2-1-2003

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Recommended Citation

St Peter, S. D., Moss, A. A., Mulligan, D. C. Effects of tacrolimus on ischemia-reperfusion injury. *Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society* 9, 105-116 (2003).

Effects of Tacrolimus on Ischemia-Reperfusion Injury

Shawn D. St. Peter, Adyr A. Moss, and David C. Mulligan

In addition to efficacious immunosuppression for the benefit of organ transplantation, tacrolimus has diverse actions that result in amelioration of ischemia-reperfusion injury. Knowledge is accumulating rapidly on the mechanisms through which tacrolimus exerts these cytoprotective effects, including alterations in microcirculation, free radical metabolism, calcium-activated pathways, inflammatory cascades, mitochondrial stability, apoptosis, stress-response proteins, and tissue recovery. Within the nucleus, actions mediating the effects of tacrolimus appear to be dominantly influenced by interactions with the transcription factor, nuclear factor-κB. Because tacrolimus is a cornerstone agent in immunosuppression regimens throughout the world and knowledge of its cellular mechanisms is evolving, it is important to update the clinical literature with this information. We reviewed the published literature with intent to portray the interactions of tacrolimus in the intricate cellular mechanisms initiated by ischemia and reperfusion. (*Liver Transpl 2003;9: 105-116.*)

Tacrolimus (FK506; Prograf; Fugisawa Healthcare Inc, Deerfield, IL), a macrolide antibiotic compound, is a metabolite of the fungus *Streptomyces tsukubaensis*, discovered March 25, 1984, in a soil sample from the base of Mount Tsukuba near Tokyo, Japan.[1] In vitro demonstration of immunosuppressive properties was published 3 years later.[2]

Tacrolimus entered the clinical world classified as a calcineurin inhibitor, and it showed suppression of in vitro proliferation of lymphocytes to alloantigen at a concentration 100 times lower than its predecessor, cyclosporine A,[1] which had revolutionized results of transplantation in the mid-1980s.[3,4] The drug was rapidly incorporated into the clinical practice of solid-organ transplantation as a backup for cyclosporine A.[5] Subsequent results from comparative clinical trials have rendered tacrolimus a mainstay in immunosuppressive regimens for kidney,[6-9] liver,[10-14] and pancreas transplantation.[15-17]

Potential applications of this multifarious agent are still under investigation. Favorable results have been generated with the use of tacrolimus for treating various immune-mediated phenomena, including rheumatoid arthritis,[18] dermatological conditions,[19] ophthalmological ailments,[20] and inflammatory bowel disease.[21]

In this review, we present currently available data addressing the impact of tacrolimus within the elaborate and injurious cellular mechanisms induced by ischemia and subsequent reperfusion (I-R).

Mechanism

A calcineurin inhibitor, tacrolimus binds with high affinity to the calcineurin-calmodulin complex, blocking its participation in calcium-dependent phosphorylation (activation) of an important intranuclear transcription-regulating factor named nuclear factor of activated T cells (NF-AT).[22] As a highly lipophilic compound, tacrolimus readily traverses the plasma membrane to gain access to intracellular spaces without dependence on cell-surface receptors.[23] Inhibition of NF-AT prevents transcription of the gene coding for interleukin-2 (IL-2), thus blunting T-cell activation.[24] Through blockade of calcineurin activity, tacrolimus also inhibits binding of NF-AT to the enhancer region of the IL-2 gene.[25] Other transcription factors that calcineurin has the capability to activate and that are thus inhibited by tacrolimus include AP-1, AP-3, Oct-1, and nuclear factor-κB (NF-κB).[26]

The intracellular target for tacrolimus is a soluble cytosolic immunophilin known as FK-binding protein (FKBP).[27] The FKBP immunophilins represent a family of binding proteins independent from cyclophilin, the binding protein for cyclosporine.[28] Several proteins in the immunophilin family with the capacity of interacting with tacrolimus have been described, including FKBP12, FKBP12.6, FKBP13, FKBP25, FKBP51, and FKBP59.[29] Recently, FKBP12 has been defined as the only FKBP activated in the pathway leading to the T-cell–suppressing effects of tacrolimus.[30]

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1527-6465/03/0902-0001$35.00/0
doi:10.1053/jlts.2003.50020

Moderately specific T-cell inhibition may be the major attributing factor to the immunosuppressive properties of tacrolimus, but the complete cellular alteration induced by tacrolimus is a more loquacious story. Early studies found tacrolimus to defend tissues from ischemic damage when administered intravenously before ischemia, although no mechanism for such effects was defined in these studies.[31-34] Readily evident from the accumulating literature is that the role of tacrolimus in tissue protection from I-R injury is not the consequence of a single pathway, but multiple interweaving mechanisms, including manipulation of microcirculation, attenuation of free radical formation, inhibition of calcium-dependent pathways, inhibition of inflammatory response, and modification of cellular responses to injury. Figure 1 schematically shows the flow of injury mediated by these variables during ischemia and reperfusion.

Microcirculatory Effects

One of the first mechanisms suspected in the effect of tacrolimus on I-R injury was alteration of hepatic microcirculation.[35] Laser Doppler scanning, which quantifies microcirculatory perfusion, has been shown to correlate with sinusoidal perfusion measured by intravital microscopy.[36] In a rat model, laser Doppler showed more rapid recovery of peripheral hepatic microcirculatory flow when tacrolimus was administered before 30 minutes of warm ischemia.[37] In this study, histological examination confirmed periportal congestion in the control group, whereas little congestion was seen in subjects administered tacrolimus.

Subsequently, it was shown that tacrolimus may be able to promote maintenance of microcirculation in the face of the normally deleterious reperfusion by suppressing endothelial expression of the potent vasocon-

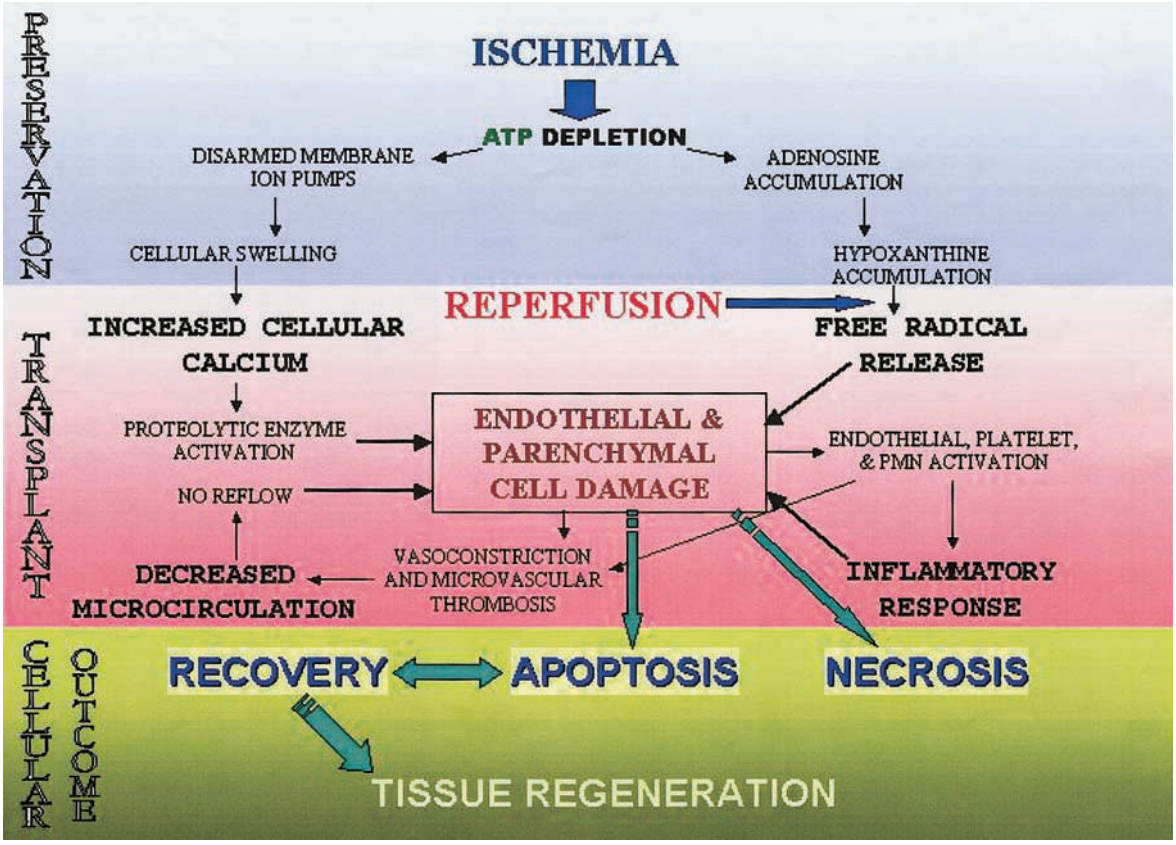

Figure 1. Cascade of I-R injury. This flow diagram shows the cumulative injury induced by multiple variables on reperfusion. Tacrolimus can ameliorate I-R injury by slowing of adenosine triphosphate (ATP) depletion, attenuation of free radical formation, and inhibition of calcium-dependent pathways in the early phase of I-R injury. Inhibition of inflammatory response and microvascular changes can help shelter tissue from the later phase of I-R injury. Finally, tacrolimus offers some modification of cellular responses to injury, favoring the outcome of recovery and regeneration over apoptosis. (PMN, polymorphonuclear neutrophil.)

strictor endothelin-1.[38] Tacrolimus also alters the nitric oxide pathway, with demonstration of such common substrates between nitric oxide and tacrolimus as AP-1 and glucocorticoid receptors.[39,40] This interaction is counteracted at least in part because tacrolimus inhibits inducible nitric oxide synthase (NOS) gene expression by blocking NF-κB activity.[39] Although interaction between these two molecules clearly exists, effects of this relationship are not currently clear.

Alterations in microcirculatory flow seem to contribute to ischemic protection offered by tacrolimus; however, it appears the majority of its action lies within the myriad of I-R pathways.

Free Radical–Mediated Injury

Products of adenosine triphosphate (ATP) breakdown normally are converted to urea by xanthine dehydrogenase (XD). However, under ischemic conditions, XD is converted to xanthine oxidase (XO). In the presence of oxygen on reperfusion, XO will convert the accumulating products of ATP breakdown into xanthine and the superoxide anion (free radical), which causes a "respiratory burst" of oxygen free radical release leading to lipid peroxidation and cellular destruction (Fig. 2).[41,42] This reaction is of particular importance in the liver, which holds the largest stores of ATP and XD in the body.[43]

Several investigators have documented decreased free radical production in association with amelioration of I-R injury when tacrolimus is administered before ischemia.[44-46]

Although these studies document an end result of tacrolimus pretreatment as suppression of free radical elaboration, they offer little insight to the mechanisms responsible for such effects. Maintenance of cellular ATP content, suppressed free radical elaboration, and possible antioxidant activities are all possible mechanisms acting to produce tacrolimus-induced free radical protection.

Cellular ATP Content

Treatment of rats with tacrolimus before occlusion of the hepatoduodenal ligament and two thirds partial

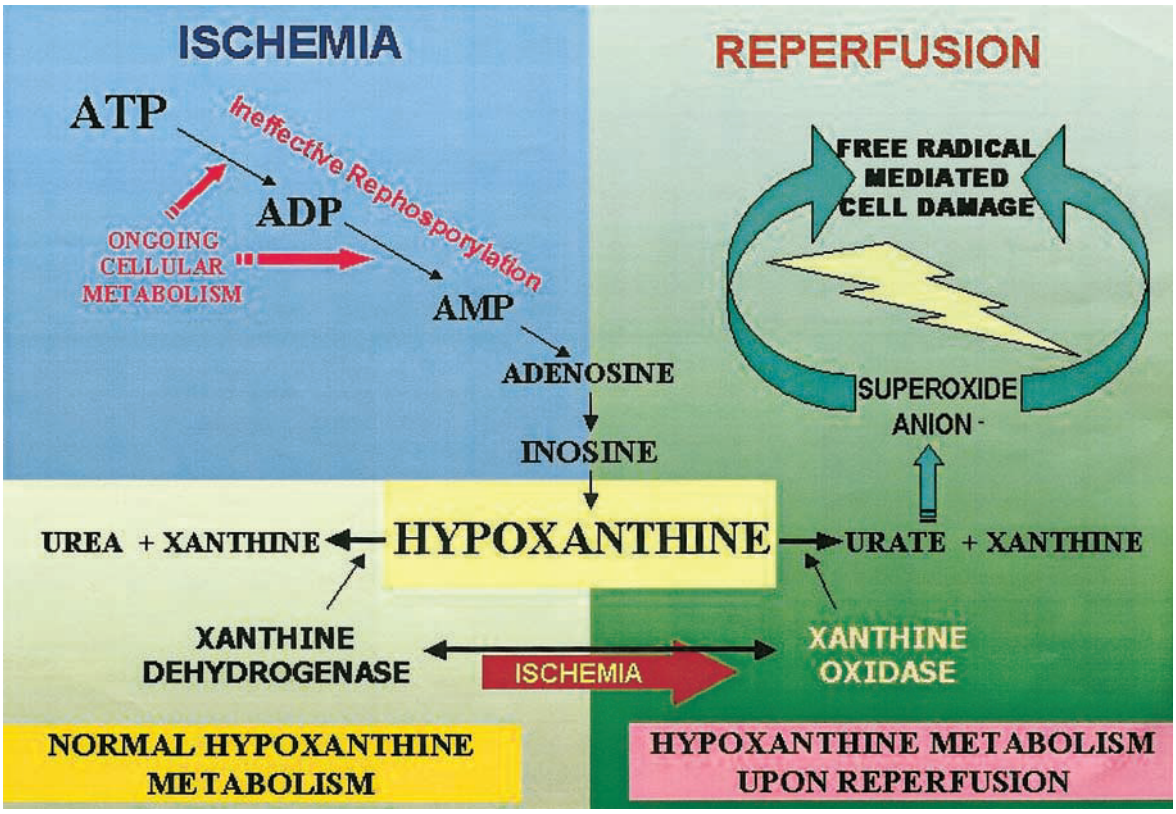

Figure 2. Hypoxanthine metabolism during normal metabolism compared with reperfusion. This flow diagram shows that conversion of xanthine dehydrogenase to xanthine oxidase during ischemia facilitates formation of free radicals on reperfusion through consumption of accumulation substrate (hypoxanthine). (ADP, adenosine diphosphate; AMP, adenosine monophosphate.)

hepatectomy resulted in improved survival and superior restoration of hepatic ATP contents.[34] Applying phosphorus 31 magnetic resonance spectroscopy to measure the phosphomonoester to inorganic phosphate ratio in a rat model of warm ischemia, the ATP–inorganic phosphate ratio was improved significantly after reperfusion when tacrolimus was administered intravenously before ischemia.[37] This study shows ATP restoration and/or preservation during ischemia, which may hold the avalanche of ischemic injury at rest. Clinical significance of this finding can be inferred because this technology has correlated with viability of renal allografts after transplantation in human recipients.[47]

Tacrolimus can improve hepatocyte oxidation/reduction states, with improved ketone body ratios, found to prevent ATP content deprivation under hypoxic conditions.[48] Interestingly, the same model failed to show a similar effect with cyclosporine A.[48]

In cerebral ischemia studies, tacrolimus has shown protective effects from I-R associated with superior recovery of mitochondrial respiration and thus superior regeneration of high-energy phosphates, although a specific mechanism for this effect was not identified.[49] More recently, mitochondrial investigations from the rat brain model have shown that tacrolimus inhibits two different complexes in the electron transport chain, complexes III and V, which result in attenuation of free radical production.[50]

Antioxidant Mechanisms

Evidence of tacrolimus showing antioxidant properties is not entirely concurrent. As in many physiological cascades, retrograde actions exist. The ligand blocked by tacrolimus-calcineurin complex, NF-AT, is bound and activated by antioxidants (pyrrolidine dithiocarbamate, *N*-acetyl-L-cysteine).[51] If NF-AT (also called AP-1) is an antioxidant-responsive transcription factor, then tacrolimus blocks the actions of these antioxidants. However, this mechanism does not speak of an injurious pathway stimulated by tacrolimus, but merely grounds a theory for antioxidant agents that act through AP-1 activation to not be effective if administered in the presence of tacrolimus. Conversely, another ligand blocked by tacrolimus, the oxidative stress-responsive transcription factor NF-κB, behaves in a manner contrary to NF-AT. NF-κB has been shown to be strongly activated by the reactive oxygen species hydrogen peroxide, whereas antioxidants alone suppressed NF-κB, providing a mechanism through which tacrolimus acts as an antioxidant.[51]

Calcium-Mediated Pathways

During ischemia, oxygen depletion leads to inefficient anaerobic metabolism, which handicaps cellular restoration of ATP, resulting in the accretion of acidic products.[52,53] Membrane ion pumps, driven by ATP, become ineffective, which compromises electrolyte gradients between intracellular and extracellular spaces, as well as between intracellular compartments. Attenuated membrane integrity results in cellular edema as ions, including calcium, move into intracellular spaces. Moreover, the developing acidic milieu liberates cytoplasmic calcium stores, normally bound to proteins at physiological pH. Intracellular calcium concentration is of critical significance because it serves as a secondary messenger capable of activating phospholipases, which commence the enzymatic cascades of inflammation and degradation pathways of cell death.

Data to date on the effect of tacrolimus on calcium-mediated cell injury have been mixed. Tacrolimus administered before ischemia has been shown to suppress intramitochondrial calcium concentration, maintain mitochondrial function, and regulate enzymatic systems that initiate inflammatory pathways.[54] However, compared with cyclosporine A, tacrolimus has been shown to be 3,000-fold less effective in the inhibition of mitochondrial release of calcium.[52] These data may imply that attenuation of calcium-mediated pathways is not among the primary effects of tacrolimus, at least compared with cyclosporine. However, mitochondrial calcium may not be the dominant variable.

In nonischemic studies using toxins to alter membrane permeability, extracellular calcium moved across damaged membranes, driven by a steep electrochemical gradient, causing an increase in intracellular calcium levels that was found to be the final common pathway of toxic cell death.[55] Thus, minimizing membrane damage and subsequent cytoplasmic calcium concentrations may be more important in preventing ischemic injury. In the canine liver model, tacrolimus pretreatment has been shown to inhibit accumulation of intracellular calcium, measured at 15 and 30 minutes after reperfusion, with a resultant decrease in hepatocellular injury.[54]

Inflammatory Response to Ischemia

Interaction between parenchymal inflammatory cells, vascular endothelium, and circulating inflammatory cells is an important factor in I-R injury. As reperfusion injury ensues, parenchymal and endothelial cell injury precipitates a chain of events that includes endothelial

expression of adhesion molecules, platelet activation, leukocyte activation, leukocyte infiltration, and subsequent activation of the entire inflammatory armamentarium, culminating in further tissue damage (Fig. 3).[53,56] Tacrolimus, a diverse immunomodulatory agent, imparts mutifaceted actions of attenuation on inflammatory damage occurring after reperfusion, shown in Figure 3.

Platelet Activation

Platelet adherence to the altered sinusoidal lining cell may contribute to microvascular changes that lead to subsequent ischemic damage in the liver.[53] After attachment to the endovascular lining, platelets are activated, releasing inflammatory communication signals that include platelet-activating factor (PAF). Platelet adhesion to vascular endothelium, mediated through fibrinogen deposition on intercellular adhesion molecule-1 (ICAM-1), has been shown to induce microvascular injury and hepatocellular apoptosis after I-R of the liver during early reperfusion.[57] PAF production, which stimulates platelet activation, has been shown in the liver within 12 hours of such an insult.[58]

In a heart model, tacrolimus was synergistic with a PAF-receptor antagonist in reducing I-R injury.[59] A combination of these two agents also has shown synergy in a non–heart-beating donor porcine liver model, allowing survival in transplant recipients after 90 minutes of warm ischemia followed by 4 hours of cold storage.[56] Tacrolimus was injected intramuscularly 18 hours before ischemia in this study.

Cytokines and Intercellular Communication

Adhesion molecules have a cornerstone role in I-R injury by instigating and maintaining the inflammatory response.[60] After endothelial injury, adhesion molecules attract leukocytes from the circulation and bind with them. This not only initiates leukocyte activation, which engages the inflammatory response, but enables the leukocyte to exit circulation through diapedesis to enter tissue parenchyma, accumulate at the site of injury, and propagate further cellular damage.[61] The selectin family is an important class of adhesion molecules that consists of three closely related cell-surface molecules with differential expression by leukocytes (L-selectin), platelets (P-selectin), and vascular endothelium (E- and P-selectin).[61] Antibodies against E-selectin and ICAM-1 have been shown to protect myocar-

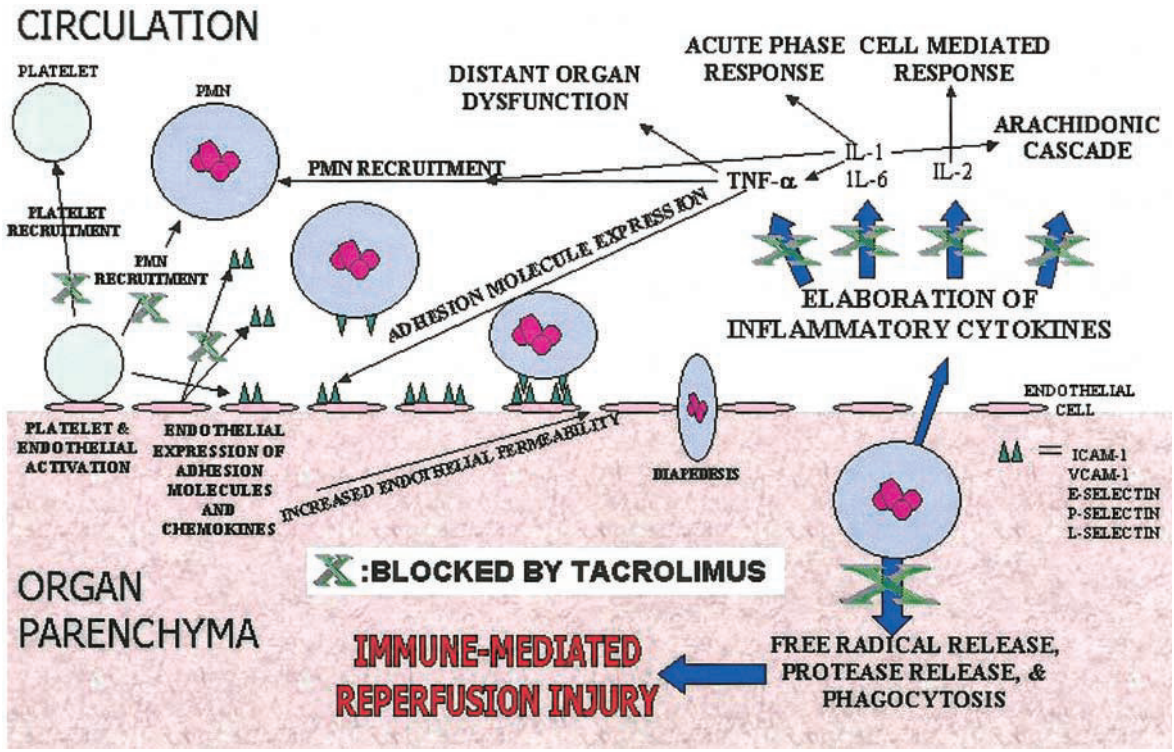

Figure 3. Tacrolimus effect on inflammatory response to the I/R insult. This diagram shows events that follow activation of endothelial cells, platelets, and PMNs, propagating further tissue injury. Tacrolimus has a diverse role in attenuating this cascade.

dium from I-R injury, providing experimental evidence to implicate this pathway as a cofactor in I-R injury.[62-64]

Tacrolimus has been shown to suppress NF-κB and thus reduce transcription of the ICAM-1 gene, which resulted in reduced leukocyte accumulation.[65] More recently, tacrolimus has been documented to reduce expression of the adhesion molecules P-selectin and ICAM-1, with a reduction in leukocyte rolling and adhesion.[66]

Tumor necrosis factor-α (TNF-α) clearly is an important mediator of I-R injury.[67] Specifically, TNF-α has been documented to activate neutrophils to produce local damage during hepatic I-R injury.[68] In addition, release of TNF-α after injury not only mediates local injury, but participates in distant organ dysfunction, as well.[67,69] Also, undefined mechanisms of tissue toxicity by TNF-α exist that are not coupled with neutrophil activity.[70] Neutralization of TNF-α has been documented to decrease hepatocellular damage after I-R injury.[70] Investigations in hepatic ischemia have shown tacrolimus pretreatment reduces IL-1 and TNF-α levels, as well as neutrophil migration.[48] Other studies have shown that tacrolimus pretreatment results in decreased expression of interferon-γ (IFN-γ), TNF-α, ICAM-1, P-selectin, IL-1α, IL-1β, IL-2, IL-3, IL-5, and IL-6.[48,65,66,71-74]

NF-κB Activation

Inflammation is based on multiple costimulatory bidirectional pathways, making it difficult to decipher the key actions of tacrolimus within this area of I-R injury. However, central to many tacrolimus-mediated cytokine alterations is NF-κB. This ubiquitous transcription factor mediates early gene expression of cytokines, chemokines, growth factors, immunoreceptors, and cell adhesion molecules during I-R injury.[65,74-84] Selective blockade of NF-κB with proline dithiocarbamate during the early phase of reperfusion resulted in remarkable tissue protection after severe ischemic stress.[85] In parallel, tacrolimus has been documented to block early activation of NF-κB.[65,74,75] In a rat lung model of I-R injury, NF-κB inactivation by low-dose tacrolimus (0.2 mg/kg) resulted in reduction in IL-1, IL-2, IL-3, IL-5, TNF-α, and IFN-γ levels, resulting in improved endothelial continuity, suppressed inflammation on reperfusion, and reduced tissue damage from I-R insults.[74]

Unfortunately, an implication of NF-κB inactivation accounting entirely for the tacrolimus effect on acute inflammation would be gross oversimplification. Some of the same cytokines elaborated consequential to gene activation by NF-κB have the ability, in return, to stimulate transcription of NF-κB.[81]

However, evidence suggests cytokine transcription is regulated through occupancy of the enhancer region by multiple promoters, and all must be bound for activation.[86] It therefore has been suggested that in such cases as ICAM-1 and IL-2 activation, NF-κB protein may be required for the persistence of stable binding with other factors.[81] If this is the case, clarity is gained on the potent effects of NF-κB blockers, such as tacrolimus, to broadly inhibit immune response.

Polymorphonuclear Neutrophils

The release of free radicals is not only a product of substrate variation, as described, but also a secondary effect of resident macrophage and circulating leukocyte activation.[53] Among circulating leukocytes, polymorphonuclear neutrophils (PMNs) possess an imposing capacity for cytodestruction through free radical release, making them a formidable presence to tissues exposed to I-R.[87,88] In addition to free radical release, activated PMNs elaborate proteolytic enzymes, and inflammatory mediators result in further tissue injury after reperfusion of ischemic tissue. Inflammatory mediators cause microvascular alteration, increase vascular permeability, activate leukocytes, and facilitate leukocyte migration.[89-92] Data from neutrophil inactivation studies have implicated PMNs to be primarily for the late phase of I-R injury.[70] Some investigators suggested that PMN infiltration and the cumulative resultant free radical production are the most important mediators of I-R injury.[66,93]

As discussed, expression of adhesion molecules on endothelial surfaces represents one of the initial steps leading to local PMN accumulation in injured tissue. Therefore, the demonstrated ability of tacrolimus to suppress cytokine release and adhesion molecule expression results in reduction of PMN infiltration and activation.[48,67,74] As such, tacrolimus pretreatment has reduced PMN infiltration into tissues exposed to I-R in small-bowel and liver models.[94,95] Beyond interrupting PMN activation by suppressing communication molecules, tacrolimus appears to show inhibitory actions specific to the PMN.

Tacrolimus has been shown to inhibit superoxide free radical production in PMNs, thus blunting I-R damage.[96] Migration of PMNs on extracellular matrix proteins occurs through cell release from the matrix protein vitronectin. This process is triggered by increased concentrations of intracellular free calcium through calcineurin-dependent processes and therefore is inhibited by the presence of tacrolimus through the formation of a tacrolimus-FKBP-calcineurin complex.[97]

Glucocorticoid Stimulation

As potent and diverse immunosuppressants, glucocorticoids should have the capacity to attenuate cell-mediated I-R injury through the suppression of inflammatory pathways. Cellular effects of tacrolimus may overlap with glucocorticoid action, which would represent a separate pathway through which tacrolimus mediates consequences of I-R injury.

Glucocorticoids have the capacity to inhibit phospholipase A_2 activity and therefore suppress the entire arachadonic acid cascade, including leukotrienes, bradykinins, kallekreins, prostaglandins, and thromboxanes. Activated glucocorticoid receptors in the cytoplasm activate the gene for IκBα, a protein that then inhibits NF-κB, resulting in broad inhibition of cellular communication proteins.[83,84] Calcineurin, which is blocked by tacrolimus, can activate NF-κB by inactivating IκB, representing an overlap in the mechanism of tacrolimus and corticosteroids.[22] More proximally in the mechanism of drug action, receptors for both tacrolimus and corticosteroids appear to be biochemically linked. One member of the immunophilin family with the capacity to bind tacrolimus (FKBP59) has been shown to congregate with heat-shock proteins (HSP90 and HSP90) during inactivation to form a glucocorticoid receptor.[98] Additional evidence has shown potentiation of glucocorticoid receptor–mediated gene expression by tacrolimus.[99] In studies of end-organ effect, tacrolimus enhances glucocorticoid-mediated suppression of histamine-induced tissue edema (capillary leakage from endothelial disruption).[40]

Cellular Response to Injury

Mitochondrial Permeability Transition

A dramatic shift in permeability of the inner mitochondrial membrane occurs when a large permeability transition (PT) pore opens.[100] Reactive oxygen species, elevated calcium concentrations, and pH shifts stimulate PT pore opening, which depolarizes the membrane completely, disabling oxidative phosphorylation of ATP.[101] Retention or recovery of membrane polarization directly correlates with cell viability.[102] At nanomolar concentrations, cyclosporine has blocked mitochondrial depolarization through permeability transition.[103] The effect of tacrolimus on the PT pore and mitochondrial PT has not been studied, but on the basis of effects exerted by cyclosporine A, this is an interesting prospect for tacrolimus-mediated tissue protection that deserves investigation.

Apoptosis Versus Necrosis

Necrosis is the culminating result of deleterious reactions initiated by I-R in which cells are mechanically destroyed, analogous to a building burning down. Conversely, apoptosis is a form of cellular differentiation concluding in the orderly resorption of the cell, analogous to evacuating the inhabitants of a building, then taking it down in a safe controlled manner. The cumulative impact of cellular changes induced by tacrolimus help prevent cells from acutely succumbing to the total injury induced by I-R (necrosis). However, as an independent mechanism to minimize cell death, tacrolimus may alter steps leading to apoptosis.

Such proteins as TNF-α, Fas-ligand, apoptosis-inducing factor, and cytochrome *c* can trigger apoptosis through activation of cysteine-aspartate proteases (caspases).[104-106]

Investigations in a rat renal model of I-R have shown significant decreases in Fas-ligand and caspases compared with controls, with a resultant reduction in apoptosis when low-dose tacrolimus is administered systemically before ischemia.[73] Interestingly, this study found tacrolimus pretreatment resulted in postreperfusion Fas-ligand levels similar to the sham group, which received no ischemic insult. Cytochrome *c*, a protein in the electron transport chain of the inner mitochondrial membrane, may be released after mitochondrial PT or rupture of mitochondrial membranes.[101] Cyclosporine A prevents the membrane PT induced by TNF-α, blocking cytochrome *c* release and subsequent caspase activation and averting apoptosis.[107] Although not specifically studied, this mechanism is a possible means for tacrolimus to inhibit apoptosis, being a well-documented inhibitor of TNF-α.[44,71-74]

Inhibition of calcineurin-mediated dephosphorylation of NOS causes decreased NOS activity.[107] Recent studies in a cancer model have shown inducible NOS activity to correlate directly with apoptosis.[108] However, tacrolimus-induced apoptosis suppression through an NOS mechanism is entirely speculative at this time.

The path of apoptosis, a product of sequential enzyme activation, is a process that can be inhibited or blocked. The same cannot be said for necrosis, which is a result of irreparable membrane damage. Therefore, diversion of distressed cells toward apoptosis may be desirable, considering distress is unavoidable during organ preservation. A likely determinant that separates the cellular courses of apoptosis from necrosis is ATP content.[109] Necrosis occurs in the presence of ATP depletion, whereas ATP levels greater than 20% result

in aversion of necrosis. After necrosis is averted, persistent nontotal deficiency (>20%) of energy substrates leads to apoptosis.[110] The aforementioned ATP-preserving actions of tacrolimus, in combination with potential apoptosis-blocking actions, provide a theoretical mechanism for tacrolimus to limit parenchymal loss after I-R injury.

The ability of tacrolimus to inhibit NF-κB represents a separate possible mechanism to promote aversion of apoptosis. Blockade of NF-κB activation has prevented apoptosis in human cell cultures, although it is unclear from these studies whether this is a primary effect of NF-κB inactivation or a consequence of blocking downstream events precipitated by NF-κB activity.[111]

Heat Shock Proteins

HSPs are a family of stress-response molecules named for discovery in heat-challenged cells, and their production after heat stress was found to protect organisms from a second thermal insult.[112] HSPs subsequently were shown to be induced in response to other physiological stresses, including ischemia.[113,114] Induction of HSPs by heat exposure has protected rat livers from ischemia.[115] A short period (15 minutes) of ischemic preconditioning has induced HSP72, significantly improving hepatic tolerance to 30 minutes of complete warm ischemia.[116] In the same study, heat-conditioned animals responded similarly to those preconditioned with ischemia. Transgenic mice with increased HSP70 expression are less susceptible to ischemic injury.[117] Tacrolimus has been found to enhance expression of the inducible form of HSP70 in cultured hepatocytes.[50] Induction of HSP70 has been seen in rat kidneys after a low nonnephrotoxic dose of tacrolimus (0.3 mg/kg) administered intravenously, and these animals subsequently showed significantly better renal function when exposed to I-R injury.[72] The same study showed that tacrolimus-pretreated animals had better postischemic renal function than animals pretreated with cyclosporine A (3 mg/kg).

Although the full spectrum of HSP action is not defined, the presence of these protective proteins may provide an independent mechanism of tacrolimus-mediated protection from cellular stresses.

Hepatotropic Effects (Tissue Regeneration)

After damage of an I-R insult is complete, organ survival can be enhanced through tissue recuperation by replacing cells lost to necrosis or apoptosis. Only the liver has a capacity for regeneration, whereas all other solid organs currently transplanted must rely on cellular recovery. Tacrolimus facilitates recovery by minimizing I-R damage through the aforementioned mechanisms, but within the liver, it has shown fascinating abilities to promote hepatic regeneration.[118,119] In these studies, ongoing ischemic injury was present during tacrolimus administration, which partly blurs true hepatotropic effects with the known I/R protective effects. However, increased mitosis was shown in tacrolimus-treated groups, implying stimulated regeneration as an independent effect of tacrolimus.

Cyclosporine A also has shown hepatotropic effects.[120,121] Comparison of low-dose cyclosporine A (0.06 to 0.6 mg/kg/d) with low-dose tacrolimus (0.01 to 0.06 mg/kg/d) directly infused into the portal vein under partially ischemic conditions showed no difference in hepatotropic properties.[121] However, when greater doses of cyclosporine A (4 mg/kg/d) are compared, significantly greater mitosis is stimulated by high-dose tacrolimus (1 mg/kg/d).[119] These doses are not reasonable to draw conclusions applicable to the clinical setting, but the prospect of hepatotropic effects are an important variable to consider in the effects of tacrolimus as we enter the era of living donor liver transplantation.

Clinical Application

Effects of tacrolimus on I-R injury unfortunately are based only on data generated from animal models. Administration of tacrolimus to the donor before harvest appears to ameliorate I-R injury in these animal models, although clinical application has yet to be examined. Supplementation of tacrolimus to the perfusate or flush solution also should provide the donor organ some protection from the pending storm. In our center, we recently showed that flushing the liver before transplantation with a solution containing tacrolimus results in superior early graft function and decreased hepatocellular injury after reperfusion compared with flushing with placebo.[122]

Summary

Calcineurin inhibitors possess diverse characteristics that limit tissue injury resulting from I-R. Tacrolimus is the most potent and effective calcineurin inhibitor in this capacity. Its ability to ameliorate I-R injury is the cumulative result of effects on microcirculation, free radical metabolism, calcium-activated pathways, inflammatory cascades, mitochondrial stability, apoptosis, stress-response proteins, and tissue recovery. Within the nucleus, actions mediating effects of tacroli-

mus appear to be more influenced by its interactions with NF-κB than with NF-AT, the factor credited for its T-cell suppression. Supplementing flush or perfusate with tacrolimus may have a similar effect. Further delineation of the precise cellular mechanisms imparted by this fascinating agent will have widespread therapeutic implications extending well beyond the boundaries of transplantation.

Acknowledgment

The authors thank Eliane Purchase, Dee Perry, and the excellent library staff at Mayo Clinic Scottsdale for their diligent efforts, which made this work possible.

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