

The Influence of Paraquat on the *in vitro* Oxygen Consumption of Rabbit Lung

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SUMMARY

The effects of paraquat on the aerobic metabolism of rabbit lung slices were investigated. The oxygen consumption of lung slices was examined at different oxygen tensions and in the presence or absence of glucose as substrate in a Krebs-Ringer phosphate medium.

In an air phase, the oxygen consumption of control lung tissue with glucose in the medium was practically the same as the oxygen consumption without glucose over a 3-hour period. In a 100% oxygen phase, glucose induced a marked increase in oxygen uptake, which persisted for about 2 hours. Thereafter, a decrease occurred in oxygen consumption which was notably faster than that of lung tissue without added glucose.

With 10 mM glucose in the medium, paraquat (10^{-5} M and 10^{-7} M) immediately stimulated the oxygen consumption of lung slices. Although the initial increase in aerobic metabolism seemed to be glucose-dependent, the secondary inhibitory effect of paraquat appeared to be of the same magnitude in the presence or absence of glucose. Both the initial stimulation as well as the secondary inhibition were much more pronounced in a 100% oxygen atmosphere than in an air phase.

These results indicate that the rabbit lung is sensitive to paraquat toxicity *in vitro*, and confirm that oxygen and paraquat supplement each other's toxicity in the lung.

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Paraquat^R (1,1'-dimethyl-4,4'-dipyridylum dichloride) is a quaternary bipyridylum herbicide.¹ Paraquat poisoning in man² and laboratory animals³ results in injury to the lung. No similar pulmonary lesions have ever been reported from other related bipyridylum compounds, e.g. diquat (1,1'-ethylene-2,2'-dipyridylum), although their herbicidal action is very similar.⁴ Furthermore, clinical observation and experimental evidence suggest that oxygen therapy aggravates the morbidity and mortality of paraquat poisoning.^{2,5}

Paraquat toxicity in mammals is characterized by delayed development of pulmonary lesions.⁶ In the first few days after exposure, paraquat produces a widespread disintegration of both type I and type II alveolar cells, followed by massive oedema and acute inflammatory changes (the destructive phase). The second or pro-

liferative phase is characterized by an intra-alveolar fibrosis, which later develops into an interstitial fibrosis.⁶

The mechanism of paraquat toxicity in mammals remains an enigma. In experimentally induced paraquat poisoning, it has been shown that paraquat accumulates in the lung,³ presumably by an energy-dependent uptake mechanism.⁷ The pulmonary toxicity may be related to the production of hydrogen peroxide (H_2O_2), superoxide anions (O_2^-) and the peroxidation of membrane lipids.^{8,9} Montgomery,¹⁰ however, presented evidence of an acute inhibition of the pulmonary fatty acid desaturase system which did not appear to be related to an increased production of H_2O_2 and O_2^- . The increased production of hydrogen peroxide which accompanies diquat intoxication is also not a prerequisite for the development of the secondary pathological changes in the lung.¹¹ Recently it was shown that both rat and rabbit lung microsomes lack superoxide dismutase activity, but that rat lung microsomes generated much higher levels of O_2^- when exposed to paraquat *in vitro* than did rabbit lung microsomes.¹² However, when rats were treated with paraquat *in vivo*, no stimulation of O_2^- or H_2O_2 production was observed, nor was the generation of these highly active molecules increased when microsomes, prepared from rats that were given paraquat for a short period, were incubated in the presence of added paraquat *in vitro*.¹⁰ Furthermore, if one considers the relatively high concentration of catalase in the lung, it seems necessary to re-evaluate the role of H_2O_2 , O_2^- and lipid hydroperoxides in the development of paraquat toxicity.¹⁰

Failure to detect gross lung abnormalities in rabbits after *in vivo* exposure to paraquat^{5,13,14} led to the generally accepted view that rabbits are resistant to the pulmonary toxicity of paraquat. Preliminary experiments, and our present *in vitro* investigations, undoubtedly confirmed that the rabbit lung responds in a very similar way to that of rats. Because any hypothesis on the mechanism of paraquat toxicity should also account for the *in vitro* susceptibility of the rabbit lung, we decided to use the rabbit as our experimental model.

MATERIALS AND METHODS

Thirty-two New Zealand White rabbits weighing about 2 kg were used. The animals were anaesthetized by injection of a sublethal dose of 2.5% thiopental sodium through the marginal ear vein, and the lungs were immediately perfused *in situ* with 50 ml cold isotonic saline. The large airways were dissected free, the normal 'white' lung tissue was blotted gently, and 1-mm tissue slices were cut with a McIlwain tissue chopper.¹⁵

Oxygen uptake was measured in a Warburg apparatus at 37°C (waterbath temperature 38°C), with a shaking

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speed of 120/min over a period of 3 hours.¹⁵ The main compartment of the flask contained 50-70 mg of lung slices in 3 ml Krebs-Ringer phosphate (KRP) buffer,¹⁵ with or without 10 mM glucose. In the centre well 0,2 ml of 20% KOH and a piece of filter paper provided for the absorption of CO₂. Paraquat (Aldrich Laboratories, Wisconsin) solutions were tipped from the side-arm to the main flask after 15 minutes of equilibration, to give final concentrations of 10⁻⁵M and 10⁻³M respectively. Equal volumes of KRP-buffer were used in the side-arm of control flasks.

The gas phase was air (20,9% O₂; P_{O₂} 149 mmHg) or 100% oxygen (P_{O₂} 713 mmHg).¹⁵ In the latter, the flasks were gassed with medical oxygen for 5 minutes. The flow-rate was checked by monitoring the flow through the outlet of the side-arm. Readings were taken at 20-minute intervals, and the results were expressed in terms of microlitres of oxygen consumed per mg wet weight per hour. Each experiment (see Figs 1 and 2) was repeated 6 times in quadruplicate, and standard methods were used to compute the mean and standard deviations. Pairwise comparisons (*P* values) were made, using a two-sided Student's *t* test.

RESULTS

In Fig. 1 the effects of different oxygen tensions and glucose concentrations on the oxygen uptake of control lung slices over a period of 3 hours are shown. In air, the oxygen consumption of lung slices with or without 10 mM glucose in the medium, was practically identical at all time intervals examined (Fig. 1A). When lung slices were incubated in a 100% oxygen phase without exogenous glucose, the rate of oxygen uptake started to decline after 60-80 minutes, and reached an oxygen quotient of 0,73 after 3 hours (20% O₂ control value *P*<0,05). In a 100% oxygen phase with glucose (10 mM), a rapid significant increase in oxygen utilization was noted which persisted for 2 hours, followed by a sharp decrease. This decline was markedly faster than that of lung slices in 100% oxygen, but exogenous glucose was not metabolized (Fig. 1B), and may represent a decreased effectiveness of the glucose-dependent metabolic pathways

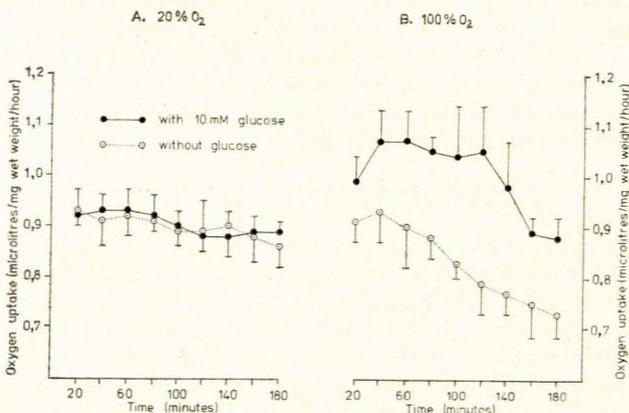


Fig. 1. Oxygen consumption of normal lung slices with or without glucose in the medium. A — gas phase 20% oxygen; B — gas phase 100% oxygen.

in protecting lung tissue against oxygen toxicity.

The effects of paraquat (10⁻⁵M and 10⁻³M respectively) in air and 100% oxygen, with and without glucose as substrate, on the rate of oxygen consumption of rabbit lung slices over a period of 3 hours are recorded in graphs in Fig. 2 A and B.

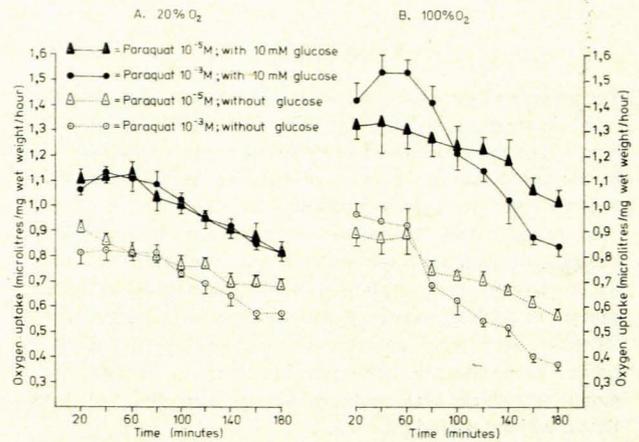


Fig. 2. The effect of various concentrations of paraquat on the oxygen uptake of lung slices. A — gas phase 20% oxygen; B — gas phase 100% oxygen.

In an air phase, without glucose in the medium, both paraquat concentrations caused a marked decline in the rate of oxygen uptake with time, compared with the control values (10⁻³M paraquat, *P*<0,01). With 10 mM glucose in the medium, paraquat significantly (*P*<0,05) increased the oxygen quotient for about 1 hour. Thereafter a steady decline in the oxygen consumption set in to reach a value of 0,81 (for both paraquat concentrations) after 3 hours. Although this value did not differ significantly from that of the control values (*P*>0,05), it is, however, interesting to note that in an air phase the rate of decrease in oxygen uptake due to paraquat is very similar — whether glucose is present or not (Fig. 2A).

In a 100% oxygen phase and without glucose in the medium, paraquat did not significantly influence the oxygen quotient of lung slices over the first hour, but over the next 2 hours a marked decline in the oxygen uptake occurred. This rate of decline was more pronounced with 10⁻³M paraquat (than with 10⁻⁵M paraquat), and reached an oxygen quotient of 0,37 at the termination of the experiment (Fig. 2B). In the presence of 10 mM glucose in the medium, paraquat induced an immediate and highly significant increase in the rate of oxygen utilization, which lasted for 60 minutes. Thereafter a steady decline was observed for the duration of the experiment. Despite the very significant increase initially, the mean oxygen quotient after 3 hours with 10⁻³M paraquat (0,84) was just lower than the mean control value (0,89).

The increase in aerobic metabolism of lung slices induced by paraquat seems to be glucose-dependent. However, the inhibitory effect of paraquat on the oxygen quotient is likely to be independent of the rate of glucose oxidation. Both the initial and the secondary effects of paraquat were much more pronounced in a 100% oxygen

atmosphere, which confirmed that oxygen potentiates the toxic effects of paraquat.

DISCUSSION

Oxygen is necessary for the toxicity of paraquat in mammals.¹⁶ Observations that oxygen therapy potentiates the morbidity and mortality of paraquat poisoning led us to study the *in vitro* effects of paraquat on lung tissue, at different oxygen tensions and in the presence or absence of added glucose. Although we undertook this study primarily to verify some of the claims regarding the proposed mechanisms of paraquat toxicity, we also found that the rabbit lung seems to be ideally suited to the investigation of some aspects of the pathophysiology of pulmonary oxygen toxicity.

Oxygen-induced pulmonary changes have been studied from ultrastructural, physiological and biochemical viewpoints, and evidence from both *in vivo* and *in vitro* experiments demonstrates that increased oxygen tensions produce changes in cellular metabolism.^{17,18} A number of enzymes containing sulphhydryl groups are inactivated; the sulphhydryl groups of smaller compounds are oxidized, hydrogen peroxide production increases, and there are significantly higher concentrations of superoxide radicals. These phenomena may all be involved in oxygen toxicity, and we therefore decided to test the effects of normal and high oxygen tensions on our *in vitro* system.

When glucose was omitted from the medium, 100% oxygen did not alter the rate of oxygen uptake of lung slices over 1 hour, whereas a significant increase in oxygen quotient was maintained for almost 2 hours when 10 mM glucose was present. This increased metabolic rate may be a reflection of multiple glucose-dependent events occurring in the lung in an effort to protect the lung from, or at least delay, the toxic effects of hyperoxia. However, the inhibition of oxygen uptake after 2 hours was much more pronounced in the presence of glucose than without it. This might indicate that a high oxygen tension in itself is toxic, or that increased amounts of toxic intermediates are produced during the period of increased glucose oxidation.

In view of our *in vitro* findings regarding the deleterious effect of a 100% oxygen phase on long-term tissue respiration, one should be extremely careful in evaluating the additional toxic effects of paraquat on the metabolic rate.

A number of biochemical and histological similarities exist between tissue injury from oxygen and that caused by paraquat.^{16,19} In the presence of glucose, paraquat increased the initial rate of oxygen consumption of lung slices. This increased rate, which was much more pronounced in 100% oxygen than in an air phase, was glucose-dependent and lasted long enough to play a substantial role in the ensuing response of lung tissue to paraquat. The higher oxygen quotient possibly reflects an increased glucose oxidation in the Embden-Meyerhof pathway, the pentose pathway, or the citric acid cycle, or is due to the uncoupling of mitochondrial oxidation and phosphorylation. Recently it was demonstrated that the pentose shunt was stimulated under the

influence of paraquat.²⁰ Although paraquat may also increase the microsomal oxidation of NADPH and other intermediates,⁴ it seems unlikely that microsomal respiration can contribute a quantitatively important fraction of this significant increase in oxygen uptake.

In the absence of added glucose in the medium, oxygen uptake was inhibited right from the start, and the rate of inhibition was very similar to that obtained in the presence of glucose. It therefore appears that the toxic effect of paraquat is not glucose-dependent, nor linked to metabolites formed in the initial glucose-dependent metabolic burst. The inhibition is, however, time- and dose-dependent.

It is premature to make projections regarding the inhibitory action of paraquat on the oxygen utilization of lung slices. The magnitude of inhibition under all four experimental conditions is such that the most probable mechanism may lie at a mitochondrial level, because 80-90% of the oxygen consumed by cells is handled by the respiratory chain in the mitochondria. Inhibition of a specific mitochondrial enzyme system is a possibility, but Gage¹¹ obtained a marked stimulation of oxygen utilization with liver mitochondria. We cannot explain this apparent contradiction, but the most likely explanation may be that their mitochondrial preparations were contaminated with microsomes. This possibility should be further explored.

Our *in vitro* system also confirmed that oxygen potentiates the effects of paraquat and vice versa. However, further research is necessary to elucidate the inhibitory effect of paraquat on the oxygen-consuming metabolic systems, as well as the possible mechanisms of increased sensitivity to oxygen. Whether they both affect the same cell and thereby produce a more severe injury (e.g. endothelial and type II epithelial cells), or disrupt a metabolic pathway which may be important for the defence mechanisms of the lung (e.g. the pentose pathway), or whether one or both might affect the scavengers of free radicals, should be further investigated.

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