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A Scientific Report on Immunosupression Used For Liver Transplantation

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A B S T R A C T

Continued advances in surgical techniques and immunosuppressive therapy have allowed liver transplantation to become an extremely successful treatment option for patients with end-stage liver disease. Beginning with the revolutionary discovery of cyclosporine in the 1970s, immunosuppressive regimens have evolved greatly and current statistics confirm one-year graft survival rates in excess of 80%. Advances in immune suppressants over the past decade have resulted in dramatic improvements in short- and long-term outcomes in organ transplantation as well as a decreased incidence of acute rejection. However, immunosuppressive drugs need to be given long term, lack specificity, and are accompanied by adverse metabolic derangements, toxicities, the risk of infection and cancer, and a myriad of other side effects.

Keywords: Immunosuppressive therapy, liver disease, organ transplant, adverse metabolic derangements.

A R T I C L E I N F O

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1. Introduction

Immuno suppression is an act that reduces the activation or efficacy of the immune system. Some portions of the immune system itself have immunosuppressive effects on other parts of the immune system, and immunosuppression may occur as an adverse reaction to treatment of other conditions. Immunosuppressants are used to control severe manifestations of allergic, autoimmune and transplant-related diseases. Some drugs have a diffuse effect on the

immune system while other drugs have specific targets. Drugs with diffuse effects are more likely to cause adverse effects, but the effectiveness of the more specific drugs may be reduced if their action can be bypassed by alternative metabolic pathways. Treatment protocols are therefore frequently use drug combinations to minimize adverse effects and to prevent resistance to treatment. Although protocols are essential to allow scientific evaluation, the clinician must be prepared to tailor treatment based on the ongoing assessment of drug effects, disease activity and the robustness of the individual patient.

Liver transplantation (LT) is a life saving procedure for patients with end stage liver disease and its complications, for liver failure. LT is also cure for some hereditary metabolic disorders like familial hypercholesterolemia and for selected cases of malignancies involving the liver, such as hepatocellular carcinoma (HCC) and hepatoblastoma. Recipients of orthotopic LT have excellent survival rate (83% for 1 year and 75% for 5 years) that has improved markedly over the past three decades [1].

Deliberately induced immuno suppression is generally done to prevent the body from rejecting an organ transplant or for the treatment of autoimmune diseases such as rheumatoid arthritis or Crohn's disease. This is typically done using drugs, but may involve surgery (splenectomy), plasmapharesis, or radiation. Many of the currently available immune suppressants were developed for use in oncology or transplantation. As this treatment is potentially life-saving desperate measures can be justified. However, there are now over 80 autoimmune diseases and several common allergic conditions in which immuno suppressants could play a role although they may not be life-saving. Clinically they are used to:

- Prevent the rejection of transplanted organs and tissues (e.g. bone marrow, heart, kidney, liver)
- Treatment of autoimmune diseases or diseases that is most likely of autoimmune origin (e.g. rheumatoid arthritis, myasthenia gravis, systemic lupus erythematosus, Crohn's disease, and ulcerative colitis).
- Treatment of some other non-autoimmune inflammatory diseases (eg. long term Allergic Asthma control).
- Cortisone was the first immunosuppressant identified, but its wide range of side effects limited its use. The more specific azathioprine was identified in 1959, but it was the discovery of cyclosporine in 1970 that allowed for significant expansion of kidney transplantation to less well matched donor-recipient pairs as well as broad application of liver transplantation, lung transplantation, pancreas transplantation, and heart transplantation. Some immune suppressants act through immune depletion of effector cells, while others are predominantly immune modulatory, affecting the activity of cells, usually through cytokine inhibition. Immunosuppressive drugs can be classified into five groups[2] **:**

I. Glucocorticoids
II. Cytostatics
III. Antibodies
IV. Drugs acting on Immunophilins
V. Other drugs

2. Glucocorticoids

Background: Corticosteroids (CS) remain the most widely used non-CNI immunosuppressant in LT. After early pioneering studies showed CS could prolong skin graft survival in rabbits, Starzl et al. and Murray et al. independently demonstrated in 1963 that CS with AZA could extend patient and allograft graft survival after human allograft renal transplantation [3-4].This combination of CS with AZA remained the cornerstone of IS for organ transplantation until the introduction of Csa in the early 1980's. However, CS continue to be used as first line therapy for the treatment of ACR and in patients transplanted for auto immune diseases, often used as an adjunct in maintenance with an agent such as CNI to prevent rejection.

Mechanism of Action

CS act primarily on T cell activation by inhibiting the production of T cell cytokines such as IL-2, IL-6, and interferon-gamma which are required to enhance the response of lymphocytes and macrophages to allograft antigens. They also suppress antibody and complement binding and stimulate the migration of T cells from the intravascular compartment to lymphoid tissue[5].

Side Effects

A variety of side effects have been reported with corticosteroids (Table 2). Osteoporosis is a common complication with an incidence greatest in the first six months post-LT and patients maintained on more than ten milligrams per kilogram per day should be regularly screened and where appropriate, offered treatment**.**

3. Cytostatics

Cytostatics inhibit cell division. In immunotherapy, they are used in smaller doses than in the treatment of malignant diseases. They affect the proliferation of both T cells and B cells. Due to their highest effectiveness, purine analogs are most frequently administered. It includes the following: Alkylating agents; Antimetabolites and Cytotoxic antibiotics.

a. Alkylating agents

The alkylating agents used in immunotherapy are nitrogen mustards (cyclophosphamide), nitrosoureas, platinum compounds and others. Cyclophosphamide is probably the most potent immunosuppressive compound. In small doses, it is very efficient in the therapy of systemic lupus erythematosus, autoimmune hemolytic anemias, Wegener's granulomatosis and other immune diseases. High doses cause pancytopenia and hemorrhagic cystitis.

b. Antimetabolites

Antimetabolites interfere with the synthesis of nucleic acids. These include: folic acid analogues, such as methotrexate; purine analogues such as azathioprine and mercaptopurine pyrimidine analogues; protein synthesis inhibitors. Methotrexate is a folic acid analogue. It binds

dihydrofolate reductase and prevents synthesis of tetrahydro folate. It is used in the treatment of autoimmune diseases (for example rheumatoid arthritis) and in transplantations. Azathioprine, is the main immunosuppressive cytotoxic substance. It is extensively used to control transplant rejection reactions. It is nonenzymatically cleaved to mercaptopurine that acts as a purine analogue and an inhibitor of DNA synthesis. Mercaptopurine itself can also be administered directly. By preventing the clonal expansion of lymphocytes in the induction phase of the immune response, it affects both the cell and the humoral immunity. It is also efficient in the treatment of autoimmune diseases.

C. Cytotoxic antibiotics

Among these, dactinomycin is the most important. It is used in kidney transplantations. Other cytotoxic antibiotics are anthracyclines, mitomycin C, bleomycin, mithramycin.

4. Antibiotics

Polyclonal antibodies

Polyclonal antibodies, including anti-thymocyte (ATG) and anti-lymphocyte globulins (ALG), have been used since the early days of liver transplantation and are prepared by inoculating rabbits or horses with human lymphocytes or thymocytes[6]**.** Their mechanism of action is rapid lymphocyte depletion due to complement-mediated cell lysis and uptake by the reticulo-endothelial system (RES) of opsonized T cells [7]**.** In addition, they may also cause partial T cell activation and blockade of T cell proliferation [8]. Polyclonal antibodies were routinely used as induction therapy in liver transplantation along with corticosteroids and AZA before the discovery of CYA. Lymphocyte depletion is believed to play a role in preparing the recipient's immune system to adapt and recognize the transplanted organ as self and prevent destruction of the allograft. Accordingly, studies have shown that ATG administration results in regulatory T cell (Treg) expansion *in vitro* and *in vivo*[9]**.** Tregs or suppressor T cells are responsible for preventing activation of the immune system and maintaining tolerance to self-antigens.

Currently, approximately 20% of transplant centers use these agents for induction purposes[10] and recent data support the administration of thymoglobulin induction to delay CNI use and avoid renal toxicity without increasing the risk of rejection or HCV recurrence[11]. A few studies have also successfully shown the benefit of using these medications as induction therapy to avoid post-transplant corticosteroid use without an increased incidence of acute rejection. This is especially important in HCV recipients where high-dose pulsed corticosteroid therapy can significantly accelerate liver fibrosis. At present, anti-lymphocyte antibodies are used extensively to treat steroid-resistant acute rejection and are successful in 70%-96% of patients [12].

The main side effect of these medications, affecting 80% of patients, is a "first-dose reaction" and febrile episode which can often be ameliorated by pre-medication with antipyretics, antihistamines and intravenous steroids. This effect is likely due to pyrogen release from the massive destruction of lymphocytes [13]. Other adverse effects include thrombocytopenia, anemia, CMV infection, PTLD, pruritic skin rashes, serum sickness and anaphylaxis [14].

Monoclonal antibodies

Monoclonal antibodies include the anti-IL-2 receptor (CD25) antibodies, anti-CD52 antibody and muromonab-CD3 (OKT3). The two anti-IL-2 receptor antibodies approved for clinical use are basiliximab (Simulect), a chimeric protein, and daclizumab (Zenapax), a humanized protein. Both antibodies are specific for the α chain of the IL-2 receptor, CD25, which is only expressed on activated Tcells[15]. These antibodies remain in the circulatory system for weeks after initiation of therapy and have been used successfully with low-dose CNIs in preventing acute rejection in the early post-transplant period [16]. They also have fewer side effects compared to the anti-lymphocyte globulins, rarely cause the typical first-dose infusion reactions and are associated with less risk of opportunistic infections and PTLD.

Muromonab-CD3 (OKT3) targets the CD3 molecule on T cells and causes depletion of lymphocytes by massive T cell lysis and cytokine release. This profound cytokine release can lead to pulmonary edema and acute respiratory distress and rarely, intra-graft thrombosis and aseptic meningitis. As a result, antihistamines and intravenous steroids are routinely used as pre-medication to reduce this "cytokine release syndrome". Several days after OKT3 administration, T lymphocytes no longer express CD3 and are considered to be immunologically incompetent.

OKT3 is primarily used in liver transplantation for steroid-resistant acute rejection [17] and has a success rate of complete recovery in 50% of patients. OKT3 use should be limited in the HCV population as several studies have confirmed exacerbation of disease recurrence with this agent. The humanized anti-CD52 antibody, alemtuzumab (Campath-1) targets lymphocytes, monocytes, macrophages, natural killer cells and thymocytes but spares plasma cells and memory lymphocytes. It is unique in that it depletes lymphocytes from the circulation as well as peripheral lymph nodes. Several studies in renal transplant patients have shown its efficacy in preventing rejection when used in combination with low-dose CNIs or sirolimus.

Tzakis et al compared the use of alemtuzumab induction therapy combined with low-dose tacrolimus in liver transplant recipients receiving standard doses of tacrolimus and corticosteroids. Although patients who received alemtuzumab had less renal dysfunction and acute rejection in the first two months post-transplant, the overall incidence of rejection was not significantly different between the two groups. Similarly, Marcos et al proposed that alemtuzumab, in conjunction with minimal CNI use, is a successful treatment strategy in liver transplant recipients, improving overall graft and patient survival, especially in HCV-infected subjects18.

5. Drugs Acting on Immunophilins

a. Cyclosporin [19]

Together with tacrolimus, cyclosporin is a calcineurin inhibitor. It has been in use since1983 and is one of the most widely used immunosuppressive drugs. It is a fungal peptide, composed of 11 amino acids. Cyclosporin is thought to bind to the cytosolic protein cyclophilin (an immunophilin) of immunocompetent lymphocytes, especially Tlymphocytes. This complex of cyclosporin and cyclophilin inhibits calcineurin, which under normal circumstances induces the transcription of interleukin-2. The drug also inhibits lymphokine production and interleukin release, leading to a reduced function of effector T-cells. Cyclosporin is used in the treatment of acute rejection reactions, but has been increasingly substituted with newer immunosuppressants, as it is nephrotoxic.

b. Tacrolimus

Tacrolimus is a fungal product (Streptomyces tsukubaensis). It is a macrolide lactone and acts by inhibiting calcineurin. The drug is used particularly in the liver and kidney, transplantations, although in some clinics it is used in heart, lung and heart/lung transplants. It binds to an immunophilin, followed by the binding of the complex to calcineurin and the inhibition of its phosphatase activity. In this way, it prevents the passage of G0 into G1 phase. Tacrolimus is more potent than cyclosporin and has less pronounced side effects.

c. Sirolimus

Sirolimus is a macrolide lactone, produced by the actinomycetes Streptomyces hygroscopicus. It is used to prevent rejection reactions. Although it is a structural analogue of tacrolimus, it acts somewhat differently and has different side

effects. Contrary to cyclosporine and tacrolimus that affect the first phase of the T lymphocyte activation, sirolimus affects the second one, namely the signal transduction and their clonal proliferation. It binds to the same receptor (immunophilin) as tacrolimus, however the produced complex does not inhibit calcineurin, but another protein. Therefore, sirolimus acts synergistically with cyclosporine and in combination with other immunosuppressants, hasfew side effects. Indirectly it inhibits several T lympohocyte kinases and phosphatases, preventing the transmission of signal into their activity and the transition of the cell cycle from G1 to S phase. Similarly, it prevents the B cell differentiation to the plasma cells, which lowers the quantity of IgM, IgG and IgA antibodies produced. It acts as an immuno regulatory agent, and is also active against tumors that involve the PI3K/AKT/mTOR pathway.

d) Azathioprine:

Azathioprine is a purine synthase inhibitor and one of the first immunosuppressive agents used in the field of solid organ transplantation. For many years, azathioprine was included in the post organ transplant immunosuppressive maintenance as the only immunomodulatory agent and then later was used as an adjunct with CNIs. However, with the introduction of newer and more potent agents such as tacrolimus, the need for azathioprine was reduced and later it was replaced by MPA when a second agent was needed. Today, azathioprine is less commonly used for LT but may be helpful when there is a need for intensifying immuno suppression and when other agents are not tolerated due to their adverse side effects. In addition, as azathioprine is less costly, in some instances where finances are limited, it is preferred over MPA. The major adverse side effects of azathioprine are related to bone marrow suppression and its hematologic consequences and hepatotoxicity. A minority of patients are at risk of developing severe bone marrow suppression due to genetically reduced or deficient thiopurine methyl transferase (TPMT) activity, the enzyme responsible for metabolizing 6-mercaptopurine, leading to over-accumulation of 6-thioguanine nucleotides (TGNs). TGNs are the active metabolites of azathioprine. Laboratory genotype or phenotype testing for TPMT may help to recognize these patients. In some other patients TGNs may fail to reach their therapeutic levels despite increasing drug dosage. In these patients who are at increased risk of hepatotoxicity due to accumulation of 6-methy-mercaptopurine (6-MMP), TGN level monitoring during treatment and the ratio of 6-MMP/TGN may be useful in helping to recognize this condition. The complexity, availability and cost of these tests should also be considered [20].

Future Direction of Immuno supression (Other Drugs):

Costimulation blockade (Belatacept): Belatacept is a soluble cytotoxic T-lymphocyte antigen-4 (CTLA-4) agent which binds CD80 and CD86 and inhibits T cell activation [4,8]. Belatacept competes with the CD28 receptor on T cells which normally binds CD80 and CD86 on the antigen presenting cell (APC) as a co-stimulatory signal required for T cell activation. Belatacept is administered intravenously once a month and does not carry the renal toxicity of CNIs. Clinical trials in liver transplant patients are currently ongoing with this agent.

Efalizumab[15]

Efalizumab is a humanized leukocyte function-associated antigen-1 (LFA-1; CD11a) specific monoclonal antibody that inhibits T cell-APC stabilization and blocks lymphocyte adhesion to endothelial cells[21]. This agent was approved for the treatment of psoriasis in 2003 and has not yet been used in liver transplantation, although a few clinical trials have been carried out in renal transplant patients with mixed results [22]. Although the results regarding immunosuppression were promising, an increased risk for PTLD was shown when efalizumab was used in combination with high-dose CYA. Other newer agents on the horizon undergoing phase II/III trials include Janus Kinase (JAK) 3 inhibitors, AEB071 (a protein kinase C (PKC) isoforms inhibitor), and Alefacept (a LFA3-IgG1 fusion receptor protein). JAKs are intermediaries between cytokine receptors and signal transducers and activators of transcription (STATs) which lead to immune cell activation[23]. JAK-3, a cytoplasmic tyrosine kinase, is primarily found on hematopoetic cells and its stimulation is specific for the IL-2 family of cytokines which makes it a very attractive target for immunosuppression. Clinical trials are underway in renal transplant patients using these agents. AEB071 (PKC inhibitor) is an oral agent that blocks early T cell activation and IL-2 production[24]. Three phase II

renal transplant trials using AEB were started, two of which had to be stopped due to increased episodes of acute rejection; the third trial is ongoing in Europe[25]. Alefacept, a LFA3-IgG1 fusion receptor protein initially approved for the treatment of psoriasis, interferes with T-cell activation and produces a dose-dependent reduction in T-effector memory cells [26]. A multi-center clinical trial in renal transplant recipients is currently underway. Managing and monitoring patients taking immuno suppressants Patients need to be under constant surveillance, usually by a partnership between the specialist and the general practitioner. Frequency of visits depends on perceived level of risk, but typical parameters to monitor are summarized. Patients may need prophylaxis against the adverse effects of their treatment. Therapeutic drug monitoring is available now for a number of drugs, for example cyclosporin, tacrolimus, sirolimus and mycophenolate. This allows for 'concentration-controlled' regimens. Some common drugs, for example corticosteroids, still have no good measure of individual bioavailability.

6. Conclusion

Rapid advances in the development of immunosuppressive medications promise that the future of LT will continue to be bright. However, in parallel with these discoveries are an urgent need to perform well-designed randomized studies and also to consider the emerging role of pharmaco-genomics which has quickly played an important role in the use of drugs such a AZA. It is likely that recurrent HCV will continue to play a pivotal role in LT for the foreseeable future and transplant professionals will need to be aware of the impact future drugs may have on this disease.

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