Cell Transplantation, Vol. 13, pp. 263–271, 2004
Printed in the USA.

0963-6897/04 $20.00 + .00

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Liposomal Formulations of Tacrolimus and Rapamycin Increase Graft Survival and Fiber Outgrowth of Dopaminergic Grafts

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The immunosuppressive drugs tacrolimus (TAC) and rapamycin (RAPA) have both been found to have neuroprotective effects on dopaminergic neurons. The purpose of the present study was to investigate whether liposomal formulations of these drugs administered directly into the brain improve cell survival and fiber outgrowth. Rats with unilateral 6-hydroxydopamine lesions were transplanted with 800,000 fetal rat ventral mesencephalic cells and randomly divided to one of four groups. Group 1 received a transplant containing cells only; group 2 received a cell suspension containing 0.68 μM liposomal RAPA (LRAPA); group 3 received a cell suspension containing 2.0 μM liposomal TAC (LTAC); and group 4 received a cell suspension containing a liposomal formulation of both 0.68 μM RAPA and 2.0 μM TAC (LRAPATAC). Rats were sacrificed after 6 weeks, and cell survival and fiber outgrowth were assessed using tyrosine hydroxylase (TH) immunohistochemistry. The animals receiving a cell suspension containing either LTAC or LRAPATAC were found to have significantly more surviving TH-immunoreactive (TH-ir) cells than the control group receiving cells only. The group receiving LTAC had significantly longer fibers, the group receiving LRAPA had significantly more fibers close to the graft, and the group receiving LRAPATAC had significantly more fibers at all distances. This study shows the feasibility of using liposomal formulations of neuroimmunophilins directly in the brain at the time of implantation to improve graft survival and fiber outgrowth. Furthermore, we have shown that the combination of LTAC and LRAPA has a synergistic effect. These compounds may play an important role in optimizing graft survival and host reinnervation in cell-mediated brain repair strategies for the treatment of neurological conditions.

Key words: Transplantation; Parkinson's disease; Tacrolimus; Rapamycin; Liposomes

INTRODUCTION

Neural transplantation is a promising restorative strategy for the treatment of Parkinson's disease (PD). However, survival of transplanted cells is limited. It has been estimated that between 3% and 20% of grafted cells actually survive in the host brain (5). Fiber outgrowth is also of concern, as it has been postulated that a critical threshold of reinnervation to the host must be met to achieve functional effects from a dopaminergic graft (15).

Various agents, such as neurotrophic factors, have been employed to increase the survival rate of grafted neurons (2,23,33). Newer drugs that have been found useful in promoting graft survival and neurite outgrowth include the neuroimmunophilin ligands. Tacrolimus (TAC) is a widely used immunosuppressive drug that binds to FK binding proteins (FKBP) present at high levels in the brain (28). TAC has been found to have neurotrophic effects in cultures of PC12 cells and sensory ganglia (16), to increase the length of tyrosine hydroxylase-immunoreactive (TH-ir) neurites extending from embryonic DA neuron cultures (8,9), and to increase the survival of cultured and grafted rat embryonic DA neurons (6). Rapamycin (RAPA) is another neuroimmunophilin ligand that also binds FKBPs. RAPA has also been found to have neurotrophic effects in cultures of PC12 cells and sensory ganglia (16), as well as increasing the number of neurites extending from PC12 cell culture (6) and from embryonic DA neuron culture (9).

In recent years liposomal formulations of the neuroimmunophilin ligands have been investigated as superior immunosuppressive strategies in solid organ transplantation. It is our hypothesis that liposomal formulations of these drugs would be superior in providing neurotrophic effects to grafted dopaminergic neurons, resulting in greater survival and fiber outgrowth. In previous work we have shown that liposomal tacrolimus (LTAC) administered to the rat host within a xenograft of embry-

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onic mouse ventral mesencephalic (VM) cell suspension in combination with daily suboptimal systemic doses of LTAC improved grafted cell survival (1). This was either due to neuroprotection from the LTAC or a local immunosuppressive effect in the brain from the LTAC in the cell suspension. It is thought that LRAPA may also provide neuroprotection in liposomal formulation, and that there may be an added benefit from a combination of the two drugs.

The present study was designed to determine whether liposomal formulations of TAC and RAPA retain the neuroprotective effects seen with the original formulations of the drugs. As well, we wanted to investigate whether a combination of the two drugs has any added benefit on cell survival and fiber outgrowth when administered within the cell suspension at the time of transplantation.

MATERIALS AND METHODS

Experimental Design

A total of 28 female Wistar rats (Charles River, St. Constant, Quebec) weighing 200–225 g were used in this study. All animals received 6-hydroxydopamine (6-OHDA) lesions of the right nigrostriatal pathway, and were randomly divided into one of four groups. All groups received grafts of 800,000 fetal rat VM cells. Group 1 ($n = 7$) received the cells only. Group 2 ($n = 7$) received a dose of LRAPA (0.68 μM) within the cell suspension. Group 3 ($n = 7$) received a dose of LTAC (2.0 μM) within the cell suspension. Group 4 ($n = 7$) received a dose of LRAPATAC (0.68 μM LRAPA and 2.0 μM LTAC) within the cell suspension. Functional recovery was assessed by amphetamine-induced rotational behavior. Graft survival and fiber growth were assessed by TH immunohistochemistry.

Animals and 6-OHDA Lesions

The rats were housed two per cage with food and water ad libitum, on a 12-h light/dark cycle, at 21°C. All animal procedures were in accordance with the guidelines of the Canadian Council on Animal Care and the University Council on Laboratory Animals. Rats were anesthetized intramuscularly with 2.0 ml/kg of a ketamine/xylazine/acepromazine mixture [25% ketamine hydrochloride (Ketalean, MTC Pharmaceuticals, Cambride, Ontario), 6% xylazine (Rompun, Miles Canada, Etobicoke, Ontario), 2.5% acepromazine maleate (Wyeth-Ayerst Canada, Montreal, Quebec), in 0.9% saline]. Two stereotactic injections of 6-OHDA (Sigma Chemical Co., Chicago, IL; 3.6 μg of 6-OHDA HBr/μl in 2.0 mg/ml of L-ascorbate in 0.9% saline) were placed into the right ascending nigrostriatal pathway at the following coordinates (mm): 1) 2.5 μl at anteroposterior (A/P) −4.4, mediolateral (M/L) −1.2, dorsoventral (D/V) −7.8, toothbar −2.4; and 2) 3.0 μl at A/P −4.0, M/L −0.8, D/V −8.0, toothbar +3.4. Coordinates were derived from bregma and the surface of the brain at the site of injection. The rate of injection was 1 μl/min, with the cannula being left in place for 5 min after the injection before being slowly retracted. Animals were allowed to recover for 2 weeks in the animal care facility before receiving an amphetamine challenge (5.0 mg/kg, IP). Rotational scores were collected over a 60-min period using a computerized video activity monitor programmed for rotational behavior (Videomex-V, Columbus Instruments, Columbus, OH). Only animals exhibiting a mean rotational score of greater than 8 ipsilateral turns per minute were included in the study.

Dissection, Preparation, and Transplantation of VM Cell Suspensions

Twenty-eight animals with greater than 8 ipsilateral full body turns per minute were chosen for transplantation. Transplants were performed on two separate days, with animals randomized to surgery day. Prior to transplantation, VM tissue was obtained from fetuses of gestational day 14 Wistar rats (Charles River). Tissue from 14 fetuses was pooled at each transplant session to form the cell suspension (1,950,000 cells on average per fetus). The pregnant rats were anesthetized with 2.4 ml/kg, IP, of the ketamine/xylazine/acepromazine anesthetic mixture, and the uterine horns were removed. The VMs were collected under aseptic conditions in Dulbecco's modified Eagle's medium (DMEM; Sigma Chemical Co.) and stored overnight at 4°C in a phosphate-buffered, calcium-free hibernation medium consisting of: 30 mM KCl, 5 mM glucose, 0.24 mM $MgCl_2$, 5 mM NaH_2PO_4, 20 mM lactic acid, 32.18 mM KOH, and 164.7 mM sorbitol, pH 7.4 (2). The next day the tissue was washed twice with 0.05% deoxyribonuclease (DNase; Sigma Chemical Co.)/DMEM and incubated in 0.05% DNase/0.1% trypsin (Sigma Chemical Co.)/DMEM at 37°C for 20 min. The tissue was rinsed four times with 0.05% DNase/DMEM before being mechanically dissociated to make a homogenous single cell suspension. The cells were centrifuged and washed three times with 0.05% DNase/DMEM. Cell viability and concentration were calculated by the trypan blue dye exclusion method, and viabilities were found to be 97% and 98% at the first and second transplant sessions, respectively.

Liposomes were prepared by dissolving 5% phosphatidylcholine (Netterman, Germany), 0.5% cholesterol, and 0.2% of either TAC, RAPA, or both, in 5% ethanol, and then adding 95 ml of 0.9% saline. Multilamellar lipid vesicles were formed by shaking the mixture at 55°C for 10 min, and the liposomes were then sized by compression through a 0.22-μm filter. The liposomes were then cooled to room temperature and stored at 4°C

(1). Before grafting, the cell suspension was divided into four volumes and diluted with cell media, plus or minus drug, for a final concentration of 300,000 cells/μl. One tube remained as is, and contained cells only for transplanting into group 1. The control group contained the cells only, as opposed to vehicle-treated liposomes, as we have previously found no difference between cells-only and vehicle-treated liposomes (unpublished work). The second tube contained cells plus LRAPA at a final concentration of 0.68 μM. The third contained cells plus LTAC at a final concentration of 2.0 μM. The final tube contained cells plus LRAPATAC at a final concentration of 0.68 μM LRAPA and 2.0 μM LTAC.

The cell suspensions were stereotactically injected using the microtransplantation technique previously described (21). Briefly, a capillary tip with an opening of 50–70 μm was attached to a 2-μl Hamilton syringe and used to stereotactically implant the cells at a rate of 100 nl/min into the striatum. Injections of 0.45 μl of cell suspension, containing approximately 133,333 cells, were place into the dorsolateral striatum at the following coordinates (mm): 1) A/P +1.3, M/L −2.1, D/V −5.5 and −4.3; 2) A/P +0.6, M/L −2.9, D/V −5.5 and −4.3; 3) A/P +0.3, M/L −3.7, D/V −5.5 and −4.3; toothbar −3.3 (coordinates from bregma and the dorsal surface of the skull at bregma). A total of 2.7 μl of suspension containing 800,000 cells was injected in each animal.

Immunohistochemistry

The rats were euthanized approximately 6 weeks after transplantation with an overdose of the ketamine/xyalazine/acepromazine mixture (3.0 ml/kg b.wt.) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M PB. The brains were removed from the cranium and postfixed with 4% paraformaldehyde in 0.1 M PB overnight, before being stored for 24 h in PBS containing 30% sucrose. Sections (30 μm) were cut with a freezing microtome and stored in Millonig's buffer solution (6% sodium azide in 0.1 M PB) until immunohistochemical staining was performed.

Staining for the presence of TH was performed using the primary rabbit anti-TH antibody (Ab) (1:2500; Pel Freez Biologicals, Rogers, AR) and the avidin-biotin complex kit (Vector Laboratories Canada Inc., Burlington, Ontario). Briefly, one in every four sections through the grafted area was selected and stained. The sections were preincubated for 30 min in a solution of 10% methanol and 3% hydrogen peroxide and blocked in 0.1 M PB containing 0.3% Triton X-100 and 5% normal swine serum for 1 h. The sections were incubated in a 1:2500 solution of rabbit polyclonal anti-TH Ab for 16 h at room temperature. A 1:500 solution of biotinylated swine anti-rabbit IgG Ab (DAKO Diagnostics Canada Inc., Mississauga, Ontario) was used as a secondary Ab, followed by a streptavidin-biotinylated horseradish peroxidase complex kit. TH immunoreactivity was visualized by the addition of 3,3′-diaminobenzidine (DAB) and hydrogen peroxide (0.01% hydrogen peroxide in DAB solution). The sections were washed in 0.1 M PB before mounting on gelatin-coated slides in entellan.

Rotational Behavior

Functional recovery was assessed at 3 and 6 weeks posttransplantation by amphetamine challenge (5.0 mg/kg, IP). Rotational scores were collected over a 60-min period as described above. Statistical analyses for between- and within-group differences were assessed at $p < 0.05$ using a two-way analysis of variance (ANOVA), followed by Tukey's post hoc test.

Cell Counts

Unbiased stereological cell counting software was used to estimate absolute cell survival within the graft area (Stereo Investigator, Microbrightfield). The optical fractionator probe was used on every fourth TH-stained section throughout the graft area (9,23). Cells within the area of the graft were counted using a 20× objective magnification; the graft area was defined as that portion of the striatum containing the cell bodies of the graft. The optical fractionator probe used a counting frame that was 120×120 μm with a height of 15 μm. A sampling grid 150×150 μm^2 was used. Accordingly, the number of frames counted per section varied depending on the area of the individual transplant within each section. The mean section thickness was estimated at every fifth dissector and then averaged for each section. All stereological analyses were performed by an investigator who was blinded to the animal's treatment. The data were subjected to statistical analyses for between-group differences at $p < 0.05$ with a one-way ANOVA followed by Tukey's post hoc test.

Cell counts have been expressed as the average number of surviving DAergic cells ± SE. Cell counts of surviving DAergic neurons were also calculated as an approximate percentage survival of grafted DAergic cells. It has previously been shown that 8–10% of dissected FVM are DAergic (20). Therefore, using 800,000 VM cells, approximately 80,000 DAergic cells were transplanted per animal, and this number was used in the percentage calculations.

Fiber Outgrowth

Fiber outgrowth was measured as previously described (2,17). Briefly, the number of fibers that crossed a 50×200-μm grid placed perpendicular to the graft–host interface was counted. The number of fibers crossing the grid at distances of 50, 100, 150, and 200 μm from the

interface was counted. A total of 30 measurements was made for each animal. The data were subjected to statistical analyses for within- and between-group differences at $p < 0.05$ using a two-way ANOVA followed by Tukey's post hoc test.

RESULTS

Rotational Behavior

All animals exhibited strong ipsilateral rotational behavior on amphetamine challenge 2 weeks following 6-OHDA lesioning. All groups showed a significant reduction in ipsilateral rotations at both 3 and 6 weeks posttransplantation ($p < 0.05$). There was no difference among groups at any of the time points tested (Fig. 1).

Cell Counts

All animals exhibited surviving grafts with TH-ir cell bodies and fibers (Fig. 2). There was a mean of 1961 ± 656 surviving TH-ir cells in the group receiving a transplant of cells only, representing an approximate survival rate of 2.45% of DAergic cells. The group receiving a dose of LRAPA in the cell suspension had mean of 2493 ± 553 surviving TH-ir cells, with an approximate survival rate of 3.12%. The group receiving LTAC had an average of 4405 ± 21018 TH-ir cells, with an approximate survival rate of 5.51% of DAergic cells. Finally, the group receiving LRAPATAC in the cell suspension had an average of 4339 ± 729 surviving TH-ir cells, representing an approximate survival rate of 5.42%. The number of surviving TH-ir cells was significantly greater in the groups receiving LTAC or LRAPATAC compared with the group receiving cells only ($p < 0.05$) (Fig. 3).

Fiber Outgrowth

All animals had TH-ir fibers extending from the grafted area to the host striatum (Fig. 4). In the group receiving cells only, the number of fibers crossing the grid reduced significantly at 150 and 200 μm compared with the number crossing at 50 μm ($p < 0.05$). The group receiving LRAPA had significantly more fibers at 50 and 100 μm compared with the number of fibers in the cells-only group ($p < 0.05$), but there was no significant difference at 150 or 200 μm. In the LTAC group the number of fibers at 50 μm was no different than in the cells-only group, but there were significantly more fibers at 100, 150, and 200 μm compared with the cells-only group ($p < 0.05$). Finally, in the LRAPATAC group, the number of fibers was significantly greater at all distances compared with the cells-only group ($p < 0.05$) (Fig. 5).

DISCUSSION

Improving cell survival and functional benefits is crucial to maximize the efficacy of neural transplantation.

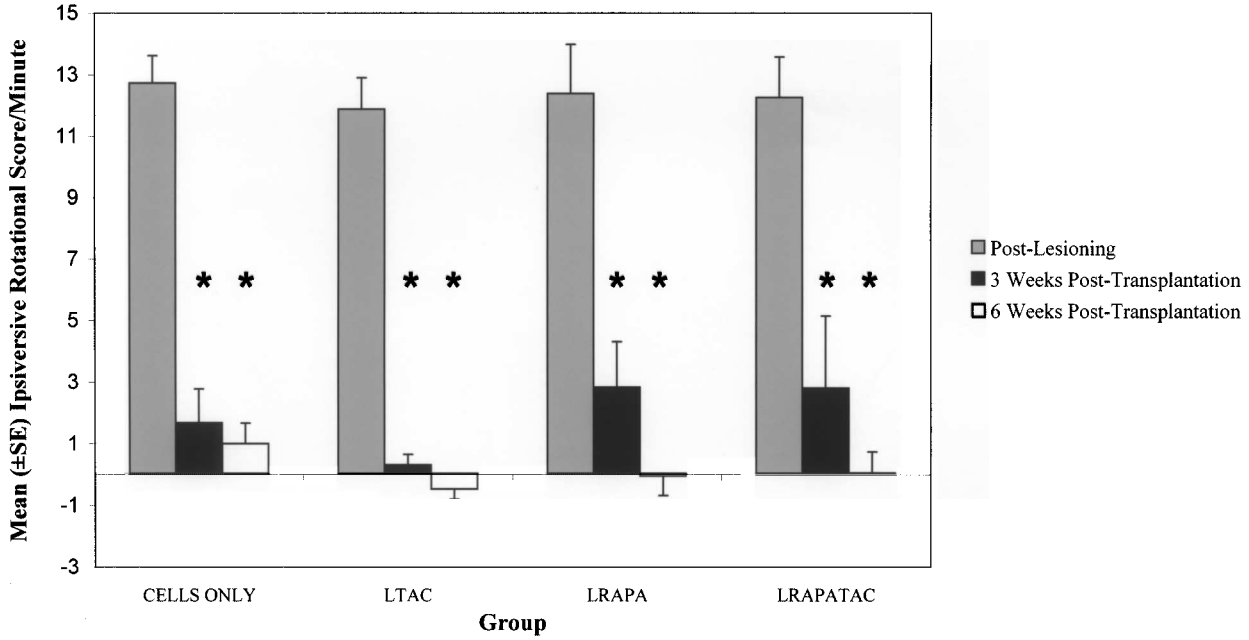

Figure 1. The mean (±SE) rotations per minute over 60 min following amphetamine challenge (5 mg/kg, IP) after 6-hydroxydopamine lesions of the right ascending nigrostriatal pathway (gray bars), 3 weeks posttransplantation of fetal rat ventral mesencephalic (VM) cells (black bars), and 6 weeks posttransplantation of rat VM cells (white bars). A significant decrease in rotational score was observed at 3 and 6 weeks posttransplantation in all groups (*$p < 0.05$ compared with postlesion scores). There was no difference between groups.

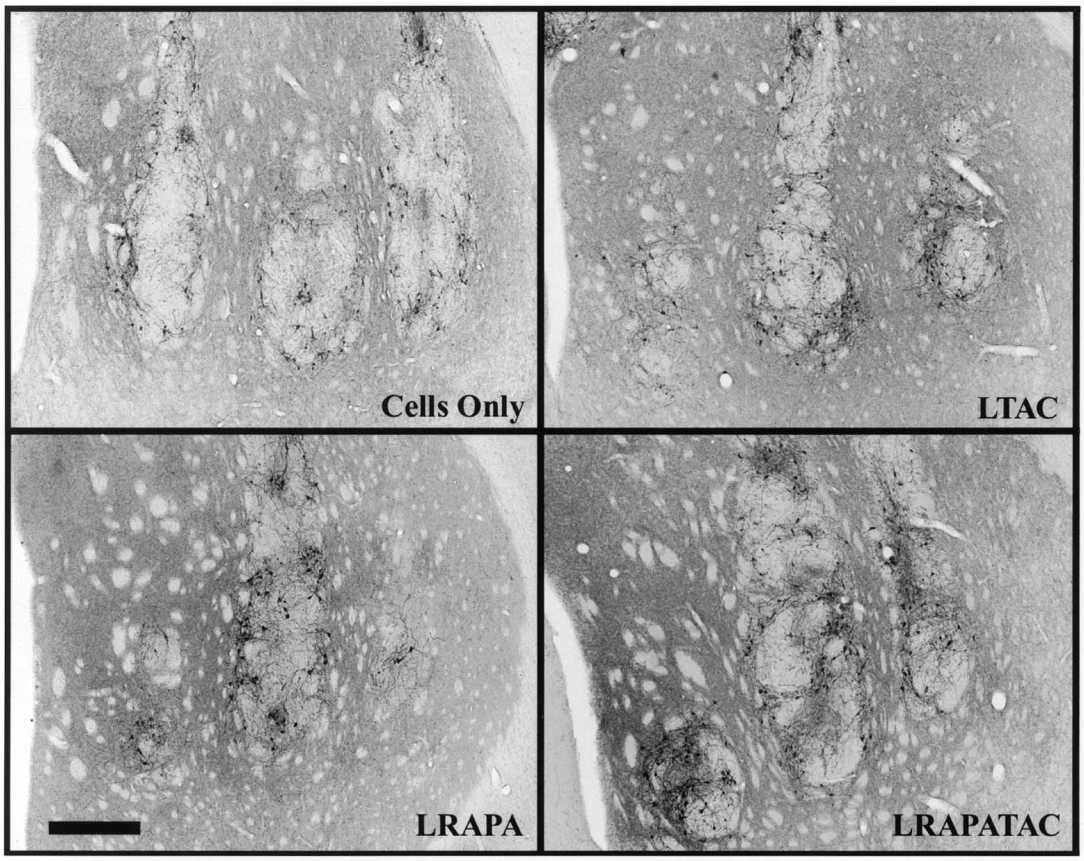

Figure 2. Representative coronal sections through the level of the striatum of 6-hydroxydopamine-lesioned rats with fetal rat ventral mesencephalic grafts immunostained for the presence of tyrosine hydroxylase (TH). All animals had surviving grafts with TH-ir cells and fibers. The grafts in the LTAC and LRAPATAC groups appear to contain more surviving TH-ir cells than the cells-only control group. There is a denser halo of TH-ir around the grafts in the LRAPA and LRAPATAC groups. Bar = 320 μm.

In this study we have found that liposomal formulations of the neuroimmunophilin ligands TAC and RAPA improve cell survival and fiber outgrowth. LTAC within the donor cell suspension was found to cause both increased cell survival and an increase in the length of neurite outgrowth. LRAPA was found to increase the number of fibers extending from the graft to the host. A combination of the two, LRAPATAC, was found to increase both cell survival and fiber outgrowth. In terms of functional benefits, no significant difference was seen in amphetamine-induced rotational behavior after grafting. It has been suggested that there is a threshold of approximately 1000 surviving DAergic neurons necessary to reduce rotations by 50%, and approximately 2000 neurons are needed to obtain complete rotational symmetry (19). One possibility why we did not observe a difference in rotational behavior between groups may be that this critical threshold of surviving neurons was met in all groups, and survival beyond this threshold provided no additional functional benefits. However, a difference may be seen in complex sensorimotor tasks such as paw reaching, the stepping test, or the cylinder test. We are currently investigating these issues.

Many attempts at improving cell survival have been made in the past, including the use of neurotrophic factors such as glial cell line-derived neurotrophic factor (2, 23) and brain-derived neurotrophic factor (25,33). Other agents, such as caspase inhibitors (26), CEP-1347 (4), and lazaroids (19), are useful in reducing the degree of apoptosis in transplanted neurons, and therefore increasing survival. As well, a combination of apoptosis and complement inhibition has been found beneficial (7).

The neuroimmunophilin ligands, such as TAC and RAPA, are promising compounds to improve graft survival. TAC binds to FKBPs, including FKBP-12 and 52, and in doing so inhibits the phosphatase activity of calcineurin (27). This inhibition of calcineurin activity prevents the dephosphorylation of nuclear factor of activated T cells (NF-AT), preventing it from entering the cell nucleus and initiating transcription (27). RAPA also binds to FKBPs; however, this complex does not interact with calcineurin (27). RAPA inhibits cellular prolifera-

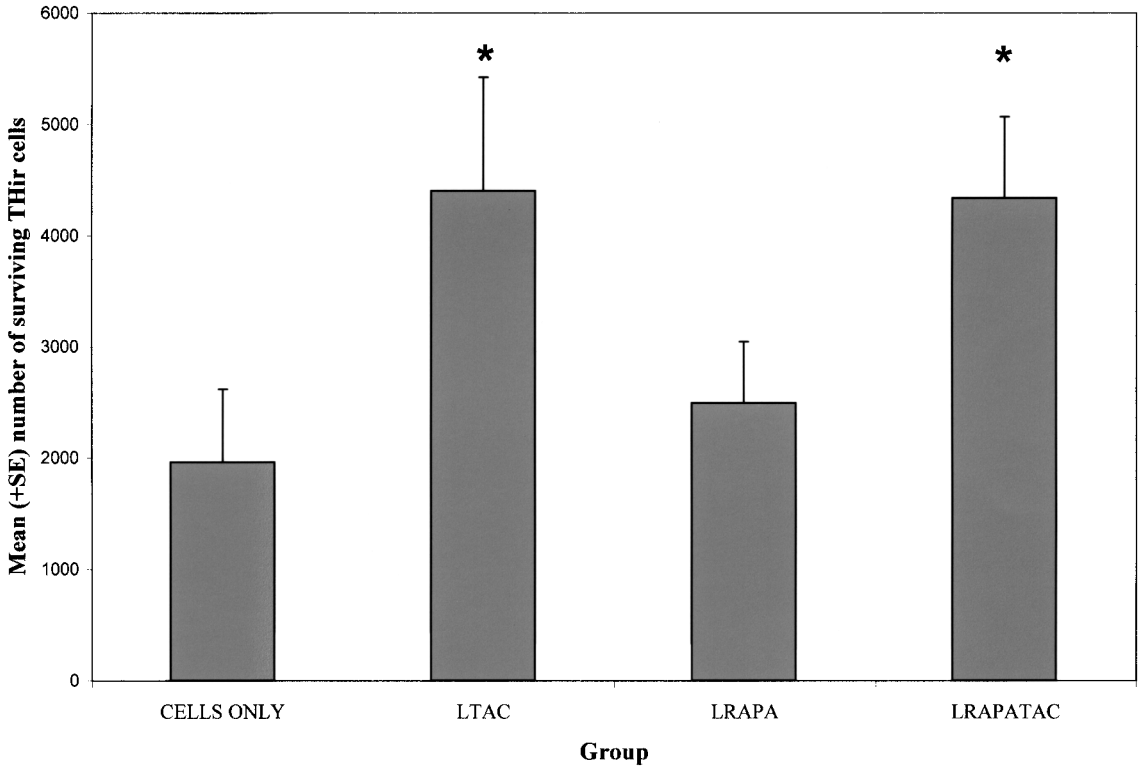

Figure 3. Bar graph demonstrating the mean (+SE) number of TH-ir cells found within the intrastriatal fetal rat ventral mesencephalic grafts of 6-hydroxydopamine-lesioned rats. There were significantly more surviving cells in the LTAC and LRAPATAC groups ($*p < 0.05$) compared with the cells-only group.

tion by inhibiting translation, thereby arresting the cell at the G_1 phase of the cell cycle (22). It is through this binding to FKBPs that both of these drugs have an immunosuppressive effect. High levels of FKBPs have also been found in the rat brain, colocalized with calcineurin (28), suggesting an alternate role for the immunophilins in the brain besides that of immunosuppression. Since then TAC has been found to enhance neurite outgrowth in PC12 cells and sensory ganglia by increasing their sensitivity to nerve growth factor (16). These effects were found to be blocked by RAPA in the sensory ganglia, possibly by competitive binding for the same immunophilin, although RAPA itself stimulated outgrowth in the PC12 cells (16).

TAC has been found to play some role in the DA system, as it has an effect on locomotor activity in rats and suppresses the behavioral response produced by methamphetamine in rats (30), suggesting TAC may play a role at the level of the DA receptor. It has recently been found that TAC probably has no role with D1-like receptors, but appears to alter the postsynaptic responsiveness of D2/D3-like receptors (24). TAC plays a role in the survival of cells, as it increases neuron survival in the substantia nigra pars compacta after axotomy of the medial forebrain bundle (31), increases the number of TH-ir neurons in embryonic rat cell culture and grafting (6), as well as increases the cell number in human brain cell culture (3). We have previously shown that LTAC given within a cell suspension in combination with daily suboptimal systemic doses improves cell survival in rats receiving xenografts of fetal mouse cells (1). In the present study, we have demonstrated that LTAC enhances cell survival and fiber outgrowth of syngeneic grafts. When LTAC was added to the cell suspension, there were significantly more surviving TH-ir cells at 6 weeks posttransplantation compared with animals receiving cells only. Interestingly, in the combination LRAPATAC group, RAPA did not block TAC from increasing the number of cells. This could imply that in increasing cell survival TAC does not bind FKBP-12, but rather another FKBP to which RAPA does not bind. Another possibility is that at the concentrations of drug used in this experiment, there was not enough RAPA to block all the FKBP-12 sites, and thus both drugs could bind. The exact mechanism for this increased cell survival is currently not well understood, although there appears to be a role in the inhibition of apoptosis. TAC suppresses levels of c-Jun in the sub-

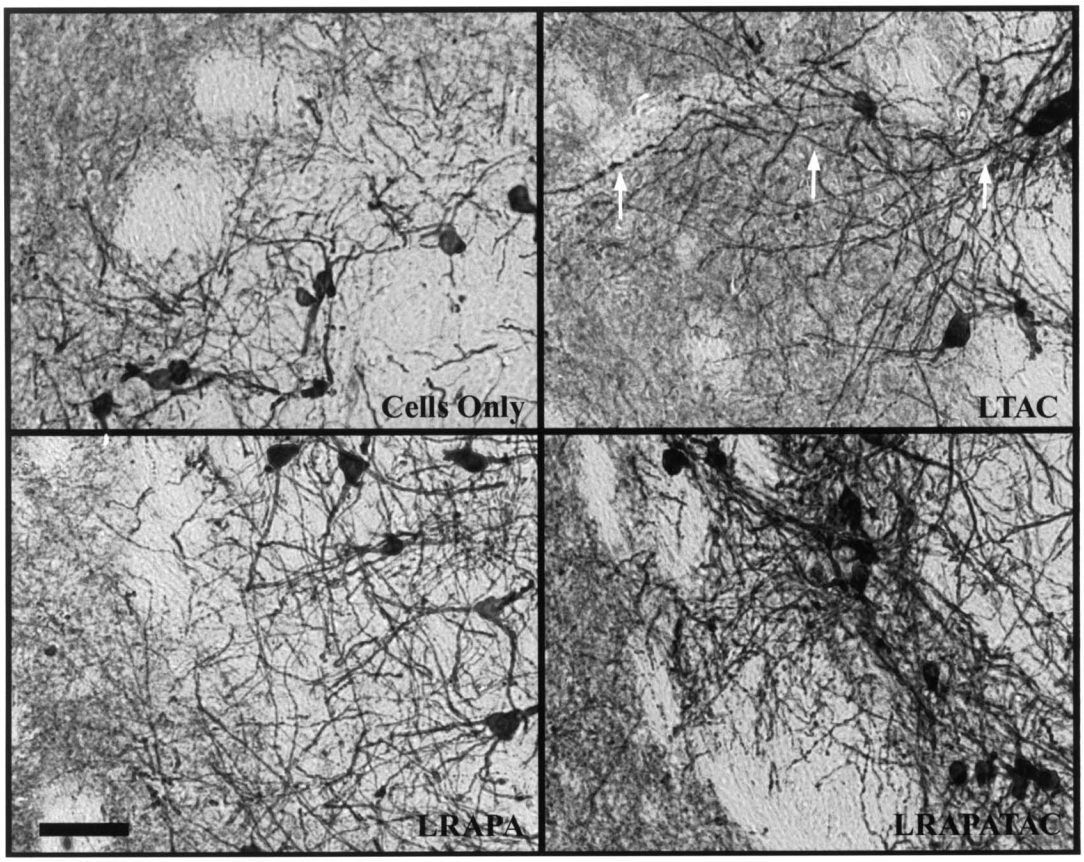

Figure 4. High-power representative coronal sections through the level of the striatum of grafted 6-hydroxydopamine-lesioned rats immunostained for the presence of tyrosine hydroxylase (TH). There are long fibers extending from the surviving TH-ir cells in the LTAC group (demonstrated by the white arrows), and a large number of fibers in the LRAPA group. The LRAPATAC group appears to have both a large number of fibers and long fibers. Bar = 40 μm.

stantia nigra after transection of the rat medial forebrain bundle (31), as well as c-Jun expression of cortical cells in culture (32). TAC also decreases levels of tumor necrosis factor-α in the striatum of the 6-OHDA-lesioned rat, which may be correlated with apoptosis (18). Calcineurin is also known to induce cell death in certain circumstances, and the inhibition of calcineurin by TAC can protect neurons from apoptosis, possibly by a mechanism involving Bcl-2, which also blocks calcineurin-induced cell death (10).

Along with cell survival, TAC has an effect on fiber outgrowth, as does RAPA. In culture, TAC was found to increase the fiber length from embryonic TH-ir neurons (8,9), while RAPA was found to increase branching and antagonize the elongation seen with TAC (9). RAPA itself has been found to increase neurite outgrowth of PC12 cells in conjunction with a low concentration of nerve growth factor (22). RAPA is known to inhibit cellular proliferation at the G_1 phase of the cell cycle via inhibition of translation, so it is hypothesized that the increased outgrowth is from shifting cells from a proliferative to a differentiative path (22). It had long been postulated that the neurotrophic effects of TAC were due to its binding with FKBP-12 and its inhibition of calcineurin. However, recent work has shown that cells from FKBP-12 knockouts still have increased neurite outgrowth when exposed to TAC, while an antibody to FKBP-52 blocks this outgrowth (11), and TAC also protects against MPP+ and 6-OHDA toxicity by increasing the process length and number in cultures of FKBP-12 knockout cells (14). A more recent theory suggests that TAC causes fiber elongation by binding FKBP-52, which is part of the mature steroid receptor complex (12). Along with FKBP-52, this complex is also composed of a receptor protein, Hsp-90, and p23. When TAC binds, it disrupts the complex and activates Hsp-90, dissociating p23. Hsp-90 is then free to activate the mitogen-activated protein kinase (MAPK) pathway, and extracellular signal regulated kinase (ERK) activates gene products, which are required for nerve regeneration (12).

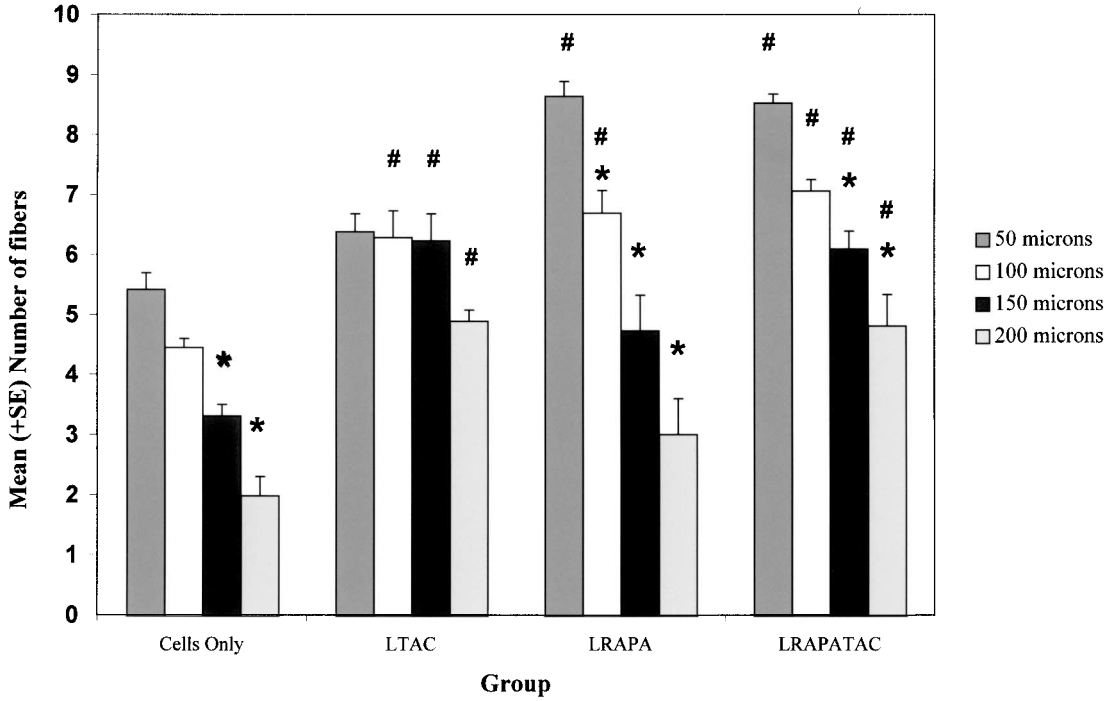

Figure 5. Bar graph demonstrating the mean (+SE) number of TH-ir fibers at various distances from the graft–host interface in grafted 6-hydroxydopamine-lesioned rats. $*p < 0.05$ compared with 50 μm within the same group; $\#p < 0.05$ compared with the same distance in the cells-only groups.

In the present study, we found that LTAC increases fiber length, as demonstrated by the increased number of longer fibers in the group receiving LTAC within the cell suspension. LRAPA was found to increase the number of fibers, as demonstrated by the increased number of fibers close to the graft. Interestingly, a combination of LTAC and LRAPA within the cell suspension, LRAPATAC, was found to increase the number of fibers at all measured distances, compared with the control, including a higher number of longer fibers. This synergistic effect of a combination of LTAC and LRAPA in increasing both the number of fibers and the number of long fibers is a novel observation.

The findings in the present study demonstrate that liposomal formulations of TAC and RAPA have strong neurotrophic effects when administered in the cell suspension at the time of implantation. Liposomes may provide a slower release of the drug in the brain, and they may allow higher concentrations of the drugs to be delivered to the brain than is possible with systemic administration. Direct brain administration of these drugs may prove to be a superior delivery strategy for immunosuppression and neurotrophic effects. Optimizing cell survival and fiber outgrowth is of crucial importance for cell restorative therapies and their potential applications to the treatment of neurological conditions in the future. Liposomal formulations of the neuroimmunophilin ligands TAC and RAPA administered directly into the brain at the time of transplantation may play an important role in improving cell survival and host reinnervation in cell-mediated brain repair strategies.

ACKNOWLEDGMENTS: The authors thank Ms. Nancy McGrath, Mr. Matthew Lewington, and Mr. Ruperto Ulalia for their technical support. This work was supported in part by the Dr. W. Alan Curry Studentship in Anatomy or Surgery awarded to A.Y.A. by the Dalhousie Medical Research Foundation.

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