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Adrenal Cortex Transplantation After Bilateral Total Adrenalectomy in the Rat

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An experimental animal model with adrenal cortex transplantation was developed to study adrenal cortex replacement therapy in patients with multiple endocrine neoplasia type 2 who have had bilateral adrenalectomy for pheochromocytomas. Adrenal cortex of syngeneic rats was isolated from the medulla by collagenase digestion and a defined sedimentation. The cell suspension of the cortical cells was implanted under the kidney capsule of untreated syngeneic rats. After two weeks the recipients were bilaterally adrenalectomized. Serum corticosterone levels were measured as an estimate of function of the grafts. All recipients were healthy throughout the observation period, whereas all adrenalectomized controls died within 18 days. Vital cortex cells could be demonstrated in the explanted grafts by immunohistochemistry. Corticosterone levels of transplanted animals were nearly normal (9.5 ng/100 mL ± 0.4) compared to the controls (0.20 ng/mL ± 0.06). This animal model of adrenal cortex transplantation allows the separation of medullary from cortical cells. After transplantation, these cortical cells survived for eight weeks and were able to replace the adrenal cortex function. (Henry Ford Hosp Med J 1989;37:154-6)

Of patients with multiple endocrine neoplasia type 2 (MEN 2), 30% to 40% have neoplasms in one adrenal gland at the time of diagnosis. In long-term observations, 60% to 70% of patients develop bilateral pheochromocytoma (1,2). When pheochromocytoma is detected at the time of MEN 2 diagnosis, the patient has a 90% chance of also developing a contralateral adrenal tumor later and must have bilateral adrenalectomy (2). Although the function of the adrenal medulla is replaced well by catecholamine production of sympathetic nerves, cortical hormones are required. Exogenous substitution of adrenal cortical hormones can prevent these patients from lethal acute Addison crises, but it cannot possibly regulate the metabolism as well as the glands themselves. Patients with inadequate substitution of adrenal cortical hormones are prone to complications, with infections and stress incompetence being the most common. There are also the risks of developing Nelson tumors of the pituitary gland as well as the difficulties of the patients' compliance to lifelong drug therapy.

Transplantation of adrenal cortical tissue is a potential answer to this problem. However, further research is necessary to study the effect and the regulation mechanisms of adrenal cortical grafts. A special problem of earlier transplant models was the separation of the hyperplastic medullary cells from the cortical cells to be transplanted. We have therefore developed an animal model of adrenal cortical transplantation. The two major conditions required of this model included 1) isolation of adrenal cortical cells and their separation from medullary cells, and 2) successful transplantation of these cells in a compartment which is well defined and easily accessible for investigation.

Animals, Materials, and Methods

Male adult LEW rats were used as donors and recipients. Two donors were used for one recipient. In the donor a bilateral adrenalectomy was performed. After freeing the adrenals from adjacent fat, they were transferred into a new petri dish, the capsule was cut, and the whole adrenal gland enucleated. Thereafter the gland was chopped into fine pieces and transferred into a centrifugation tube. After a short centrifugation the supernatant was discarded and 5 mL of collagenase solution was added to the pellet. The glands were then digested in a shaking waterbath at 38°C for 20 minutes. Following two washes in cold Hank's solution and centrifugation for five minutes at 100 g, the pellet was transferred into a 100 μL syringe.

For transplantation a 26 G catheter was placed transrenally from the lower pole of the recipient's left kidney ventrally under the kidney capsule. The entry into the kidney was electrocoagulated to prevent bleeding and reflux of the grafted tissue. To allow engraftment of the tissue without temporary substitution,
the bilateral adrenalectomy in the recipient was performed seven days after transplantation. Thereafter the animals were followed for four weeks.

Results

Following collagenase digestion the adrenal parenchyma was nearly completely dissolved. On cytologic examination viable cortical cells were observed but medullary cells were rare (less than 10%).

Following centrifugation at 20 or 50 g, only 2% to 5% of all cells were of medullary origin, and at 100 g no medullary cells were observed. The viability was 91% as tested by a trypan blue stain. Although all of the untreated control rats survived and gained 62 g of weight within four weeks, all rats that underwent bilateral adrenalectomy suffered from severe hypoaldosteronism and hypocorticism and died within two weeks after the operation (Fig 1). Following isogenic transplantation of collagenase-digested adrenal tissue all rats survived and were completely healthy (Fig 1) throughout the observation period and had a mean weight gain of 59 g. Basal corticosterone levels in the serum five weeks after transplantation of adrenal tissue and four weeks after bilateral adrenalectomy were slightly diminished as compared to normal controls. Histologic and immunohistologic staining was performed on all grafts. Light microscopy revealed vital tissue of adrenal cortical origin without signs of cortical organization. The transplants showed no binding of neuron-specific enolase in immunohistochemical staining (Fig 2). One week following bilateral adrenalectomy corticosterone was barely detectable in the nontransplanted animals (0.20 ng/mL ± 0.06).

Discussion

Adrenal autotransplantation has been performed frequently, but successes in clinical models have been rare (3). During our experience with the treatment of MEN 2 we have frequently been confronted with problems from bilateral adrenalectomy and the necessity of subsequent substitution of adrenal cortical hormones. In our view, the best chances of an adequate substitution therapy are given by cortex transplantation. A special problem of transplantation lies with the separation of the cortical from the medullary cells. Animal studies on adrenal cortical transplantation (3,4) have demonstrated adequate function, but strict separation of cortical from medullary cells was not possible. Using collagenase digestion and a well-defined centrifugation, we have been able to separate out cortical cells, as demonstrated by cytologic studies. These grafts are able to produce corticosterone and prevent Addison crises in bilaterally adrenalectomized laboratory rats. Whether hormone production of these transplants can be regulated by humoral factors to enable recipients to adapt to stress situations such as infection or trauma remains to be established.

Histologically the adrenal cells were not organized in a cortical structure as they are following transplantation of whole adrenal glands (5). It has been speculated that a cortical structure is necessary for an adequate graft function. Our study shows that cellular adrenal cortical grafts have a sufficient graft function without being organized into a cortex-like structure. It is possible that a cortical structure reorganizes later on, but this will require long-term studies for verification. Whether or not steroids other than corticosterone are adequately produced by this type of adrenal cortex grafting also remains to be established.

One prerequisite of a valid transplantation model is the complete elimination of the original organ function. In our model, after complete bilateral adrenalectomy (including the organ capsule), corticosterone was still detectable after two weeks. However, the hormone levels were lowered to near the detection limit and all adrenalectomized animals died from acute Addison crisis. On histology, no accessory adrenal glands were found. Measurable corticosterone levels despite a complete bilateral adrenalectomy are well known (6,7). The underlying mechanism of this phenomenon is not understood.

Conclusions

This new model of adrenal cortical transplantation allows for separation of medullary and cortical cells and successful trans-
plantedation of the latter. Normal corticosterone levels indicate a good graft function. Whether other steroids are produced sufficiently remains to be determined. This model may prove helpful in the study of regulation mechanisms in the adrenal cortex.

References