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SHORT COMMUNICATIONS

Pulmonary oxygen toxicity in the rat and the role of histamine

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The interaction of free radicals with biological membranes as a primary mechanism for explaining pulmonary oxygen (O_2) toxicity has focused on the ability of O_2 free radicals to destroy cells that comprise the lung air–blood interface [1]. Suggestions also have been proposed that O_2-induced lung damage may be due to specific free radical-induced inflammatory responses of the lung. Such studies have implicated both inflammatory cells [2] as well as chemical mediators [3–5] in the development of the O_2-induced lung damage. Histamine, a primary mediator of immediate hypersensitivity reactions, has been considered previously for its role in pulmonary O_2 toxicity [6, 7]. More recently *in vivo* hyperoxic exposure has been found to increase the histamine concentration in blood [8] and plasma [9] of rats, and to increase histamine release from isolated peritoneal mast cells [10]. In earlier studies [9], a correlative relationship between the duration of exposure of rats to 100% O_2 and the concentration of histamine released from the isolated perfused lung (IPL) was found. These findings and others [7] suggested that various antihistamines might be used to delay the onset, or decrease the severity, of pulmonary O_2 toxicity in rats. Studies were therefore carried out to investigate further the effect of both the duration of O_2 exposure and O_2 pressure on the spontaneous histamine release and malondialdehyde (MDA) formation from the rat IPL. In addition, specific H_1- and H_2-receptor antagonists, *d*-chlorpheniramine and metiamide, respectively, were administered to rats to determine their effects on blocking O_2-induced pulmonary edema.

Materials and methods

S-Adenosyl-L-methyl-[^{3}H]methionine (sp. act. 14 Ci/mmole) (New England Nuclear, Boston, MA), thiobarbituric acid (TBA), histamine free base (Sigma Chemical Co., St. Louis, MO) and malondialdehyde tetramethyl acetal (Eastman Kodak Co., Rochester, NY) were purchased from the sources indicated. Metiamide was provided by the Smith Kline Beckman Corp. (Philadelphia, PA) and *d*-chlorpheniramine by the Schering Corp. (Bloomfield, NJ). In the *in vivo* experiments, *d*-chlorpheniramine or metiamide was administered to rats intraperitoneally every 6 hr. A 0.5% methyl cellulose/0.9% saline solution was used as the vehicle in the *in vivo* experiments, while modified Krebs–Henseleit buffer was used as the vehicle in the IPL experiments.

Adult, respiratory disease-free female Sprague–Dawley rats (190–230 g, 5- to 7-weeks-old), bred and raised in the Animal Care Facility at the University of Kansas, were used in all experiments. In the IPL studies, rats were exposed to either 1 atmosphere of air, or 100% O_2, for 12, 24, or 48 hr, or to 1.0, 1.5, or 1.75 atmospheres of 100% O_2 for 12 hr. After the appropriate exposure period, the IPLs were prepared [9], and the IPL perfusate was analyzed for histamine [11] and malondialdehyde [12]. Metiamide and *d*-chlorpheniramine, when used to study the maximal release of histamine from the IPL, were added in solution form directly to the IPL perfusate at the onset of perfusion. In the experiments to investigate the protective effect of these antihistamines *in vivo*, *d*-chlorpheniramine or metiamide was given to rats every 6 hr while exposed to either 1 atmosphere of air or 100% O_2 during the 24- or 48-hr exposure period. After exposure, the rats were killed, their lungs were removed, and the lung dry weight/wet weight ratio was determined, which was used as an index of pulmonary edema formation [13].

Statistical comparisons were carried out employing a one-way analysis of variance (ANOVA) followed by a Student–Newman–Keuls *a posteriori* multiple comparison of means test.

Results and discussion

The effects of varying the duration of O_2 exposure and O_2 pressure on IPL maximal histamine release and perfusate MDA are shown in Fig. 1, A and B. A linear relationship was found between both IPL perfusate histamine and MDA content and the duration of 100% O_2 exposure. IPL perfusate histamine and MDA from lungs of rats exposed to both 24 and 48 hr of 100% O_2 were significantly higher than corresponding controls. As the absolute pressure of O_2 was increased, IPL perfusate histamine and MDA increased exponentially. Maximal histamine release and perfusate MDA content in rats exposed to 1.75 atmosphere of 100% O_2 were significantly higher than controls. It also was found that *d*-chlorpheniramine and metiamide, when added to the IPL perfusate, markedly altered IPL histamine release (Table 1). *d*-Chlorpheniramine significantly decreased perfusate histamine content, whereas metiamide significantly increased perfusate histamine content when compared to control in IPLs of both air- and O_2-exposed rats. In the *in vivo* experiments, *d*-chlorpheniramine (11.4 mg/kg i.p.) attenuated the O_2-induced edematogenesis in rats, while equimolar doses of metiamide had no significant effect (Fig. 2).

The cause and origin of O_2-induced IPL histamine release are not clearly defined. From studies by others, it is suggested that IPL histamine release may be due to O_2-damaged pulmonary mast cells as a result of a free radical mechanism. For example, intrapulmonary instillation of O_2 free radical generators has been shown to produce acute inflammatory pulmonary edema in rabbits [14]. Xanthine and xanthine oxidase, which generate superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), have also been shown to cause histamine release from isolated peritoneal mast cells [15]. Similarly, hyperoxic exposure produces acute inflammatory pulmonary edema in mammals. Hyperoxic exposure also can stimulate O_2^- and H_2O_2 production in rat lung tissue [16, 17], and cause histamine release from isolated peritoneal mast cells [10]. Mast cells are the primary storage site for histamine in lung tissue. Their membranes contain a large proportion of phospholipids, thus making mast cells particularly susceptible to free radical attack by O_2 reactive species. Although the use of MDA as an index of non-enzymatic lipid peroxidation is controversial [18, 19], the present studies provide a good correlation between increases in the duration of O_2 exposure or O_2 pressure, and IPL histamine release and MDA formation. This seems to suggest that O_2-induced histamine release is a free radical-mediated event.

d-Chlorpheniramine inhibited, while metiamide enhanced, IPL histamine release (Table 1). Both inhibition of release by H_1-receptor antagonists [20] and enhancement of release by H_2-receptor antagonists [21, 22] also have

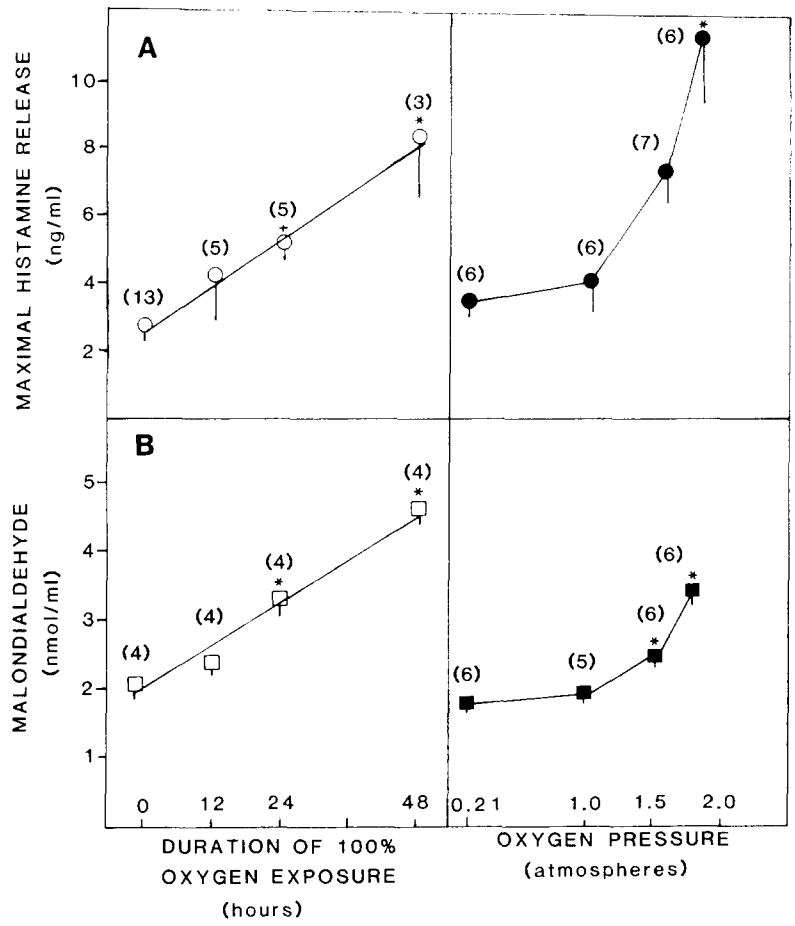

Fig. 1. Effect of increased duration of O_2 exposure (open symbols) and O_2 pressure (closed symbols) on maximal histamine release (A) and perfusate MDA (B) from the IPL. Values are the mean ± S.E.M., with the number of rats in each group shown in parentheses. Key: (*) $P < 0.01$, and (†) $P < 0.05$ when compared to controls.

Table 1. Effect of *d*-chlorpheniramine or metiamide on histamine release from rat IPL

Treatment group	Perfusate histamine (ng/ml)
Air + vehicle	2.8 ± 0.4 (13)
Air + *d*-chlorpheniramine	0.0 ± 0.0* (3)
100% O_2 + vehicle	4.6 ± 0.5† (7)
100% O_2 + *d*-chlorpheniramine	0.3 ± 0.4‡ (3)
Air + vehicle	2.1 ± 0.6 (8)
Air + metiamide	4.8 ± 0.8† (6)
100% O_2 + vehicle	5.7 ± 0.7† (5)
100% O_2 + metiamide	8.4 ± 1.0§ (5)

Rats were exposed to 1.0 atm of either air or 100% O_2 for 24 hr, the rats were killed, and the IPLs were prepared. *d*-Chlorpheniramine (100 μM) or metiamide (100 μM) was added to the perfusion fluid and the isolated lung was perfused for 30 min. Values are the mean ± S.E.M., with the number of rats shown in parentheses.

* $P < 0.001$ when compared to air + vehicle.
† $P < 0.05$ when compared to air + vehicle.
‡ $P < 0.001$ when compared to O_2 + vehicle.
§ $P < 0.05$ when compared to O_2 + vehicle.

been reported previously by others using different tissue systems. The mechanism of action of these histamine-receptor antagonists on IPL histamine release is unclear. *d*-chlorpheniramine does not appear to inhibit release via an anticholinergic effect [9], or its capacity as an H_1-receptor blocker [20]. Rather, the mechanism may be one of mast cell stabilization [20]. The mechanism by which metiamide enhanced IPL histamine release is also tenuous at this time. Others have similarly reported enhancement of immunologic [21] and non-immunologic [22] histamine release by H_2-receptor antagonists using different tissue preparations. The mechanism by which this occurs has been suggested to be through inhibition of histamine's autoregulatory influence on the release process. Exposure to 100% O_2 caused a progressive increase in lung fluid accumulation in rats (Fig. 2). A significant change in lung dry weight/wet weight ratios was observed only for rats exposed to 48 hr of 100% O_2. This finding is in agreement with those of others who have employed alternative techniques for the assessment of O_2-induced changes in lung vascular permeability [23]. Repetitive treatment with 0.11 mg/kg *d*-chlorpheniramine or 7.1 mg/kg metiamide had no effect on the formation of lung fluid accumulation in rats during hyperoxic exposure (Fig. 2). However, multiple treatments of rats with 11.4 mg/kg of *d*-chlorpheniramine significantly attenuated lung water retention in rats exposed to 100% O_2 for 48 hr.

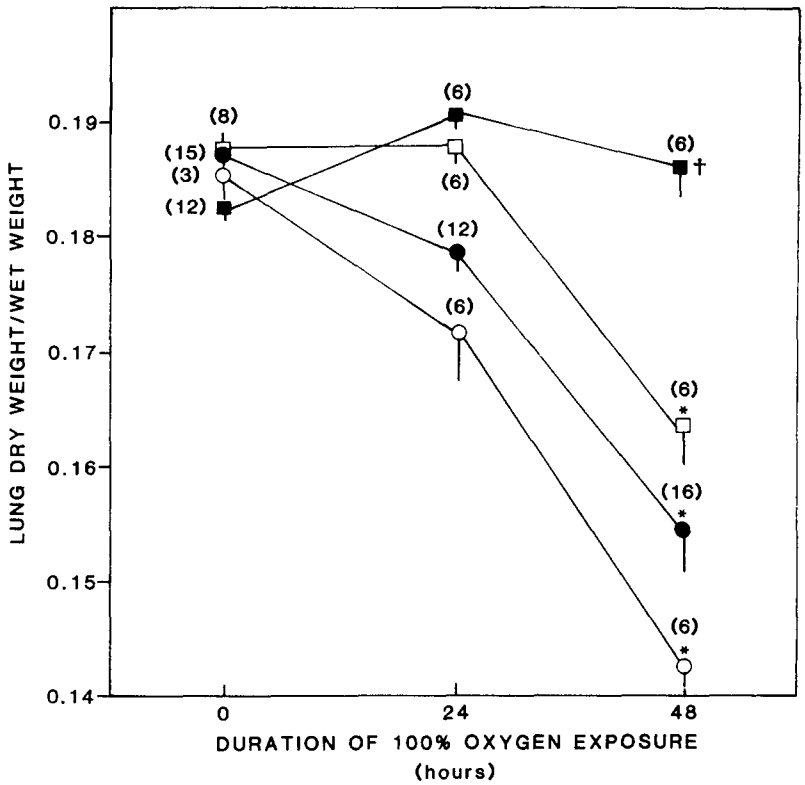

Fig. 2. Lung dry weight/wet weight ratios from rats treated with *d*-chlorpheniramine or metiamide and exposed to 100% O_2. Values are the mean ± S.E.M. with the number of rats in each group shown in parentheses. Key: (●) O_2 plus vehicle, (○) O_2 plus 0.11 mg/kg *d*-chlorpheniramine, (■) O_2 plus 11.4 mg/kg *d*-chlorpheniramine, and (□) O_2 plus 7.1 mg/kg metiamide; (*) $P < 0.01$ when compared to control; and (†) $P < 0.05$ when compared to other treatment groups at similar O_2 exposures.

The reason *d*-chlorpheniramine attenuated O_2-induced pulmonary edema development in the rat is unclear. One possibility is that *d*-chlorpheniramine inhibited histamine H_1-receptor-mediated changes in lung vascular permeability [24]. Another explanation is that *d*-chlorpheniramine inhibited mast cell degranulation and subsequent endogenous histamine release (Table 1). Inhibition of mast cell degranulation by *d*-chlorpheniramine could further attenuate inflammatory reactions of the lung if the release of mast cell contents other than histamine [25], is similarly inhibited by the H_1-receptor antagonist. Prostaglandin and serotonin vascular effects, and inflammatory cell chemotaxis are all factors previously reported to be important in both lung hypersensitivity reactions and the development of pulmonary O_2 toxicity in the rat [2, 26, 27].

Both IPL perfusate histamine and MDA increased as the duration/pressure of O_2 exposure was increased. It also was observed that the histamine H_1-receptor antagonist *d*-chlorpheniramine significantly inhibited release, whereas the histamine H_2-receptor antagonist metiamide enhanced spontaneous histamine release from the rat IPL. Furthermore, in *in vivo* studies, *d*-chlorpheniramine administration was found to attenuate O_2-induced pulmonary edema development in rats. These studies help to support the idea that endogenous histamine release or mast cell degranulation may be an important factor in the exudative phase of pulmonary O_2 toxicity.

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Lack of hepatotoxic interaction between the anticonvulsant drugs phenytoin, sodium valproate and phenobarbital in the rat

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Phenytoin and valproic acid are widely used anticonvulsant drugs that have been implicated in rare cases of idiosyncratic hepatotoxicity in human beings [1–3]. Liver damage attributed to phenytoin usually occurs in the context of a hypersensitivity (allergic) reaction [1, 2], whereas the hepatotoxicity caused by valproate is believed to result from a metabolic idiosyncrasy in susceptible individuals [2, 3]. Anticonvulsant-induced hepatotoxicity frequently occurs in epileptic patients taking two or more anticonvulsant drugs [2]. Indeed, polytherapy-induced interactions have been suggested to be a major cause of many types of chronic toxicity associated with anticonvulsant drug therapy, and this has resulted in an advocacy for monotherapy [4]. However, control of seizures with a single drug is not always possible and, therefore, a knowledge of potential interactions is essential to devise therapeutic regimens which minimize the risk of adverse reactions. We have set up potential interactions between phenytoin, sodium valproate and phenobarbital, at doses approximately ten times human therapeutic levels, as a possible cause of hepatotoxicity in rats and thus a model for liver damage that can occur in human beings.

Materials and methods

Forty male albino Wistar rats (200 g, Charles River, Canada Inc.) were divided into ten treatment groups. Water and Purina Certified Rodent Chow No. 5002 were supplied *ad lib.* Anticonvulsant drugs, as their sodium salts, valproic acid (300 mg/kg), phenytoin (100 mg/kg) and phenobarbital (65 mg/kg), and 0.9% sodium chloride (saline control) were given orally by gavage as aqueous solutions on each of five successive mornings. Nine hours after the fifth dose all animals received a single (acute challenge) dose of a different anticonvulsant drug, sodium valproate (300 mg/kg) or phenytoin (100 mg/kg), or saline. Animals were killed 16 hr after the acute dose.

Heparinized blood was collected when the animals were killed, and plasma was analyzed for alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total protein (Worthington Kits) using a Roto-chem CFA 2000 centrifugal analyzer, and ornithine carbamyl transferase [5]. For histopathologic evaluation, liver tissue was fixed in formalin, embedded in paraffin, and sections were stained with hematoxylin and eosin or periodic acid-Schiff. The remaining liver was homogenized in 0.25 M sucrose (pH 7.4). Reduced glutathione (sulfosalicylic acid non-precipitable reduced sulfhydryls) concentration was determined in the liver homogenate [6]. Microsomes prepared with calcium chloride [7] were assayed for: cytochrome(s) P-450 [8], aniline hydroxylase [9], aminopyrine demethylase [9], glucose-6-phosphatase [10], and protein [11]. Enzyme activities are expressed as nmoles or μmoles of product formed per mg of microsomal protein per min.

The data were analyzed by two-way analysis of variance (ANOVA). Significance ($P < 0.05$) was determined for each of the subacute and acute treatment regimens, as well as their potential interaction, for each variable measured. Statements of significance with respect to a particular drug and regimen are made in reference to control (saline) animals given the same regimen.

Results and discussion

The rationale for our approach in setting up potential hepatotoxic interactions between phenytoin, sodium valproate, and phenobarbital was based on two factors. First, both phenytoin and valproic acid are converted in the liver by the cytochrome(s) P-450-linked mixed-function oxidase system to potentially reactive metabolites [3, 12–14]. Such metabolites could trigger injury either directly, in the case of valproate, or indirectly, in the case of phenytoin by acting as a hapten leading to secondary immune responses [12] or by causing mild subclinical hepatotoxicity upon which a superimposed hypersensitivity reaction leads to overt liver damage [1]. Second, there are many ways in which combinations of drugs may cause or enhance a toxic response. Damage may result from direct interaction at the cellular targets of toxicity or may be secondary to pharmacokinetic interactions. The latter interactions between anticonvulsant drugs include induction of oxi-