ABSTRACT:
The delivery and pharmacokinetics of cyclosporine A (CyA) given locally to the airways or iv was evaluated in young and adult rats. After intratracheal (i.t.) instillation of saline suspended CyA to adult rats, the CyA plasma levels peaked at 30 min with a bioavailability of 78.1 ± 6.9%. After the i.t. instillation of CyA with micelles forming surfactant, Cremophor EL, in adult and young rats, the plasma levels peaked at 5 min with a bioavailability of 77.5 ± 7.2% and 66.3 ± 4.5%, respectively. The bioavailability of aerosolized CyA was 80.1 ± 4.1% in adults. Thus, CyA is absorbed by the lungs into the systemic circulation of the rat in high amounts, independent of age and type of delivery system. Long-term treatment with i.t. instillations did not affect body weight gain in young and adult rats, and no histopathological changes were found in the lungs. It is important to emphasize that CyA plasma clearance in young rats was lower and elimination half-life longer than in adults. The slow elimination of CyA in young rats indicated profound pharmacokinetic age differences for this species.

Materials and Methods
Two types of CyA preparations were used: CyA as a dry powder (Sandz Pharma, Basle, Switzerland) and CyA for iv infusion (Sandimmum, Sandz Pharma) containing 50 mg/ml CyA and 650 mg/ml polyoxyethylated castor oil (Cremophor EL, Sandz Pharma) in 33% (v/v) ethanol as vehicle. Sprague-Dawley rats (Mollegard, Skensved, Denmark) of both sexes, 28 days old weighing 80–100 g (young rats), and 90–120 days old weighing 220–320 g (adult), were used. The animals were kept in standard laboratory conditions. For repeated blood sampling right jugular veins were chronically catheterized under 50 mg/kg BW ketamine (Ketalar, Parke-Davies, Barcelona, Spain), and 10 mg/kg azaperon (Stresnil, Janssen, Beerse, Belgium) anesthesia. The animal experiments were approved by the Ethical Review Committee for animal experiments at Lund University.

Two groups of rats, young (N = 4) and adult (N = 6), were i.t. instilled with CyA (Sandimmum) in a micellar solution 1:50 in 0.9% saline (CyA concentration 1mg/ml). A third group of rats (adult, N = 6) was instilled with a suspension of pure CyA in 0.9% saline (1mg/ml) prepared immediately prior to each instillation with extensive mixing. Under brief ether anesthesia the CyA preparations were administered via the mouth into the trachea using a modified syringe needle (1×80mm), in a volume of 1ml/kg BW(11). To check the precision of the i.t. instillation procedure, 5 mg/ml bovine serum albumin (BSA, Sigma, St. Louis, MO) was added to the control mixtures as an accessory macromolecular marker of pulmonary passage (12).

One group of adult rats (N = 5) was exposed to aerosolized CyA. Pure CyA was dissolved in 99.6% ethanol (20 mg/ml) and an aerosol was generated by an air jet nebulizer (MA2s, Viasol, Malmo, Sweden) having 4 mg CyA/min output and 5 liters/min flow rate. Rats were placed individually in polycarbonate tubes connected to the exposure Battelle chamber (13) and nose-only output and 5 liters/min flow rate. Rats were placed individually in polycarbonate tubes connected to the exposure Battelle chamber (13) and nose-only.

For the evaluation of new peptides involved in the regulation of the immune response, an interest in new routes for administration, e.g. via the airways, has been increasing. The cyclic undecapeptide, CyA, has proven to be therapeutically effective not only for organ transplantation, but also during an abnormal immune reaction in progress. Local intra-arterial delivery or aerosolization of low-subtherapeutic CyA doses not only reduces airway hyper-responsiveness (1), and T cell infiltration and proliferation (2) but also protects heart (3) or lung (4–7) allografts from rejection at a site where the concentration of the substance is high. Inhalation of aerosolized CyA has been examined to some extent (8–10), but it was 80.1 ± 6.9%

6.9%. After the i.t. instillation of CyA with micelles forming surfactant, Cremophor EL, in adult and young rats, the plasma levels peaked at 5 min with a bioavailability of 77.5 ± 7.2% and 66.3 ± 4.5%, respectively. The bioavailability of aerosolized CyA was 80.1 ± 4.1% in adults. Thus, CyA is absorbed by the lungs into the systemic circulation of the rat in high amounts, independent of age and type of delivery system. Long-term treatment with i.t. instillations did not affect body weight gain in young and adult rats, and no histopathological changes were found in the lungs. It is important to emphasize that CyA plasma clearance in young rats was lower and elimination half-life longer than in adults. The slow elimination of CyA in young rats indicated profound pharmacokinetic age differences for this species.

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Two groups of rats, young (N = 4) and adult (N = 5), were injected (bolus) via the jugular vein (15) with CyA (Sandimmum) dissolved 1:50 with 0.9% saline at a concentration of 1 mg/ml in a volume of 1 ml/kg BW. Blood samples (0.15–0.4 ml) were taken from the jugular catheter before
Fig. 1. Plasma levels (mean ± SE) of CyA i.t. instilled or administered as aerosol (dose = 1 mg/kg BW) in young and adult rats, respectively.

and after administration at 2.5, 5, 15, and 30 min, 1, 2, 4, 8, 12, 24, 48, 72, and 96 hr. The blood samples were collected in heparinized tubes, and after allowing 2 hr for CyA redistribution between plasma and erythrocytes, centrifuged at 3000g for 15 min (+20°C). Plasma was harvested and stored at −20°C until analysis.

To determine if the instillation procedure or the aerosol exposure per se had affected the respiratory tract, two groups of adult rats (N = 4) were i.t. instilled with either the micellar solution (group 1) or the saline suspension of CyA (group 2). A third group (N = 5) was exposed to the CyA aerosol. A fourth group (N = 4) was untreated. Immediately after the rats were sacrificed, a bronchoalveolar lavage was performed (11). After centrifugation of the lavage fluid at 500g for 15 min (+4°C) the supernatant was collected and stored (−20°C). The lavage cells were differentially stained with May-Grünwald-Giemsa and counted. Routine light microscope histological examination of the lung tissue was also done. In a long-term study (51 days), young (N = 8) and adult rats (N = 12) were randomly divided into equal groups and treated with i.t. instilled CyA in saline suspension (1 mg/kg every 48 hr) or with vehicle (saline). CyA toxicity was monitored by weighing the rats frequently (16). Examination of lung tissue histology was done on animals from each group. To study the contents of CyA in the lung tissue, lungs were homogenized and assayed at 24, 48, and 72 hr after the i.t. instillation of micellar CyA to adult rats (N = 5). CyA concentration in the lung homogenates was calculated from tissue weight and initial dilution of the sample.

The CyA levels in the plasma were determined using a Fluorescence Polarization Immunoassay FPIA (TDx/TdxFLx Cyclosporine and Metabolites Serum Assay Abbott, Abbott Park, IL) (17).

Bioavailability of CyA was calculated using the formula: $F = \frac{AUC(lung)}{dose(iv)AUC(iv) \times dose(lung)}$, where $AUC$ was determined by the trapezoidal rule and extrapolated to infinity by dividing the last blood concentration by the elimination rate constant ($k$), as estimated by the log-linear least-square fit of the last 4–6 concentrations on the elimination part of the level-time curve. Plots of cyclosporine concentrations vs. time were also analyzed by a three-component open model system and the adequacy of the kinetic model was confirmed by examining the plots of residuals and the precision of parameter estimates. The kinetic behavior of CyA was estimated by the use of the computer program TopFit (Version 2.0, Gustav Fisher Verlag, Stuttgart, Germany). Statistical evaluations were made using Student’s t-test and Mann-Whitney U-test, $p < 0.05$ was considered statistically significant. Values are given as mean ± SE.

Results

In adult rats, after the i.t. instillation of the CyA (saline micellar solution), the CyA plasma concentrations rapidly increased, reaching a maximum at 5 min (fig. 1). After i.t. instillation of the CyA saline suspension, the plasma levels time curve was not as sharp, peaking at 30 min. The total passage over the respiratory tract was 77.47 ± 7.2% and 78.1 ± 6.9% respectively. Bioavailability of the inhaled dose of CyA in adult rats was 80.13 ± 4.1%.

The pharmacokinetic parameters for CyA of the young rats, after iv administration, except for the volume of distribution ($V_{\text{arc}}$ and $V_{\text{sv}}$), were significantly different from those of the adult rats (table 1): the $t_{1/2}$ and $MRT$ were longer, and the $CL$ was lower. The passage time curves obtained after the i.t. instillation of CyA to young rats were different than those of the adults (fig. 1). The CyA plasma levels peaked at 5 min, with a significantly longer $t_{1/2}$ than in the adult rats (table 1).

CyA lung-tissue concentrations in the lung homogenates averaged 1251.63 ± 79.2 ng/g at 24 hr, 163.78 ± 15.3 ng/g at 48 hr, and 47.2 ± 10.3 ng/g at 72 hr after instillation. There were no significant differences in the total cell number, cell differential counts, and total protein concentrations in the bronchoalveolar lavage performed in rats i.t. instilled with CyA micellar solution, saline CyA suspension, or rats exposed to the CyA aerosol when compared with untreated control rats. In the long-term study, young and adult rats treated with i.t. instilled CyA did not show any effects on body weight gain in comparison to that of control rats. None of the rats receiving CyA locally developed signs of pulmonary inflammation or infections. Necropsies performed on rats i.t. instilled with CyA in the short and long-term studies showed no differences between rats treated with CyA and the control rats. The evaluation of lung histology did not reveal any accumulation of inflammatory cells in the intravascular, interstitial, and intraalveolar spaces. No focal alveolar collapses, no signs of pulmonary edema or fibrotic alterations, or vascular destruction could be observed in the lung parenchyma and extra-alveolar vessels.

Discussion

This investigation showed that after both i.t. instillation and aerosol exposure, CyA will be absorbed from the lung into the systemic circulation of young and adult rats. The total absorption of CyA in rats was generally high and similar in all the investigated groups independent of age, method of drug delivery, or type of formulation used. This is in contrast to the pulmonary passage of more hydrophilic drugs, peptides, proteins (11,12) and to casual data of CyA absorption in two patients (0% and 9% respectively) with single-lung transplant during chronic rejection (6). However, lipid-soluble drugs, e.g. procarbazine and sulfisoxazole, are known to pass through the lungs in similar amounts in young and adult rats (18). The bioavailability of CyA observed in rats after pulmonary delivery (66–78%), appears to be greater than after oral administration (10–30%) (19,20) and similar to bioavailability observed after sc injection (43–77%) (21). A limited number of the observations (data not shown) suggests a linear relationship between dosage and absorption after i.t. instillation of CyA as proved after oral administration of the drug (19). After the i.t. instillation of CyA micellar solution, the plasma concentrations increased rapidly, whereas after the instillation of CyA saline suspension, the plasma levels peaked later, which suggests that the initial absorption rate depends on the type of formulation, not the total amount absorbed. The possible influence of the micelle forming surfactant (Cremophor EL) on lung permeability mechanisms cannot be excluded (22).

The CyA volumes of distribution in young and adult rats were similar. However, young rats showed a lower total plasma clearance of CyA when compared with the adult rats. This explains the differences in elimination/passage plasma-level time curves and values for $t_{1/2}$ and conclusively suggests a lower elimination capacity of CyA.
and probably CyA metabolites in young rats. This may be why identical doses of CyA administered to developing rats leads to higher plasma CyA concentrations in younger rats than in older ones (23). Thus, to elucidate differences in CyA elimination, further studies in which changes in microsomal enzyme activity and the generation of CyA metabolites in growing animals should be conducted.

The CyA concentrations in lung tissue during the period 24–72 hr after instillation were initially high but diminished with time. Only minimal CyA plasma levels could be detected in that time. These results corresponded with the suggestion that the lung has an avidity for CyA and may also support the concept that a greater efficacy of local therapy could be achieved, in part, because of high tissue concentrations. Local delivery of the drug might also act via pathways not accessible from the systemic route alone (3.6). Furthermore, the pulmonary delivery of CyA might have important advantages, not only in terms of diminished systemic toxicity, but for providing enhanced antioxidant activity at the alveolar epithelial surface in the time of reperfusion during transplantation (9).

Similar to the observations of a previous study of rats receiving multiple treatments of aerosolized CyA (12), the long-term treatment using i.t. instillation did not provoke any signs of inflammation in the airways.

In conclusion, this study suggests that in rats CyA is absorbed by the lungs into the systemic circulation in high amounts, independent of the age of the rat and the type of delivery system used. This supports the suggestion by several investigators that the pulmonary delivery of CyA could be effective as a regimen for reducing immunological reactivity in autoimmune and allergic lung diseases and may be useful for lung transplantation. The finding that the elimination of CyA observed in young rats was twice as slow as in adults indicate profound pharmacokinetic age differences for this species.

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References


