PHARMACOLOGICAL IMMUNOSUPPRESSIVE AGENTS

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A vast range of different compounds seem capable of depressing the immune response, however, it has been disappointing that so few of these have shown potential as immunosuppressive agents in man. The action of many has been to suppress lymphocyte reactivity in vitro and of those that are capable of prolonging graft survival in animals in addition, just a handful have been found to be safe and effective in man. It is not intended in this paper to discuss in great detail the properties of most of these compounds since many excellent reviews already exist,1-5 rather I would wish to concentrate on those drugs that have proved useful as immunosuppressive agents in man.

ANTI-METABOLITES

These compounds interfere with protein synthesis by competing for and blocking specific receptors. They include the purine antagonist 6-mercaptopurine and azathioprine, the pyrimidine antagonist 5-fluorouracil, cytosine arabinoside, and the folic acid antagonist methotrexate. Since these agents are cycle specific and only effective against proliferating cells, they are most effective when given after, rather than before, the exposure to antigen.

6-mercaptopurine and azathioprine

6-mercaptopurine is an analogue of the purine base hypoxanthine in which the 6-hydroxil group has been replaced by a thiol group. Azathioprine is the same compound with an imidazol group attached to the sulphur atom. It is rapidly converted back into 6-mercaptopurine and other compounds following ingestion and for this reason the activity of the two compounds are largely the same. Nonetheless, various differences have been described in the actions of the two compounds and these have been summarized by Berenbaum.5 During the breakdown of 6-mercaptopurine thioinosinic acid is produced which competes with its analogue inosinic acid for the enzyme which converts inosinic acid to xanthylic acid. This latter step is important in the synthesis of DNA, and its inhibition profoundly affects RNA synthesis as well. All immune responses requiring cell proliferation may be inhibited including antibody production, graft rejection and the induction of auto-allergic disease. Azathioprine and 6-mercaptopurine also exert a non-specific anti-inflammatory effect but this is probably not an important part of its immunosuppressive action. As has been mentioned previously the optimum time for administering these drugs is after exposure to antigen and it has been shown that antibody production in man is effected very little if they are given before.6 Nonetheless “pretreatment” with azathioprine has been shown to be effective in
prolonging renal transplant survival in dogs and as a result some transplant centres elect to start treatment a few days before transplantation in those patients who are planned to receive a kidney from a living relative. Azathioprine and 6-mercaptopurine have been shown to be capable of prolonging the survival of organ allografts in many experimental animals although the effect varies considerably between species. Rats for example are affected very little by these drugs. Even in human organ transplantation azathioprine is rather ineffective on its own. It has been the practice in some kidney transplant centres in the past to give azathioprine alone but graft survival was on the whole rather poor. Kreis et al, described a series of 54 patients in whom only azathioprine was administered after transplantation. Because of a high incidence of early renal failure episodes, 88% of these patients subsequently received steroids during the first week although not all these episodes were likely to have been due to rejection. 6-mercaptopurine and azathioprine exert their main toxic effects on the bone marrow to cause leukopenia, thrombocytopenia and occasionally anaemia. Approximately 20% of kidney transplant patients experience leukopenic episodes, the frequency of which are related to the dose of azathioprine given as well as the degree of function of the transplant. Fortunately the bone marrow usually recovers quickly when the drug is withdrawn or the dosage reduced. Azathioprine is more toxic when administered with allopurinol since the degradation of azathioprine is blocked by the drug. Very occasionally azathioprine can cause liver dysfunction and when this occurs it is common practice to substitute cyclophosphamide for azathioprine.

*Methotrexate*

Methotrexate is an analogue of folic acid in which a methyl and amino group respectively replace a hydrogen atom and a hydroxyl group. It binds to the enzyme folic reductase which has the effect of blocking the recycling of folic acid derivatives. Since these derivatives are involved in the conversion of deoxyuridine to thymidine, DNA synthesis and cell proliferation are impaired.

Apart from its immunosuppressive activity, the drug is also an inhibitor of inflammation due to the way it can block responses to histamine and other mediators of inflammation.

Like azathioprine, methotrexate is active against dividing cells and is more effective as an immunosuppressant when given shortly after the antigen. Antibody responses are affected more than cell mediated immunity although methotrexate is incapable of suppressing responses in previously sensitized individuals.

The drug has been shown to prolong skin graft survival in some animals but perhaps because of this rather weak immunosuppressive effect, it has not found a place in routine immunosuppression in man, although it has been employed in bone marrow transplantation. Its principal use is in the treatment of cancer when it is given in a high dose followed by a “folic acid rescue”.

**ALKYLATING AGENTS**

These compounds possess an alkyl radical with active end groups (usually chlorine atoms) which can bind to two or more different molecules causing them to become cross linked. The alkylating agents are mostly cycle specific but their activity is in
general not confined to just one phase. Some agents, such as nitrogen mustard, sulpha mustard and cyclophosphamide are also active against resting (G0) cells. With most alkylating agents DNA synthesis is inhibited to a greater extent than is RNA synthesis but the alkylation of DNA does not necessarily lead to cell death since repair is possible. Although alkylating agents have shown to be most useful in treating malignancies they have been of little value on the whole as immunosuppressants.

Cyclophosphamide

Cyclophosphamide is inactive in vitro but is oxidised in the liver into active metabolites which reach peak serum levels one hour after ingestion. These are excreted in the urine together with a small amount of unchanged drug. In patients with severe renal insufficiency, the reduced clearance of the metabolites can cause increased toxicity. The activation of cyclophosphamide can be slowed if other drugs are given which are metabolised through the same pathway, eg. steroids and barbiturates, although repeated administration of these drugs will have the opposite effect as the result of enzyme induction. By cross linking DNA, cyclophosphamide interferes with the reproduction of immunologically competent cells and it is most effective in depressing antibody responses in animals if given 24-48 hours after immunisation. Santos and his colleagues have studied the effects of cyclophosphamide administration in man by challenging patients who were to receive cyclophosphamide for malignant disease with bacterial antigens. He also found that antibody responses were best inhibited if cyclophosphamide was given shortly after the antigen. In this respect cyclophosphamide resembles the antimetabolites. However, resting cells can also be damaged and small lymphocytes can be killed by a process unrelated to cell proliferation. Turke and Poulter have suggested that the drug acts more against B-cells than T-cells (at least in the guinea pig), and this would explain the proficiency with which cyclophosphamide can suppress antibody responses in animals. High doses will also suppress cell mediated immunity and prolonged skin graft survival has been noted when cyclophosphamide has been administered to mice, rats, guinea pigs and rabbits. Under certain defined conditions, cyclophosphamide can be used to make animals tolerant to a variety of antigens including allo-antigens but unfortunately these very promising results have never been reproduced in man. Nonetheless cyclophosphamide is still used to prepare patients for bone marrow transplantation. In 1971 Starzl proposed that cyclophosphamide might be substituted for azathioprine with advantage in cadaveric renal and hepatic transplantation. Patient follow-up was only two to three months however and there was no comparable control group. The increased toxicity of cyclophosphamide has probably been responsible for dissuading other transplant centres from using the drug in this way. Cyclophosphamide has been combined with azathioprine and prednisolone in animal experiments and found to have a superior immunosuppressive effect than just azathioprine and prednisolone. Such a combination has been tried in human kidney graft recipients following transplantation but it is not very effective and undoubtedly toxic. Uldall et al, have used cyclophosphamide for treating chronic steroid resistant rejection with some benefit although some serious complications were seen. Like azathioprine, cyclophosphamide can cause leukopenia and thrombocytopenia, and haemorrhagic cystitis, testicular atrophy, nausea and vomiting are other side effects.
STEROIDS

In organ transplantation, steroids are frequently administered in high concentrations as a prophylaxis against rejection or for treatment of rejection after it has occurred. The side effects of such treatment are well documented and it is therefore surprising that the dosage is still largely empirical with different centres using very contrasting regimes. Although many corticosteroids have been synthesized, prednisone and prednisolone are the two most commonly used in transplantation and their actions are comparable. Unlike the antimetabolites, steroids have a large number of actions at the biochemical level. They bind to specific cytoplasmic receptors which transport them to intranuclear receptors where, at toxic levels they inhibit a variety of enzymes with a resulting depression of protein, RNA and DNA synthesis. There is extensive death of small lymphocytes both in the blood and in the thymus, lymph nodes and spleen, although the mechanism for this last effect is not well understood. In some species of animals steroids are able to suppress antibody production but there is little evidence for this in man. Cell mediated immunity however is depressed in most species but the evidence that steroids protect tissue allografts is curiously sparse considering how essential steroids are in clinical transplantation. It is often assumed that in clinical transplantation high dose steroid therapy must be started immediately rejection has been diagnosed if the graft is to be saved, and yet this may not be true. Using a rat heart allograft model we have found to the contrary that a single pulse of methylprednisolone is more effective in prolonging graft survival when given late than when given early in the rejection process.

It is usual practice to give maintenance doses of steroids from the day of organ transplantation, increasing the dose whenever rejection is suspected. Traditionally, steroid therapy is commenced at a high dose (150-250 mg of prednisolone/day) which is gradually reduced over the following weeks to a maintenance dose of 10-30mg/day. Such high starting doses may be quite unnecessary since excellent results can be obtained for cadaver kidney transplantation when patients are given just 20mg of prednisolone/day after grafting. A controlled clinical trial comparing a high and low dose regime has demonstrated no advantage from using the higher dose. Even the large steroid dose that is customarily given on the day of transplantation seems to be unnecessary. Steroids seem to be the only agents which can reliably reverse rejection episodes. They can be administered to patients as tablets orally or as an intravenous “bolus” injection. There is some evidence that intravenous therapy gives fewer complications but this has not been borne out in clinical trials.

The numerous toxic effects of steroids have already been eluded to. The stunting effect of steroids in children may be lessened by administering the drug on alternate days although the evidence for this is not very convincing.

DRUG TREATMENT OF THE GRAFT DONOR

It has been argued by Guttmann and others that much of the antigenicity of a transplanted kidney is contributed by a population of “passenger leukocytes” that inhabit the graft. They have shown, in some elegant experiments, that rat kidney allografts deprived of their passenger leukocytes are tolerated by the host, and kidney isografts populated with allogenic leukocytes are “rejected”. After
experimenting with many cytotoxic agents they found that high doses of cyclophosphamide and methylprednisolone given to the donor animals five hours before the removal of the kidney gave the most graft protection. Accordingly they used such a regime to treat human cadaver (brain dead) kidney donors.\textsuperscript{31} The dose of methylprednisolone given was 5g and cyclophosphamide 3g, although this was later increased to 7g.\textsuperscript{32} Kidneys from treated donors fared very well with 71\% functioning one year after transplantation. However, kidneys from non-pretreated donors also did well in this centre and no attempt was made to compare the two in a controlled way. Another poorly controlled study was reported by Zincke and Wood\textsuperscript{33} in which a similar scheme was used to prepare the donors of kidneys used to transplant 21 recipients. These grafts survived better than did those harvested from two groups of untreated donors. Such reports caused considerable interest and more controlled trials of donor pretreatment were soon carried out in other centres.\textsuperscript{34-38} Unfortunately none of these studies were able to confirm these results, and graft survival at one year in both pretreated and control groups was barely 50\% in each of the trials. The value of donor pretreatment in cadaveric renal transplantation therefore remains in doubt at the present time.

**DISCONTINUANCE OF IMMUNOSUPPRESSION**

It has been known for many years that kidney transplants will often survive for many months in dogs and rats following the withdrawal of all immunosuppressive treatment. Patients with long surviving kidney transplants are frequently maintained on very small doses of immunosuppressive drugs and it has been debated as to whether this treatment is really necessary in view of these experimental findings. Occasionally patients have stopped their own treatment and apparently come to no harm.\textsuperscript{37} Owens et al, have reported six patients (five of which had kidney transplants from living related donors) whose immunosuppression was stopped between 3 and 108 months after transplantation.\textsuperscript{38} Only two patients subsequently experienced rejection episodes but one kidney was lost. Similar reports have come from other transplant centres,\textsuperscript{39, 40} but on the whole most people's experience has been much less favourable\textsuperscript{41, 42} and total withdrawal of immunosuppression is not often attempted. It would appear however that azathioprine can be safely withdrawn two or more years after transplantation\textsuperscript{43} and this is sometimes necessary in cases of bone marrow intolerance. Having successfully withdrawn azathioprine in ten patients, Naik et al,\textsuperscript{44} attempted to withdraw prednisolone as well but found that rejection episodes occurred when the dose went below 7 mg/day. Thus steroids, at least, are required indefinitely for long term function of renal transplants in man.

**CONCLUSION**

Pharmacological immunosuppression has been a neglected field for many years. As the result of the efforts of large pharmaceutical companies, many new and effective remedies have been introduced for the treatment of infection, malignant disease, peptic ulcer, etc., but the needs of transplantation have been overlooked. Many hundreds of compounds possess immunosuppressive activity of sorts but none has been found until recently to challenge azathioprine and steroids as the basis for immunosuppression in man. Fortunately recent experimental and clinical studies with Cyclosporin-A have proved the exception and the use of this and similar
compounds will undoubtedly lead to an improvement in pharmacological immuno-suppression in the years ahead.

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