Review

Effects of non-steroid immunosuppressive drugs on insulin secretion in transplantation

Effets des immunosupresseurs non stéroïdiens sur l’insulinosécrétion en transplantation

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Résumé

Le diabète post-transplantation (DPT) représente une complication importante des greffes d’organe. Il s’associe à un risque accru de dysfonctionnement du greffon, mais surtout de morbidité et de mortalité cardiovasculaire. L’incidence du DPT est corrélée à l’âge, à l’ethnicité non caucasienne, aux antécédents familiaux de diabète, au poids, à la présence d’une infection à VHC, et aux bolus de corticoïdes administrés en cas de rejet. Différents mécanismes peuvent expliquer ces troubles du métabolisme glucidique après transplantation. Les lésions d’ischémie reperfusion, quel que soit l’organe greffé vont favoriser une insulinorésistance, aggravée par la corticothérapie postgreffe. Le rôle délétère des immunosuppresseurs non stéroïdiens sur l’insulinosécrétion est également mis en cause, notamment celui des inhibiteurs des calcineurines. Les études in vivo et in vitro ont montré l’effet inhibiteur du tacrolimus sur l’insulinosécrétion, tandis que ces effets sont moins nets pour la ciclosporine et surtout mis en évidence in vitro. Le mycophénolate n’a pas d’effet démontré sur l’insulinosécrétion. Les travaux sur les inhibiteurs de mTOR, sirolimus et éverolimus, montrent des résultats controversés. Les effets du sirolimus, principal inhibiteur des mTOR étudié, pourraient dépendre de la concentration testée, du type cellulaire (cellules \( \beta \) ou lignées), de l’espèce humaine ou animale et également de facteurs nutritifs d’environnement. À concentrations thérapeutiques, sur des cellules humaines, un effet stimulant sur l’insulinosécrétion a été signalé, ce qui pourrait participer au succès du protocole d’Edmonton, en greffe d’îlots pancréatiques. Globalement, les stéroïdes sont essentiellement délétères du fait d’une augmentation de l’insulinorésistance, tandis que les anticalcineurines, et principalement le tacrolimus induisent une diminution de synthèse d’insuline.

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Abstract

Post-transplantation diabetes (PTD) is a serious complication in organ transplantation: not only does it increase the risk of graft dysfunction; it also increases cardiovascular morbidity and mortality. PTD incidence is correlated with age, non-Caucasian ethnic background, a family history of diabetes, excess weight, hepatitis C infection and steroid boluses for potential rejection. Different mechanisms might explain post-transplantation glucose metabolism disorders: ischemia–reperfusion disorders, whether renal, hepatic or cardiac, are responsible for insulin-resistance, which is increased by post-transplantation steroids; the detrimental effect of non-steroid immunosuppressive drugs on insulin-secretion could also be involved, especially with calcineurin inhibitors. In vivo and in vitro studies have shown that tacrolimus has inhibitory effects on insulin-secretion, while these effects are less obvious for cyclosporin, and were mainly demonstrated in vitro. Mycophenolate has no overt effect on insulin-secretion. Sirolimus and everolimus, two mTOR inhibitors, have shown controversial results in this realm. The effects of sirolimus (most often studied mTOR inhibitor) appear to depend on serum levels, cell type (\( \beta \) cell or cell line), species (human or animal) and

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also environmental nutrients. At therapeutic concentrations, a stimulatory effect on insulin secretion was observed on human β cells. This might explain the success of islet cell transplantation with the Edmonton protocol. Finally, steroids are mainly detrimental because they accentuate insulin resistance whereas anticalcineurins, in particular tacrolimus, lower insulin synthesis.

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**Keywords:** Immunosuppressive drugs; Insulin-secretion; β cell; Cyclosporine; Tacrolimus; Mycophenolate; Sirolimus; Inhibiteurs des calcineurine; Inhibiteurs des mTOR

1. Introduction

Post-transplantation diabetes (PTD) is a severe complication, in particular after kidney transplantation [31]. Not only does it correlate with higher rates of organ dysfunction, but it also increases cardiovascular morbidity and mortality by as much as 3.27 after 8 years of graft survival [23]. PTD costs an additional 21 500 US$ per patient over the first two post-transplant years [64]. PTD incidence correlates with age, non-Caucasian origin, a family history of diabetes [13,31,40], excess weight [34] and VHC infection [3]. Some of these risk factors often cause complications immediately after transplantation on account of immunosuppression, even more so when steroids are used concomitantly [39].

PTD incidence is extremely variable from one study to another, from 2% in some reports to as much as 53% in others [39]. Fluctuations are due to the difference in populations and the variety of immunosuppressive regimens used, but also to the definition of diabetes that is at the very least hazy, and often not even provided [8]. Last, age is important to consider, as the risk of developing diabetes increases with age, just as in the non-transplanted population, and not with the number of transplant years [6]. PTD incidence is extremely variable from one study to another, from 2% in some reports to as much as 53% in others [39]. Fluctuations are due to the difference in populations and the variety of immunosuppressive regimens used, but also to the definition of diabetes that is at the very least hazy, and often not even provided [8]. Last, age is important to consider, as the risk of developing diabetes increases with age, just as in the non-transplanted population, and not with the number of transplant years [6].

The effect of immunosuppressive drugs on insulin secretion might also modulate the loss of insulin independence observed after islet transplantation in type 1 diabetes [52,54]. Indeed, metabolic studies after islet graft have shown that the early post-glucose insulin-secretion peak decreases and tends to disappear after 3 years despite persisting insulin-independence and normal hemoglobin A1c [54].

Different mechanisms can explain post-transplantation glucose metabolism disorders: ischemia/ reperfusion disorders after organ transplantation can cause insulin-resistance, which is accentuated by post-transplantation steroid treatments. Non-steroid immunosuppressors might also be detrimental to insulin secretion. Calcineurin inhibitors, in particular tacrolimus, have been pointed out as particularly detrimental.

The object of our study was to review in vivo and in vitro studies on the effects of calcineurin inhibitors (tacrolimus and cyclosporin A), mTOR inhibitors (sirolimus and everolimus) and mycophenolate on insulin secretion in pancreatic β cells. We deliberately omitted epidemiology studies on the prevalence of PTD in different types of transplantation according to immunosuppressive regimen.

2. Tacrolimus

2.1. Clinical cases

Les effets délétères du tacrolimus sur l’insulinosécrétion ont été suspectés dès 1996 avec la publication de cas cliniques de diabètes apparemment induits par le tacrolimus. [59].

2.2. In vivo studies in man

Based on these clinical cases, clinical trials were designed to test the effect of tacrolimus on insulin secretion after kidney, pancreas and liver transplantation (Table 1). PTD after kidney transplantation has been more often studied, though on smaller patient groups. Results tend to show that glycemia and insulinemia are more markedly increased with tacrolimus [4,10,61]. Only one study including 136 type 1 diabetic patients with a kidney-pancreas transplantation reported no significant difference in metabolic parameters between patients initially treated with tacrolimus or sirolimus [9].

Metabolic data after liver transplantation, though more delicate to interpret, showed that insulin clearance increased [35]. In addition to trials with transplanted patients, a small group of 7 non-transplanted, non-diabetic patients was studied with euglycemic hyperinsulinemic clamps before and after beginning tacrolimus treatment for an auto-immune disease. Results showed that insulin secretion decreased, regardless of glucose tolerance. Tacrolimus did not, however, modify insulin sensitivity [57]. Actually, when tacrolimus was used without steroids, PTD incidence was little different from that observed in patients treated with cyclosporin, except for those with a VHC infection [3,51]. PTD incidence is highly dependent upon tacrolimus serum concentrations [39]. But immunosuppressors have also been studied in animals.

2.3. In vivo studies in animals

2.3.1. In rodents

Human islets were transplanted under the renal capsula of nude diabetic mice, and increasing doses of intra-peritoneal tacrolimus (0.03, 1 and 3 mg/kg of FK506 for 1 week) were injected. As a result glucose tolerance deteriorated and E-peptide response to intra-peritoneal glucose decreased when tacrolimus doses increased [48]. Similar findings were reported in rat treated with increasing doses of tacrolimus (1.5 and...
10 mg/kg): results found a dose-dependent vacuolization of islets that disappeared once treatment was interrupted [24].

2.3.2. In large mammals

In normal dog, a glucose tolerance test before, after 2-4 weeks of tacrolimus, and after weaning, showed that glucose tolerance decreased regardless of treatment duration, but that it returned to normal once treatment was interrupted. However insulin secretion remained significantly lower at a distance from the longest periods of treatment [56].

2.4. In vitro studies

In vivo investigations are difficult to interpret because of the variation in insulin sensitivity of peripheral tissues. That is why in vitro studies at therapeutic tacrolimus doses (5 to 10 ng/ml) were required. At these doses, tacrolimus decreased glucose-stimulated insulin secretion in β pancreatic rat cells, in cell lines (HIT-T15 and β TC3) and in human β cells [24,44,45]. Yet responses varied with the species, age [28], and the type of stimulus. The mechanisms potentially involved in decreased insulin-secretion are given in Table 2 [11,15,27,43,46,47,60].

2.5. Conclusion

In vivo studies in human and in animal along with in vitro studies on β cells or cell lines concord: tacrolimus decreases insulin secretion and ultimately leads to glucose tolerance disorders that are dose-dependent. The underlying mechanisms are not clearly understood.

3. Ciclosporin A

The effects of cyclosporin A on insulin secretion are more difficult to evidence. The first studies of cyclosporin showed a remission of type 1 diabetes within the first year of the diagnosis [58]. There was no incidence on glucose homeostasy in non-diabetic patients that were treated with cyclosporin for multiple sclerosis during one year, whether on serum glucose or on serum insulin and insulin response to IVGTT [50].

Table 1
Results of in vivo studies in humans. IVGTT: intravenous glucose tolerance test; HGPO: OGTT oral glucose tolerance test; AIR: Acute Insulin response

<table>
<thead>
<tr>
<th>KIDNEY</th>
<th>Patients</th>
<th>Methods</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dmitrewski [10]</td>
<td>12 randomized for tacrolimus or cyclosporin A 6 controls</td>
<td>OGTT</td>
<td>Fasting serum glucose and insulin significantly &gt; in immunosuppressor groups vs. controls</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serum insulin after OGTT significantly&gt; in tacrolimus group vs. controls and cyclosporin A group</td>
</tr>
<tr>
<td>Van Duijnhoven [61]</td>
<td>23 randomized for tacrolimus vs. cyclosporin A</td>
<td>IVGTT/ 6 months for 3 years</td>
<td>hyperinsulinism apparently linked with increased resistance</td>
</tr>
<tr>
<td>Boots [4]</td>
<td>15 non-diabetic</td>
<td>IVGTT</td>
<td>Increased fasting serum glucose insulin-resistance during the first 6 months, then stable levels in both groups</td>
</tr>
<tr>
<td>Kidney and pancreas.</td>
<td>136 type 1 diabetics treated with tacrolimus or cyclosporin A</td>
<td>OGTT at 3 months and at 3 years</td>
<td>Once steroids were stopped (though low doses: 10 mg): decreased insulin resistance, significantly increased serum insulin and lower insulin/fasting glucose ratio</td>
</tr>
<tr>
<td>Dieterle [9]</td>
<td></td>
<td></td>
<td>After subsequent reduction of tacrolimus doses, lowering serum concentrations from 9.5 to 6.4ng/ml, elevated serum insulin and lowered HbA1c</td>
</tr>
<tr>
<td>Liver. Konrad [35]</td>
<td>10 controls 10 transplanted with tacrolimus 10 transplanted with cyclosporin A</td>
<td>Minimal Model</td>
<td>No difference in age, body mass, creatinin levels, or sex ratio in both groups</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 months: fasting serum glucose, HbA1c, basal and stimulated insulin secretion, and frequency of glucose tolerance disorders did not differ between both groups</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>3 years fasting serum glucose, HbA1c and glucose tolerance disorders &gt; in the tacrolimus group (non significant)</td>
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<tr>
<td></td>
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<td></td>
<td>No deterioration in insulin sensitivity, AIR or insulin secretion in the long term</td>
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<td></td>
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<td></td>
<td>But normal insulin sensitivity and higher 2nd insulin secretion peak suggesting accelerated liver clearance of insulin compensated by increased insulin secretion</td>
</tr>
</tbody>
</table>

Table 2
Mechanisms susceptible to mediate decrease of insulin secretion by tacrolimus

<table>
<thead>
<tr>
<th>KIDNEY</th>
<th>Methods</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct effect on insulin secretion mechanisms by closing ATP-sensitive potassium channels via calcineurin-independent effect [15]</td>
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<tr>
<td>Caspase 8 activation causes fat apoptosis, intolerance to carbohydrates and decreased inflammation [43]</td>
<td></td>
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<tr>
<td>Calcineurin pathway inhibition (calcium-dependent calmodulin) with decreased expression of insulin gene at multiple cell signaling levels [21,47,60]</td>
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<tr>
<td>Fewer anti-apoptotic factors [27]</td>
<td></td>
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<tr>
<td>reduced activity of glucokinase ⇒ decreased ATP production and mitochondrial dysfunction [46]</td>
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</table>
3.1. Clinical trials

The clinical study of post-transplantation insulin secretion is more divergent than for tacrolimus. In heart transplantation, serum cyclosporin concentrations correlated negatively with serum insulin levels [66].

But in a series of live donor kidney transplantation, cyclosporin increased insulin secretion beyond what tacrolimus does after transplant, but also beyond the initial levels of insulin secretion, in particular in patients at high risk for PTD [53].

Meanwhile previously mentioned trials [28,61] (Table 1) in kidney and liver transplantation showed that tacrolimus and cyclosporin both had similar effects on metabolism. A body of in vivo studies in animals have been added to these contradictory results.

3.2. In vivo studies

3.2.1. In rodents (rat)

Degranulation and degeneration of β cells was induced by therapeutic and supra-therapeutic doses of cyclosporin administered over a 2-week period [19,20]. Intolerance to carbohydrates developed, along with a drop in β cell count and DNA synthesis, all of which progressively regressed after treatment was interrupted [18]. A decrease in insulin secretion and an increase in insulin resistance via a post-receptor mechanism were also evidenced [16,29,38].

3.2.2. In large mammals (dog)

A glucagon test and a euglycemic hyperinsulinemic clamp performed in 6 dogs that were treated with cyclosporin showed that glucose regulation was altered in a dose-dependent way via the inhibition of insulin secretion and increased peripheral insulin resistance that both progressively regressed after treatment was interrupted. Return to normal levels was quicker and more complete when treatment was shorter [62]. Irreversible alterations of insulin secretion were detected with fairly sophisticated tests such as the IVGTT after treatment with therapeutic doses of cyclosporin (500 ng/ml) [1].

In auto-grafted dogs studied with the IVGTT and a clamp, Kneteman did not find any effect of cyclosporin on insulin secretion or sensitivity in either the liver or peripheral tissues [32].

3.3. In vitro studies

Results in non-transplanted laboratory animals tend to confirm the deleterious effect of cyclosporin on insulin secretion, once again in a dose-dependent way.

These in vivo results have been confirmed in vitro on isolated cultured β cells, on cell lines and on perfused pancreas.

Pancreatic β cells from different species were used (rat, mouse, and hamster) [6,11,18,44,49,63]. Note that Hahn [14] studied rat pancreatic β cells that were treated with cyclosporin in vivo while in all the other trials, cyclosporin was added to the culture media. Wang [54] confirmed the data after glucose stimulation. In dog β cells, the first and second insulin peaks after glucose stimulation both decreased, even at therapeutic doses [30]. The same was shown in human β cells, both at base level and after glucose stimulation. Insulin content, however, increased in human β cells [42].

Decreased insulin secretion was also noted in different cell lines: HIT with doses of 100ng/ml of cyclosporin, NIT with doses of 10 mmol/l, MIN6 after glucose stimulation [12,49]. Recent investigations on RIN cells also showed that the expression of the insulin gene and the glucose transporter GLUT2 gene dropped [45].

3.4. Mechanisms

The mechanisms by which cyclosporin affects insulin secretion have been under less scrutiny than for tacrolimus. Yet both immunosuppressors seem to share similar mechanisms: it has been hypothesized that they are calcium-dependent via voltage-dependent calcium channels [16,17], independent from calcium-neurins using ATP-dependent potassium channels, and that there is a mitochondrial dysfunction [11,15].

4. Mycophenolate

Few authors have investigated mycophenolate. The few that have focused on in vitro studies. A study on human islets cultured for 5 days showed a base level insulin secretion that was close to levels with cyclosporin but less than levels with tacrolimus [45]. On the HIT-T15 cell line, post-stimulation insulin secretion was inhibited by 60 to 83% compared to controls by 24-to-48 hours of exposition to mycophenolate at the lower and upper therapeutic doses. In identical experimental conditions, insulin secretion of murine pancreatic cells was reduced by 0 to 48% compared to control cultures [44].

It seems therefore that the effect of mycophenolate on insulin secretion depends on the species tested. We did not find any in vivo studies. It seems that mycophenolate has little detrimental effect on insulin secretion.

4.1. mTOR inhibitors

mTOR inhibitors such as sirolimus (also called rapamycine) and everolimus are much more recent immunosuppressors. They link with a cytosolic protein, FKPB-12, forming a complex that inhibits the mTOR (mammalian target of rapamycine). The inhibition of the kinase involved in cell cycle inhibits signal transduction and induces immunosuppression. The effects of mTOR inhibitors on β cells have mainly been studied with sirolimus and results are very much controversial.

4.2. Clinical trials

The main clinical contribution of sirolimus was that β cell grafts became successful in diabetes for the first time. The
Edmonton immunosuppression regimen for pancreatic islet graft relies on the absence of steroids, sirolimus associated with low doses of tacrolimus, and induction with daclizumab [25,50].

4.3. Animal studies

Studies in small mammal [14] and in dog [33] showed that sirolimus was not detrimental to glucose metabolism, except in two cases: at concentrations that were ten-fold those used for treatment and in a very recent study on mouse [5]. Knetemans’s results on auto-transplanted islets in the spleen of dog show that base and stimulated insulin levels increase at therapeutic concentrations of mTOR inhibitors, concordant with results in healthy minipigs [37]. Elevated insulin levels might be due to the direct stimulation of sirolimus on β cells or to altered insulin sensitivity. Yet Knetemans’s results with a minimal model failed to demonstrate any change in insulin sensitivity by sirolimus. It even appears that insulin sensitivity may improve with sirolimus, and this may explain the higher glucose clearance found in auto-transplanted dog as well as in healthy mini-pigs.

Further experimental data in mouse receiving either Edmonton’s immunosuppressive regimen (low dose tacrolimus and sirolimus) or sirolimus alone after auto-transplantation of β cells that were previously transfected with Hepatocyte Growth Factor showed that insulin levels increased in both groups [36]. Insulin levels were, however, higher in the “sirolimus alone” group whereas glucose tolerance was altered in the “tacrolimus and sirolimus” group, most likely on account of tacrolimus.

4.4. In vitro study

In order to investigate the specific effect of sirolimus on β cells, in vitro studies were required to break free from the modifications in liver and peripheral insulin sensitivity.

Most in vitro studies on cultured animal β cells and on MIN6 or HIT cell lines have highlighted the deleterious effect of sirolimus on insulin secretion [2,14,27]. And both sets of experimental data on cultured human cells were obtained at supra-therapeutic doses [5,44]. The negative effect of sirolimus on viability and apoptosis were found in MIN6 type cell lines at therapeutic doses and may be dose-dependent. Similar effects have been found on human islets, ductal human cells and new-born pig islets at supra-therapeutic doses [5,22,26,41,55]. The pro-apoptotic effects of sirolimus were not found in blood cells such as monocytes or macrophages, nor were they found in human pancreatic cancer cell lines or in human cultured β cells, at therapeutic doses [37,65]. Thus the effect of sirolimus on β cells, a crucial point in cell therapy of diabetes, might depend on species, cell line and most of all on serum levels of sirolimus.

4.5. Mechanism

The mechanisms by which sirolimus affects insulin secretion are still unclear. Sirolimus may affect insulin signaling, as its target, mTOR, plays a key part in the transduction of receptor signals to insulin, but sirolimus might also be detrimental on fat metabolism, which might in turn increase insulin resistance.

5. Conclusion

The detrimental effects of cyclosporin A and especially of tacrolimus on insulin secretion have been shown in vivo in the animal and in vitro on human β cells, even at therapeutic concentrations. They do, however, appear to be dose-dependent. These results condone with the elevated occurrence of diabetes in epidemiological studies of patients treated with calcineurin inhibitors, first and foremost with tacrolimus. But the genesis of DPT involves multiple factors and the benefit: risk ratio should naturally be taken into account, as well as risk factors of pretransplantation diabetes, immunosuppressive treatment, in particular steroids and the effect on insulin resistance of concomitant dyslipidemia. The effects of sirolimus are much more controversial: several animal trials and a study on human β cells seem to demonstrate that sirolimus bears no toxicity and that it might even be beneficial for insulin secretion at therapeutic serum levels. In any case the characteristics of sirolimus contrast with those of other immunosuppressors and might have contributed to the positive results of islet transplantation in the Edmonton protocol. Nevertheless these experimental data need to be confirmed by clinical trials in human organ transplantation. If confirmed by large scale studies, the characteristics of sirolimus could find new applications in the treatment of DPT patients, in particular those whose other risk factors for diabetes have been dealt with. A better understanding of the mechanisms by which immunosuppressors affect glucose metabolism would help identify chemicals with immunosuppressive characteristics but no detrimental effect on metabolism.

6. French version

A French version of this article is available at doi: 10.1016/j.ando.2007.02.005.

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