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PUTRESCINE AND PARAQUAT UPTAKE IN HUMAN LUNG SLICES AND ISOLATED TYPE II PNEUMOCYTES

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Abstract—Paraquat is accumulated into the lungs of various species by an active uptake system which also appears to mediate the uptake of endogenous polyamines, such as putrescine. The accumulation of putrescine in the human lung has been previously shown to be mainly located in the type II cells. In the present study, we have studied the mutually competitive inhibition of putrescine and paraquat in human lung slices and the inhibition of putrescine by paraquat or cystamine in isolated human type II pneumocytes. Peripheral lung tissue taken from patients undergoing pneumectomy or lobectomy was used. The initial steps of the cell isolation procedure differed from the literature in that the tissue was first sliced in 0.7 mm thick slices, which were washed in phosphate buffered saline without calcium and magnesium (PBS^-), followed by incubations with trypsin. The type II cells were purified and isolated by differential adherence on plastic followed by Percoll gradient centrifugation. Uptake was determined 48 hr after cell isolation. The accumulation of radiolabelled putrescine showed saturation kinetics, with the following apparent kinetic parameters: K_m 6.7 and 6.2–7.6 μM and V_{max} 2.7 and 3.0–3.4 μmol/g prot/hr for slices and isolated cells, respectively. In the presence of paraquat, putrescine uptake was reduced, in both systems, in a manner compatible with competitive inhibition, with calculated inhibition constants (K_i) of 549–614 and 659–895 μM paraquat for slices and isolated cells, respectively. The accumulation of putrescine in isolated human pneumocytes was strongly reduced in the presence of cystamine, with calculated K_i of 3.7 μM cystamine. These data indicate that putrescine, paraquat and cystamine accumulate in the human lung by the same uptake system, but that the affinities for the three substrates differ. The presence of an uptake system for putrescine in cultured human pulmonary type II is probably useful as a functional viability test.

Key words: polyamines; putrescine; paraquat; cystamine; human type II pneumocytes; *in vitro* toxicology; cell culture

The contact herbicide paraquat (1,1-dimethyl-4,4′-bipyridilium; methyl viologen) is highly toxic to both humans and animals. The lung is the primary target organ, with acute and/or delayed toxicity, depending on the dose [1]. The acute toxic damage is characterized by disruption of the alveolar epithelial cells, and is associated with haemorrhage, edema and infiltration of inflammatory cells into the interstitial and alveolar spaces; the delayed toxic phase is characterized by a proliferative intra-alveolar fibrosis. At the cellular level, the toxicity of paraquat is considered to be due to its cyclic reduction–oxidation, resulting in the production of toxic species of oxygen and a depletion in reducing equivalents [2]. Morphological studies have indicated that, regardless of the route of exposure to paraquat, the most damaged cells were the alveolar epithelial type I and type II cells (or pneumocytes) [2].

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† Abbreviations: TCA, trichloroacetic acid; FCS, foetal calf serum; KRPB Krebs–Ringer phosphate buffer; dpm, disintegrations per minute; PBS^+ and PBS^-, with or without calcium and magnesium, respectively; V_{max}, the maximal rate of uptake; K_m, the medium concentration at which the rate of uptake is half V_{max}; $K_m app$, the K_m in the presence of an inhibitor; K_i, the concentration of the inhibitor at which the K_m is doubled.

The basis for the selective toxicity of paraquat for the lung is essentially its active accumulation in the lung [3]. This accumulation can be competitively inhibited, at least *ex vivo*, by several exogenous and endogenous compounds, including the oligoamines (putrescine, spermidine and spermine) which are possibly the natural substrates for the carrier [4–7]. Further *in vivo* and *in vitro* studies, in the rat, have demonstrated using autoradiography that the cells damaged by paraquat are also the sites of uptake of paraquat and oligoamines [8–10].

The alveolar type II cell, one of the lung cell-types accumulating paraquat *in vivo*, comprises 16% of the human lung [11]. As reviewed by Massey [12] and Dobbs [13], the type II cells of many species, including man [14–18], can be isolated and cultured in primary cultures to study their functions and biochemical properties. *In vitro*, paraquat has been shown to be accumulated in primary cultures of alveolar type II cells from the rat [19] and rabbit [20], but no data were available for human cells.

In this study, we have characterized the uptake of putrescine and its competitive inhibition by paraquat, and vice versa, in human lung slices and the uptake of putrescine in the presence of paraquat or cystamine in isolated human alveolar type II cells.

MATERIALS AND METHODS

Reagents. [1,4-^{14}C]Putrescine dihydrochloride

(110 mCi/mmol), [1,4*n*-^{3}H]putrescine dihydrochloride (24 mCi/mmol) and L-[4,5-^{3}H]leucine (140 mCi/mmol) were purchased from Amersham International Ltd. (Brussels, Belgium) and [methyl-^{14}C]paraquat dihydrochloride (111 mCi/mmol) was purchased from Amersham International Ltd. (Amersham, U.K.). Soluene 350 tissue solubiliser, Emulsifier Safe and Ultima Gold scintillants and plastic scintillation vials (20 mL and 5 mL) were purchased from Packard N.V. (Zellik, Belgium). Paraquat dichloride (99.9% pure, Plant Protection Division, ICI plc, now Zeneca) was a gift from Dr L. L. Smith (Central Toxicology Laboratory, ICI plc, now Zeneca). Putrescine dihydrochloride, cystamine, glucose, pyruvate, reduced NADH, trizma base (Tris), BSA, DNAse I, Percoll, TCA†, sodium carbonate, sodium azide, glutaraldehyde, osmium tetroxide, tannic acid, DMSO, naphthol AS-B1 phosphate sodium salt, fast red violet LB and 2-amino-2-methyl-1-propanol were purchased from Sigma Germany (Filter Service NV/SA, Eupen, Belgium). Waymouth's 752/1 medium, trypsin Rega 5 (trypsin 1:300; 0.275% w/v), fungizone (amphotericin 250 μg/mL), penicillin–streptomycin solution (10,000 units–10,000 μg per mL), L-glutamine (200 mM), Hepes and FCS were purchased from Gibco (Merelbeke, Belgium). Protein assay dye solution was purchased from Bio-Rad (Brussels, Belgium). All other chemicals were obtained from U.C.B. Belgium (Vel NV/SA, Leuven, Belgium).

Preparation of the human lung tissue. Lung tissue was obtained from patients undergoing lobectomy or pneumectomy, mostly for lung cancer. Within minutes after resection, a large portion of macroscopically non-tumoural tissue was cut from the surgical specimen, the pleura was removed and the tissue was placed in KRPB containing NaCl (130 mM), KCl (5.4 mM), $CaCl_2$ (1.9 mM), $MgSO_4$ (1.29 mM), Na_2HPO_4 (10 mM), glucose (11 mM) (pH 7.4) at room temperature.

Accumulation of putrescine and paraquat in lung slices. Within 1 hr, slices of lung parenchyma (i.e. without visible bronchi) of 0.7 mm thickness and cut surfaces of about 0.5 cm^2 were prepared with a McIlwain tissue chopper (Mickle Laboratories, Surrey, U.K.). The slices were weighed and incubated at 37° or 4° in lots of approximately 30 mg in 3 mL KRPB in 40 mL polyethylene flasks placed in a shaking water bath (120–140 strokes/min) [21]. Putrescine and paraquat uptake were determined by the method of Smith *et al.* [22], as described previously [23]. In a first set of experiments, the slices were incubated for various lengths of time (15, 30 and 45 min) with putrescine (2.5, 5, 10, 20, 40 or 80 μM) containing [1,4-^{14}C]putrescine (0.1 μCi per incubation flask) and paraquat (0, 500, 1000 or 5000 μM), or with paraquat (7.5, 30, 75, or 240 μM) containing [methyl-^{14}C]paraquat (0.1 μCi) and putrescine (0 or 10 μM). In a second set of experiments, slices were incubated in paraquat (50, 250, 1000 or 5000 μM) containing [methyl-^{14}C]-paraquat (0.1 μCi).

At the end of the incubation, the slices were removed, briefly immersed in KRPB, blotted, weighed and dissolved in Soluene 350, whereafter 10 mL of Ultima Gold scintillant were added. In addition, a 0.1 mL aliquot of medium was added to 0.4 mL water and 4.5 mL Emulsifier Safe scintillant. The tissue and medium radioactivity were determined by liquid scintillation spectrometry using a Beckman ls5000ce counter, fitted with appropriate quench correction curves to convert cpm to dpm. The accumulation of putrescine (nmol/g/hr) was obtained from the tissue-associated radioactivity, the specific activity, post-incubation weight (all lung weights are wet weights) and the incubation time. The passive diffusion of putrescine or paraquat was obtained from incubations at 4°, and subtracted from the accumulation at 37° to calculate the active uptake.

Measurement of metabolic parameters in lung slices. Protein synthesis was determined as the stable incorporation of radioactivity into acid precipitable material after 30 min incubation with L-[4,5-^{3}H]-leucine (0.07 μCi/mL) [24]. The incubated tissue was removed and rinsed in KRPB, homogenized (Ultra-Turrax T25 Homogenizer, 24,000 rpm, 30 sec) in 5% TCA (w/v) and centrifuged (2000 *g*, 4°, 30 min). The pellet was dissolved in 1 mL 5% TCA, centrifuged and washed twice in 1 mL ether/acetone/chloroform (2/2/1) at 60° for 30 min and finally dissolved in 1 M NaOH. The radioactivity was determined as described above.

In order to measure the LDH leakage from the slices into the incubation medium, an aliquot of 50 μL of the incubation medium was added to 2.5 mL of 80 mM Tris/200 mM NaCl containing NADH (assay concentration 0.2 mM), mixed and brought to 30°. Pyruvate (assay concentration, 1.6 mM) in Tris/NaCl was added (total volume 3 mL), and the decrease in absorbance at 339 nM was followed for 2 min. The LDH activity in the medium, calculated per gram tissue, was expressed as a percentage of the maximum releasable activity (corresponding to the activity measured in the supernatant fraction obtained by centrifugation of homogenized control tissue).

Isolation of human type II pneumocytes. The isolation procedure was based on that of Kikkawa and Yoneda [25] and Robinson *et al.* [14]. Because it is impractical to try and perfuse surgical lung specimens, the lung tissue was first sliced (0.7 mm thickness) with the McIlwain tissue chopper; aliquots of approximately 1 g of slices were washed four times at 4° in 10 mL PBS^- (130 mM NaCl, 5.4 mM KCl, 11 mM glucose, 10.6 mM Hepes and 2.6 mM Na_2HPO_4 adjusted to pH 7.4) by vigorous shaking (±15 times by hand), and then pouring the medium through a cotton gauze, from which the slices were collected. To dissociate the cells, the washed slices were incubated four times (2 × 5 min and 2 × 20 min) at 37° in 10 mL fresh trypsin with gentle shaking (waterbath 60 strokes/min). The protease activity was stopped by the addition of 4 mL FCS per gram tissue and 100 μg DNAse I per gram tissue. This primary digest was, from hereon, handled under sterile conditions. It was filtered through Nylon filters with a mesh of 80 and 25 μm (Heleine Cavenaile PVBA/SPRL, Brussels, Belgium), centrifuged for 10 min at 250 g in 50 mL tubes at 4°, resuspended in 25 mL Waymouth's medium containing DNAse 50 μg/mL, FCS 2%, fungizone 2% and penicillin–

streptomycin solution 2%, and incubated for 1 hr in a bacterial Petri dish placed in a CO_2 incubator (10% CO_2, 37°) in order to let macrophages attach. The non-adherent cells were then layered onto discontinuous Percoll layers of density 1.089 g/mL (1 mL 10× concentrated PBS^-, 6.49 mL Percoll and 2.51 mL water) and 1.040 g/mL (1 mL 10× concentrated PBS^-, 2.72 mL Percoll and 6.28 mL water) in 50 mL centrifuge tubes (1 per 2 or 3 g tissue) and centrifuged 20 min at 250 *g* at 4°. Finally, the creamy cell layer above the heavy gradient was collected, rinsed twice with PBS^+ (PBS^- with 1.9 mM $CaCl_2$ and 1.29 mM $MgSO_4$) and plated (150,000 cells in 200 μL) in 96-well cell-culture plates pre-coated with extra-cellular matrix (Biological Industries, Glasgow, U.K.) in Waymouth's medium containing 10% (v/v) FCS and 1% (v/v) fungizone, penicillin–streptomycin solution and L-glutamine.

Accumulation of putrescine in cultured type II pneumocytes. Two days (44–48 hr) after plating, putrescine uptake was determined in triplicate by incubating cells, at 37° or 4°, for 60 min in PBS^+ containing putrescine (2.5, 10, 25 or 80 μM) and [1,4*n*-^{3}H]putrescine (0.1 μCi per incubation well) in the presence of paraquat (0, 500, 1000 or 5000 μM) or cystamine (0 or 10 μM). After incubation, the cells were rapidly rinsed three times with 200 μL PBS^+ and then dissolved in 200 μL NaOH (0.1 M) containing 2% Na_2CO_3 at 37° for 1 hr. Uptake was derived from the radioactivity, measured as for the homogenized slices, and related to protein concentration, measured in cells from a similar fourth incubation by the method of Bradford [26] using the Bio-Rad dye solution (the dissolved cell mixture being neutralised with an equal amount of 0.1 M HCl before protein measurement).

Identification, viability and metabolic parameters of cultured type II pneumocytes. The cells obtained immediately after the isolation procedure and cells cultured after 44–48 hr culture were stained for alkaline phosphatase [18] and with tannic acid [27] to identify the alveolar type II cells.

Plating efficiency was calculated as the percentage of cells remaining on the plate after gentle rinsing divided by the number of (Trypan-Blue negative) cells originally put into each multiwell.

Protein synthesis was determined after 1 hr of incubation on the basis of [^{3}H]leucine (0.35 μCi/well) incorporation [24]. The medium was removed, the cells were rinsed and dissolved (carried out as above) and transferred to 200 μL 15% TCA (w/v). Radioactivity was measured as for the homogenized slices.

Viability was verified by Trypan-Blue exclusion and LDH release into the medium. The LDH leakage was measured, as described above, with the maximum releasable activity being defined as the activity measured in control cells dissolved in 0.1% Triton.

Analysis of data. When duplicate or triplicate determinations were available, the average value was taken. The apparent kinetic parameters, V_{max} and K_m, were calculated from a Hanes–Woolf plot [28].

Competitive inhibition was verified by a Cornish–Bowden plot [29], plotting S/V (substrate concentration/rate of uptake) against I (inhibitor concentration) and linear regressions were calculated per substrate concentration. The inhibitor constant K_i was determined using the equation: $K_m app = K_m (1 + I/K_i)$ with $K_m app$ representing the K_m calculated in the presence of inhibitor.

Data from control and treated slices or cells were compared by Student's *t*-test for paired data, or by analysis of variance with Duncan grouping, using the SAS/STAT package (6th version).

RESULTS

Lung slices

Accumulation of putrescine and paraquat. As found previously [23], the uptake of putrescine in slices followed Michaelis–Menten kinetics, with a mean V_{max} of 376 nmol/g/hr and a mean K_m of 6.7 μM. Uptake in the presence of paraquat was linear with time up to 45 min (data not shown). The active uptake of putrescine was decreased in the presence of paraquat, the magnitude of the decrease being dependent on the paraquat concentration (Fig. 1a). A Cornish–Bowden plot (Fig. 1b) indicated that the inhibition of putrescine uptake by paraquat was competitive, because the slopes of the linear regression lines at each putrescine concentration were equal, whereas the intercepts were significantly different. The apparent K_m increased with increasing paraquat concentrations from 6.7 μM, without paraquat, to 67.6 μM with 5000 μM paraquat. The calculated K_i values were 614 and 549 μM for 1000 and 5000 μM paraquat, respectively; the K_m value for the incubation in the presence of 500 μM paraquat (7.5 μM) did not differ significantly from the value without paraquat.

Two sets of paraquat uptake experiments were performed. Initially, paraquat accumulation was only measured in medium containing up to 240 μM paraquat; no saturation was apparent at this concentration, but putrescine (10 μM) strongly inhibited the uptake of paraquat (insert Fig. 2). (Fitting the data of these first experiments to a Michaelis–Menten kinetic model gave an apparent V_{max} of 476 nmol/g/hr and K_m of 336 μM.) When the data of both the initial experiments and subsequent experiments using paraquat concentrations up to 5 mM were plotted, the accumulation of paraquat into human lung slices fitted the Michaelis–Menten kinetic model, giving an apparent V_{max} of 370 nmol/g/hr and a K_m of 244 μM paraquat.

The passive diffusion, as evaluated from incubations at 4° in all experiments, increased linearly with the medium concentration and never exceeded a tissue/medium ratio of 1. At the highest concentrations of paraquat used, the relative proportion of this passive component became relatively large compared to the total accumulation, as measured at 37°, but it always remained lower than the total accumulation.

Measurement of metabolic parameters in lung slices. Within the time course of the incubations, i.e. 45 min maximum, the toxicity of paraquat, even at 5000 μM, was not detectable by means of LDH leakage (9.8 ± 5.2% of maximum for control incubation and 10.2 ± 4.3% of maximum for

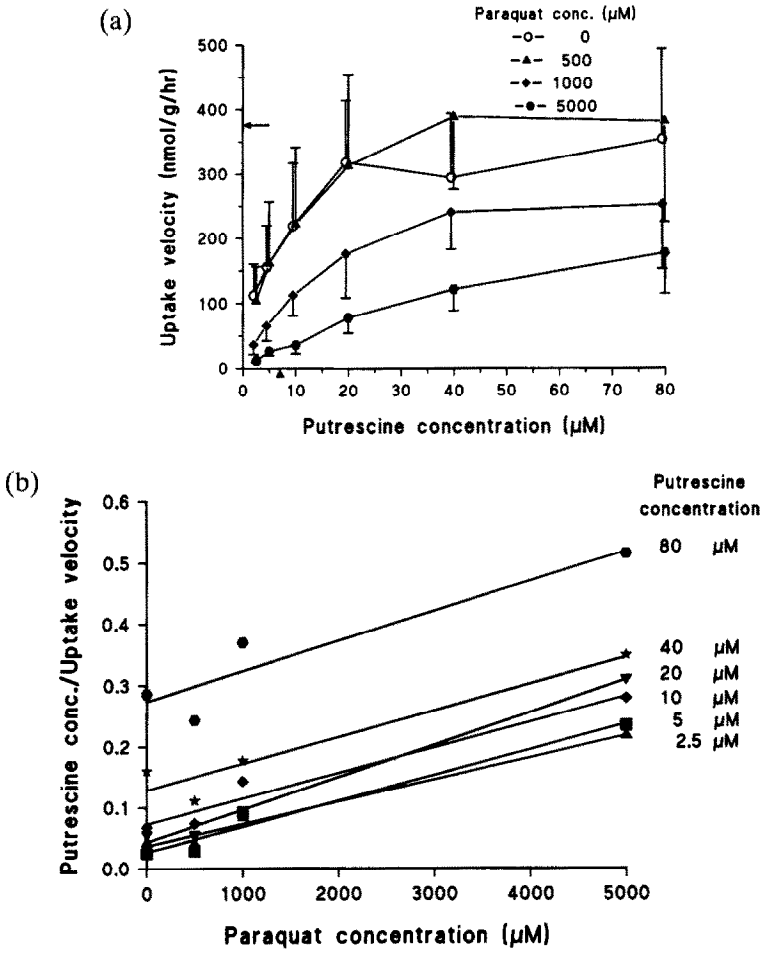

Fig. 1. Inhibition of putrescine uptake by paraquat in human lung slices. Slices were incubated for 30 min, at 37° or 4°, in various medium concentrations of [^{14}C]putrescine in the presence of 0, 500, 1000 and 5000 μM paraquat. Values obtained at 4° (not shown) have been subtracted from those obtained at 37°. (a) Data are means (±1 SD), N = 10 without paraquat, otherwise N = 5. Calculated kinetic parameters for putrescine uptake: K_m (▲ 6.7 μM and V_{max} (←) 376 nmol/g/hr). (b) Cornish–Bowden plot of the mean values. The slopes of the linear regression lines (correlation coefficients $R > 0.95$) do not differ ($P > 0.85$), whereas the intercepts do ($P < 0.0005$).

incubations with 5000 μM paraquat, N = 4). Leucine incorporation (147,398 ± 19,454 dpm/100 mg/hr for control) was not altered in the presence of 5000 μM paraquat (101 ± 15% of control, N = 4).

Cultured human type II pneumocytes

Identification, viability and metabolic parameters of cultured type II pneumocytes. Of the freshly obtained cells, 77 ± 10% stained positive for alkaline phosphatase and 77 ± 13% for tannic acid, giving a yield of 1.5 ± 0.4 million type II pneumocytes per gram of lung tissue; of these cells, 83 ± 5% showed exclusion of Trypan Blue. After 2 days, these parameters were 84 ± 1%, 84 ± 3% and 97 ± 2%, respectively.

The plating efficiency was 51 ± 3% after 2 days of culture and the cells did not detach after 1 hr of incubation with 5000 μM paraquat (53 ± 3%). The percentage of cells excluding Trypan Blue (97 ± 1%) was also unaltered in these circumstances.

The LDH leakage into the culture medium was 3.4 ± 2% for control and 4.1 ± 1.9% for paraquat-treated cells. The incorporation of leucine at 5000 μM paraquat was not altered (108.5 ± 12.8%) compared to control (40,280 ± 4005 dpm/mg prot/hr).

Accumulation of putrescine in cultured type II pneumocytes. In isolated human type II pneumocytes, the uptake of putrescine and the competitive inhibition with paraquat showed essentially the same picture as in slices (Fig. 3). The calculated kinetic parameters of putrescine uptake were a V_{max} of 3.4 μmol/g protein/hr and a K_m of 6.2 μM without paraquat, increasing to 41.6 μM in the presence of 5000 μM paraquat. Thus, the calculated K_i values were 659, 895 and 874 μM for incubations in the presence of 500, 1000 and 5000 μM paraquat, respectively.

In the presence of cystamine, the uptake of putrescine was strongly inhibited (Fig. 4). In these experiments the kinetic parameters of putrescine

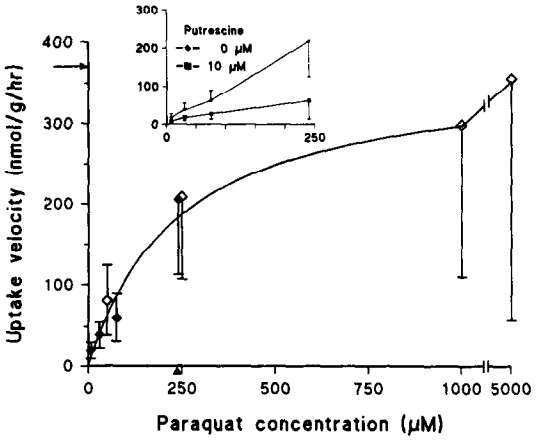

Fig. 2. Inhibition of paraquat uptake by putrescine in human lung slices. Slices were incubated for 30 min, at 37° or 4°, in various medium concentrations of [^{14}C]paraquat (insert: with or without 10 μM putrescine). Values obtained at 4° (not shown) have been subtracted from those obtained at 37°. Data are means (±1 SD), N = 4. Open and solid symbols indicate separate sets of experiments. Calculated kinetic parameters for paraquat uptake: K_m (▲) 244 μM and V_{max} (→) 370 nmol/g/hr.

uptake were a V_{max} of 3.0 μmol/g protein/hr and a K_m of 7.6 μM increasing to 28.3 μM in the presence of 10 μM cystamine (V_{max} remaining at 2.7 μmol/g protein/hr), thus giving a K_i of 3.7 μM.

DISCUSSION

The accumulation of paraquat and putrescine and the reciprocal competitive inhibition for this uptake have been demonstrated previously in rat lung slices and in rat alveolar type II cells in primary culture [19, 30, 31]. The human lung has also been shown to accumulate putrescine, with the sites of accumulation being the alveolar type I and type II cells [23]. In the present study, we have studied the reciprocal inhibition of paraquat and putrescine uptake in human lung slices, and the accumulation of putrescine and its inhibition by paraquat or cystamine in cultured human type II pneumocytes. The uptake of paraquat was not studied in the isolated cells because radiolabelled (e.g. tritiated) paraquat of sufficient specific activity was not available.

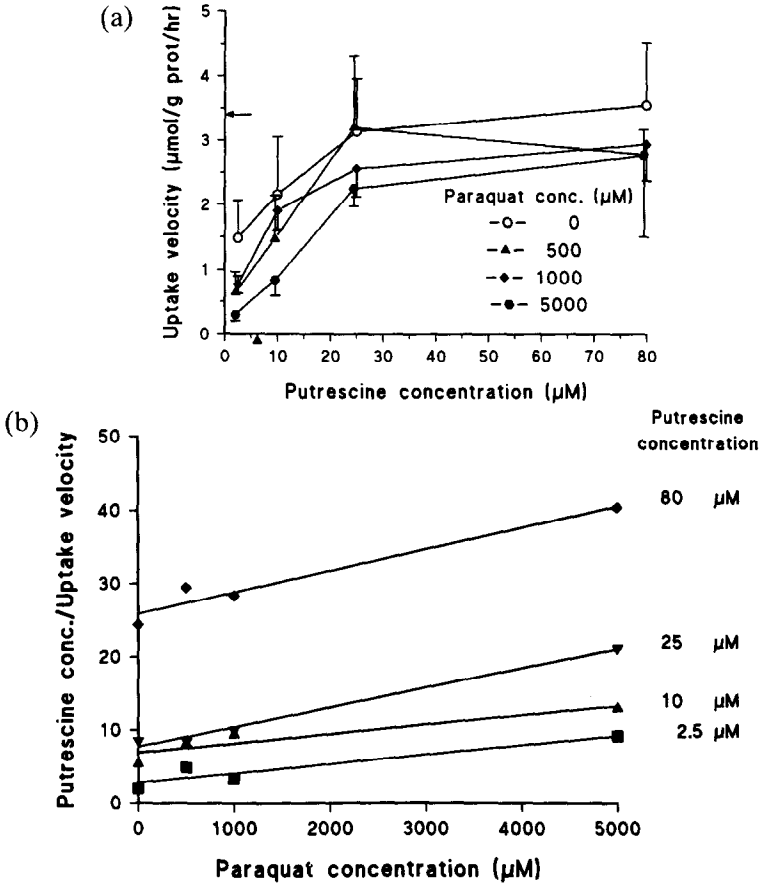

Fig. 3. Inhibition of putrescine uptake by paraquat in cultured human type II pneumocytes. Two days after their isolation, human type II pneumocytes were incubated for 1 hr, at 37° or 4°, in various medium concentrations of [^{3}H]putrescine in the presence of 0, 500, 1000 and 5000 μM paraquat. Values obtained at 4° (not shown) have been subtracted from those obtained at 37°. (a) Data are means (±1 SD), N = 6 without paraquat, otherwise N = 4. Calculated kinetic parameters for putrescine uptake: K_m (▲) 6.2 μM and V_{max} (←) 3.4 μmol/g protein/hr. (b) Cornish–Bowden plot of the mean values. The slopes of the linear regression lines (correlation coefficients $R > 0.94$) do not differ ($P > 0.8$), whereas the intercepts do ($P < 0.0001$).

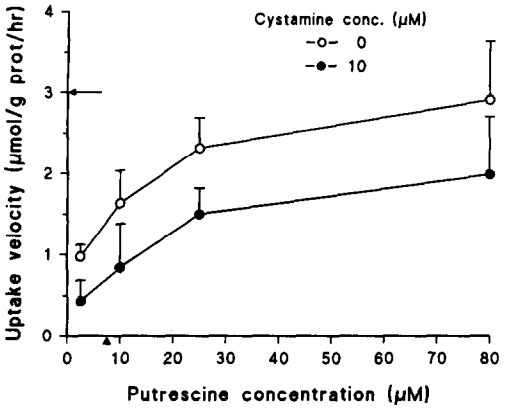

Fig. 4. Inhibition of putrescine uptake by cystamine in cultured human type II pneumocytes. Two days after their isolation, human type II pneumocytes were incubated for 1 hr, at 37° or 4°, in various medium concentrations of [^{3}H]putrescine in the absence or presence of cystamine (10 μM). Values obtained at 4° (not shown) have been subtracted from those obtained at 37°. Data are means (+1 SD), N = 3. Calculated kinetic parameters for putrescine uptake: K_m (▲) 7.6 μM and V_{max} (←) 3.0 μmol/g protein/hr.

The kinetic parameters of putrescine uptake in the lung slices (V_{max} of 376 nmol/g/hr and K_m of 6.7 μM) were similar to the values reported earlier [23]. The inhibition of putrescine uptake by paraquat was shown to be competitive by a Cornish–Bowden plot, with inhibition constants of 549–614 μM and 659–895 μM in slices and cultured cells, respectively. When the inhibitor concentration is lower than the K_i, the increase in K_m is lower than two-fold and it becomes difficult to detect such changes experimentally and statistically; this is presumably why no meaningful K_i value could be derived at 500 μM paraquat using slices, where K_m and $K_m app$ did not significantly differ (6.7 vs 7.5 μM), although it was possible to do so in the cells, where K_m and $K_m app$ did differ significantly (6.2 vs 10.9 μM). No excessive reliance, however, should be placed on "exact" figures for kinetic parameters; the important thing is probably their consistency and the orders of magnitude, i.e. 5 to 10 μM for the K_m of putrescine uptake and 0.5 to 1 mM for the K_i of paraquat on putrescine uptake. The latter values are comparable to values calculated, for the rat lung, by Oreffo *et al.* [31] for simple competitive inhibition (458 μM and 460 μM in slices and isolated type II cells, respectively), but much higher than their value when they took a model of partially competitive inhibition. The latter model, and that of Chen *et al.* [32], is, however, not consistent with our experimental data, which show similar *linear* slopes per putrescine concentration but different intercepts.

Initially, we had measured paraquat uptake in medium containing up to 240 μM paraquat, a concentration at which no saturation was achieved, in agreement with the data obtained from the inhibition of putrescine uptake, which suggested that the K_m for paraquat uptake could be around 500 μM. These first experiments, nevertheless, clearly showed that the uptake of paraquat was markedly diminished in the presence of a low concentration of putrescine (insert Fig. 2). A second series of experiments extending the paraquat concentrations to 5000 μM did show saturation. Pooling all the available data yielded values of 244 μM for K_m and 370 nmol/g/hr for V_{max} for the uptake of paraquat. These estimates are compatible with the K_i values found for the inhibition of putrescine uptake, and the V_{max} is comparable to that of putrescine. Overall, our slice data thus concur to indicate that putrescine and paraquat accumulate in the human lung by the same uptake system, but that the affinity for paraquat is much lower (approximately 40-fold) than that for putrescine.

The kinetic characteristics of the uptake of putrescine in the isolated human type II cells are in agreement with those found in slices and there was also competitive inhibition of putrescine uptake by paraquat. Here too, paraquat appeared to be a rather poor inhibitor of the putrescine uptake, with K_i values in the range of 650 to 900 μM. Cystamine, which is a much more potent inhibitor of putrescine uptake in rat [33] and human [23] lung slices, was used to obtain a more marked inhibitory response. It is not entirely appropriate to use only one inhibitor concentration to establish competitive inhibition, but since in human lung slices competitive inhibition with cystamine had been demonstrated convincingly, it was reasonable to calculate a K_i for the competitive inhibition of putrescine uptake by cystamine. The apparent K_i thus determined in cells (3.6 μM) did not differ from the value obtained in slices (3.2 μM).

In Table 1, uptake data obtained in different species using either lung slices or isolated cells have been summarized. All data have been expressed in relation to protein concentration (using a factor of 140 mg protein per gram wet weight and 4.2 g protein per g DNA). Looking at species differences, it seems that the K_m for putrescine uptake determined in the hamster lung may differ from that in rat and human, but in general the kinetic parameters are very similar in the two systems used.

Putrescine uptake could have been affected by the cellular toxicity of paraquat. Indeed, the depletion of NADPH caused by paraquat [2] affects the energy status of the cells, which can in turn have its repercussions on the uptake. In freshly cultured murine Clara cells, the LD_{50} of paraquat is very low (0.8–5 μM) after 20 hr incubation [34]. However, in our experiments the incubation time was only 1 hr at most and, therefore, presumably too short to cause manifest cellular toxicity. Another possible, but hitherto unchecked factor could be depletion of NADPH-cytochrome P450 reductase activity in cultured cells, thus causing less paraquat reduction than *in vivo*. In any case, the levels of LDH leakage and protein synthesis were not significantly altered by the different experimental conditions, indicating that the uptake was measured before the onset of overt toxicity. For the cultured cells, we also used Trypan Blue exclusion and total cells attached, and these indices remained unaltered by paraquat.

Technically, our isolation of human alveolar type II cells differs from previously published methods

Table 1. Putrescine and paraquat uptake in lung slices and isolated type II cells in different species

	K_m μM	V_{max} μmol/g prot/hr	Reference
Slices			
Mouse			
Putrescine	15	8.4	Smith *et al.* [35]
Paraquat	209	9.2	Smith *et al.* [35]
Rat			
Putrescine	7	2.4	Smith and Wyatt [4]
	13.1	5.2	Smith *et al.* [22]
	19.8–30.6	5.0–6.9	Nemery *et al.* [8]
	7.98	3.4	Karl and Friedman [5]
Paraquat	70	2.1	Rose *et al.* [3]
	210	5.1	Ross and Krieger [36]
	119	4.5	Karl and Friedman [5]
Hamster			
Putrescine	35	5.5	Own observations (unpublished)
Human			
Putrescine	2–11	0.7–1.8	Brooke-Taylor *et al.* [37]
	7.2	3.0	Hoet *et al.* [23]
	6.7	2.7	Present study
Paraquat	244	2.6	Present study
Cells			
Rat			
Putrescine	8–14	14	Richards *et al.* [30]
	14.7	31	Oreffo *et al.* [31]
	2.5	8.1	Chen *et al.* [32]
Paraquat	88	6.9	Chen *et al.* [32]
Hamster			
Putrescine	22.5	2.3	Own observations (unpublished)
Human			
Putrescine	6.2–7.6	3.0–3.4	Present study

[14–17] with respect to the initial treatment of the tissue. Rather than trying to cannulate the surgical specimen to remove blood and free macrophages, the tissue was cut into slices. We believe that this approach is much easier and allows a much more rapid processing of the tissue. The incubation time of the tissue with trypsin (2 × 5 min and 2 × 20 min) was chosen to achieve a reasonable yield of relatively undamaged cells, although occasionally this yield can be very low or cells can appear profoundly damaged, possibly depending on the "quality" of the tissue received. One of the possible, but hitherto unverified, determinants may be the time the lung has been clamped during the operation. In the present experiments, however, only "good" preparations were used.

In conclusion, our experiments have demonstrated that the human lung possesses an active uptake system for putrescine which is located in specific epithelial cells and which is competitively inhibited by paraquat; the kinetic parameters which characterize this uptake system are comparable in lung slices and isolated type II cells. In these cells, as in slices, putrescine uptake is also strongly inhibited in the presence of a low cystamine concentration. One of the purposes of our experiments is to use the accumulation of putrescine in cultured human alveolar type II cells as a measurement of cell viability and functionality after isolation. Experiments are in progress to study the maintenance of this function with time in culture.

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