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SPECIFIC POLYCLONAL AND MONOCLONAL ANTIBODY PREVENTS PARAQUAT ACCUMULATION INTO RAT LUNG SLICES

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Abstract—Sheep polyclonal and mouse monoclonal antibodies have been produced that bind to the bipyridyl herbicide, paraquat. The binding capacities and affinities of the various antibody solutions (serum, ascites, purified tissue culture supernatant) to paraquat were determined using a radioimmunoassay. All antibody solutions bound paraquat with high affinity ($K_a = 10^9$–10^{10} l/mol). The sheep polyclonal antisera, the mouse ascites fluid, and the purified culture supernatant had mean binding capacities of 8, 1 and 22 μg paraquat/ml respectively. All the antibody preparations were able to prevent the *in vitro* accumulation of paraquat into rat lung tissue. The amount of antibody to achieve this was dependent upon the binding capacity of the antibody solution, i.e. when the binding capacity of the antibody was equal to the amount of paraquat present in the incubation medium a total blockade of uptake was achieved. When antibody was added to lung tissue that had been accumulating paraquat for 1 hr, the inhibition of uptake was immediate and was complete for at least 2 hr. Both the radioimmunoassay and lung slice experiments indicate that an equivalent of 1 mg of IgG is required to bind 2.5 μg of paraquat ion. Preincubation of lung tissue with antibody did not affect the subsequent accumulation of paraquat, nor did it result in a detectable degree of intracellular neutralisation of paraquat as measured by paraquat's ability to stimulate the pentose phosphate pathway. The rate of efflux of paraquat from lung slices prepared from rats dosed intravenously with paraquat was not increased by the presence of antibody in the incubation medium. In conclusion, neutralising antibodies to paraquat have been produced. They bind to paraquat in solution with high affinity and render the paraquat unavailable for its *in vitro* accumulation into lung cells.

Paraquat (1,1′-dimethyl-4-,4′-bipyridilium) is a contact herbicide used in many countries throughout the world. Although safe when used properly [1, 2], over the last two decades there have been several hundred fatalities attributed to paraquat poisoning, largely as a consequence of the intentional ingestion of the concentrated commercial product for suicidal purposes [3]. When paraquat is ingested, the symptoms of poisoning depend largely on the amount consumed. Patients who die within 1–5 days have generally ingested extremely large amounts of paraquat, death resulting from multi-organ failure. In patients who die after 5 days (and up to several weeks), the most characteristic features of poisoning are damage to the lung and kidney [4].

After paraquat is administered orally to rats, the plasma paraquat concentration remains relatively constant over 30 hr, whereas the concentration in the lung rises progressively to several times that in the plasma [5]. In no other organ studied is this time-dependent accumulation of paraquat seen.

Rose *et al.* [6], using rat lung slices, discovered an accumulation process for paraquat which was energy dependent and obeyed Michaelis–Menten kinetics. The accumulation cannot be explained as a consequence of binding of paraquat to the tissue [7, 8]. It appears that the process of accumulation is a consequence of the transport of paraquat into an intracellular compartment. In addition, the diamines (putrescine, cadaverine) and polyamines (spermidine and spermine) are effective inhibitors of paraquat accumulation [9, 10]. Additional work has indicated that all these compounds, including paraquat, are accumulated as a result of a single process within specific cell types of the lung [11].

Exogenous antibodies have a well-established place in the treatment of many disease states. It is likely that frequent administration of xenogeneic antibodies may lead to serum sickness and anaphylaxis. However, the treatment of drug toxicity would be likely to require only a single administration of antibody. Only in the case of the cardiac glycosides have both *in vitro* and *in vivo* studies of antibody reversal of drug effects been carried out fully, and clinical efficacy established in man [12–14]. Neutralisation, but not toxicity reversal studies have been reported for antibodies raised specifically to morphine and barbiturates [15] and the organophosphorus hapten soman [16].

Many *in vitro* inhibitors of paraquat accumulation into the lung have been identified but they have not proved effective *in vivo*. At present, treatment consists of (a) reducing the absorption of paraquat from the gastro-intestinal tract into the plasma [17] and (b) removing paraquat from the plasma before it has accumulated into the lung [18]. As an initial study, polyclonal and monoclonal antibodies have been produced to determine their ability to (1) specifically bind to paraquat, (2) neutralise paraquat

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in vitro and (3) cause efflux of paraquat from lung tissue.

MATERIALS AND METHODS

Materials

[^{14}C]Methyl paraquat dichloride (111 mCi/mmol), [^{3}H]methyl paraquat chloride (3 Ci/mmol), [1-^{14}C]glucose, and Na^{125}I were purchased from Amersham International (Amersham, U.K.). Analytical grade paraquat dichloride was supplied by Plant Protection Division, ICI plc (Surrey, U.K.). Halothane was obtained from Pharmaceuticals Division, ICI plc (Cheshire, U.K.). The scintillants Optiphase MP and Dimilume were purchased from LKB (Surrey, U.K.) and Packard Ltd (Dorset, U.K.), respectively. The tissue solubiliser, Soluene 350, was supplied by Packard Ltd. Keyhole Limpet Haemocyanin (KLH) was supplied by Calbiochem (La Jolla, CA), bovine serum albumin (BSA) by BDH (Poole, U.K.), ovalbumen (OVA) by Sigma Chemical Co. (London, U.K.). Polystyrene wells were obtained from Dynatech (Billingshurst, Sussex, U.K.). Pristane (2,6,10,14-tetra-methylpentadecane) was obtained from Aldrich Chemical Company (Dorset, U.K.). Non-ulcerative Freund's adjuvant (Morris) was obtained from Guildhay Antisera (University of Surrey, Guildford, Surrey, U.K.).

Balb/C mice (body wt 20–30 g) and Alderley Park Wister derived rats (body wt approx. 200 g) were maintained in a clean, temperature-controlled room with a 12 hr light, 12 hr dark cycle and were allowed free access to food and water. Suffolk cross-bred sheep were maintained by Guildhay Antisera (University of Surrey, Guildford, Surrey).

Methods

Synthesis of paraquat/protein conjugates. KLH, BSA and OVA were coupled to 6-bromohexanoic acid paraquat derivative (4,4′-bipyridinium, 1-(5-carboxypentyl)-1′-methyl-bromide methyl sulphate) via carbodiimide. This has previously been described and characterised [19].

The BSA conjugate (15 mol paraquat/mol BSA) was used for immunising mice and the OVA conjugate (6 mol paraquat/mol OVA) for immunising sheep. The KLH conjugate (662 mol paraquat/mol KLH) was used to detect for paraquat specific antibodies in immunoassays.

Production of polyclonal sheep antiserum. Immunisations and antiserum preparation were performed by Guildhay Antisera. Three mature sheep were immunised with a priming dose of 6 mg paraquat–OVA conjugate emulsified with non-ulcerative Freund's complete adjuvant (Morris) in the ratio of 1 vol. aqueous solution to 2 vol. oily adjuvant. Each sheep was injected intramuscularly with 3 ml of emulsion in six divided doses. Blood samples were taken twice monthly from the jugular vein of each sheep and the paraquat binding capacity of each serum sample determined. Nine weeks after priming, the sheep were boosted with 2 mg paraquat–OVA conjugate emulsified with non-ulcerative Freund's incomplete adjuvant (Morris) and the emulsion again injected intramuscularly in six divided doses. Large volumes of blood (600 ml) were then taken from the jugular vein of each animal from 10 days onwards to coincide with the peak antibody response. The blood was allowed to clot overnight. The serum was then separated aseptically from the blood clot, filtered and stored at 4° in the presence of 0.1% sodium azide.

Generation of paraquat-specific hybridomas. Spleen cells from mice immunised with paraquat–BSA were fused with the NSI mouse myelomas using a protocol based on a modification of the method of [20]. Our methodology has previously been reported [21]. The hybridomas, BP1A12G6 generated for this study was recloned from the hybridoma BP1A1A3 [21].

Production of ascites tumours. Balb/C mice were injected with a single 0.5-ml i.p. injection of pristane 10 days prior to injection of 5×10^6 hybridoma cells. The ascites fluid was harvested 10–14 days later, separated from cells by centrifugation and stored at −20°.

Bulk production of antibody from culture supernatant. The cell line BP1A12G6, was cultured under contract to Celltech Ltd (Slough, Berks, U.K.).

Quantitation of specific antibody by [^{3}H] paraquat binding assay. Constant amounts (25 μl) of suitably diluted antibody solutions (1:5, 1:10) were incubated with increasing concentrations of a radioactive paraquat solution (10–160 μl of 1.594 μg paraquat ion/ml containing 40 μl [^{3}H] paraquat), made up to a total volume of 200 μl with saline. The reaction mixtures, in 1.5-ml centrifuge tubes, were incubated at 37° for 2 hr, and terminated by the addition of saturated ammonium sulphate (200 μl) and allowed to precipitate for 30 min. The tubes were microcentrifuged for 3 min, the supernatants discarded, 200 μl of saline added to each, and 50 μl of the dissolved precipitates taken for radioactivity determination in plastic vials containing 10 ml Dimilume. Total radioactivity in the initial [^{3}H]paraquat solution was similarly determined. Non-specific binding was assessed by assaying non-relevant ascites fluid or non-relevant hyperimmune sheep serum.

Scatchard [22] plots were derived by plotting the molar bound:free ratio against the molar concentration of paraquat bound to antibody (Fig. 1). The paraquat binding capacity of the antibody and the specific antibody concentration were calculated from the antibody combining sites, making assumptions that (1) 1 mol of paraquat binds 1 mol of combining site, (2) the mol. wt of IgG is 155,000, and (3) each IgG molecule is able to bind 2 molecules of paraquat. All data handling was computer programme assisted. Scatchard data were manually selected but final calculations were made using linear regression parameters.

Measurement of accumulation of paraquat into rat lung slices. Rats were killed with halothane, the lungs removed immediately after cessation of breathing, and slices (0.5 mm thick) prepared using a McIllwain tissue chopper. Freshly prepared lung slices (20–40 mg) were incubated in 3 ml of media containing modified Krebs–Ringer phosphate (KRP), which consists of NaCl (130 mM), KCl (5.2 mM), $CaCl_2$ (1.9 mM) $MgSO_4$ (1.29 mM), Na_2HPO_4 (10 mM), glucose (11 mM). (The pH of the buffer was adjusted

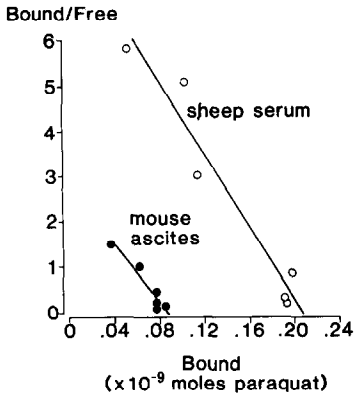

Fig. 1. Suitably diluted serum or ascites solutions were assayed as described in the [^{3}H]paraquat binding assay method. The data were plotted according to the method of Scatchard [22]. The slope of the straight line, obtained by linear regression, enables derivation of the binding association constant (K_a) and the intercept on the ordinate the total number of antibody combining sites.

to 7.4 with 1 M HCl), 1 μM [^{14}C]paraquat or 9.2 μM [^{14}C]paraquat (8.2 μM carrier paraquat plus 1 μM [^{14}C]paraquat) and various volumes (see legends to figures) of sheep polyclonal antisera, mouse ascites fluid or protein A purified antibody. Non-relevant sheep hyperimmune antiserum or mouse ascites fluid were used as controls. Incubations were carried out for 2 hr at 37° in a shaking water-bath.

Lung slices were also incubated in medium containing ascites fluid for 1 hr and transferred to flasks containing 1 μM [^{14}C]paraquat, and the effect on uptake studied. Also, lung slices were incubated in 1 μM [^{14}C]paraquat for 1 hr and then sheep polyclonal antisera or purified antibody added and incubations continued for 1–2 hr.

Tissue slices were removed from the incubation medium, washed with KRP, dissolved in 1 ml of Soluene and 10 ml of Dimilume, and radioactivity determined. Paraquat has been shown not to be metabolised in the rat [23]. Thus the level of [^{14}C]radioactivity has been used as a measure of the amount of paraquat present.

Measurement of [1-^{14}C]*glucose oxidation.* Lung slices were incubated for 1 hr at 37° in 3.0 ml modified KRP containing 100 μl sheep polyclonal antisera or protein A purified antibody with KRP as control. After incubation the slices were washed with KRP and transferred to centre well flasks containing 3 ml of modified KRP with 1 μCi [1-^{14}C]glucose, with and without 1 μM paraquat. The centre well contained 0.2 ml 20% w/v KOH, and a 2-cm square rolled wick of hard filter paper and the flask was sealed with a rubber serum cap. Incubations were carried out for 2 hr at 37° and the experiment terminated by the instillation of 0.3 ml 50% w/v trichloroacetic acid (TCA) into the incubation media. The $^{14}CO_2$ produced was determined by measuring the radioactivity trapped in the centre well.

Efflux of paraquat from lung slices. Rats were dosed (via the tail vein) with 65 μmol [^{14}C]paraquat/kg (4.2 μCi/μmol). Two hours later the rats were killed with halothane, and the *in vitro* efflux of paraquat from slices of perfused lung tissue determined over 4 hr as previously described [24]. Efflux was determined in the presence or absence of antibody. The antibody binding capacity in each flask was sufficient to bind all of the paraquat calculated to be present in the lung tissue (0.2 mg specific antibody/flask). The retention of paraquat in the slice with time was expressed as a percentage of the total paraquat in the slice and medium. The rate of efflux was

$$\frac{\log \%\text{ retention at 1 hr} - \log \%\text{ retention at 4 hr}}{3}$$

and

$$t_{\frac{1}{2}} = \frac{\log_2}{\text{slope}}.$$

RESULTS

Details of sheep polyclonal antiserum

The second immunisation resulted in a large secondary antibody response, sheep serum maximally binding 11.5 μg paraquat/ml. Presuming that all the binding capacity was derived from immunoglobulin G with two active binding sites/molecule, the peak secondary response represents a specific antibody concentration of 4.5 mg/ml. The mean association affinity constant (K_a) for the three peak antibody levels was $1.8 \pm 0.4 \times 10^9$ l/mol.

Details of hybridoma and antibody

The hybridoma used in this study, BP1A12G6, was recloned from BP1A1A3, described in [21]. Ouchterlony analysis using sub-class specific antisera confirmed that the hybridoma secreted IgG_1 antibody. In static culture the cell line produced an equivalent of 15 μg specific antibody/ml with a mean K_a of 1.4×10^{10} l/mol. The specific antibody comprised approx. 30% of the total antibody secreted by this hybridoma (data not shown). In ascites fluid from seven mice the mean K_a was 1.1×10^{10} l/mol. The mean specific antibody concentration was 0.40 mg/ml (0.08–1.36) with approx. 3–5 ml of fluid being obtained from each mouse.

Large-scale culture (100 l) of the same hybridoma yielded 7 g of specific antibody after purification with protein A affinity purification. The mean K_a of this product was 1.4×10^{10} l/mol.

Effect of anti-paraquat antibody on in vitro *accumulation of paraquat into lung tissue*

Approximately 2 μg paraquat/g wet wt accumulated into the lung tissue (Figs 2 and 3) when rat lung slices were incubated with 1 μM [^{14}C]paraquat in the presence of non-relevant sheep antiserum or mouse ascites fluid over a period of 2 hr. In the presence of increasing volumes of anti-paraquat sheep serum or mouse ascites fluid this accumulation of paraquat was progressively inhibited (Figs 2 and 3). The sheep serum used in this study had a paraquat binding capacity of 7.7 μg paraquat/ml, whereas the mouse ascites fluid binding capacity was 3.0 μg paraquat/ml. For 1 μM paraquat, each incubation flask contained 0.186 μg paraquat/ml. The degree of inhibition of accumulation was proportional to the binding

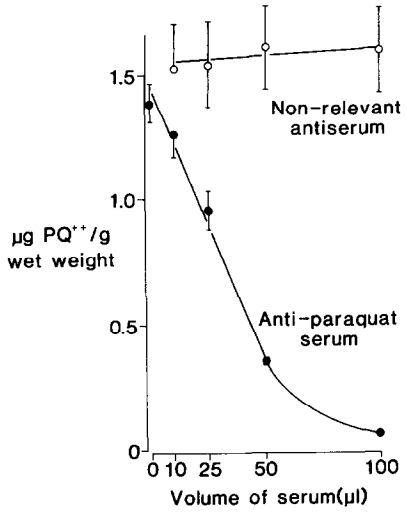

Fig. 2. Lung slices were incubated in 1 μM [^{14}C]paraquat in the presence of 10, 25, 50 or 100 μl of sheep anti-paraquat serum (binding capacity 8 μg paraquat/ml) (●) or non-relevant sheep antiserum (○) for 2 hr at 37°.

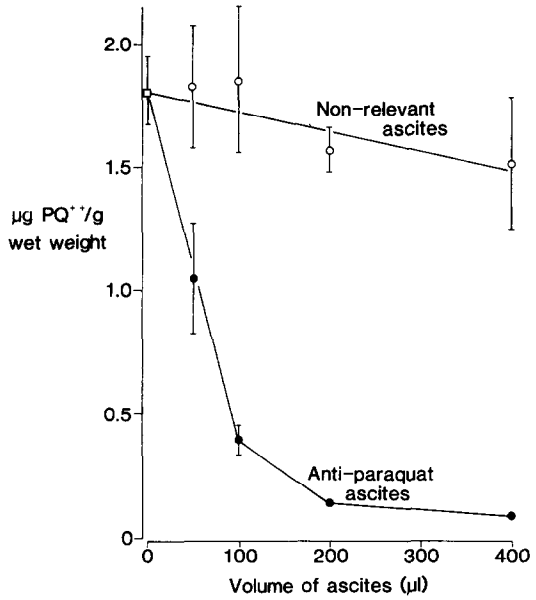

Fig. 3. Lung slices were incubated in 1 μM [^{14}C]paraquat in the presence of 0.05, 0.1, 0.2 or 0.4 ml of anti-paraquat ascites fluid (binding capacity 3.1 μg paraquat/ml) (●) or non-relevant ascites fluid (○) for 2 hr at 37°. The amount of paraquat in the lung slice was calculated from the level of radiolabel present. The results are expressed as the mean ± SEM with three observations per measurement.

capacity of the antibody added. The protein A purified material from Celltech had a considerably greater binding capacity (21.6 μg paraquat/ml) than either the sheep serum or mouse ascites fluid. Thus 30 μl of this product totally inhibited the accumulation of 1 μM paraquat, and 300 μl totally inhibited the accumulation of 9.2 μM paraquat into rat lung slices (Table 1).

In the previous experiments, antibody and paraquat were preincubated for approx. 15 min prior to the addition of lung tissue. In order to investigate

Table 1. The effect of protein A purified antibody on the accumulation of [^{14}C]paraquat by lung slices

Treatment	μg paraquat/g wet wt
1 μM PQ	1.62 ± 0.30
+5 μl	1.35 ± 0.12
+10 μl	0.75 ± 0.19
+20 μl	0.31 ± 0.09
+30 μl	0.11 ± 0.01
9.2 μM PQ	18.2 ± 1.59
+50 μl	15.1 ± 1.47
+100 μl	7.85 ± 0.90
+200 μl	1.01 ± 0.14
+300 μl	1.14 ± 0.10

Lung slices were incubated in 1 μM or 9.2 μM [^{14}C]paraquat containing 5, 10, 20, 30 μl or 50, 100, 200, 300 μl of purified antibody, respectively. The purified antibody had a binding capacity of 22.4 μg paraquat/ml. Incubations were carried out at 37° for 2 hr and the amount of paraquat in the slice was calculated from the level of radiolabel present. The results are expressed as the mean ± SEM with 3 observations per determination.

the rapidity and longevity of the binding, lung slices were allowed to accumulate paraquat for 1 hr prior to the addition of anti-paraquat sheep serum or purified antibody. The addition of control antibody did not affect the accumulation of paraquat, whereas the addition of 100 μl of anti-paraquat sheep antiserum or 30 μl of purified antibody (sufficient antibody to bind all the paraquat in solution) resulted in no further paraquat being accumulated into the rat lung slices (Figs 4 and 5). With both sets of data it was apparent that antibody must bind very rapidly to paraquat as no further accumulation was detectable after 1 hr. The inhibition of accumulation was complete for at least 2 hr after the addition of antibody (Fig. 4).

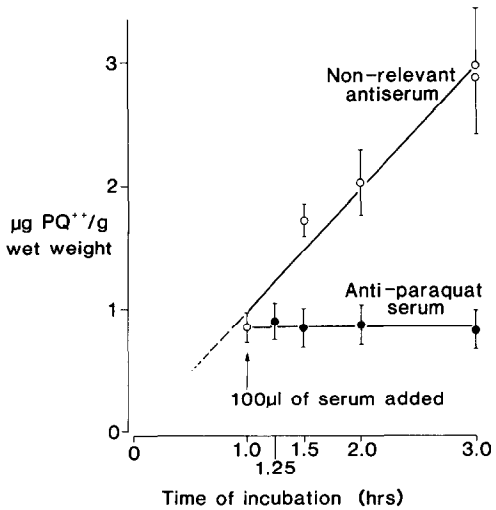

Fig. 4. Lung slices were incubated at 37° in 1 μM [^{14}C]paraquat and 1 hr after the start of incubation, 100 μl of sheep anti-paraquat serum (binding capacity 8 μg paraquat/ml) (●) or non-relevant antiserum (○) was added. The level of paraquat accumulated by the lung slice was studied at 0, 0.25, 0.5, 1 and 2 hr after the addition.

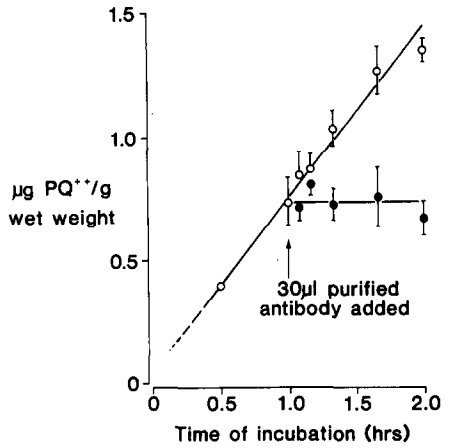

Fig. 5. Lung slices were incubated at 37° in 1 μM [^{14}C]paraquat and 1 hr after the start of incubation, 30 μl of protein A purified antibody (binding capacity 22.4 μg paraquat/ml) (●) or KRP (○) were added and the level of paraquat accumulated by the slice was studied at −30, 0, 5, 10, 20, 40 and 60 min after addition. The amount of paraquat in the lung slice was calculated from the level of radiolabel present. The results are expressed as the mean ± SEM with 3 observations per time point.

Preincubation of lung slices with antibody does not have the ability to inhibit the subsequent accumulation of paraquat (Table 2). In addition, the preincubation of lung slices with antibody for 1 hr did not reduce the ability of paraquat to increase the production of $^{14}CO_2$ from [1-^{14}C]glucose (Table 3). Antibody alone did not affect the amount of $^{14}CO_2$ produced from [1-^{14}C]glucose in control lung slices (Table 3).

Effect of anti-paraquat antibody on the efflux of paraquat from lung tissue

When rats were dosed with paraquat intravenously, and lung tissue from these animals incubated in medium containing anti-paraquat antibody, the rate of efflux of paraquat $t_{\frac{1}{2}}$ ($\bar{x} \pm$ SD) = 17.2 ± 10.6 hr from the lung tissue was not significantly different from the rate of efflux in the absence of antibody ($t_{\frac{1}{2}}$ = 10.7 ± 5.6 hr).

DISCUSSION

Paraquat presents a challenging study for antibody-induced reversal of chemical toxicity. Its lethal toxicity is the result of ingestion of several grams of compound, it is rapidly absorbed from the gastrointestinal tract into the plasma and is accumulated specifically by lung tissue, the major site of damage. Paraquat toxicity could be (1) prevented, by not allowing the paraquat to be accumulated by the lung or, (2) reversed, by neutralising or causing the efflux of the paraquat once accumulation into the lung had occurred.

Sheep polyclonal and mouse monoclonal antibodies have been produced that have the ability to bind to paraquat. Paraquat-specific rabbit polyclonal antibodies and a mouse monoclonal antibody have previously been reported [19, 21]. Whilst these products have proved useful in assays of paraquat levels in human samples, a well-characterised product in large quantities is required in order to quantitatively investigate the neutralisation of paraquat *in vitro* and eventually *in vivo*.

Sheep polyclonal antiserum

The sheep immunisation programme has demonstrated that a significant secondary antibody response to paraquat can be achieved within a few weeks of initiation of dosing. Although the component subclasses of this polyclonal antisera have not been defined, it is likely that with large bleeds, several grams of specific immunoglobulin can be obtained within 3–4 months. Although linearisation of the Scatchard data derives an average affinity association constant (K_a) of the heterogeneous population of antibodies present, the polyclonal antibody can be considered to bind paraquat with high affinity (1.8×10^9 l/mol).

Table 2. The effect of preincubating lung slices in ascites fluid on the ability of the slice to accumulate 1 μM PQ

Treatment	Time (min)	μg paraquat/g wet wt	
Volume of ascites in medium:		0.2 ml	0.4 ml
Anti-paraquat ascites	30	0.46 ± 0.05	0.47 ± 0.07
	60	1.00 ± 0.09	0.09 ± 0.09
	120	1.64 ± 0.18	1.58 ± 0.10
Non-relevant ascites	30	0.57 ± 0.02	0.50 ± 0.07
	60	0.86 ± 0.09	0.98 ± 0.06
	120	1.71 ± 0.21	1.69 ± 0.25

Lung slices were preincubated for 1 hr at 37° in the presence of 0.2 or 0.4 ml of anti-paraquat ascites (binding capacity 3.1 μg paraquat/ml) or non-relevant ascites. The slices were then transferred to 1 μM [^{14}C]paraquat and the accumulation studied after 2-hr incubation at 37°. The amount of paraquat on the slice was calculated from the level of radiolabel present. The results are expressed as the mean ± SEM with 3 observations per determination.

Table 3. The effect of preincubating lung slices in sheep polyclonal antiserum or protein A purified antibody on the ability of paraquat to stimulate [1-^{14}C]glucose oxidation

	dpm $^{14}CO_2$/100 mg wet wt		
	Control	1 μM PQ	Δ
KRP	24,359 ± 2755	50,735 ± 4616	26,376 ± 2298
Non-relevant sheep antiserum	20,875 ± 2701	52,285 ± 6466	31,410 ± 4074
Anti-paraquat serum	21,600 ± 2203	48,629 ± 4996	27,028 ± 9029
Protein A purified antibody	20,523 ± 2563	51,994 ± 4980	31,471 ± 3823

Lung slices were preincubated for 1 hr at 37° in the presence of 100 μl non-relevant sheep antiserum, sheep polyclonal anti-paraquat serum (binding capacity 10.4 μg paraquat/ml), protein A purified antibody (21.6 μg paraquat/ml), transferred to flasks containing [1-^{14}C]glucose with or without 1 μM paraquat and a centre well with 0.2 ml 20% w/v KOH and a filter paper wick. Incubations were carried out in sealed flasks for 2 hr, and the reaction stopped by instillation of 0.3 ml 50% w/v TCA into the media. The level of radiolabel trapped in the centre well—$^{14}CO_2$—was determined. The results are expressed as the mean ± SEM with 4 observations per determination.

Mouse monoclonal antibodies

Antibody produced from BP1A12G6, as ascites fluid or culture supernatant (small and large scale), all have very similar binding affinities for paraquat, and can be considered to bind paraquat with high affinity (1×10^{10} l/mol). A study of cross-reactivity has previously demonstrated that the antibody significantly cross-reacted with the very closely related diethyl paraquat, but failed to significantly cross-react with three other bipyridyls [21]. This slight cross-reactivity is unlikely to present a problem in the neutralisation of paraquat as the compound is not metabolised and is not available commercially as mixtures with other bipyridyls (with the exception of diquat with which the antibody does not cross-react).

The level of antibody secretion was low, both in culture supernatant and in ascites fluid. Whilst milligram quantities of product can be obtained from ascites fluid, the only practical route to obtain gram quantities from such a cell line is from large-scale cell culture. A 100-l fermentation in combination with Protein A purification at Celltech, has produced a product containing the equivalent of 7 g of specific antiparaquat antibody.

The central conclusion of this paper is that polyclonal antibody and monoclonal antibody, whether as ascites fluid or purified tissue culture supernatant, are equally able to inhibit the accumulation of paraquat into rat lung slices, in proportion to their binding capacity for paraquat. The effect is restricted to paraquat-specific antibodies—no effects on accumulation being observed with control hyperimmune sheep antisera or control mouse ascites fluid. The binding capacity of the antibody has been determined from a Scatchard plot of data from the [^{3}H]paraquat binding assay. Total blockade of paraquat accumulation occurs at a point when this binding capacity is equal to the amount of paraquat in the incubation flask. This confirms that, despite some of the limitations inherent in the Scatchard data, the derived binding capacity is a useful and reliable measure of the ability of antibody to bind paraquat. The inhibition of paraquat accumulation *in vitro* is indicative that the specific antibody binds to paraquat rapidly, with high affinity and is stable for at least 2 hr. The results indicate that 1 mg of IgG antibody is required to bind 2.5 μg paraquat ion.

It is most likely that the blockade of paraquat accumulation is the result of antibody binding paraquat in the incubation medium, but the possibility exists that the blockade could be due to the antibody binding to the uptake receptor for paraquat. Since lung slices preincubated with antibody did not prevent the uptake of paraquat, it is apparent that antibody simply binds paraquat in the incubation medium, to prevent uptake.

Another possibility was that the antibody would have the capacity to influence the intracellular toxicity of paraquat. The intracellular toxicity of paraquat almost certainly involves its cyclical reduction and reoxidation, which results in the production of superoxide anion, and oxidation of NADPH to $NADP^+$ [25]. Although the precise control of biochemical pathways subsequent to this is still not totally understood it is clear that the pentose phosphate pathway in the lung is stimulated by paraquat both *in vitro* and *in vivo* [25, 26]. No evidence of intracellular neutralisation has been obtained, as preincubation of lung slices with antibody did not provide a detectable degree of protection against the ability of paraquat to stimulate the pentose phosphate pathway.

Another possible mode of action for antibody neutralisation of paraquat toxicity would be for the antibody, in high concentration in the plasma, to cause an accelerated efflux of paraquat from lung tissue. This possibility has been examined *in vitro* using lung slices. The efflux of paraquat from lung slices prepared from rats dosed intravenously with paraquat is biphasic [24]. The slow component is first order and has a $t_{\frac{1}{2}}$ of 17 hr [24]. This half-life is similar to that seen *in vivo* (24 hr) following i.v. dosing. Antibody, present in the incubation medium, does not appear to increase the rate of efflux of paraquat from the lung despite there being an excess of antibody binding capacity for paraquat. This confirms that the efflux is independent of the extracellular concentration of free paraquat.

In summary, antibody is able to bind paraquat in solution with high affinity and is thus able to prevent

the *in vitro* accumulation of paraquat into lung tissue. The antibody does not appear to have the ability to influence either the efflux or the intracellular fate of paraquat. Preincubation of lung tissue with antibody does not affect subsequent paraquat uptake into the lung.

In order to demonstrate prevention of paraquat toxicity *in vivo* it will be necessary to test intact antibody and antibody fragments (Fab) for their ability to bind paraquat and therefore protect the tissues from lethal concentrations of paraquat. Having determined the binding capacity of antibody for paraquat, both in a radioimmunoassay and in lung slices, it is clear that large amounts of antibody will be needed to prevent paraquat toxicity using antibody neutralisation *in vivo*, even in laboratory animals. From these experiments we will be able to ascertain whether problems in the treatment of chemical poisoning with antibody are solely one of antibody quantity or whether such antibody therapy presents more complex problems.

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