INTRODUCTION
Autogenous nerve grafting is the commonest method for treating peripheral nerve injuries. However, it is associated with the limit of nerve source and neurological deficits where the donor nerve innervated.

We previously demonstrated successful nerve regeneration in peripheral nerve allografts that had been immersed in green tea polyphenol solution for one month. Our present concerns focus on whether nerve xenografts that have been treated in polyphenol solution could be transplanted successfully. In this study, a segment of rabbit sciatic nerve that had been immersed in polyphenol solution was transplanted to a nerve deficit created in rat sciatic nerve. We also examined the effects of administration of an immunosuppressant (FK506, tacrolimus; Astellas Pharma Co. Ltd, Tokyo, Japan) on nerve regeneration in the nerve xenografts.

METHODS
A polyphenol mix extracted from green tea was purchased from PFI Inc. (Kyoto, Japan). It was composed mainly of (-)-epigallocatechin-3-O-gallate (28%), (-)-gallocatechin-3-O-gallate (11.6%), (-)-epicatechin-3-O-gallate (4.6%), (-)-epigallocatechin (15.0%), (+)-gallocatechin (14.8%), (-)-epicatechin (7.0%) and (+)-catechin (9.5%); its purity exceeded 99%. Sciatic nerve segments were harvested from male Japanese house rabbits. They were split into funicules of about 1 mm, then stored in polyphenol solution (1 mg/mL) for one month at 4°C, and transplanted into recipient female Lewis rats to bridge 15 mm-long sciatic nerve gaps. Animals were divided randomly into three groups: one received FK506 subcutaneously at 0.2 mg/kg per day (“polyphenol-0.2”); one received 2 mg/kg per day (“polyphenol-2”) and one was left untreated as a control. In an additional experiment, fresh 2 g segments were harvested immediately without any polyphenol storage. Twelve weeks after surgery, electro-physiological and morphological studies were performed to assess nerve regeneration in the transplanted nerve segment. Transverse sections (1 Am thick) were taken from the most distal region of the transplanted nerve for the morphological studies. As genomic studies, genomic DNA was extracted from each nerve segment using phenol-chloroform extraction and quantified spectrophotometrically. Two types of polymerase chain reaction (PCR) studies (PCR 1 and 2) were carried out on each DNA sample. PCR 1 was specific to the rat β-actin gene. As PCR 2, semiquantitative polymerase chain reaction (PCR) amplification specific to the sex-determining region of the Y-chromosome (Sry) was carried out to confirm the presence of transplanted male rabbit tissues.

RESULTS
Electrophysiological study. The amplitudes of action potentials evoked in the pedal adductor muscle and motor nerve conduction velocity (MNCV) of the operated limb were expressed as percentages of the contralateral nonoperated limb in each rat. The mean amplitudes of the polyphenol control (0%), and polyphenol-0.2 (4.82% ± 6.9%) groups were significantly less than that of the fresh-2 group (18.4% ± 8.8%; P = 0.00059 and 0.010599, respectively). However, the polyphenol-2 group (24.2% ± 7.0) was similar to the fresh-2 control group (18.4% ± 6.7; P = 0.221494). The mean MNCVs (%) of the polyphenol control, polyphenol-0.2, the polyphenol-2 and the fresh-2 groups were 0%, 0%, 0.231 ± 0.055 and 0.478 ± 0.071, respectively. There was no significant difference between the polyphenol-2 and fresh-2 xenograft groups (P = 0.6955).

DISCUSSION
It is known that immune response in the xenografting is mediated by complement factors. This response is usually occurred within several minutes, which is so called a hyperacute rejection. FK506 suppresses the cell cycle of T lymphocytes and the activation of antibody production for xenograft. FK506 also showed to fokolcin binding protein 12(FKBP12) leading to promoted peripheral nerve regeneration. Thus, FK506 is used peripheral nerve allografting and xenografting. On the other hand the mechanism of immune reduction induced by polyphenol is not fully investigated. We had hypothesized that the combination therapy of FK506 and polyphenol treatment might reduce the rejection including HAR, acute and subacute rejection, leading to successful xenografting of peripheral nerve. The PCR studies demonstrated that about 23% of the nerve cells were derived from polyphenol-treated nerve xenografts in the group receiving FK506 at 2 mg/kg per day. By contrast, 46% of the nerve cells were derived from fresh xenografts in the group receiving FK506 at 2 mg/kg per day. The different number of transplanted cells between polyphenol-treated nerves and fresh nerves might explain these differences. Because of one-month polyphenol preservation, the number of cells has been decreased. But we could not detect donor-originated cells in polyphenol-treated nerve xenografts without FK506 administration or those receiving FK506 at 0.2 mg/kg per day in the present genomic study. This indicates that poly-phenol was not effective enough to suppress these rejections for surviving transplanted cells, even if rats were received FK506 at 0.2 mg/kg per day. Considering the results of our studies, polyphenols might not be able to reduce the immune reactions mediated by complements, but T-lymphocytes. According to the electrophysiological analyses, no significant difference was found in nerve regeneration between polyphenol-treated nerve xenografts and fresh nerve xenografts in rats receiving FK 506 at 2 mg/kg per day. However, the mean amplitude and MNCV were significantly lower in the rats enjoying polyphenol-treated nerve xenografts without FK506 administration than those receiving FK506 at 2 mg/kg per day. Moreover, significantly fewer myelinated axons were regenerated in the polyphenol-control group and polyphenol-0.2 group than in the fresh-2 xenograft group, but there was no significant difference between the polyphenol-2 group and the fresh-2 xenograft groups. There were no significant differences between the polyphenol-0.2, polyphenol-2 and fresh-2 xenograft groups in terms of mean axon diameter or myelinated axon densities. These results indicated that the number of myelinated axons depended on the dosage of FK506, however neither the mean axon diameter nor myelinated axon densities depended on the dosage of FK506. These results indicated that FK506 might have accelerated nerve sprouting in the transplanted nerve segments. In conclusion, polyphenols acted as immunosuppressants and can be effective in nerve allograft transplantation, but their effect was not sufficient to reduce the rejection of nerve xenografts.

REFERENCES