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Acute effects of different immunosuppressive drugs on pancreatic, islet, renal, and arterial hepatic blood flow in anesthetized rats

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Abstract The effects of four different immunosuppressive drugs on organ blood flow were investigated. Sprague-Dawley rats were injected intravenously with 0.2 ml of either 15-deoxyspergualin (DSG; 5 mg/kg body weight), RS 61443 (80 mg/kg body weight), FK 506 (0.5 mg/kg body weight), cyclosporin A (9.5 mg/kg body weight), or the vehicles used. At 15 or 60 min after injection of the drugs, the blood perfusion of the whole pancreas, the pancreatic islets, and the kidneys, as well as the arterial blood flow to the liver, were measured in anesthetized animals using a microsphere technique. Fifteen minutes after administration, both FK 506 and DSG decreased the fraction of whole pancreatic blood flow diverted through the islets. FK 506 and cyclosporin A reduced renal blood flow, but only 60 min after injection of the drug. None of the drugs influenced hepatic blood flow. RS 61443 did not affect the blood flow of the organ systems investigated. These differences in the effects of the drugs tested on blood flow might have some important implications on their efficacy and side effects. Thus, in view of its lack of influence on organ blood flow, RS 61443 seems to be preferable, at least when compared with cyclosporin A and FK 506 in the context of organ transplantation.

Key words Cyclosporin A, pancreatic blood flow, rat · RS 61443, pancreatic blood flow, rat Deoxyspergualin, pancreatic blood flow, rat · FK 506, pancreatic blood

Introduction

Recent advances within the field of organ transplantation have largely been due to the development of potent and selective immunosuppressive drugs such as cyclosporin A (CyA). However, a major drawback associated with continuous CyA treatment is the nephrotoxicity of the drug, which may affect not only transplanted kidneys but also previously healthy kidneys in patients receiving other organ grafts [9]. This nephrotoxicity may partially be explained by a direct vasoconstrictive effect on renal blood vessels [12, 16, 18]. Therefore, new potent and safer immunosuppressive drugs are presently being sought. One such drug is the macrolide immunosuppressant FK 506, a drug with a mode of action similar to that of CyA [11]. However, there are recent reports that indicate this drug may also be nephrotoxic [1, 10], possibly as a consequence of decreased renal blood flow [10].

In view of this coupling between blood flow effects and side effects of immunosuppressants on organ function, we investigated the acute effects of four different immunosuppressive drugs on organ blood flow in rats. Since several of these drugs also have adverse effects on the endocrine pancreas [2, 17, 19, 20], we deemed it of particular interest to study islet and whole pancreatic blood perfusion. In view of the high frequencies of renal and hepatic complications caused by immunosuppressive drugs, we also measured renal and arterial hepatic blood flow after acute administration of CyA, FK 506, 15-deoxyspergualin (DSG), or RS 61443.

Materials and methods

Animals

Male Sprague-Dawley rats ($n = 112$) weighing 346 ±5 g were obtained from a local breeding colony at the Biomedical Centre, Uppsala, Sweden. The animals had free access to tap water and pelleted food (Type R34; AnalyCen, Lidköping, Sweden) at all time points throughout the experiments.

Drugs and chemicals

Cyclosporin A (CyA; Sandimmun) was purchased from Sandoz (Täby, Sweden) and Cremophor EL from Sigma Chemical (St Louis, Mo., USA). FK 506 was a gift from Fujisawa Pharmaceutical (Osaka, Japan), 15-deoxyspergualin (DSG) from Research Laboratories of Behringwerke (Marburg, Germany), and RS 61443 from the Institute of Clinical Medicine, Syntex Research (Palo Alto, Calif., USA).

Administration of drugs

The animals were randomly chosen to be injected in a tail vein with 0.2 ml of one of the various vehicles – 0.9 % (w/v) saline, 1 M NaOH neutralized to pH 7.4 with concentrated HCl, or 22 % (w/v) Cremophor EL (CEL) – or with one of the drugs to be tested: DSG (5 mg/kg body weight, dissolved in saline), RS 61443 (80 mg/kg body weight, dissolved in the NaOH-solution just referred to), FK 506 (0.5 mg/kg body weight, suspended in saline), or CyA (9.5 mg/kg body weight, dissolved in CEL). All blood flow measurements were performed 15 or 60 min after the injection.

Blood flow measurements

The animals were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg body weight; Mebumal vet; NordVacc, Stockholm, Sweden) either before (15-min group) or after (60-min group) administration of one of the drugs and then placed on a heated operating table. Arterial catheters were inserted into the ascending aorta via the right carotid artery, and into the abdominal aorta via the left femoral artery. The first of these catheters was connected to a pressure transducer (PDCR 75/1; Druck, Groby, UK), and throughout the rest of the experiments the mean arterial blood pressure was continuously recorded. Fifteen or 60 min after the initial administration of one of the immunosuppressive drugs, approximately 1.5×10^5 nonradioactive microspheres (NEN-Trac; DuPont Pharmaceuticals, Wilmington, Del., USA), with a diameter of 11 μm, were injected into the catheter with its tip in the ascending aorta. Simultaneously, an arterial reference sample was withdrawn from the catheter in the femoral artery for 60 s (withdrawal rate 0.50 ± 0.03 ml/min; $n = 111$). After obtaining the reference sample, approximately 250 μl of arterial blood was removed, centrifuged, and subsequently stored frozen (–18 °C) as serum samples. The animals were then sacrificed and the whole pancreas, the adrenal glands, and approximately 150 mg each of the left kidney and the liver were removed, blotted, and weighed. The part of the kidney chosen for further processing was taken as a slice approximately through the middle of the organ, containing both cortical and medullary tissues. A peripheral part of the lower lobe of the liver was removed.

The number of microspheres present in the reference blood sample, the whole pancreas, the islets, the adrenal glands, the kidney, and the liver was then determined using a freeze-thawing technique, as previously described in detail [6]. The blood flow values of each of these organs were calculated according to the formula $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$, where Q_{org} is organ blood flow (ml/min). Q_{ref} is withdrawal rate of the reference sample (ml/min), N_{org} is number of microspheres present in the organ, and N_{ref} the number of microspheres in the reference sample.

The number of microspheres in the right and left adrenal glands, respectively, was compared in each individual animal. A difference of less than 10 % was taken to indicate that a sufficient mixing of the microspheres had taken place in the arterial circulation.

Blood glucose and serum insulin concentrations

The blood samples were analyzed for their contents of glucose with an ExacTech blood glucose meter (Baxter Travenol Laboratories, Deerfield, Ill., USA) and for insulin with radioimmunoassay [4].

Statistical calculations

All values are given as means ± SEM. Probabilities (P) of chance differences between the experimental groups were compared with a single factor factorial ANOVA in conjunction with Fischer's least significant difference test using the StatView (version 4.0) software package from Abacus Concepts and BrainPower (Calabasas, Calif., USA).

Results

A total of 22 % of the animals were excluded from the study because of anesthetic or surgical failures, or because microsphere content of their adrenal glands differed by more than 10 %. The substances injected affected neither the blood glucose and serum insulin concentrations nor the mean arterial blood pressure (data not shown).

Whole pancreatic blood flow was not affected by any of the test substances (Tables 1 and 2). However, 15 min after administration of FK 506, islet blood flow in itself was decreased (Table 1), whereas no such effect could be discerned after 60 min (Table 2). In general, fractional islet blood flow constituted 6 %–12 % of the whole pancreatic blood flow. Both FK 506 and DSG decreased the fractional islet blood flow compared with saline 15 min after administration (Table 1). Likewise, CEL, the CyA vehicle, caused a reduction in the fractional islet blood flow (Table 1). This was, however, not observed when CyA dissolved in CEL was injected. The decrements had disappeared when the blood flow measurements were performed 60 min after drug administration (Table 2).

Renal blood flow was decreased 60 min after administration of FK 506 or CyA, whereas the other drugs caused no such effect at either 15 min (data not shown) or 60 min (Table 3) after drug administration. Interestingly, there was a clear tendency for CEL itself to decrease the renal blood flow, but this difference did not attain statis-

Table 1 Whole pancreatic blood flow (PBF), islet blood flow (IBF), and islet blood flow expressed as a fraction of whole pancreatic blood flow (fIBF) in Sprague-Dawley rats 15 min after an intravenous injection of 0.2 ml of either saline, 15-deoxyspergualin (DSG), FK 506, Cremophor EL dissolved in distilled water (vehicle for cyclosporin A), cyclosporin A, RS vehicle (vehicle for RS 61443), or RS 61443. All values represent means ± SEM. * $P < 0.05$ and ** $P < 0.01$ when compared with the corresponding vehicle-treated rats; *** $P < 0.05$ when compared with the saline-injected rats

Substance	n[a]	PBF (ml/min × g)	IBF (μl/min × g)	fIBF (% of RBF)
Saline (0.9 %)	6	0.40 ± 0.05	48 ± 9	11.8 ± 1.4
DSG (5 mg/kg body weight)	7	0.40 ± 0.03	29 ± 3	7.5 ± 1.0*
FK 506 (0.5 mg/kg body weight)	5	0.34 ± 0.04	21 ± 5*	5.9 ± 0.7**
Cremophor EL (22 % w/v)	7	0.49 ± 0.09	39 ± 8	8.0 ± 0.9***
Cyclosporin A (9.5 mg/kg body weight)	8	0.44 ± 0.05	52 ± 7	11.8 ± 0.8*
RS vehicle (1 M NaOH neutralized to pH 7.4 with concentrated HCl)	7	0.44 ± 0.06	40 ± 9	8.8 ± 1.2
RS 61443 (80 mg/kg body weight)	8	0.39 ± 0.04	44 ± 7	11.8 ± 1.6

[a] Number of experiments within each group

Table 2 Whole pancreatic blood flow (PBF), islet blood flow (IBF), and islet blood flow expressed as a fraction of whole pancreatic blood flow (fIBF) in adult Sprague-Dawley rats 60 min after an intravenous injection of 0.2 ml of either saline, 15-deoxyspergualin (DSG), FK 506, Cremophor EL dissolved in distilled water (vehicle for cyclosporin A), cyclosporin A, RS vehicle (vehicle for RS 61443), or RS 61443. All values represent means ± SEM

Substance	n[a]	PBF (ml/min × g)	IBF (μl/min × g)	fIBF (% of PBF)
Saline (0.9 %)	7	0.40 ± 0.06	35 ± 6	8.8 ± 1.4
DSG (5 mg/kg body weight)	8	0.47 ± 0.07	39 ± 4	9.1 ± 1.1
FK 506 (0.5 mg/kg body weight)	10	0.28 ± 0.04	29 ± 4	11.5 ± 1.3
Cremophor EL (22 % w/v)	8	0.52 ± 0.09	37 ± 8	7.2 ± 0.8
Cyclosporin A (9.5 mg/kg body weight)	8	0.53 ± 0.08	38 ± 8	7.4 ± 1.3
RS vehicle (1 M NaOH neutralized to pH 7.4 with concentrated HCl)	7	0.55 ± 0.12	39 ± 10	7.0 ± 0.5
RS 61443 (80 mg/kg body weight)	8	0.42 ± 0.10	30 ± 6	7.3 ± 0.5

[a] Number of experiments within each group

tical significance ($P < 0.087$). The arterial hepatic blood flow was similar in all experimental groups at both time points investigated (data not shown and Table 3).

Discussion

Side effects caused by immunosuppressive treatment after organ transplantation are still a major problem. These effects can partially be explained by vasoconstrictive effects of the drugs [10, 12, 16, 18]. In the present study we examined the circulatory effects of FK 506, DSG, and RS 61443, all of which represent a new generation of immunosuppressive drugs, and compared them with CyA, presently the most widely used immunosuppressive drug in organ transplantation [9, 11, 15, 23]. The findings in this study confirm previous reports of a decreased renal blood perfusion after administration of CyA or FK 506 [12, 16, 18]. In contrast, however, neither DSG nor RS 61443 influenced the renal blood perfusion. It has previously been suggested that CyA-induced nephrotoxicity may be explained, at least partially, by vasoconstriction, which is probably mediated by an effect on calcium channels [13]. A similar mechanism has recently been suggested for the nephrotoxicity of FK 506 [10]. It is unclear to what extent the blood flow decrease seen after CyA administration is due to the effects of CEL, a derivative of castor oil and ethylene oxide. Our finding that FK 506, a drug with a mode of action similar to that of CyA but not dissolved in CEL, also induced a decrease in renal blood flow [10] suggests that the CyA molecule itself has detrimental effects in this context.

Pancreatic blood flow was not affected by any of the drugs or vehicles investigated in the present experiments. With regard to CyA, there are some reports that indicate an unchanged blood flow [7], others that indicate a decreased pancreatic blood flow, as manifested by an increased flow resistance [8], and still others that indicate an increased pancreatic blood flow [22]. Jennings and Corry [8] used single-dose administration to anesthetized rats and looked at earlier time points after administration. Youngelman et al. [22] administered CyA perorally to sheep for several weeks, whereas in our previous study [7] CyA was given perorally to pancreas-

Table 3 Renal blood flow (RBF) and arterial liver blood flow (LBF) in adult Sprague-Dawley rats 60 min after an intravenous injection of 0.2 ml of either saline, 15-deoxyspergualin (DSG), FK 506, Cremophor EL dissolved in distilled water (vehicle for cyclosporin A), cyclosporin A, RS vehicle (vehicle for RS 61443), or RS 61443). All values represent means ± SEM. * $P < 0.05$ when compared with the corresponding vehicle-treated rats; ** $P < 0.05$ when compared with the saline-injected rats

Substance	n[a]	RBF (ml/min × g)	LBF (ml/min × g)
Saline (0.9 %)	7	4.78 ± 0.87	0.18 ± 0.04
DSG (5 mg/kg body weight)	8	4.16 ± 0.70	0.16 ± 0.02
FK 506 (0.5 mg/kg body weight)	10	2.77 ± 0.41*	0.16 ± 0.04
Cremophor EL (22 % w/v)	8	3.02 ± 0.46	0.16 ± 0.03
Cyclosporin A (9.5 mg/kg body weight)	8	2.61 ± 0.45**	0.13 ± 0.03
RS vehicle (1 M NaOH neutralized to pH 7.4 with concentrated HCl)	7	4.49 ± 0.80	0.22 ± 0.05
RS 61443 (80 mg/kg body weight)	8	4.30 ± 0.86	0.17 ± 0.03

[a] Number of experiments within each group

transplanted rats for 14 days. The present findings of an unchanged pancreatic blood flow 60 min after administration of CyA confirm the results of our previous study [7], as well as the findings of Jennings and Corry [8]. The latter group, however, observed an initial increase in vascular resistance that we did not notice in the present study.

In terms of islet transplantation it is worth noting that CyA has been reported to produce severe degranulation and hydropic degeneration of islet β cells in rats [5]. It should be noted that rats given FK 506 had slightly, although not significantly, lower pancreatic blood flow values at both time points studied. Both FK 506 and DSG also reduced fractional islet blood flow 15 min after administration. Studies performed by Doi et al. [3] suggest that FK 506, even at low doses, induces a functional impairment of the exocrine rat pancreas. In addition, other investigators have reported increased blood glucose levels in baboons and rats given FK 506 [14, 21]. RS 61443 caused no discernible effects on pancreatic blood flow 15 or 60 min after administration and, thus, seems to be inert with respect to circulation.

Arterial liver blood flow was unaffected by all substances investigated. However, this finding should be interpreted with caution. This is because the microsphere technique measures only the flow through the hepatic artery and not that through the portal vein, which usually contributes 70 % or more of the total liver blood flow. Likewise, none of the substances examined in this study caused any significant changes in mean arterial blood pressure, blood glucose, or serum insulin concentrations. CyA has been reported to cause impairments in carbohydrate metabolism, but this was seen after prolonged exposure to the drug and not, as in the present study, after acute administration [9].

Thus, considering the connection between the effects of immunosuppressive drugs on organ blood flow and the adverse effects, DSG, despite is influence on the pancreatic islet blood flow, and RS 61443 seem to be preferable to CyA and FK 506, both of which affect the renal blood flow.

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