after the start of the exposure was an effective suppressor of O_3-produced lung edema in mice. These discrepancies are difficult to explain. They may be attributed to species difference and/or to a high dose and/or to different regimen of aspirin administration employed in the present study. The release of hydrolytic enzymes from the lysosome is one of the suggested mechanisms for O_3-induced lung damage (20). It has been demonstrated that aspirin at the high dose labilizes and at low dose stabilizes the lysosomal membrane (21). This would then explain why aspirin at the high dose (125 mg/kg) accentuated the O_3-induced lung damage, but this does not explain why aspirin failed to offer any protection against O_3 at the low dose (10 mg/kg).

There is evidence in the literature that aspirin-like anti-inflammatory drugs not only decrease the biosynthesis of PGs but also decrease their biotransformation (22). One may hypothesize that the increased lung damage following O_3 exposure in rats pretreated with a high dose of aspirin may also be due to inhibitory effects of aspirin on PG-inactivating enzyme. In contrast to aspirin, the apparent protective effect of indomethacin at the high dose against the O_3-induced lung damage might be explained by a greater ability of this compound to inhibit PG-synthesis (23) and/or to antagonize PG at the receptor level (24, 25).

Our finding that hydrocortisone pretreatment failed to have any effect on O_3-induced pulmonary edema was in agreement with the findings reported by Matzen (26) that hydrocortisone treatment had no beneficial effect against O_3-induced lung damage. This was not surprising since steroidal anti-inflammatory drugs are known to have a feeble effect on the enzymes responsible for PG-synthesis and degradation (13). The protective effect of heparin against O_3-induced lung damage was a great surprise. How heparin accomplishes this effect is not presently known. It is conceivable that heparin increases the activity of PG-dehydrogenase by chelating calcium ions in the lung, and thus facilitates the rapid bioinactivation of PGs. This hypothesis is based on the findings of Limas and Cohn (27) that myocardial PG-dehydrogenase is inhibited by Ca^{2+} ions in concentrations of 10^{-7} *M* and above.

Summary. The effects of pretreatment with acetylsalicylic acid (aspirin), hydrocortisone, indomethacin, and heparin administered ip against the pulmonary edema produced by O_3-exposure (4 ppm for 4 hr) were studied in rats. These anti-inflammatory drugs were found to alter the injurious effect of O_3 on lung differently. First, aspirin at the high dose (125 mg/kg) accentuated O_3-induced lung injury, and had no effect at the low dose (10 mg/kg); second, hydrocortisone (50 mg/kg) failed to have any effect; third, indomethacin at a high dose (20 mg/kg) offered a significant degree of protection, but had no effect at the low dose (2.5 mg/kg); and fourth, heparin (1000 units/kg) also offered a significant degree of protection against the lung damage normally induced by O_3-exposure. Several mechanisms for the favorable and unfavorable interactions of anti-inflammatory drugs with O_3-exposure are discussed.

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