Published in final edited form as:

Transplantation. 2013 March 15; 95(5): . doi:10.1097/TP.0b013e31826e592e.

Calcineurin Inhibition and New-Onset Diabetes Mellitus After Transplantation

Harini A. Chakker^{1,4} and Lawrence J. Mandarino^{2,3}

¹Division of Nephrology and Hypertension, Mayo Clinic, Phoenix, AZ

²Department of Medicine, Mayo Clinic, Phoenix, AZ

³Department of Physiology, Mayo Clinic, Phoenix, AZ

Abstract

New-onset diabetes after transplantation independently increases the risk of cardiovascular disease, infections, and graft loss and decreases patient survival. The required balance between insulin sensitivity/resistance and insulin secretion is necessary to maintain normal glucose metabolism. Calcineurin inhibitors are standard immunosuppression drugs used after transplantation and have been implicated in the development of new-onset diabetes after transplantation partially by pancreatic β -cell apoptosis and resultant decrease in insulin secretion. The ability of muscle to take up glucose is critical to blood glucose homeostasis. Skeletal muscle is quantitatively the most important tissue in the body for insulin-stimulated glucose disposal and is composed of diverse myofibers that vary in their properties between healthy and insulin-resistant muscle. Various signaling pathways are responsible for remodeling of skeletal muscle, and among these is the calcineurin/nuclear factor of activated T-cell pathway. The mechanism of action of the calcineurin inhibitors is to bind in a complex with a binding protein to calcineurin and inhibit its dephosphorylation and activation of nuclear factor of activated T cells. In this review, we will provide a detailed discussion of the hypothesis that inhibition of calcineurin in tissues involved in insulin sensitivity/resistance could be at least partially responsible for the diabetogenicity seen with the use of calcineurin inhibitors.

Keywords

Insulin resistance; Insulin secretion; Glucose intolerance

IMPACT AND PATHOPHYSIOLOGY OF NEW-ONSET DIABETES MELLITUS AFTER TRANSPLANTATION

New-onset diabetes mellitus after transplantation (NODAT) is a frequent complication after kidney transplantation, with an incidence of 15% to 30% (1, 2). Development of NODAT undermines the benefits of kidney transplantation by decreasing survival of the graft and the patient and decreasing the quality of life (1, 3, 4). In addition to the human costs, NODAT results in an extra Medicare cost of U.S. \$21,500 per case by 2 years after transplantation (5). Detection of NODAT after it develops is straightforward, but why or how NODAT develops is not well understood. The very high incidence of diabetes mellitus in the first

year after transplantation supports the concept that NODAT occurs when patients with a baseline pretransplantation risk of diabetes are exposed to the unique posttransplantation milieu that somehow results in lower insulin sensitivity or insulin secretion, tipping the patient toward development of NODAT.

NODAT and type 2 diabetes mellitus (T2DM) share common risk factors of decreased insulin sensitivity and decreased insulin secretion. Abnormalities in both insulin sensitivity and insulin secretion are central to the development of T2DM. In T2DM, defects in β -cell function are present in high-risk patients long before hyperglycemia sets in (6). The required balance between insulin sensitivity and insulin secretion necessary to maintain normal glucose metabolism is well described (7, 8). The hyperbolic curve (Fig. 1) demonstrates the relationship between the insulin secretion and insulin sensitivity to maintain glucose homeostasis (9).

Traditional T2DM risk factors, including older age, ethnicity (African American, Hispanic, and Native American), family history of T2DM, and obesity, join risk factors unique to the posttransplantation environment, including immunosuppression, cytomegalovirus infection, hepatitis C seropositivity, and weight gain, after transplantation as risk factors for the development of NODAT. In addition, immunosuppression drugs commonly used have been implicated to be diabetogenic, including calcineurin inhibitors (tacrolimus and cyclosporine), corticosteroids, and mammalian target of rapamycin inhibitors (sirolimus and everolimus) (10, 11), although earlier studies had raised the possibility that rapamycin inhibition of mammalian target of rapamycin may reduce the risk of diabetes (12).

The diabetogenic effects of calcineurin inhibitors are partially attributed to pancreatic β -cell apoptosis and impaired insulin secretion (13, 14); additionally, there is sparse literature describing calcineurin inhibitor-induced insulin resistance (15–17). In this review, we will provide a hypothesis-driven discussion describing recent advances in our understanding of potential mechanisms involved in the diabetogenicity of calcineurin inhibitors focusing on its contribution to increased insulin resistance.

STRUCTURE AND FUNCTION OF CALCINEURIN

Calcineurin is a two-subunit structure, with a 58 to 64-kDa catalytic and calmodulin-binding subunit calcineurin A that is tightly bound to a regulatory 19-kDa calcium-binding regulatory subunit, calcineurin B (18). This calcium/calmodulin-dependent serine/threonine protein phosphatase is unique among the protein phosphatases and has been conserved from yeast to man. Calcineurin is widely distributed among tissues, including islet cells of the endocrine pancreas (19–21), skeletal muscle, heart, neurons, and adipocytes (22, 23). Calcineurin is a key enzyme in many cellular processes including antigen-stimulated T-lymphocyte activation, skeletal-muscle gene expression and remodeling, and the formation of the embryonic cardiovascular system (24). In this review, we will provide a detailed discussion describing potential mechanisms involved in the diabetogenic properties of calcineurin inhibitors.

Activated calcineurin dephosphorylates many substrates, including nuclear factor of activated T cell (NFAT) 1 to 4 transcription factors. NFAT initially was identified as an inducible nuclear factor that could bind to the interleukin 2 promoter in T cells (25). However, subsequent work has demonstrated the ubiquitous presence of NFAT with one NFAT family member expressed by almost every cell type including both immune and nonimmune cells (23, 24, 26, 27). There have been five identified members in the NFAT family, with four of these being regulated by calcium signaling (28). Dephosphorylated NFAT translocates to the cell nucleus. The nuclear NFAT is a key component of cytokine gene expression stimulation that triggers T-cell activation (28).

IMPACT OF CALCINEURIN INHIBITION ON INSULIN LEVELS

Calcineurin inhibitors (cyclosporine A and tacrolimus or FK506) are commonly used immunosuppression drugs after transplantation. Cyclosporine A and FK506 form complexes with the immunophilins cyclophilin A and FKBP12 (FK506-binding protein), respectively (29–32), and inhibit calcineurin phosphatase activity. The immunosuppression effect is the result of prevention of the dephosphorylation of NFAT transcription factors in T cells and other immune cells, thus preventing the translocation of dephosphorylated NFAT into the cell nucleus and inhibiting the initiation of cytokine gene expression that is responsible for T-cell activation (33).

With regard to possible mechanisms of effects of calcineurin inhibition on insulin secretion, calcineurin is expressed in pancreatic insulin-secreting β cells, where it has two well-described molecular targets: (1) the cAMP responsive element binding protein (CREB) transcriptional coactivator and (2) the NFAT family of transcription factors. The transcription factor CREB is believed to mediate survival and proliferative effects of glucagon-like peptide (GLP-1) and glucose-signaling pathways in the pancreas (34). Transgenic mice expressing a dominant negative CREB polypeptide in islets develop diabetes with apoptosis of insulin-producing β cells because of reduced expression of IRS2, a direct target of CREB (35). A family of coactivators called the transducer of regulated CREB activity 2 (34, 36, 37) modulate CREB activity. Recent experiments in a rodent model demonstrated a downstream gene responsive to calcineurin activation to be *IRS2*, whose promoter is engaged by NFAT in a calcineurin-sensitive manner. In addition, these investigators demonstrated that calcineurin inhibition resulted in reduced phosphorylation of Akt substrates in both rodent and human islets, implying that calcineurin could regulate replication and survival of β cells by the P13K/Akt pathway (14). These effects are likely to be indirect because knockout of IRS2 by itself also produces diabetes because of β -cell apoptosis/failure in mice (38).

ROLE OF PERIPHERAL SKELETAL MUSCLE IN GLUCOSE DISPOSAL

Skeletal muscle is quantitatively the primary site for glucose uptake (39), accounting for up to 70% to 80% of the total glucose disposal after insulin stimulation (40). The ability of muscle to take up glucose in response to insulin is critical to blood glucose homeostasis (41). Human skeletal muscle is composed of diverse myofibers with variations in structural and functional properties. Most human muscles are composed of a mixture of three myofiber types, IId/x, IIb, and I. Type I (slow twitch fibers) have greater insulin sensitivity, are rich in mitochondria, exhibit oxidative metabolism, have low velocity of shortening, and have a high resistance to fatigue. Type IId/x (fast twitch) are less sensitive to insulin, exert quick contractions, fatigue rapidly, and is predominated by glycolytic metabolism (42, 43). The slow and fast fiber phenotypes result from the concerted activation of complex gene programs comprising various contractile proteins and metabolic enzymes. Tonic motor activity is characteristic of type I fibers and results in a sustained elevation of Ca^{2+} (100–300 nM) in a pattern predicted to activate calcineurin (44, 45). In type II, insulin-resistant myofibers, resting Ca^{2+} is maintained only at 50 nM (46), which is insufficient to evoke calcineurin signaling. Chronic stimulation at 10 Hz of the motor nerve innervating fast myofibers results in sustained increase of calcium and promotes transformation from fast to slow fibers.

Insulin resistance in skeletal muscle characterizes T2DM (39, 47, 48). Various signaling pathways have been shown to be responsible for remodeling and regeneration of skeletal (and cardiac) muscle (49–52). Three major pathways contribute to the regulation of gene transcription events that lead to muscle remodeling, and studies have demonstrated

interactions among these pathways (53–56). The first pathway includes myocyte enhancer factor 2 (MEF2) and a family of basic helix-loop-helix transcription factors that couple with class II histone deacetylases (HDACs) to regulate skeletal muscle development (57). Studies using genetically altered mice showed that deletion of MEF2 leads to loss of slow, oxidative, and insulin-sensitive muscle fibers (58). Deletion of class II HDAC results in increased slow fiber proportion, whereas overexpression of HDACs leads to loss of slow fibers (59). These studies indicate the importance of MEF2/HDAC signaling in muscle fiber remodeling. The second pathway involved in formation of type I fibers is the peroxisome proliferator activated receptor gamma activator-1 (PGC-1) (α and β) pathway. PGC-1 is a transcriptional coactivator, the overexpression of which increases slow fiber formation, although its lack does not result in reduced slow fiber proportion (51, 60). PGC-1 expression (and type I fiber proportion) is reduced in insulin-resistant muscle (61), and PGC-1 gene expression in response to exercise is reduced in insulin-resistant muscle (62). PGC-1 also is involved in mitochondrial biogenesis and maintenance of expression of nuclear encoded mitochondrial genes, which are decreased in insulin-resistant states (62, 63). The third pathway is the calcineurin/NFAT pathway (Fig. 2). Activated calcineurin dephosphorylates NFAT, and dephosphorylated NFAT translocates to the nucleus to activate genes involved in muscle remodeling (23, 49, 53), but this is a complex pathway in differentiated muscle, with different NFAT isoforms (c1–c4) exhibiting different functions and differing patterns of phosphorylation and nuclear/cytoplasmic shuttling (64). Mice overexpressing a constitutively active calcineurin have increased oxidative fibers and endurance capacity (65). The specific phosphoregulation of NFAT is complex. Dephosphorylation of NFAT exposes a nuclear localization signal sequence, promoting its translocation to the nucleus. Once activated, NFAT then orchestrates changes with other transcription factors including MEF2, GATA-2, four and a half LIM domains protein 1, activator protein 1, and PGC-1 for coordinated transcriptional activity (66, 67). The previously mentioned NFAT isoforms are involved in regulating muscle fiber phenotype and morphology. The specific role of each NFAT isoform is only beginning to be understood. NFATc1 has been reported to be responsible for the transcription of myosin I slow fibers and the inhibition of myosin II fast fibers (64, 68, 69). Thus, it could be proposed that pharmacological inhibition of the calcineurin/NFAT pathway in skeletal muscle could promote the transcription of insulin-resistant myosin fast fibers contributing adversely to the development of insulin resistance.

Evidence is emerging that insulin-resistant skeletal muscle is characterized by changes that are reminiscent of a greater type II fiber phenotype. For example, an enhanced reliance on glycolysis for metabolism in insulin-resistant muscle was demonstrated in work by Simoneau et al. (70). More recently, Lefort et al. (71) showed that mitochondria isolated from insulin-resistant muscle have characteristics reminiscent of mitochondrial from type II glycolytic muscle, such as a more negative free energy of hydrolysis of adenosine triphosphate. Moreover, changes in structural protein content of insulin-resistant muscle, revealed by a proteomics approach, also point toward differences suggestive of reduced muscle differentiation and regeneration (72). Hints as to how this might be occurring come from studies showing that insulin-resistant muscle does not respond normally to a bout of exercise, with respect to the pattern of changes in gene expression (62). Insulin-sensitive muscle, after exercise, has robust increases in expression of PGC-1 α and nuclear-encoded mitochondrial genes, whereas insulin-resistant muscle shows dramatically reduced responses (62). Because contraction activates the calcineurin pathway, it is conceivable that these data indicate that contraction-mediated calcineurin signaling is abnormal in insulin-resistant muscle. As described previously, exercise enhances type I (slow twitch fibers), which have greater insulin sensitivity, thus it is conceivable that increase in insulin-sensitive myofibers and decrease in insulin-resistant myofibers will improve whole-body insulin sensitivity and decrease NODAT. Patients with chronic kidney disease self-report low levels of physical activity (73). Objectively measured physical activity in predialysis patients was negatively

correlated with fat mass and positively correlated with fat-free mass (74). This indicates that an increase in physical activity would likely be beneficial. However, the previous data suggest that, when calcineurin is inhibited, the normal response of gene transcription to aerobic exercise and a switch to type I muscle fibers would be inhibited. One approach to bypass this problem might be to prescribe a mixed aerobic and resistance training regimen that would also build fat-free mass. Along with this, it might be important to advise patients to consume a balanced diet to supply sufficient protein to accomplish this while at the same time not compromising renal function.

It should be noted that uremia associated with chronic kidney disease can cause increased protein degradation and muscle wasting, likely independent of the fiber-type switching mechanism of calcineurin activation effects (75). It is suggested that this occurs through activation of the ubiquitin proteasome system and is reminiscent of the increase in proteasome abundance in muscle from patients with type 2 diabetes without renal disease (72). Thus, kidney transplant patients are potentially at a double risk of muscle function and insulin sensitivity abnormalities. However, amelioration of inflammation and uremia after transplantation with an optimally functioning allograft should reduce the negative impact of proteasome activation.

In summary, the current literature supports the concept that pharmaceutical inhibition of calcineurin-dependent NFAT, transducer of regulated CREB activity 2 activation, and P13K/Akt pathway activity contribute adversely by diminishing β -cell survival in murine models. Moreover, data from mouse models, albeit sparse, suggest that calcineurin signaling may be involved in insulin sensitivity in skeletal muscle. In addition, proteomics studies of human insulin-resistant muscle show patterns of changes suggesting that muscle remodeling, fiber-type switching, response to exercise, and mitochondrial function are altered in a way that suggests the involvement of the calcineurin pathway. Table 1 describes a composite of studies suggesting the proposed effect of calcineurin inhibition on known pathways related to insulin secretion and insulin sensitivity. Robust studies in human models to study the correlation of calcineurin inhibition and diminished β -cell survival and its independent effect on insulin resistance merit further investigation.

Acknowledgments

The authors thank Mr. Marvin Ruona of the graphic design office and Ms. JoAnn McBroom of the academic office in Mayo Clinic Arizona for their assistance in the preparation of this article.

This work was supported by Grant numbers DK047936 and DK066483 (L.J.M.) from the National Institutes of Health.

H.A.C. and L.J.M. participated in making the research design, reviewing the literature, and writing the article.

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FIGURE 1.

Natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. The dashed lines represent the prediction line and the lower and upper limits of the 95% confidence interval of the regression between acute insulin secretory response and the rate of total insulin-stimulated glucose disposal with low-dose insulin infusion as derived from a reference population with normal glucose tolerance. IGT, impaired glucose tolerance; NGT, normal glucose tolerance. Adapted from *J Clin Invest* 104:6 doi: 10.1172/JCI7231.

FIGURE 2.

Proposed mechanism of the calcineurin/nuclear factor of activated T cells (NFAT) pathway and regulation of gene transcription in muscle remodeling. CaM, calmodulin; CnA, calcineurin A; CnB, calcineurin B; MEF, myocyte enhancer factor.

Table 1

Effect of calcineurin inhibition on factors related to insulin secretion and insulin sensitivity

Target Molecule/pathway	Insulin Secretion	Insulin Sensitivity
IRS2	Reduced IRS2 produces β -cell apoptosis (39) cAMP promotes pancreatic β -cell survival by CREB-mediated induction of IRS2	Possible in liver
MEF2	N/A	Deletion of MEF2 leads to loss of insulin-sensitive muscle fibers (58)
PGC-1	Unknown	Increase in expression is seen in insulin-sensitive skeletal muscle (62)
P13K/Akt pathway	Calcineurin inhibition resulted in reduced phosphorylation of Akt substrates in both rodent and human islets (14)	Possible but not directly examined

Calcineurin is expressed in pancreatic insulin-secreting β cells, where it has two well-described molecular targets: (1) the cAMP responsive element binding protein (CREB) transcriptional coactivator and (2) the nuclear factor of activated T cells (NFAT) family of transcription factors. Activated calcineurin de-phosphorylates NFAT, and dephosphorylated NFAT translocates to the nucleus to activate genes involved in muscle remodeling. Activated NFAT results in changes in other transcriptional factors including myocyte enhancer factor (MEF) 2, PGC-1, GATA-2, FHL1.