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Role of ^{201}Tl in the management of cardiac transplantation

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Abstract. Serial percutaneous endomyocardial biopsy is the standard for diagnosis of cardiac rejection; it is generally done on a weekly basis for the first month and then as indicated by ECG voltage; but, this method is invasive, can be done only in specialized centers, not available for the many patients who return home, requires 18–24 h before a result is obtained, delaying institution of therapy, and it is aggressive for the endomyocardium of the graft. A reliable, rapid, and noninvasive test for detection of cardiac rejection is still not available. The aim of this work was to determine whether ^{201}Tl uptake was significantly correlated with the histological findings presenting in cardiac rejection. Auxiliary heart transplantation was carried out on 60 male rats. Graft viability was evaluated by direct palpation, ECG voltage, and ^{201}Tl uptake during the study (from 4–6 h after transplantation to 1 month). Syngenic rats (without rejection) were used as a control group. Histological studies were done at the end of the isotope study in all animals. The statistical significance of all results was determined by means of Student's *t*-test. A very significant correlation between the severity of the histological findings of rejection and ^{201}Tl uptake was found ($P<0.001$). Our results have demonstrated that ^{201}Tl uptake detected rejection earlier than ECG voltage; moreover, ^{201}Tl uptake was more sensitive in detecting a mild degree of rejection while ECG only detected a moderate degree. We believe that ^{201}Tl uptake should be the screening method for the follow-up of cardiac transplantation; it offers to surgeons the opportunity to select patients for endomyocardial biopsy, with a higher accuracy than the other noninvasive methods.

Key words: Cardiac rejection – Cardiac transplantation – ^{201}Tl

Cardiac transplantation has achieved remarkable progress since the introduction of serial percutaneous endomyocardial biopsy for the diagnosis of cardiac rejection (Caves et al. 1974; Copeland and Stinson 1979; Hastillo et al. 1981). But this procedure has some limitations and complications. This technique is an invasive method, takes 18–24 h to obtain results, must be done in specialized laboratories, not available for the many patients who return home, and repetitions are limited (Baumgarter et al. 1979; Billingham 1981).

A reliable, rapid, and noninvasive method for the detection of cardiac rejection is still not available. Electrocardiography, echocardiography, hemodynamic explorations, measurement of circulating T-cell (E-rosette) levels, and serum enzymes of myocardial origin have not achieved the efficacy of endomyocardial biopsy (Schoeder et al. 1969; Baumgarter et al. 1979; Copeland and Stinson 1979; Hess et al. 1982; Billingham 1981).

The aim of this work was to determine whether ^{201}Tl scintigraphy was correlated with the histological findings presenting in rejection and, therefore, if it can be used in the assessment and management of cardiac transplantations.

Material and methods

Male Wistar and Sprague-Dawley rats (300–350 g) were selected (20 syngenic and 100 nonsyngenic), 60 as donors and 60 as recipients. Auxiliary heart transplantation was carried out using the technique described by Ono and Lindsay (1966) (Fig. 1). The average total graft ischemic time was aproximately 45 min. No immunosuppressive therapy was used.

Graft viability function was evaluated by: (a) direct palpation of the transplanted heart, and (b) electrocardiograms of both host and graft hearts. These tests were done daily within the first week after the operation and weekly until the study was completed (1 month). The electrical activity of both transplanted and natural hearts were registered in standard derivation. The recordings were done at a speed of 100 mm/s and 10 mV voltage amplification.

Direct palpation was carried out following the scheme of Tauber et al. (1978): degree 4, optimal beat; 3, suboptimal; 2, less vigorous; 1, hardly palpation; and 0, no palpation. Degrees 3 and 4 are not really different from each other in a functional way. Degree 3 only characterizes the findings when the heart is embedded in surrounding tissues while still contracting vigorously. Degree 2 is interpreted as a definite sign at the beginning of rejection; not only are the contractions less vigorous, but also a considerable increase in consistency and size of the graft tissue can be felt. Degree 1 is the terminal finding when no real contrac-

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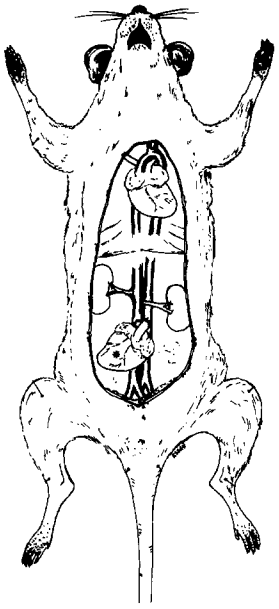


Fig. 1. Ono and Lindsay's technique for heterotopic heart transplantation in rats

tion can be felt, but yet some kind of tickling sensation is still present. At degree 0, complete arrest has occurred.

For ^{201}Tl studies, the rats were divided into five study groups and a control group. Group 1 ($n=10$) was formed by the animals explored 4–6 h after the transplantation; group 2 ($n=10$): 72 h; group 3 ($n=10$): 1 week; group 4 ($n=10$): 15 days; and, group 5 ($n=10$): 1 month after the operation. The control group ($n=10$) was formed by the syngenic rats, in which rejection was absent; the isotope study was carried out 1 month after the transplantation in all these control rats.

The ^{201}Tl studies were done following IV injection of 50 μCi/kg; 15 min later, both host and graft hearts were removed. Cardiac scintigraphy ex vivo of both hearts was carried out with an Anger camera provided with a pin-hole collimator, and recorded with an on-line computer. Afterwards, the uptake per gram of tissue was calculated with a well-counter, and the accumulation of activity in each heart was calculated as a percentage of the sum of both host and graft hearts.

Hematoxylin + eosin stain studies were done at the end of the isotope study in all animals. The rejection signs were evaluated according to the parameters described by Stanford's group (Kosek et al. 1968; Bieber et al. 1970; Billingham et al. 1973) as: mild, moderate, and severe.

The statistical significance of all results was determined by means of the Student's *t*-test.

Results

In the control group, the degree of direct palpation of the transplanted heart was significantly constant (4) during the study, from a few hours after the surgical intervention until the date of isotope exploration (1 month). The degree was 4 in all rats of group 1 and 3 in all rats of group 2. In group 3, five rats showed degree 3, and five rats degree 2. In group 4, only one rat showed degree 3, three rats degree 2, and six rats degree 1. In group 5, two rats showed degree 2, four rats degree 1, and four rats degree 0.

Table 1. ^{201}Tl uptake by graft hearts as a percentage of the total of both natural and transplanted hearts

Group 1 (4–6 h after the transplantation)	43.4% ± 8.2%
Group 2 (72 h)	35.8% ± 6.2%
Group 3 (1 week)	29.2% ± 7.6%
Group 4 (15 days)	25.1% ± 12.2%
Group 5 (1 month)	19.5% ± 9.7%
Control group (1 month)	45.2% ± 4.6%

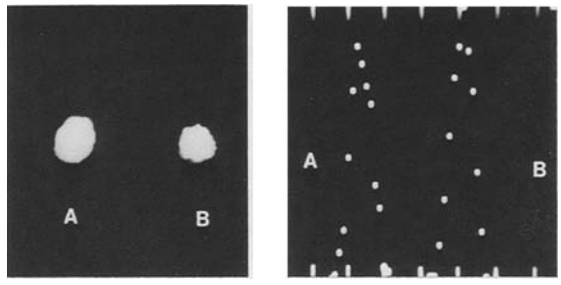


Fig. 2. No significant differences between natural (*B*) and transplanted (*A*) hearts in group 1. *Left*: scintigraphy ex vivo; *right*: uptake profiles (percentage of accumulation of activity of each heart with regard to the sum of both natural and transplanted hearts)

In rats with degree 3 of direct palpation, edema with lymphocyte infiltration was observed. In rats with degree 2 an increased amount of lymphocytes, perinuclear vacuolization, and a little myocytolysis were observed. In rats with degree 1 there were necrosis, more myocytolysis, and marked cellular infiltration. In rats with degree 0, there was fibrosis and calcification.

Anomalies in the ECG examination were observed in all groups 24 h after the surgical intervention, which, in our opinion, were a consequence of cellular damage induced by the surgical intervention; these anomalies disappeared a few hours later.

In all rats in the control group, the ECG examination did not show differences between both host and graft hearts during the remaining study. In groups 1 and 2 (4–6 h and 72 h after the operation, respectively), the ECG examination showed similar results to the control group. In group 3 (1 week after the transplantation), a fall of 20%–30% in QRS voltage was observed; this finding corresponded to moderate histological signs of rejection, and was only seen in rats with degrees 2 and 1 of direct palpation.

In groups 4 and 5 (15 days and 1 month after the operation, respectively), the fall in the ECG voltage was at least 40%, with a mean of 60%. This finding corresponded to severe histological signs of rejection, and to degrees 1 and 0 of direct palpation.

No differences were observed in ^{201}Tl uptake between the control group and group 1 in both host and graft hearts (Table 1). The ex vivo scintigraphy of both hearts was similar, and there were no differences either in activity per gram of tissue or in the percentage accumulation (Fig. 2).

However, a progressive diminution in ^{201}Tl uptake by graft hearts was observed during the study, in comparison with host hearts, in groups 2, 3, 4 and 5 (Fig. 3) (Table 1). This diminution in ^{201}Tl uptake was significantly correlated with the histological findings observed in graft hearts as the study progressed (Table 2).

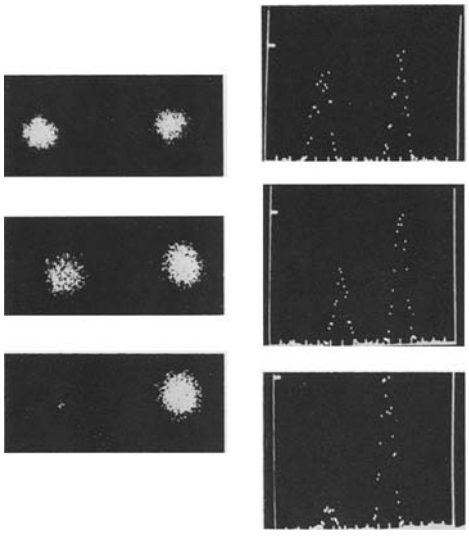


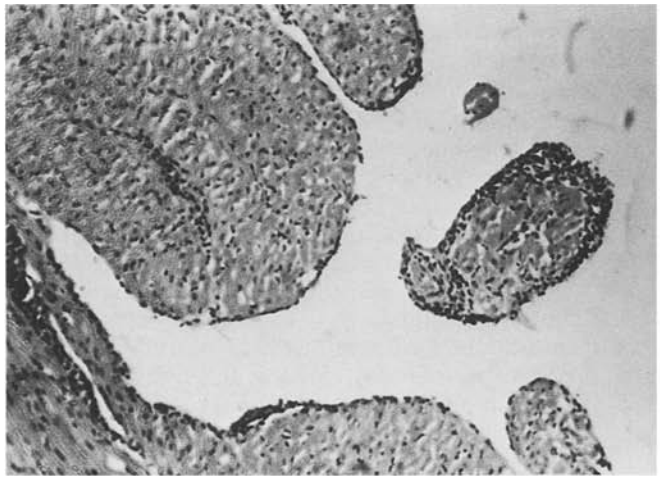
Fig. 3. Progressive diminution in ^{201}Tl uptake in graft hearts compared with host hearts, along the whole study. *Left*: scintigraphy ex vivo; *right*: uptake profiles (percentage of accumulation of activity of each heart with regard to the sum of both natural and transplanted hearts). From top to bottom: group 2, group 3, and group 5

No signs of rejection were observed in either control group or group 1. Mild signs of rejection were observed in group 2, consisting of perinuclear vacuolization, mild fibrinous exudate, small lymphocytes, edema, and endothelial swelling (Fig. 4). In group 3 there were moderate signs of rejection, consisting of lymphocyte infiltration, prominent perinuclear vacuolization, focal myocyte necrosis adjacent to clumps of inflammatory cells, and fibrinous exudate (Fig. 5). In group 4 there were signs according to severe rejection: marked endothelial swelling, heavy cellular and perivascular infiltrate, myocytolysis in focal areas, hemorrhage, and heavy fibrinous exudate. In group 5 there was calcification and fibrosis, with the normal pattern of the myocardium entirely lost (Fig. 6).

In our study, ^{201}Tl uptake decreased when mild signs of rejection were present (group 2); however, the ECG was entirely normal, with no fall in QRS voltage, and direct palpation showed a degree 3 (normal) (Table 2). Therefore, ^{201}Tl was the only test to detect mild signs of rejection (Table 2). ECG and direct palpation detected rejection when only moderate signs were present (Table 2).

Table 2. Relationship between direct palpation, ECG, ^{201}Tl and histological signs

Degree of direct palpation	Fall in ECG	% uptake of ^{201}Tl in grafts	Histological signs
3	0%	43.4% ± 8.2%	No acute rejection
3	0%	35.8% ± 6.2%	Mild
2	20%–30%	25.1% ± 12.2%	Moderate
1, 0	40%–100%	19.5% ± 9.7%	Severe



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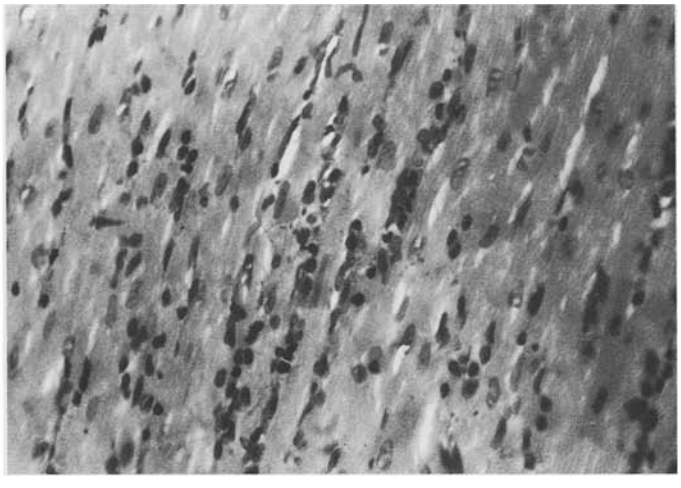

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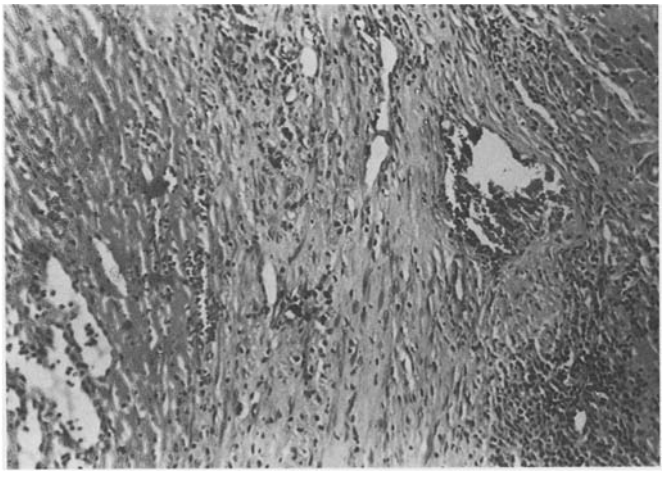

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Fig. 4. Allograft with mild acute rejection. Note the early endocardial mononuclear infiltrate. Hematoxylin + eosin. × 100

Fig. 5. Allograft biopsy showing acute moderate rejection with mononuclear cells extending into the interstitium. Hematoxylin + eosin. × 250

Fig. 6. Severe rejection: myocardial study showing fibrosis with mixed inflammatory infiltrate and myocyte necrosis. Hematoxylin + eosin. × 100

Discussion

Orthotopic heart transplantation has achieved remarkable progress as a therapeutic modality since its introduction by Barnard in 1967. The 1-year survival is today 70%, while in 1978 it was 56%. In patients younger than 40, the 5-year survival is 40%, while in 1975 it was 27%. This evolution has been attributed to more careful selection of the candidates, better knowledge of surgical technique and postoperative complications, especially the early diagnosis and treatment of acute rejection (Resnekov 1972; Stinson et al. 1973; Baumgarter et al. 1979; Hunt and Stinson 1981; Griffith et al. 1982).

Heterotopic heart transplantation has a wide life in the experimental field, but it was only attempted in humans 10 years ago (Barnard and Losman 1979; Cooper 1968; Losman and Barnard 1977). The donor heart acts solely as a left ventricular assist device (Barnard and Losman 1979; Barnard and Cooper 1981; Barnard et al. 1981). Heterotopic heart transplantation connects the donor heart in parallel with the recipient heart (Beck and Gersh 1976).

The original purpose of heterotopic heart transplantation was to permit heart transplantation in patients with high pulmonary vascular resistance in whom an orthotopic transplant would lead to right failure and death.

Early diagnosis of rejection is essential in the postoperative period, in order to increase the dose of immunosuppressive therapy, which is accompanied by its toxic effects.

Techniques for the diagnosis of cardiac rejection are based on three types of data, namely, physical and electrocardiographic findings, endomyocardial biopsy and, immunologic monitoring techniques. But, all three types of data have associated disadvantages.

The immunologic monitoring techniques which has proved most useful in cardiac transplantation, measurement of circulating T-cell levels, is associated with a nontrivial false-positive rate (20%–25%), and therefore cannot be used alone for clinical decisions (Baumgarter et al. 1979; Copeland and Stinson 1979).

A technique for serial percutaneous right ventricular endomyocardial biopsy was modified for use in cardiac transplantation and described first by Caves et al. in 1973. This technique has become the standard for diagnosis of rejection and provides a morphological index for evaluation of the efficacy of the therapy (Baumgarter et al. 1979; Caves et al. 1974, 1973; Hunt and Stinson 1981).

Although few complications have been reported (premature ventricular contractions in approximately 20% of the cases, transient supraventricular arrhythmias in 30%, pneumothorax in 0.4%), this procedure has some limitations: it requires about 18–24 h before a result is obtained, delaying institution of therapy for acute rejection, it must be done in specialized laboratories not available for the patients who return home, and it is necessary to carry the patients to the operating room, which is not always possible (Baumgarter et al. 1979; Billingham 1981).

Furthermore, cardiac biopsy is much more difficult in heterotopic heart transplantation, with isolated left-sided anastomoses requiring an arterial access site and retrograde entrance across the aortic valve into the left ventricle. It is less easily used in day-to-day management.

Biopsy examinations are generally done on a weekly basis for the first month and then as indicated by ECG voltage. Currently, the sum of the peak-to-peak QRS voltage for leads I, II, III, V_1, and V_6 is used. A fall of 20% or more is an indication for heart biopsy (Copeland and Stinson 1979).

But, histological changes of rejection are always present at least 2 days before a decrease in ECG voltage (Copeland and Stinson 1979; Golitsin et al. 1984). The experience of other authors has demonstrated that ECG voltage, although highly sensitive, is not entirely specific for rejection (Copeland and Stinson 1979). Changes in thoracic impedance (pneumonia, pneumothorax, pleural and pericardial effusion) may lower QRS voltage. Voltage can be decreased by a sudden rise in body weight (fluid retention), a fall in hematocrit, sepsis with fever, and problems in electrode contact (Copeland and Stinson 1979).

Other techniques, such as echocardiography, hemodynamic exploration, and serum enzymes of myocardial origin, have been used for the management of cardiac recipients and diagnosis of acute rejection; but, none have achieved the efficacy of endomyocardial biopsy in the diagnosis of cardiac graft rejection and the assessment microscopically of response to modulation of immunosuppressive therapy (Schoeder et al. 1969; Billingham 1981; Hess et al. 1982).

Two histologically distinct patterns of rejection, acute and chronic, have been studied in detail in the animal model (Kosek et al. 1968; Billingham et al. 1973). In acute rejection, the first abnormalities were found to occur at the host-graft interface, the capillary endothelium. Endothelial cells were enlarged with numerous pinocytotic vesicles and became closely related to marginated monocytes; some were occluded with thrombi and ruptured, permitting hemorrhage, or became shrunken and necrotic. The heart was noted to be edematous and focally hemorrhagic. Interstitial edema, exudation of monocytes, and myocytolysis were seen next, followed by phagocytosis of necrotic myocytes by monocytes and polymorphonuclear cells.

Chronic rejection took the form of destructive arteritis and proliferative intimal fibrosis leading to luminal narrowing and, ultimately to foci of myocardial infarction (Kosek et al. 1968, 1971; Bieber et al. 1970). In chronic rejection intimal hyperplasia and atheromatous plaques nearly identical to the abnormalities seen in spontaneous coronary artery disease can be seen, resulting in myocardial infarction and congestive heart failure (Kosek et al. 1968, 1971; Bieber et al. 1970). Chronic rejection, in the form of progressive graft arteriosclerosis, is similar histologically to coronary arteriosclerosis (Kosek et al. 1968, 1971; Bieber et al. 1970).

The clinical experience had led to many refinements in diverse areas of cardiac transplantation technology. Availability of donor hearts has improved by virtue of wide acceptance of a definition of brain death. The recognition and acceptance of the concept of brain death has been a prerequisite to successful heart transplantation. The Harvard Criteria (1968) has been accepted widely: (a) unreceptivity and unresponsitivity; (b) no movements or breathing; (c) no reflexes; and (d) flat ECGs 24 h apart.

In our study, we have found a very significant correlation between the severity of the histological findings of rejection and ^{201}Tl uptake ($P<0.001$), shown on scintigraphy, specific activity, and percentage of accumulation in both natural and transplanted hearts (Table 2).

The ^{201}Tl uptake in the control group was somewhat lower in transplanted hearts (syngenic rats) than in natural

hearts, but with no significant difference (45% ± 4% vs 55% ± 5%, respectively) (Table 1).

In the host hearts, ^{201}Tl uptake remained constant in the five groups during the study (1 month). However, a significant progressive reduction in the ^{201}Tl uptake was observed during the time of the study in the transplanted hearts, according to the worsening of the histological lesions (Tables 1 and 2) (Figs. 2 and 3).

Our results demonstrated that uptake of ^{201}Tl detected rejection earlier than ECG; furthermore, ^{201}Tl uptake was able to identify a mild degree of rejection while ECG detected only a moderate degree (Table 2). Perhaps, the false-negative results of ECG examination in the presence of mild signs of rejection were due to edema.

The findings obtained by the ^{201}Tl studies can be explained because immunologic injury of the capillary endothelium and myocardium leads to edema, capillary microthrombosis, loss of perfusion, increased vascular resistance, and ischemic injury.

201-Thallium offers several advantages in the management of cardiac transplant patients: It has a more widespread application, the patient can be followed more easily and safely, and repeated studies can be obtained to follow the patient's course obviating the minor risk of endomyocardial biopsy and the delay in its interpretation.

201-Thallium is a rapid, simple, and noninvasive method for diagnosis of cardiac rejection and for evaluating the outcome of treatment. It can be obtained repeatedly without risk to the patient or delay in initiating therapy for rejection if indicated. Moreover, it is not necessary to move the patients from their beds.

We believe that it should be a useful adjunct for the follow-up of cardiac transplant patients and it offers to surgeons the opportunity to select patients for endomyocardial biopsy, with a higher degree of accuracy than other noninvasive methods.

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