Intralymphatic Injection of Radioisotopes as Immunosuppressive Therapy in Renal Transplantation

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Lymphocytes of the host play an important role in the cell mediated as well as the humoral stage of the transplantation reaction.

Lymphocytes have a very long life, a fraction of the lymphocytes is constantly recirculating between blood and lymphoid organs, and the lymphocytes are the most radiosensitive cells of the blood. For these reasons it is possible to influence a large part of the total lymphocyte population with a prolonged effect, by producing protracted damage to the lymphoid organs.

Intralymphatic injections of radioisotopes to deplete lymphocytes have already been described in dogs (Tilak & Howard, 1964; Tilak et al, 1966), in rabbits (Wheeler et al, 1965) and in dogs and in patients undergoing renal transplantation (Shikata, 1968). Because we had prior experience of this type of treatment in cancer therapy, we decided to examine whether the method could be used as an immunosuppressive measure in kidney transplantation.

The purposes of our investigation were to find out, whether it was possible to give a safe intralymphatic injection of a suitable radioisotope, whether this isotope would remain in the lymph nodes for a suitable period, and whether a reasonable, prolonged effect could be obtained.

METHODS

We wanted to use an isotope with a short half-life to limit possible unknown secondary effects. We wanted to obtain a radiation effect on the lymph nodes without giving an internal wholebody irradiation, and therefore chose a beta-emitter; but with sufficient gamma-irradiation to make its distribution visible on a gamma camera. For these reasons we decided to use $^{198}$Au. The isotope was kept in a colloidal solution, and was concentrated so that the amount for our purposes was contained in a volume of 0.3 ml.

We injected the isotope into lymph vessels on the dorsum of the foot. The usual lymphography technique was used with omission of the indicator dye.
In our first experiments, we injected first an X-ray contrast material to ensure that the isotope injected next was given intralymphatically. This was soon abandoned because it became evident that this method produced too much interference with the normal functioning of the lymphatic system, causing extravasation of the subsequent injection of radioisotope. We experimented with different pressures on the piston of the syringe during the injection, and finally found out, that the best way was to give the injection manually, because we could then assess the ability of the lymphatic system to absorb the isotope. The slightest resistance caused extravasation, and therefore injections were only given in lymph vessels with no resistance. Administered in this way, the injected radioisotope always passed to the inguinal and pelvic lymph nodes.

Figure 1. Gamma camera scintigram over the pelvic lymph nodes
The distribution of the radioisotope was controlled by a gamma camera just after the injection, and repeated after one day, and again after a week (Figure 1). The radioactivity of the blood was also measured.

RESULTS

A total of 14 treatments was given to 10 patients, of whom 2 received two treatments and one, three treatments. The total activity per treatment was 1-4 mCi. The patients included 7 women (all infertile) and 3 elderly men. During 8 treatments the patients received $^{198}$Au as their only immunosuppressive therapy. The remaining 6 injections, were given to transplanted patients, who received conventional immunosuppressive therapy as well.

On the basis of the repeated measurements of the radioactivity in the inguinal, iliac and aortic lymph glands by means of the gamma camera, it could be shown that the activity in the lymph nodes was nearly constant during the period of measurements, when correction was made for the half-life of the isotope (Figure 2).

![Activity in lymph node](image)

Figure 2. Radioactivity in the lymph node

In all cases radioactivity was found in the blood, but only about one per cent of the injected amount per litre plasma, corrected for the half-life of the isotope. In some cases a small concentration of activity could be demonstrated in the liver (Figure 3).

On the basis of the injected amounts of activity, assuming an activity on 1 mCi and a volume of 1 cm$^3$ for a lymph gland, it could be calculated that the whole body dose would be 2-8 rad; the dose to a lymph gland 60,000 rad; the dose to the ovaries 200 - 300 rad at maximum (assuming a close relation between ovary and lymph node); to the testicles 5 rad; and to a renal trans-
Figure 3. Radioactivity in the blood

Figure 4. A typical lymphocyte depletion curve. After the initial decrease, the pre-injection values are reattained after two or three weeks.
plant a maximum of 200 - 300 rad (assuming a close relation between graft and lymph node).

We did not see any change in the leucocyte, erythrocyte, thrombocyte counts or in the haemoglobin concentration, but in all cases a selective lymphocytopenia of differing degree and duration was observed.

The decrease in lymphocyte count took place during the first week following the injection. The minimal lymphocyte count obtained, expressed as per cent of preinjection value, was 40 - 80% in those cases where Au was the only kind of immunosuppression, and 20 - 60% in the transplanted cases where other kinds of immunosuppressive therapy were used, in addition. The depletion lasted in no cases more than three weeks (Figure 4).

In a few cases a transient increase of the eosinophil granulocytes was seen following the injection.

We examined the influence of the radioisotope on serum immunoglobulin

![Graph of serum immunoglobulin concentrations](image)

Figure 5. Serum immunoglobulin concentrations during the treatment. No significant change was seen
concentrations, by making immunochemical determinations of IgA, IgG and IgM before the treatment, three days and one week after the treatment. No significant changes were seen in relation to the injections. (Figure 5)

DISCUSSION

As we set out to do, we have found a safe method by which intralymphatic injections of radioisotopes may be given. We have proved that the activity remains in the lymph nodes for a suitable period, and that this will cause a selective lymphocytopenia for a short time.

On the basis of assumptions already mentioned, it can be calculated, that the accumulated dose to a lymphocyte passing the lymph node through the blood stream would be about 6 rad, and drawing on our experience from extracorporeal irradiation of the blood, one can say that the decrease in lymphocytes count is not caused in this way.

The effect seems to be due to damage of those lymphocytes which pass through the tissue of the lymph nodes, and during this prolonged transit period receive a large dose of radiation. The lymphocytes pass from the blood to the lymph through the postcapillary venules in the lymph nodes. There is a large and rapid migration of lymphocytes from the blood to the lymphoid tissues and to the lymph, and the number of recirculations seems adequate for the individual lymphocyte to visit most of the lymphatic tissue of the body.

With the injected doses, which we consider to be maximum permissible in one treatment, a transient lymphocyte depletion was obtained. If a prolonged lymphocytopenia is desired, injections could be repeated every two to three weeks; this is technically possible for a staff with routine experience in lymphography. The method would be unsuitable as pretransplantation treatment of patients waiting for cadaver grafts, but could be of value in the post-transplantation period, and in the treatment of rejection.

It is possible that the use of another radioisotope with longer half-life might cause more prolonged lymphocytopenia, and in this way could be more suitable, perhaps even given in smaller doses than $^{198}$Au.

The advantages of the method are several. It can been given quickly without any discomfort to the patient, and it does not require shunts or fistulas as does extracorporeal irradiation of blood. It does not have any systemic effects – (for example on the bone marrow) although the risk of later outbreak of cancer or leukaemia is unknown. Drawbacks are the possibility of radionecrosis of the involved skin and tissue, the impossibility of making isotope renography studies for three weeks following the injection, and the fact that the method is limited to the relative small group of infertile women and elderly men undergoing renal transplantation.

The risk of possible unwanted secondary effects is considered minimal
in relation to the other risks for the patients, who are receiving RDT or who are undergoing renal transplantation, especially if it can be used as a routine method of immunosuppression.

REFERENCES

Shikata, T. (1968) Tohoku Journal of Experimental Medicine, 94, 55
Tilak, S. P. and Howard, J. M. (1964) Surgical Forum, 15, 160

OPEN DISCUSSION

J MOORHEAD (London): I may have missed this, but did you find this extremely interesting approach effective in the treatment of rejection?

BIRKELAND: Well it is very difficult to answer that. We have so few cases that it is not possible to make a statistical analysis of the results. But we will go on with further studies and can perhaps find something.

MOORHEAD: What was your impression?

BIRKELAND: Well, I hope we can find another isotope. We are looking for something like $^{32}$P, and with this we could perhaps get more prolonged lymphocyte depletion and in this way obtain lasting immunosuppression.

J DORMONT (Paris): Did you check the bone marrow? Was it normal or depressed after the injection of gold? I ask this question because (some years ago I think) a similar study was done in dogs by Calne's group. They found that they had lots of trouble with the bone marrow in the dogs and had to decrease the dose of drugs in the animals. Did you use the usual immunosuppressive regimen or did you decrease the dosage of the drugs?

BIRKELAND: On the basis of the peripheral blood count we have not seen any suppression of the bone marrow; only the lymphocytes were depleted. To answer the second question, we use ordinary drugs in anti-rejection treatment and the gold is an extra. We use nearly the same doses of azathioprine as we used without using the radioactive gold, and I think that with this very small and controlled dose we will not get into trouble with the bone marrow.