



Immunosuppressants – mechanisms of action and monitoring

Peter Pillans, Associate Professor and Medical Specialist, Director of Clinical Pharmacology, Princess Alexandra Hospital, Brisbane

Summary

Although corticosteroids and drugs such as azathioprine still have a role, there is increasing use of newer potent immunosuppressants. Many of these drugs act on T-lymphocytes. Tacrolimus is a calcineurin inhibitor which has a similar mechanism of action to cyclosporin, reducing T-cell differentiation. Sirolimus and everolimus bind to the same protein as tacrolimus, but have a different mechanism of action. As some of these drugs have a narrow therapeutic range, drug concentrations must be monitored. Mycophenolate is an inhibitor of purine synthesis. Another approach is to block the receptors on T-cells with immunosuppressant antibodies such as basiliximab, daclizumab and muromonab-CD3.

Key words: azathioprine, cyclosporin, everolimus, sirolimus, tacrolimus.

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Introduction

Immunosuppressants are essential for successful organ transplantation and the treatment of many autoimmune disorders. They suppress rejection and dampen the autoimmune process, but they also lead to the undesired consequences of immunodeficiency, such as infection or malignancy, and non-immune toxicity.¹ Glucocorticoids and thiopurines such as azathioprine are still widely used, but newer potent drugs have become the cornerstone of many treatments.

Azathioprine

Azathioprine is a prodrug which is converted to 6-mercaptopurine and metabolised to cytotoxic thioguanine nucleotides which are responsible for immunosuppression and inhibiting DNA synthesis. Both cell-mediated and antibody-mediated immune reactions are depressed. Although its use in transplantation has declined, azathioprine is still

widely used as an immunosuppressant or corticosteroid-sparing drug in immune disorders. The main toxicities are bone marrow suppression (particularly agranulocytosis) and hepatotoxicity.

Improved understanding of its pharmacogenetics has led to the safer use of azathioprine. A major influence on thiopurine therapy is the inherited activity of thiopurine methyltransferase. This enzyme shunts thiopurine to relatively inactive compounds. A deficiency of thiopurine methyltransferase is associated with grossly elevated concentrations of thioguanine nucleotides and severe haematological toxicity (agranulocytosis). Where laboratory assays are available, measuring thiopurine methyltransferase activity before starting azathioprine therapy may be advisable to identify patients at risk of acute haematological toxicity.² The other major purine metabolic pathway involves xanthine oxidase. In patients taking azathioprine, use of a xanthine oxidase inhibitor such as allopurinol may result in severe myelotoxicity.

Calcineurin inhibitors

Calcineurin catalyses some of the intracellular processes associated with the activation of T-lymphocytes. When calcineurin inhibitors bind to intracellular proteins called immunophilins, they block the effect of calcineurin. This results in reduced production of interleukin-2 and reduced proliferation of T-cells.

The nephrotoxicity of calcineurin inhibitors has emerged as an increasing cause of late renal allograft loss. The pathogenesis appears to be multifactorial and includes calcineurin-induced vasoconstriction, calcineurin-induced release of endothelin-1 (a potent vasoconstrictor), decreased production of the vasodilator nitric oxide, and increased expression of transforming growth factor beta₁ (a key cytokine associated with interstitial fibrosis).³ Reducing the dose of calcineurin inhibitor, or using protocols including mycophenolate and sirolimus, may minimise the risk of nephrotoxicity and improve allograft and patient survival.

Cyclosporin

Since the early 1980s, cyclosporin has been the primary immunosuppressant used in transplantation. It binds with cyclophilin to inhibit calcineurin.

Cyclosporin has a narrow therapeutic range with large inter- and intra-subject pharmacokinetic variability. Target concentration strategies are therefore used to monitor its use. Traditionally, trough concentrations (C_0) are measured, but measurement of whole blood concentrations two hours post-dose (C_2 monitoring) has been recently promoted. There is no good evidence that C_2 monitoring is superior to C_0 monitoring. Timing of the blood sample for cyclosporin monitoring is critical and, generally, less convenient with C_2 monitoring.

Cyclosporin is a substrate for cytochrome P450 3A4 and the multidrug efflux pump, P-glycoprotein. Absorption and subsequent elimination may therefore be influenced by drugs that affect CYP3A4 or P-glycoprotein. Inhibitors of P-glycoprotein may decrease the efflux of drug from intestinal cells and therefore increase blood concentrations. The whole blood concentration of cyclosporin should be carefully monitored whenever inducers or inhibitors of CYP3A4 are concurrently administered and following their discontinuation. Important inhibitors and substrates of CYP3A4/P-glycoprotein include the azole antifungals (ketoconazole reduces cyclosporin dose requirements by up to 80%), calcium antagonists (diltiazem), ergots, fluvoxamine, HMG CoA reductase inhibitors (atorvastatin and simvastatin), protease inhibitors and macrolides such as erythromycin and clarithromycin. Important CYP450 inducers which may be associated with a significant fall in calcineurin inhibitor concentrations include rifampicin, isoniazid, carbamazepine, phenytoin, barbiturates and St John's wort.

Although some brands of cyclosporin are bioequivalent on a population basis and therefore interchangeable, cyclosporin has a narrow therapeutic range. There is therefore a potential that individual variations in pharmacokinetics could lead to significant alterations in blood concentrations if the patient is prescribed a different preparation. Unplanned generic substitution should not occur. For transplant patients in particular, consult an appropriate specialist before any substitution is considered. If patients are switched from one brand to another brand of cyclosporin, increased monitoring is indicated.

Concentration-related adverse effects include nephrotoxicity, hypertension, gingival hyperplasia, hirsutism, tremor and hyperlipidaemia. Haemolytic uraemic syndrome and post-transplantation diabetes mellitus may also occur.

Tacrolimus

Tacrolimus (FK506) is a macrolide antibiotic but is also a calcineurin inhibitor. It is more potent than cyclosporin and binds to a different immunophilin (FK-binding protein) to inhibit calcineurin.

Adverse effects in common with cyclosporin include hypertension, nephrotoxicity and the haemolytic uraemic syndrome. Tacrolimus is less likely to cause hyperlipidaemia, hirsutism and gingival hypertrophy, but diabetes is more

commonly associated. Trough whole blood concentrations should be monitored along with renal and hepatic function. As with cyclosporin, tacrolimus is also a substrate of CYP3A4 and subject to the same interactions.

Antiproliferative drugs

Sirolimus (rapamycin) and everolimus are structurally very similar and have the same mechanism of action. Like tacrolimus, they bind to FK-binding protein, but they have no effect on calcineurin. Instead, the complex inhibits a protein kinase that is critical for cell cycle progression. This kinase is known as the mammalian target of rapamycin (mTOR). Inhibition of mTOR suppresses cytokine driven T-lymphocyte proliferation and activation, resulting in immunosuppression.

The main difference between sirolimus and everolimus is that the half-life of sirolimus (60 hours) is approximately double that of everolimus (30 hours). Both drugs are cleared by hepatic metabolism and, like cyclosporin and tacrolimus, they are substrates for cytochrome P450 3A4 and P-glycoprotein, so have similar interactions.

As with tacrolimus, monitoring of whole blood concentrations of sirolimus and everolimus is essential because of their significant toxicity, narrow therapeutic window, large inter-individual variations in bioavailability and clearance and the potential for drug-drug interactions. Furthermore, efficacy in preventing acute rejection correlates with blood concentrations. Whole blood is the preferred medium because the concentration in red blood cells is 10–30 times higher than in plasma. For sirolimus (and tacrolimus) the effective therapeutic concentrations can be less than 5 microgram/L, so HPLC-mass spectrometry provides the most specific and accurate results.⁴ Enzyme-linked immunosorbent assay and microparticulate enzyme immunoassay are non-specific and less precise methods.

Mycophenolate

Mycophenolate is the prodrug of mycophenolic acid which inhibits purine synthesis by inhibiting inosine monophosphate dehydrogenase. Currently two products are available in Australia – mycophenolate mofetil and mycophenolate sodium. Both are converted to mycophenolic acid.

Mycophenolic acid is 97–98% protein bound and it is the unbound or free mycophenolic acid that is pharmacologically active. Multiple factors, including hypoalbuminaemia, uraemia and the accumulation of the inactive glucuronide metabolite influence the protein binding of mycophenolic acid in renal failure, and thus alter the free fraction. As with other highly protein-bound drugs, the free fraction of mycophenolic acid inversely correlates with albumin concentrations. The free fraction is increased by reduced renal function and mycophenolic acid glucuronide (which displaces mycophenolic acid from albumin).⁵

Mycophenolate is currently used in a fixed dosage regimen. However, the pharmacokinetics of mycophenolic acid are complex with up to a 10-fold variation in the area under the concentration time curve (AUC) for a given dose. The AUC for mycophenolic acid has a predictive value for the risk of acute rejection.⁶ Concentrations of mycophenolic acid can be measured by some laboratories and can be considered if efficacy or toxicity are in question. A limited four-point AUC with samples at 0, 1, 3 and 6 hours can be used, but this test can only be realistically performed with inpatients.

Immunosuppressant antibodies

Antithymocyte globulin

This is a polyclonal IgG antibody from horses or rabbits immunised with human thymocytes. Infusions of antithymocyte globulin cause profound T-cell depletion and the lymphopenia typically persists beyond one year. An unwanted effect is the release of cytokines. This is associated with the 'cytokine release syndrome' characterised by fever, rigors and hypotension.

Antibodies against CD25

Basiliximab and daclizumab are monoclonal antibodies against CD25, a receptor on the surface of T-lymphocytes. They are indicated for prophylaxis of acute rejection in renal transplantation. The antibodies bind to and block the interleukin-2 receptor α -chain (CD25 antigen) on activated T-cells. This results in inhibition of interleukin-2 induced T-cell activation. These antibodies appear to be relatively well tolerated and hypersensitivity reactions are uncommon. No monitoring is required.

Muromonab-CD3

This is a mouse-derived monoclonal antibody which binds to the CD3 component of the T-cell receptor complex leading to T-cell depletion. Muromonab is also associated with the cytokine release syndrome which can range from a mild self-limiting flu-like illness to more serious manifestations including pulmonary oedema and neuropsychiatric adverse reactions. Neutralising antibodies can develop which block the effect and limit the re-use of muromonab-CD3. A longer-term concern is the increased incidence of lymphoma.

Conclusion

Advances in transplantation and the treatment of immune disorders have paralleled the development of immunosuppressant drugs. While the newer drugs are associated with superior efficacy, this may come at the cost of a greater incidence of opportunistic infections and malignancy, and adverse effects such as chronic allograft nephropathy, hyperglycaemia and hyperlipidaemia. Accurate concentration monitoring of cyclosporin, tacrolimus, sirolimus, everolimus and probably mycophenolate is necessary to improve outcomes and minimise toxicity.

References

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Conflict of interest: none declared

Self-test questions

The following statements are either true or false (answers on page 115)

3. Sirolimus and tacrolimus bind to the same protein, but have different mechanisms of action.
4. Sirolimus, tacrolimus and everolimus are all substrates for cytochrome P450 3A4.

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