Importance of endogenous prostaglandins for the toxicity of cyclosporin A to rat endocrine and exocrine pancreas?

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Abstract

Previous work has shown that cyclosporin A is toxic to the endocrine and exocrine pancreas. The aim of this study was to examine whether endogenous eicosanoids play a role in controlling cyclosporin A induced toxicity. Rats were treated for eight days with indomethacin (2 mg/kg, twice daily) in addition to cyclosporin A (5 or 10 mg/kg daily). Effects of drug treatments on exocrine (as assessed by amylase and protein secretion into the pancreatic juice) and endocrine (as assessed by the glucose dependent insulin release) pancreatic functions, and pancreatic formation of prostaglandins and thromboxane were evaluated. Treatment with cyclosporin A in the doses used did not inhibit eicosanoid formation by the pancreatic tissue *ex vivo*. Indomethacin caused significant inhibition of pancreatic formation of prostaglandin E₂, 6k prostaglandin F_{1α} and thromboxane B₂. Combined treatment with indomethacin and cyclosporin A (5 or 10 mg/kg) augmented cyclosporin A induced pancreatic toxicity with further impairment of insulin release, amylase secretion, and pancreatic juice protein content, but did not result in more pronounced inhibition of pancreatic eicosanoid formation. The increased toxicity of the combined treatment was, however, associated with raised cyclosporin A whole blood concentrations. The data suggest that the potentiation of pancreatic toxicity of cyclosporin A observed during coadministration of indomethacin is not the result of suppression of endogenous pancreatic eicosanoid biosynthesis, but more likely results from altered cyclosporin A pharmacokinetic which may be caused by an interference of indomethacin with the hepatic cytochrome P-450 dependent monooxygenase involved in cyclosporin A metabolism. The possibility that coadministration of non-steroidal antiinflammatory drugs aggravates toxic effects in cyclosporin A treated patients should be considered.

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Cyclosporin A is a unique cyclic undecapeptide of fungal origin with potent immunosuppressive properties used to prevent the rejection of transplanted organs and to treat autoimmune diseases.¹ This drug has a narrow therapeutic window between inadequate immunosuppression and toxicity.² The best known clinical side effects of cyclosporin A are nephrotoxicity and hepatotoxicity.^{3,4} We have recently shown in rat pancreas that adverse effects include pancreatic

toxicity.⁵ Chronical application of cyclosporin A in immunosuppressive doses caused dose dependent impairment of endocrine and exocrine pancreatic functions. In addition, it has been shown that cyclosporin A induced toxicity in rats corresponds with severe degranulation and hydropic degeneration of islet B-cells⁶ and a decrease in pancreatic insulin content and release.⁷ Concomitant administration of prostaglandins partially alleviated cyclosporin A induced nephrotoxicity.^{8,9} In addition we have recently shown that in rats treatment with a synthetic prostaglandin E₁ analogue (Rioprostil) protects the pancreas against cyclosporin A induced damage.¹⁰

This study was designed to investigate the role of endogenous eicosanoids in the sensitivity of endocrine and exocrine pancreatic cells against the toxic effects of cyclosporin A. Depletion of pancreatic eicosanoids was achieved by use of the cyclooxygenase inhibitor, indomethacin.

Methods

ANIMALS

Male Wistar rats (mean body weight 300 g), housed under conditions of constant temperature and a 12 hour lighting cycle, were used for the study. They were allowed free access to food (standard diet purchased from Altromin, Germany) and water. Before operation, the rats were fasted for 18 hours with free access to water and then anaesthetised with pentobarbital (60 mg/kg, intraperitoneally).

MEDICATION

Groups of 10-12 rats received the following drug treatments: (i) 5 mg/kg or 10 mg/kg cyclosporin A intragastrically once daily; (ii) 2 mg/kg indomethacin subcutaneously twice daily; (iii) 2 mg/kg indomethacin subcutaneously twice daily in addition to 5 or 10 mg/kg cyclosporin A intragastrically once daily.

Indomethacin (Merck, Sharp and Dohme, Germany) was suspended in saline. Cyclosporin A (Sandimmun®; Sandoz, Switzerland) in pure olive oil was administered intragastrically using a metal feeding tube. Controls were treated with the corresponding vehicles (saline subcutaneously and/or olive oil intragastrically). Drugs or vehicles were administered for eight days. Pancreatic perfusion experiments and incubation experiments to determine pancreatic eicosanoids formation were performed on day 9. In cyclosporin A treated rats whole blood concentrations of cyclosporin A were determined 24

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hours after the last dose applied of cyclosporin A. Pancreatic eicosanoid formation was determined about 18 hours after the last administration of cyclosporin A and four hours after the last dose of indomethacin.

OPERATION

The isolated arterially perfused rat pancreas model was used to evaluate effects on exocrine and endocrine pancreatic functions. Briefly, the perfused preparation consisted of pancreas with a small remnant of duodenum according to the method as described elsewhere.¹¹ The pancreas was perfused in situ through the superior mesenteric artery and the coeliac trunc at a constant flow of 4 ml/minute without recirculation. The vascular effluent was collected in one minute fractions through the portal vein. The exocrine secretions were collected in 10 minute fractions through the cannulated pancreatic duct.

PERFUSATES AND TEST SOLUTIONS

The perfusate consisted of Krebs-Ringer bicarbonate buffer containing 0.2% bovine serum albumin (Serva, Germany), 3% dextran T 70 (Pharmacia, Germany), 7.9 or 15.8 mmol/l glucose, gassed with 95% O₂-5% CO₂ to achieve a final pH of 7.4. The perfusion rate was 4 ml/minute with a perfusion pressure between 50 and 60 cm H₂O.

The preparation was equilibrated by perfusion for 15 minutes with a buffer containing 7.9 mmol/l glucose. After the equilibration period the perfusion medium was changed to buffer containing 15.8 mmol/l glucose and sampling started for a further 30 minute period. Synthetic cholecystokinin-8 (100 pg/ml final concentration; Sigma, Germany) was additionally infused over the last 20 minutes of the sampling period at a constant flow of 0.2 ml/minute to stimulate the exocrine pancreas. Infusion of cholecystokinin-8 did not change the perfusion pressure.

ASSESSMENT OF PANCREATIC EICOSANOIDS FORMATION

The pancreata were carefully removed and cut into fragments of 2-5 mg wet weight. The fragments were blotted, weight and tissue aliquots of 100 mg were immediately incubated in 1.0 ml of oxygenated Tyrode solution at 37°C for 10 minutes. Release of prostaglandin E₂, 6k prostaglandin F₁α, and thromboxane B₂ into the incubation media was determined radioimmunologically as described previously.¹² Validation experiments have ascertained that standard prostanoids added to aliquots of pancreatic tissue media are recovered quantitatively. Furthermore, thin layer chromatography has shown that the immunoreactivity released into pancreatic tissue incubation media co-chromatographs exclusively with the corresponding standard prostanoids.

ANALYSES

Insulin concentrations in the portal vein effluent

were determined by a specific radioimmunoassay as described elsewhere.¹¹ Pancreatic juice volume was sampled in 10 minute collection periods and the total amount collected over a 10 minute period was defined as pancreatic volume output. Amylase secretion into the pancreatic juice was measured according to the method of Street and Close,¹³ protein content of the pancreatic juice according to the method of Lowry *et al.*¹⁴ Whole blood concentrations of cyclosporin A were measured with the TDx fluorescent polarisation immunoassay (Abbott Diagnostics, Germany) according to the method of Schroeder *et al.*¹⁵ Enzyme determinations were done immediately after the end of the experiments. Radioimmunoassays of insulin and cyclosporin A were performed after storage of the samples at -20°C within two weeks after the experiments were done.

STATISTICAL ANALYSIS

Results are expressed as mean (SEM). Comparisons between different groups of data were made using Student's *t* test for unpaired data and differences with *p* values <0.05 were considered significant.

Results

EFFECTS OF CYCLOSPORIN A AND/OR INDOMETHACIN ON GLUCOSE DEPENDENT INSULIN RELEASE

Treatment with cyclosporin A (5 or 10 mg/kg) alone significantly reduced (*p*<0.05 and *p*<0.001, resp) insulin release. Treatment with indomethacin (2 mg/kg) had no significant influence on insulin release. Combined administration of cyclosporin A and indomethacin caused a further significant decrease of insulin release as compared with cyclosporin A treated rats only (cyclosporin A 5 mg/kg 1091 (187) μU *v* cyclosporin A 5 mg/kg+indomethacin 490 (70) μU, *p*<0.01; cyclosporin A 10 mg/kg 447 (44) μU *v* cyclosporin A 10 mg/kg+indomethacin 222 (60) μU, *p*<0.01; Fig 1).

EFFECTS OF CYCLOSPORIN A AND/OR INDOMETHACIN ON CHOLECYSTOKININ-8 STIMULATED AMYLASE SECRETION

Amylase secretion was not clearly affected after treatment with 5 mg/kg cyclosporin A alone. 10 mg/kg cyclosporin A alone significantly (*p*<0.05) reduced amylase secretion. While indomethacin alone did not affect amylase secretion, together with cyclosporin A it caused significant (*p*<0.01 *v* controls) inhibition of enzyme secretion (Fig 2).

EFFECTS OF CYCLOSPORIN A AND/OR INDOMETHACIN ON CHOLECYSTOKININ-8 STIMULATED PROTEIN CONTENT OF THE EXOCRINE JUICE

Pancreatic juice protein content was not reduced after treatment with 5 mg/kg cyclosporin A, but was significantly reduced after 10 mg/kg cyclosporin A (*p*<0.001). Indomethacin given alone

Figure 1: Effect of 5 and 10 mg/kg cyclosporin A and/or 2 mg/kg indomethacin on insulin release during isolated arterial perfusion of the pancreas with 15.8 mM glucose (mean (SEM); $n=10-12$ per group): Significant decrease of insulin release at 5 and 10 mg/kg cyclosporin A ($p<0.05$ and $p<0.001$), and more severe impairment after additional treatment with indomethacin ($p<0.01$ v each other; $p<0.001$ v controls). Indo=indomethacin, CyA=cyclosporin A.

had no significant effect. Cyclosporin A applied together with indomethacin significantly decreased pancreatic juice protein content with both doses used (5 mg/kg cyclosporin A + indomethacin $p<0.05$; 10 mg/kg cyclosporin A + indomethacin $p<0.001$; p v controls; Fig 3).

EFFECTS OF CYCLOSPORIN A AND/OR INDOMETHACIN ON THE EXOCRINE PANCREATIC VOLUME OUTPUT

Both doses of cyclosporin A did not diminish the volume output (control 29.6 (5.6) μ l, 5 mg/kg cyclosporin A 26.6 (5.9) μ l, and 10 mg/kg cyclosporin A 27.0 (5.3) μ l). Indomethacin alone caused a significant reduction of the volume output (18.6 (2.2) μ l, $p<0.05$). Indomethacin plus 5 or 10 mg/kg cyclosporin A tended to further decrease volume output (5 mg/kg cyclosporin A + indomethacin 14.0 (3.1) μ l, $p<0.01$ v controls; 10 mg/kg cyclosporin A + indomethacin 12.2 (2.2) μ l, $p<0.01$ v controls). Differences between indomethacin treated and indomethacin + cyclosporin A treated rats, however, did not reach statistical significance.

Figure 2: Effect of 5 and 10 mg/kg cyclosporin A and/or 2 mg/kg indomethacin on cholecystokinin-8 stimulated (100 pg/ml) amylase secretion in the presence of 15.8 mM glucose (mean (SEM); $n=10-12$ per group): Significant decrease of amylase secretion in rats treated with 10 mg/kg cyclosporin A alone ($p<0.05$) and at both doses of cyclosporin A after additional treatment with indomethacin ($p<0.01$ v controls). CCK-8=cholecystokinin-8.

Figure 3: Total protein content in pancreatic juice during stimulation with 100 pg/ml cholecystokinin-8 in the presence of 15.8 mM glucose in rats treated with 5 and 10 mg/kg cyclosporin A and/or 2 mg/kg indomethacin (mean (SEM); $n=10-12$ per group): Significant decrease of protein content at 10 mg/kg cyclosporin A ($p<0.05$) and at both doses of cyclosporin A after additional treatment with indomethacin ($p<0.001$ v controls).

EFFECTS OF INDOMETHACIN ON WHOLE BLOOD CONCENTRATIONS OF CYCLOSPORIN A

Treatment with 5 or 10 mg/kg cyclosporin A once daily for eight days resulted in blood concentrations of 401 (88) ng/ml and 1353 (145) ng/ml, resp, 24 hours after the last dose. In rats with combined treatment with indomethacin and cyclosporin A over the same period cyclosporin A blood concentrations were significantly higher than in rats treated with the corresponding dose of cyclosporin A only (Fig 4).

EFFECTS OF CYCLOSPORIN A AND/OR INDOMETHACIN ON SYNTHESIS OF PROSTAGLANDINS AND THROMBOXANE B2 BY PANCREATIC TISSUE EX VIVO

As shown in Figure 5, treatment with cyclosporin A (5 or 10 mg/kg for eight days) did not affect synthesis of prostaglandin E₂, 6k prostaglandin F_{1a}, and thromboxane B₂ by pancreatic tissue ex vivo. Formation of all three eicosanoids was inhibited after treatment with indomethacin (2 mg/kg) for eight days. Inhibition of eicosanoid formation after combined administration of indomethacin and cyclosporin A (5 or 10 mg/kg) was not more pronounced than after indomethacin alone.

Discussion

This study confirms previous reports of our group⁵ and others¹⁶ that immunosuppressive doses of cyclosporin A¹⁷ impair endocrine and exocrine pancreatic functions in rats. While the glucose dependent insulin release was reduced by treatment with 5 and 10 mg/kg cyclosporin A for eight days, the cholecystokinin-8 stimulated amylase secretion was only impaired at the higher dose of 10 mg/kg cyclosporin A. Insulin

Figure 4: Relationship between intragastrically applied 5 and 10 mg/kg cyclosporin A and whole blood concentrations of cyclosporin A determined 24 hours after the last application (measurement with the TDX fluorescent polarisation immunoassay; mean (SEM); n=10–12 per group): Significant increase of cyclosporin A blood concentrations after additional application of cyclosporin A treated rats with 2 mg/kg indomethacin ($p<0.05$ and $p<0.01$, resp, v rats treated with the corresponding cyclosporin A dose only).

promotes the synthesis of amylase by a direct effect on exocrine pancreatic cells via the insulino-acinar axis.¹⁸ Whether the noxious effect of cyclosporin A on amylase secretion is the consequence of reduced insulin release, is not known.

Treatment with indomethacin at a dose of 2 mg/kg twice daily had no influence on pancreatic functions. Indomethacin treatment, however, significantly aggravated the toxic action of cyclosporin A on the endocrine and exocrine pancreas. Reports on the effect of cyclosporin A on prostaglandin synthesis in the kidney are controversial.^{19–21} Most studies have, however, reported that cyclosporin A comparably inhibited renal prostaglandin formation. In con-

trast, treatment of rats with 5 or 10 mg/kg cyclosporin A in our study did not reduce the ex vivo synthesis of prostaglandin E₂, 6k prostaglandin F_{1α}, and thromboxane B₂ by pancreatic tissue. The more pronounced inhibition of formation of thromboxane B₂ as compared with formation of 6k prostaglandin F_{1α} and particularly prostaglandin E₂ may be the result of different cellular sources of the eicosanoids studied. Thus, thromboxane B₂ released during incubation of pancreatic fragments may be derived mainly from trapped platelets which are known to be particularly sensitive to the effect of cyclooxygenase inhibitors.

Although the time interval between the last dose of cyclosporin A and the assessment of pancreatic eicosanoid formation was 18 hours, this cannot explain the lack of inhibitory action of cyclosporin A on pancreatic eicosanoid formation. High concentrations of circulating cyclosporin A have been measured in our study 24 hours after the last dose of cyclosporin A. Furthermore, tissue concentrations of cyclosporin A in the pancreas are still high after such a period of time.⁵ Indomethacin in the dose used significantly reduced eicosanoid formation in the pancreas ex vivo. Combined treatment with indomethacin and cyclosporin A did not further increase the inhibitory action of indomethacin on pancreatic eicosanoid formation. This finding supports the concept that cyclosporin A has no inhibitory action on pancreatic prostaglandin and thromboxane formation under the experimental conditions used.

The discrepancy of our results to the reported inhibitory action of cyclosporin A on renal prostaglandin formation may be caused by an organ selective effect of cyclosporin A or to the higher doses of cyclosporin A – that is, 50 mg/kg, used in studies on renal prostaglandin formation.¹⁷

Figure 5a-c: Effect of 2 mg/kg indomethacin and/or 5 and 10 mg/kg cyclosporin A on pancreatic eicosanoid formation ex vivo (mean (SEM); n=10–12 per group): Significant inhibition with 2 mg/kg indomethacin for prostaglandin E₂, 6k prostaglandin F_{1α}, and thromboxane B₂ ($p<0.05$, <0.01 , and <0.005 , resp, v controls). Both doses of cyclosporin A given alone have no influence (p v controls) on pancreatic eicosanoid formation. Combined treatment with cyclosporin A and indomethacin has no further significant inhibitory action as compared with indomethacin alone on pancreatic eicosanoid formation (p v indomethacin). PG= prostaglandin; TX= thromboxane.

Administration of indomethacin caused no decrease in the glucose induced insulin release in our preparation confirming previous studies.²²⁻²⁴ Investigations in man have shown controversial results. Thus, treatment with cyclooxygenase inhibitors has been reported to augment²⁵⁻²⁷ or to suppress²⁸ glucose induced insulin release. In our investigations, cholecystokinin-8 induced amylase secretion and total protein content of the pancreatic juice were not altered by indomethacin, findings that are in agreement with studies in man²⁹ and dogs.³⁰ We have not studied whether indomethacin affects arterial blood flow. A dose of 5 mg/kg indomethacin has, however, been reported to be without influence on pancreatic arterial blood flow.³¹ It is therefore not likely that the aggravation of cyclosporin A induced pancreatic toxicity by indomethacin is caused by vascular effects. When two potentially toxic agents are coadministered the resultant toxicity may be greater than that observed with either agent when given alone. Treatment with the immunosuppressive agent cyclosporin A is associated with pancreatotoxicity,^{5,10} nephrotoxicity,^{8,32} and hepatotoxicity.³³⁻³⁶ Other agents that alter the degree of cyclosporin A induced toxicity – that is, nephrotoxicity, have been suggested to act by altering circulating cyclosporin A blood concentrations as a consequence of induction or inhibition of cytochrome P-450 dependent (new nomenclature: P-450III_A; 36) hepatic drug metabolism.^{37,38} Both cyclosporin A and indomethacin are known to decrease cytochrome P-450 dependent monooxygenase activity in primary cultures of hepatocytes of rats,^{9,37-40} rabbits, and man,³⁶ although the mechanism whereby cyclosporin A causes the decrease is not known. The hepatic monooxygenase system is supposed to be involved in the detoxication of cyclosporin A.⁹ Conjoint treatment of rats with cyclosporin A and indomethacin was found to be associated with increased whole blood concentrations of cyclosporin A^{33,38} as a consequence of the decreased cyclosporin A metabolism. These reports are in agreement with our findings showing increased cyclosporin A blood concentrations, but not increased inhibition of pancreatic eicosanoid formation after combined treatment with cyclosporin A and indomethacin. In our study cyclosporin A whole blood concentrations were measured with the new cyclosporin A TDx fluorescent polarisation immunoassay which recognises also the cyclosporin A metabolites.¹⁵ This assay has been proved to provide reliable whole blood and serum concentrations that correlate well with monoclonal or polyclonal radioimmunoassay measurements carried out in renal transplant recipients.^{15 41-43}

In conclusion, coadministration of indomethacin aggravated the noxious effects of immunosuppressive doses of cyclosporin A on rat endocrine and exocrine pancreas. This effect does not seem to be the result of inhibition of pancreatic eicosanoid formation as indomethacin alone exhibited no pancreatic toxicity and combined treatment with indomethacin and cyclosporin A did not further augment the inhibitory action of indomethacin on pancreatic eicosanoid formation. The indomethacin induced increase

in cyclosporin A toxicity to the pancreas is associated with raised whole blood concentrations of cyclosporin A possibly resulting from an interference with P-450 dependent hepatic drug metabolism by indomethacin. Thus, the protection conferred by administration of exogenous prostaglandins of the E-series¹⁰ may not be caused by substitution of endogenous prostaglandins, but involves other as yet unknown mechanisms. Finally, the possibility that coadministration of non-steroidal antiinflammatory drugs results in increased toxic effects in cyclosporin A treated patients should be considered.

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