RAT ALLOTRANSPLANTATION OF EPIGASTRIC MICROSURGICAL FLAPS: A STUDY OF REJECTION AND THE IMMUNOSUPPRESSIVE EFFECT OF CYCLOSPORIN A

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SUMMARY: The rejection of allotransplantation of epigastric microsurgical flaps and the effect of immunosuppression have been studied in 58 rats. Three sets of experiments were planned: (1) Wistar Furth isogenic donors and recipients (control set); (2) Brown Norway donors and Wistar Furth recipients (rejection set); and (3) Brown Norway donors and Wistar Furth immunosuppressed recipients (cyclosporin A set). Cyclosporin A (10 mg/kg/d) treated rats had a transplantation survival rate of up to 30 days: 83.3% among isogenic animals and 60% among allogeneic. There was 100% rejection by the 9th day after the transplantation in allogeneic non-immunosuppressed rats. Biopsies embedded with historesin were taken from the flap and normal contralateral skin (used as control) on the 3rd, 7th, 15th, and 30th days after the surgery. A quantitative study of infiltrating lymphocytes in the flaps, with and without cyclosporin A, was done by evaluating the local inflammatory infiltrate. A significant increase in the number of lymphocytes among the rejection and immunosuppressed groups was seen, as compared to the isogenic set. Local lymphocytosis in allogeneic non-immunosuppressed transplantations reached its highest level on the 3rd day after surgery, before gross findings of rejection, which could only be seen by naked eye on the 5th or 6th day. Therefore, we conclude that cyclosporin A is effective in preserving allogenic transplantation in rats. Biopsies of transplanted areas may contribute to earlier diagnosis of the need for immunosuppressive therapy.

DESCRIPTORS: Allotransplantation. Cyclosporin A.

Organs and tissue transplantations have always been one of the alternatives suggested for the correction of congenital acquired diseases. However, tissues coming from autogenous donor areas are relatively scarce.

Thus, there are clinical circumstances in which it would be desirable to obtain tissues from donors (alive or dead), as in very large burned areas, complex oncologic or traumatic tissue loss, and limb reconstruction due to trauma or aplasia.

The improvement of skin graft survival with the use of cyclosporin A has already been proved. However, attempts at achieving full integration of skin grafts have not always been successful.

The use of immunosuppressing drugs, usually accompanied by the concurrent adverse side effects, has fewer restrictions with organ transplantations that are essential to the patient’s survival. Limb and tissue transplantations, however, have more restraints. Therefore, allogeneic transplantations of tissues and limbs, despite their technical feasibility, have not been done in humans, and research has been limited.

In order to contribute to the study of rejection of cutaneous allotransplantations with and without the use of cyclosporin A, we developed an experimental model. Survival indexes and lymphocyte quantitative studies were performed.

MATERIAL AND METHOD

Fifty-eight epigastric flap microsurgical cutaneous allotransplantations were performed. Three sets of experiments were designed as follows:

1. isogenic I (transplantation: Wistar Furth as donors and receptors), used as controls for the surgery effects;

2. rejection R (allogeneic transplantation: Wistar Furth donors and receptors and
Brown Norway donors), used to study rejection;
– cyclosporin Ci (allogenic transplantation with cyclosporin A treatment, 10 mg/kg/day intramuscular, Wistar Furth receptors and Brown Norway donors).

Both the donor and the receptor were anesthetized with intraperitoneal sodium pentobarbital 30 mg/kg). A flap from the right lower abdominal skin of each animal was obtained, according to the technique.

A detailed description of gross changes of the skin was recorded during the 30 days following surgery. On the third day after surgery, a microscopic review of the anastomoses was performed.

Biopsy specimens of the flap of the contralateral skin (control) were obtained from groups I and Ci on the 3rd, 7th, 15th, and 30th days after the surgery, and from R group on the 3rd and 7th postoperative days.

The specimens were prepared in methacrylate historesin and stained with toluidine blue, and are labelled as follows:
ICo = control skin from the isogenic group;
IT = isogenic group transplantation;
RCo = control skin from the rejection group;
RT = rejection group transplantation;
CiCo = control skin from the cyclosporin group;
CiT = cyclosporin group transplantation.

The postoperative day when the biopsies were taken was added to the code.

A double-blind study was performed for lymphocyte quantitation in the tissue. Lymphocyte quantitation was performed through comparison of means obtained in 18 fields. A graticule adapted to the ocular (10x) with 50 randomly distributed lines was used, and lymphocytes that were intersected by the lines were counted. Due to the great individual variations, a non-parametric statistical analysis was performed with a Mann-Whitney-Wilcoxon test. Statistical significance was declared at p < 0.05.

**RESULTS**

Among the 58 animals that underwent transplantation, 45 (77.6%) presented pervious anastomoses when evaluated on the 3rd postoperative day. Thirteen animals (22.4%) presented thrombosis in artery, vein, or both near the anastomoses, and therefore these individuals were excluded from the study.
Among the 45 remaining rats, 30 survived to the end of the 30 day follow-up period (12 from I, 8 from R and 10 from Ci group) (Table 1).

Daily microscopic analysis of the flaps from I group showed moderate edema, although color and hair development remained similar to the adjacent skin area that had not undergone surgery, other than local moderate edema.

On the 1st and 2nd postoperative days, Group R transplantations resembled those of group I. Subsequently, local edema was much more intense, and on the 4th or 5th day, hyperemia and local signs of tissue involvement were evident, which evolved from local ulceration and necrosis to full necrosis between the 6th and 9th days.

Among the 10 rats from the Ci group, 60% of the flaps survived. Four (40%) presented total necrosis at different times, observed on the 10th, 18th, 19th, and 24th postoperative days (Table 1). These animals showed an intense early edema after surgery (3rd and 4th days after surgery).

Gross examination of rats with 30-day graft survival showed significant differences between the three studies sets, group I= 83.3%, group R = 0 and group Ci = 60%.

Table 1 - Analysis of microsurgic cutaneous transplantations survival in the individuals within 30 days

<table>
<thead>
<tr>
<th></th>
<th>Group NEC</th>
<th>% NEC</th>
<th>N NEC</th>
<th>% N NEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2/12</td>
<td>16.7</td>
<td>10/12</td>
<td>83.3</td>
</tr>
<tr>
<td>R</td>
<td>8/8</td>
<td>100.0</td>
<td>0/8</td>
<td>0</td>
</tr>
<tr>
<td>Ci</td>
<td>4/10</td>
<td>40.0</td>
<td>6/10</td>
<td>60.0</td>
</tr>
</tbody>
</table>

NEC = necrosis; N NEC = non necrosis; R = rejection; I = isogenic; Ci = cyclosporin A.

Figures 2 and 3 are examples of the features observed in the presence and absence of rejection. Microscopic findings are shown on Figs. 4 and 5.

There was a significant difference in the histologic analysis of normal skin and flap biopsies in all postoperative days for groups R and Ci, and only on the 15th day for I group. Lymphocytes in control skin among the 3 groups were scarce or absent in all studied postoperative days.

We have compared counting of lymphocytes among the different groups for each studied postoperative day. There were statistically significant differences between of groups I and R.
and between the controls of $I$ and $Ci$ groups on the 7th day (Table 3).

**DISCUSSION**

The feasibility of a future use of allogeneic 60% tissue and limb transplantations in reconstructive plastic surgery justifies the development of an experimental model for the systematic study of skin rejection.

A 77.6% vascular permeability was obtained—a satisfactory rate, according to the literature, in which 75 to 90% permeability has been seen.

The isogenic flap survival rate, 83.3%, was considered the basal line for evaluation of the other sets of experiments.

Mortality, although high (33.3%), occurred in different postoperative phases before we observed any gross sign of rejection. Therefore, we were able to evaluate transplantation for 30 days among the remaining 30 animals without having to consider mortality as a variant for the rejection factor.

In group $R$, flap loss occurred in 100% of the cases after 6 to 8 days, a rate which is significantly different from group $I$ (Table 1). Gross findings for group $R$ clearly characterized a rejection delay occurring according to the reported literature. However, rejection occurred earlier than the ones observed in organ transplantations, such as heart, kidney, and small bowel rejection in rats. Similarly in the $Ci$ group, rejection occurred earlier than for other tissues and organs, even with the use of cyclosporin A. A higher skin antigenicity may be the reason why flaps are rejected earlier than other tissues, although the mechanism is not yet fully understood.

A 30-day follow-up survival was observed in six flaps from $Ci$ Group. Four flap losses occurred on the 10th,
Therefore cyclosporin A was effective, increasing survival rates of cutaneous allogeneic transplantations.

Third day post-op biopsy lymphocyte counts had significant differences between the allogenic non-treated transplantation group (R) and isogenic transplantation group (I) (Table 2). Comparison between gross rejection findings and lymphocytic infiltration showed that lymphocyte infiltration precedes gross signs of necrosis. The lymphocyte infiltration occurred on the 3rd day post operative, whereas symptoms of gross rejection were first seen on the 6th post-operative day. Therefore, as reported in the literature, biopsy specimens must be taken and examined for early signs of rejection in order to prevent full necrosis of the transplanted tissue.

In group Ci, there was a significant difference in lymphocytes counted between transplanted and control sets. Thus, it was observed that even though cyclosporin A had a satisfactory action on rejection control, its use did not result in a decrease in the number of “in situ” lymphocytes with the method used.

Based on what was found, the authors continued researching immuno-histochemical methods with monoclonal antibodies to identify the “in situ” lymphocytes found in this experimental model.

CONCLUSION

1) The rate of survival of epigastric flap used as microsurgical cutaneous transplantation to isogenic Wistar Furth rats was 83.3% up to 30 days, confirming the efficacy of the adopted experimental model.

2) In our experiments, when flaps of Brown-Norway rats are transplanted to Wistar-Furth receptors, a 100% rejection rate in non-immunosuppressed transplantation occurs before the 9th postoperative day.

3) Cyclosporin A immunosuppression (10 mg/kg/d) increased the allogeneic transplantation success up to 30 days post-operation.

4) Non-immunosuppressed allotransplantation shows a significant increase of infiltrating lymphocytes on the 3rd day as compared to isogenic transplantation. Symptoms of gross rejection findings are preceded by lymphocytic infiltration.

5) Despite the use of cyclosporin A, an increased rate in the lymphocytic infiltration was seen, as compared to the control isogenic transplantation group. Therefore, it may be assumed that cyclosporin A induces rejection delay, blocking the cytotoxic cell effect or humoral immune reaction, even though cell migration does occur.

6) The use of plastic embedded tissue enables a better characterization of the histological findings of rejection. Further studies with immunohistochemical cell sorting are ongoing in our laboratories to enhance our knowledge in this field.

<table>
<thead>
<tr>
<th>Group and POday</th>
<th>ICo-7 Isogenic set control</th>
<th>CiCo-7 Cyclosporin set control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CiCo-7</td>
<td>7.919 x 10⁻³</td>
<td>-</td>
</tr>
<tr>
<td>RCo-7</td>
<td>0.693*</td>
<td>0.476</td>
</tr>
</tbody>
</table>

(-) = comparison not performed
* significant values when p≤0.05
Rco = rejection set control

RESUMO

Os efeitos da rejeição e da imunossupressão com ciclosporina A em retalhos epigástricos utilizados como transplantes cutâneos alógenos microcirúrgicos, foram estudados em 58 ratos, distribuídos em três grupos: Wistar-Furth isogênicos doadores e receptores; e Brown-Norway doadores e Wistar-Furth receptores, imunossuprimidos com ciclosporina A(10mg/kg/dia). Biópsias dos retalhos e da pele normal contralateral utilizadas...
como contrôle foram colhidas no terceiro, sétimo, 15º e 30º dias pós transplante. As biópsias foram preparadas em historesina, e coradas em azul de toluidina, para avaliação do infiltrado inflamatório local, permitindo estudo quantitativo dos linfócitos infiltrantes nos retalhos. O modelo revelou-se eficiente, obtendo-se sobrevivência dos transplantes por 30 dias, de 83,3% entre animais isogênicos, e de 60% nos alogênicos sob ação da ciclosporina A. Em ratos alogênicos não imunossuprimidos houve 100% de rejeição dos retalhos, até o nono dia pós-operatório. Nos transplantes desses animais, verificou-se aumento significativo do número de linfócitos infiltrantes no terceiro dia, quando comparados aos ratos isogênicos. A linfocitose observada antecedeu os achados macroscópicos da rejeição, verificados apenas no quinto ou sexto dias.

DESCRITORES: Transplante de tecidos. Ciclosporinas.


27. FURNAS DW, BLACK KS & HEWITT CW - Cyclosporine and long-term survival of composite tissue allografts (limb transplants) in rats (with historical notes on the role of plastic surgeons in allotransplantation). *Transplant Proc* 1983;15:3063-3068.


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