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Dissolving intravenous cyclosporin A in a fat emulsion carrier prevents acute renal side effects in the rat

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Abstract. Cyclosporin A (CyA)-induced nephrotoxicity is still a serious clinical problem. The nephrotoxicity seems to be more pronounced when CyA (solubilized in water with Cremophore EL, as in Sandimmun) is given intravenously than when it is given orally. Using soybean oil in which CyA was dissolved, we prepared an intravenous fat emulsion without an artificial detergent, such as Cremophore EL. The renal effects of our new formula were compared with those of Sandimmun and Cremophore EL in a rat model. CyA in the fat emulsion did not significantly affect the glomerular filtration rate (GFR). Both Sandimmun and Cremophore EL significantly reduced the GFR. These results suggest that a change in the vehicle may obviate the acute nephrotoxic side effects caused by intravenous administration of CyA solubilized by Cremophore EL.

Key words: Cyclosporin A, carrier, in the rat – Soybean oil, cyclosporin A – Cremophore EL, cyclosporin A

Introduction

Cyclosporin A (CyA) is an immunosuppressant routinely used in organ and bone marrow transplantations and presently being evaluated for the treatment of autoimmune diseases. Its most significant side effect is nephrotoxicity, which can be divided into acute reversible and chronic irreversible types. CyA is usually given orally, but in critically ill patients it must be administered intravenously. To achieve the same serum concentrations, approximately 30% of the oral dose is recommended. However, the intravenous administration of CyA seems to cause more side effects than the oral administration does [8].

The mechanism underlying acute CyA nephrotoxicity is still not clear, but changes in plasma renin activity and in renal prostaglandin production, nerve-dependent blood flow impairment, and activation of the mechanism of tubulo-glomerular feedback have been discussed [15]. The direct toxicity of CyA to cultured rat microvascular endothelial cells that has been demonstrated [9] may result in the release of the potent vasoconstrictor endothelin. The view that endothelin may be involved is favored by Kon et al. [7], who have shown that the vasoconstriction is counteracted by a simultaneous infusion of anti-endothelin. Nephrotoxic side effects of orally administered CyA have also been counteracted by dopamine infusion [2], a fact that also supports the hypothesis that acute CyA nephrotoxicity is mediated by a vascular mechanism.

Acute histological changes are found mainly in the proximal tubuli [11]. With scanning electron microscopy, however, constriction of the afferent arterioles to the glomeruli has been demonstrated after a few days' administration of oral CyA to rats [3].

Clearance studies show an acute impairment of the glomerular filtration rate (GFR) within minutes after starting a CyA infusion [1, 14]. Similar changes have been found after the infusion of Cremophore EL alone [1], which suggests that the solvent may play a role in the acute nephrotoxicity seen during intravenous CyA administration.

Amphotericin B is another drug in which the vehicle has been demonstrated to have a major impact on side effects. Administration of the drug in liposomes has diminished the nephrotoxicity, but fungicidal efficiency seems to be maintained [13].

On the basis of these findings, we hypothesized that if CyA were dissolved in a nontoxic fat emulsion carrier, the acute renal side effects would be reduced.

Materials and methods

Test substances

CyA, in the form of a crystalline powder, was kindly supplied by the Sandoz Company (Basel, Switzerland). The fat emulsion contained 10% purified soybean oil and 1.2% purified egg phospholipids. The CyA was dissolved in the lipid phase. The water phase contained distilled water, glycerol (to give an isotonic emulsion), and purified

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egg phospholipids. The water phase was heated to 60°–70°C and the lipid phase was added in a mixer at high speed. A fine emulsion was prepared in a valve homogenizer at high pressure and was then dispensed in glass vials and heat-sterilized. The emulsion was supplied by Professor Arvid Wretlind, Stockholm, Sweden. The final concentration of CyA in our fat emulsion was 3 mg/ml.

Commercially available Sandimmun infusion substance (Sandoz, Basel, Switzerland) was dissolved in 5% glucose, as recommended. The final concentration was 3 mg CyA + 39 mg Cremophore EL + 16.5 mg ethanol/ml. Cremophore EL (Sigma Chemical, St. Louis, Mo., USA) was dissolved in 5% glucose to a final concentration of 39 mg/ml.

Preparation of the rats

Male Wistar FU rats (A-lab, Stockholm, Sweden) weighing 200–250 g were anesthetized with Inactin (Byk Gulden, Konstanz, FRG), 120 mg/kg body weight, intraperitoneally. Each rat was placed on a servocontrolled heating pad to maintain its body temperature at 37.5°–38°C. A tracheostomy was performed. Polyethylene catheters were inserted into both femoral veins for intravenous infusions and into both femoral arteries, one for blood sampling and one for continuous recording of systemic arterial pressure (GRASS Polygraph, Model 7D and Gould P231D transducer, Quincy, Mass., USA). A polyethylene catheter for urine sampling was inserted in the bladder via a midline incision. The rat was then placed on its side and not moved during the experiment.

Clearance studies

The GFR was measured by inulin clearance (C_{in}). After a priming dose of 2 μCi 3 H-inulin (New England Nuclear, Boston, Mass., USA) in 0.9% saline, a continuous intravenous infusion of 1 μCi/h per 100 g body weight 3 H-inulin in 0.9% saline was given at the rate of 0.68 ml/h per 100 g body weight. The infusion was administered by a constant speed infusion pump (TeruFusion Syringe Pump model STC-52, Terumo, Tokyo, Japan). Before the measurements were made, an equilibration period of 60 min was allowed in order to achieve a steady-state condition. Thereafter, urine was collected in preweighed polyethylene vials during three periods of 20 min. Blood samples (75 μl) were taken in heparinized capillary tubes at the midpoint of each urine collection period.

After three baseline periods, a bolus dose of 2 mg CyA in fat emulsion/100 g body weight (group 1, $n = 6$) or 2 mg CyA as Sandimmun/100 g body weight (group 2, $n = 6$) was given for 5 min. The third group ($n = 7$) received only Cremophore EL, 26 mg/100 g body weight. Thereafter, 2.0 mg/h per 100 g body weight CyA in fat emulsion or 2.0 mg/h per 100 g body weight CyA as Sandimmun or 26 mg/h per 100 g body weight Cremophore EL was given as a continuous infusion for 2 h. This dose schedule has previously been shown to cause acute nephrotoxicity in rats [12]. To establish the effect of a pure fat emulsion on the GFR, a fourth group of six rats was later added. They were given 0.67 ml fat emulsion/100 g body weight as a bolus followed by 0.67 ml/h per 100 g body weight as a continuous infusion for 2 h. These volumes corresponded to the volumes given when the fat emulsion was used as a vehicle for CyA. Six clearance periods of 20 min each were obtained during the continuous infusion.

The blood samples were centrifuged and 5 μl of plasma was blown into 3 ml of the liquid scintillation solution (Scintilator 299 TM, Packard, USA). Then, the radioactivity of 3 H-inulin was determined in a liquid scintillation counter (Beta-Rack, LKB, Stockholm, Sweden). All plasma samples were taken in duplicate.

The urine vials were weighed and centrifuged. At each sampling time, 1 μl of urine was taken in duplicate for the determination of radioactivity.

The radioactive samples were correlated to a quenching calibration curve. The clearance rate of inulin was calculated using standard formulas. The GFR response was expressed as delta GFR area above (+) or below (−) the preinfusion level (mean of three clearance periods).

Statistical analyses

Values are expressed as the arithmetic mean ± SEM. The differences between the baseline values in the four study groups were analyzed by ANOVA, followed by the unpaired t-test, as were the differences in GFR (delta GFR area) in response to the infusions of the test substances. Changes from the basal values in the four groups were analyzed by ANOVA (repeat measurements), followed by a paired t-test. The Bonferroni procedure was applied to adjust for repeated comparisons. A P value less than 0.05 was accepted as statistically significant.

Results

As shown in Fig. 1, a stable GFR was observed during the basal period in all four study groups. Calculated for all rats, the coefficient of variation for the three basal GFR determinations was 9.3% (± 1.4%). In rats given CyA in fat emulsion carrier (group 1), the GFR remained unchanged during the whole experiment. In rats given conventional Sandimmun infusion substance (group 2), a significant ($P < 0.05$) reduction in GFR was observed. From a basal level of 1.04 (± 0.08) ml/min per 100 g body weight (mean of the three baseline determinations), GFR decreased to a nadir of 0.62 (± 0.07) ml/min per 100 g body weight during the first clearance period after the CyA bolus dose and it remained impaired throughout the 2-h study period. Rats receiving Cremophore EL (group 3) also reduced their GFR significantly ($P < 0.05$). From a baseline level of 1.08 (± 0.04) ml/min per 100 g body weight, it decreased to 0.80 (± 0.03) ml/min per 100 g body weight at 30 min and remained at approximately this level throughout the study. In rats receiving pure fat emulsion (group 4), a tendency to an increased, though not sig-

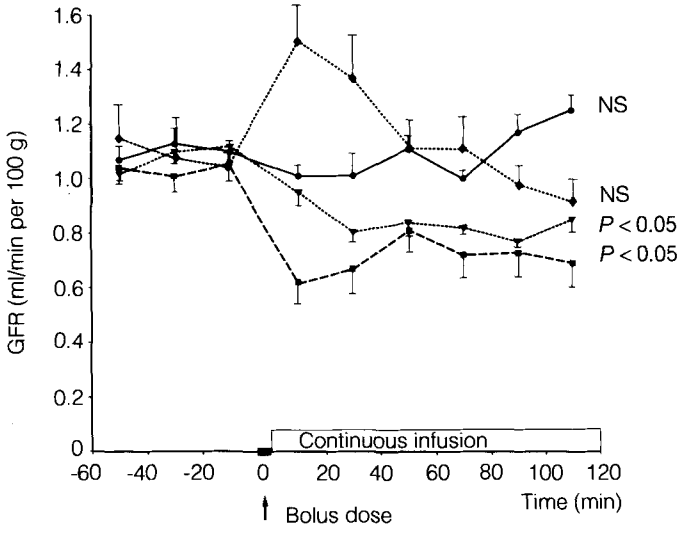

Fig. 1. Glomerular filtration rate (mean ± SE) before and during infusion of the various test substances. The GFR decreased significantly in the animals given CyA in Cremophore EL (■---■) or Cremophore EL only (▼---▼), as compared to the basal period, but did not change significantly in those given CyA in fat emulsion (●---●) or the pure fat emulsion (◆---◆)

Table 1. Glomerular filtration rate (GFR) during infusion of the test substances expressed as delta GFR area. The GFRs were significantly lower in animals given CyA in Cremophore EL or Cremophore EL only than in those given CyA in fat emulsion or the pure fat emulsion. The delta GFR areas in groups 2 and 3, but not in groups 1 and 4, were significantly different from zero

Group	Infused substance	No. of animals	Basal GFR (ml/min)	Δ-GFR area (ml)
1	CyA in fat emulsion	6	1.1 ± 0.05	−3.8 ± 3.4[a]
2	CyA in Cremophore EL (= Sandimmun)	6	1.0 ± 0.05	−34.2 ± 4.2[c]
3	Cremophore EL	7	1.1 ± 0.04	−26.1 ± 5.0[c]
4	Fat emulsion	6	1.1 ± 0.07	5.6 ± 10.1[b]

[a] $P < 0.001$ and $P < 0.02$ compared with groups 2 and 3, respectively
[b] $P < 0.05$ compared with groups 2 and 3
[c] Significantly different from zero ($P < 0.001$)

nificant, GFR was observed during the initial two clearance periods following the bolus injection, after which GFR returned to the preinfusion level and remained constant until 120 min.

Prior to the infusion of test substances, the GFR was almost identical in the four groups. However, during the infusion of test substances, the course of GFR varied significantly between the groups (Table 1). The GFR was significantly lower in rats given Sandimmun infusion substance and in those given Cremophore EL than it was in rats given CyA in the fat emulsion carrier ($P < 0.001$ and $P < 0.02$, respectively). The decrease in GFR in these two groups was also significant ($P < 0.05$ for both comparisons) compared to the GFR in the group given pure fat emulsion. When comparing rats given CyA in fat emulsion with rats given pure fat emulsion, the GFR did not differ significantly.

Discussion

CyA in our new fat emulsion carrier did not affect GFR, while Sandimmun and Cremophore EL reduced GFR to approximately 70% and 75%, respectively, of the baseline level. These results indicate that the acute nephrotoxicity caused by intravenously administered CyA may, to some extent, depend on the vehicle used.

During the two initial clearance periods following bolus injection of the pure fat emulsion, there was a transient rise in GFR (although not statistically significant), after which GFR returned to and remained at the baseline level. One can, therefore, speculate that CyA lowered GFR when it was given in the new fat emulsion carrier, which could have been counterbalanced by an opposing effect of the fat emulsion itself. However, the tendency to an increased GFR following the injection of the pure fat emulsion was of short duration. In contrast, the GFR remained stable at preinfusion levels in animals given CyA in the new fat emulsion carrier, whereas in animals given CyA in Cremophore EL, the GFR remained low at approximately 70% of the basal level throughout the 120-min observation period. It is therefore unlikely that the nontoxic effect of CyA on GFR, when given in the fat emulsion carrier, was due to an effect of the fat emulsion itself on GFR.

An important role of the vehicle is further suggested by our observation that the infusion of Cremophore EL alone led to a marked reduction in GFR. Our results are in accordance with findings by Thiel et al. [12] who, in a rat model, demonstrated that the GFR (measured by creatinine clearance) was impaired to the same extent by the administration of Cremophore EL alone as by the administration of conventional Sandimmun infusion substance (containing both Cremophore EL and CyA).

In humans, the doses of CyA necessary for adequate immunosuppression frequently cause nephrotoxic side effects. In rats, the therapeutic window is wider and, to explore nephrotoxicity, doses much higher than those used in humans have to be administered. As a consequence, the amount of Cremophore EL given in the Sandimmun infusion substance was increased to a corresponding extent. Rat studies therefore do not necessarily reflect the role that Cremophore EL plays in acute nephrotoxicity in humans. However, in a study by Luke et al. [10] on the isolated, perfused rat kidney, a marked deterioration in kidney function was noted with a Cremophore EL concentration of 703 mg/l, a concentration that may be attained in humans by intravenous CyA administration. On the other hand, Weir et al. [14] found no change in renal hemodynamics when Cremophore EL was given intravenously to healthy volunteers. This, however, does not exclude the possibility that Cremophore EL acts synergistically with CyA to induce renal impairment.

Luke et al. [10] have also demonstrated that, in the isolated, perfused rat kidney, the administration of Sandimmun infusion substance causes a 63% decrease in GFR (measured by inulin clearance), while CyA delivered by nontoxic liposomes has no negative effect, despite the same accumulation of CyA in the kidney. Górecki et al. recently published a study showing that the liposomal incorporation of CyA reduced the side effects and increased graft survival in a mouse model [4]. These findings resemble the results achieved in humans by administering amphotericin B in a new vehicle. As mentioned earlier, the use of a new liposomal preparation of amphotericin B prevented the previously observed renal side effects of the drug [13].

Pharmaceutical preparations of lipid-soluble drugs for intravenous administration normally require the use of solvents or detergent systems to solubilize the drugs. To avoid the negative effects of these systems, liposomes or fat emulsions have been used as alternative vehicles. They both contain vesicles with a shell of phospholipids. In fat emulsions the particles contain fat; in liposomes, they contain water.

In liposomes, lipid-soluble drugs are solubilized in the hydrophobic regions of the bilayer structures. In fat emulsions, the lipid-soluble drug can occur in the fat drop inside the phospholipid shell, but it may also be solubilized in the hydrophobic core of the emulsion droplets. Fat emulsions have a potential advantage over liposomes in that they are easy to manufacture and have a good long-term stability.

It is possible that the use of certain liposomes or fat emulsions as carriers, in addition to excluding a harmful

vehicle, in itself somehow presents the drug in a more favorable way. The carrier may also influence the tissue or cellular distribution of a drug. A decreased concentration of CyA in the kidney, or in some parts of the kidney, should also reduce the renal side effects. Preliminary tissue distribution studies indicate no differences in CyA concentration in renal tissue or blood when comparing Sandimmun infusion substance and CyA in our fat emulsion carrier.

In addition to its potentially nephrotoxic effects, Cremophore EL is considered to cause the anaphylactic reactions noted during the infusion of Sandimmun [6]. Fat embolism caused by Cremophore EL has been proposed as the cause of side effects in the central nervous system [5]. Fat emulsions of the type used in our new formulation may also prove beneficial in these respects. Such fat emulsions have been extensively evaluated in research on parenteral nutrition, and side effects in the doses used here are extremely rare.

In summary, CyA was dissolved in a nontoxic fat emulsion carrier. When tested in a rat model, this new formulation was found not to cause the acute impairment of renal function produced by Sandimmun infusion substance and by Cremophore EL. We propose that the use of a fat emulsion carrier is a promising way of reducing the acute, nephrotoxic side effects of intravenously administered CyA. Further studies are needed to ensure that the immunosuppressive potential is preserved in this new formulation.

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