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The effect of a novel immunosuppressive drug, a PAK-2 inhibitor, on macrophage differentiation/polarization in a rat small intestinal transplantation model

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ABSTRACT

Objective: PQA-18 (Prenylated quinolinecarboxylic acid-18) has been reported to be a novel immunosuppressant that attenuates the production of various cytokines, and the differentiation of macrophages by inhibiting PAK2. In this study, we investigated the function of this drug mainly on macrophages using a rat small intestinal transplant model.

Methods: Male Dark Agouti (DA) and Lewis rats (LEW), 7–9 weeks of age, were used as donor and recipient, respectively. Approximately 15 cm intestinal grafts were heterotopically transplanted to the recipient rats. The recipient rat was treated with PQA-18 (4 mg/kg/day) by intraperitoneal injection (ip) from postoperative day 1 for 2 weeks. The in vivo effects of this drug were evaluated based on changes in body weight, and the population of each type of blood cell. Mixed lymphocyte reaction (MLR) was also assessed, using the T cells from intestinal mesenteric lymph nodes (MLN) of the grafts on POD6. Total cells from MLN and graft Payer's patch (PP) were next collected on POD6, and the number of infiltrated macrophages was determined.

Results: While the survival time was 7.0 ± 0.77 days for the control group ($n = 9$), that for the PQA-18 group was 10.7 ± 1.26 days ($n = 10$) ($p < .001$). Histological examinations showed a relatively clear difference in the grafts for both groups. In addition, the MLR response was significantly lower in recipients treated with PQA-18, suggesting PQA-18 well suppressed the T cells. Moreover, while a significant increase of both MHC class II and CD11b/c positive cells, estimated as differentiated/polarized macrophages, in MLN & PP was observed in the control group, PQA-18-administration significantly suppressed the differentiation of macrophages in the MLN & PP.

Conclusion: PQA-18 significantly prolonged the survival of the rats with intestinal grafts, and also suppressed the infiltration of lymphocytes, and macrophages to the grafts.

1. Introduction

Recent advances in immunosuppressive drugs have improved allogeneic transplant results. The appearance of calcineurin inhibitors (CNI) such as cyclosporine (CsA) and tacrolimus (TAC; FK506) has substantially increased the performance of organ transplantation [1,2]. However, although CNI have significantly improved short-term outcomes after organ transplantation by minimizing the incidence of acute graft rejection, long-term graft and patient survival have not markedly increased [3]. This evidence suggests that drug-induced toxicity such as nephrotoxicity is a determining factor for graft survival in allogeneic transplantation.

Furthermore, because the small intestine is one of the largest lymphoid organs in the body, the rejection of an allograft in the small

Abbreviations: CNI, calcineurin inhibitors; PQA-18, Prenylated quinolinecarboxylic acid-18; PAK2, p21-activated kinase 2; Ma, macrophage; DA, dark Agouti; LEW, Lewis; POD, postoperative day; DMSO, Dimethyl sulfoxide; MLR, Mixed Lymphocyte Reaction; BMDM, Bone marrow-derived macrophages; MLN, mesenteric lymph nodes; PP, Peyer's patch; Mo, monocyte; DC, dendric cells; MDSC, myeloid derived suppressor cells

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intestine is usually very severe and therefore represents a significant immunogenic burden to manage. The recent International Intestinal Transplant Registry (ITR) reported the 1-, 5-, and 10-year graft survival as 71%, 50%, and 41%, respectively, for intestinal grafts transplanted since 2000 [4]. This evidence suggests that, although short-term survival is high, the long-term results continue to be poor. Therefore, the development of a new immunosuppressive drug with a different suppressive mechanism from CNI and with less adverse effects would be highly desirable in the field of organ transplantation.

Prenylated quinolinecarboxylic acid-18 (PQA-18), a unique p21-activated kinase 2 (PAK2) inhibitor, has been recently reported to be an agent that exerts an immunosuppressive effect with less impairment in renal function [5].The investigators demonstrated, in an in vitro study, that PQA-18 suppresses the release of various cytokines from peripheral leukocytes. Furthermore, in an in vivo study using Nc/Nga mice, PQA-18 exhibited a therapeutic effect against dermatitis without any adverse effect in the liver and kidney. In addition, we recently demonstrated that PQA-18 suppresses the differentiation/polarization of macrophages (Ma) and induces a clear immunosuppressive effect in an in vitro xenogeneic model [6]. These findings suggest that PQA-18 has the potential to serve as a novel immunosuppressant that functions via a different suppression mechanism from CNI.

In this study, we report on an investigation of the effect of PQA-18 on the survival of small intestinal grafts, using an allogeneic rat intestinal transplantation model, focusing on the immunosuppressive function of this compound on macrophages.

2. Materials and methods

2.1. Animals and cells

Male dark Agouti (DA) rat (RT-1a) and Lewis (LEW) rats (RT-1l) weighting 230–280 g, 7–9 weeks old, were purchased from Japan SLC (Shizuoka, Japan). DA small intestines were heterotopically transplanted into LEW rats. The study was approved by the animal care committee of Osaka University Graduate School of Medicine (approval No.26-041-010), and was carried out in accordance with guidelines for the care and use of laboratory animals issued by the institute. Mouse fibroblast, L929 cells, were purchased from the Riken Cell Bank (RCB1422, Tsukuba, Japan) to gather the supporting cytokines to develop rat macrophage in vitro [7].

2.2. Surgical procedures of small intestinal transplantation

A heterotopic small intestinal transplantation was performed from DA rats to LEW rats precisely as was outlined in our previous study [8–11]. Briefly, after anesthesia was induced, an approximately 15 cm intestinal segment was removed from the donor. End-to-side anastomosis was performed with 8–0 proline between the donor superior mesenteric artery and the host aorta, and between the donor portal vein and the host inferior vena cava (IVC). A Thiry-Vella loop was placed in the right abdominal flank.

2.3. Experimental design

The recipient rats were treated with PQA-18 (4 mg/kg/day) by intraperitoneal injection (i.p.) from postoperative day 1 (POD1) for 2 weeks. The Dimethyl sulfoxide (DMSO, SIGMA) group, without PQA-18, was treated with exactly the same amount of DMSO as the solvent for PQA-18 in Figs. 3A and 5B. The rejection was manifested by progressive stoma coloring from ischemia and necrosis and the development of an abdominal mass, based on our previous studies [9]. On POD6, the recipients were sacrificed and graft mesenteric lymph nodes (MLN) and graft Peyer's patch (PP) were collected for use in a number of analyses. In addition, the appearance of grafts, the histology, and the number of each type of blood cells in the recipient rat were investigated.

2.4. Graft appearance, histology and blood sample

The damage of the intestinal graft was first compared by their appearance on POD6, and graft intestinal tissue from both groups was then obtained. For histopathological evaluation, formalin-fixed, paraffin embedded intestinal graft tissues were stained with hematoxylin and eosin. The slides were then analyzed according to our previous reports [9–11].

Blood samples were next collected from the rats before transplantation and at POD6. White blood cells (WBC), lymphocytes, granulocytes and monocytes were prepared from them, and the numbers of each type of cell were determined.

2.5. Mixed Lymphocyte Reaction (MLR) assay

Responder CD3+ cells (2×10^5) from the MLN of recipient LEW rats were prepared, using an Easy Sep™: Rat T cell isolation kit (STEMCELL Technologies, Vancouver, BC, Canada), and mixed with naïve DA rat spleen cells (1×10^5), treated with Mitomycine-C and cultured for 4 days in triplicate wells of round-bottomed microtiter plates. The RPMI1640 was suspended in 20 mM HEPES, 10% rat serum, non-essential amino acids, pyruvic acid and 2ME (50 μ mol/l) [10,11]. The WST-8 reagent solution (10 μl: Nakalai Tesque, Kyoto, Japan) was added to each well, followed by the 1h incubation. The absorbance was measured at 450 nm using a microplate reader (CORONA, Tokyo, Japan).

2.6. Generation of bone marrow derived macrophages

Bone marrow-derived macrophages (BMDM) were prepared using tibia and femurs of LEW Rats. Briefly, LEW rats were sacrificed and tibia and femurs were collected. Bone marrow cells were flushed using a 22-gauge needle and resuspended in PBS. Red blood cells were lysed using ACK buffer (Lonza, Basel, Switzerland) and cell mixtures were filtered using a cell strainer followed by centrifugation. After the removal of mesenchymal stromal cells by plastic adherence, the cells were resuspended in complete RPMI1640 medium supplemented with 20% L929-conditioned media [7], and then incubated for 4 days in the presence of DMSO or PQA-18. Additional feedings were performed at day 3 of culture.

2.7. Flow cytometry

Cells from MLN and PP and BMDM were stained with FITC anti-rat MHC class II antibody (RT1B, Biolegend, Japan KK., Tokyo, Japan) and APC anti-rat CD11b/c antibody (Biolegend, Japan KK.). The cells were analyzed using a FACS Canto flow cytometer (BD biosciences, Franklin Lakes, NJ). MHC class II and CD11b/c double positive cells were determined as differentiated/polarized macrophages [6].

2.8. Statistical analysis

Results are expressed as the mean ± SEM. The difference in survival curve was statistically evaluated using the Log-rank test. Other data were statistically evaluated using the paired Student's *t*-tests for comparisons between the two groups. Values of $p < .05$ were considered to be statistically significant.

3. Results

3.1. Suppression of graft rejection by PQA-18 in small intestinal transplantation

To evaluate the in vivo effect of PQA-18 on allogeneic intestinal

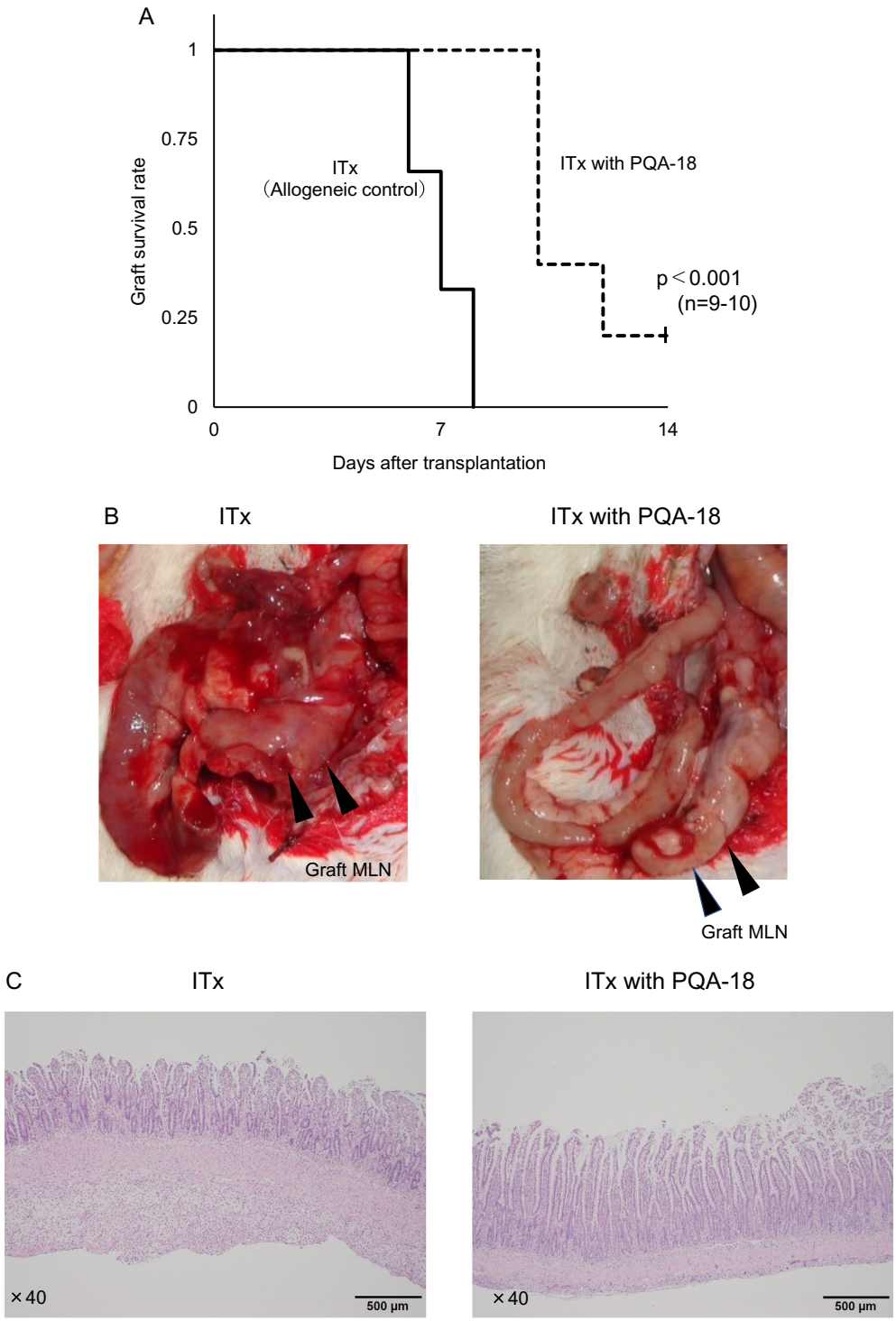


Fig. 1. Cumulative survival of grafts of small intestinal transplants. (A) Graft survival after rat small intestinal transplantation, DA to LEW, was compared between the allogeneic control group (ITx, solid line, $n = 9$) and the PQA-18 group (ITx with PQA-18, dotted line, $n = 10$). A significant prolongation in graft survival was observed in the PQA-18 group, compared with the control group. $p < .001$ by the Log-rank test. (B) The appearance of the grafts on POD6 in both groups was shown. While the control group shows progressive severe rejection, the PQA-18 group indicates a relatively good condition. (C) Histological findings in intestinal grafts on POD6 were next collected. In the control group, extensive mucosal destruction and decreased crypts were observed. On the other hand, the PQA-18 group showed relatively mild acute rejection.

transplantation, small intestines from DA rats were transplanted to LEW rats which were then treated with PQA-18. Graft survival was significantly prolonged in the PQA-18 group (Fig. 1A). While the survival time was 7.0 ± 0.77 days in the allogeneic control group ($n = 9$), the corresponding value for the PQA-18 group was 10.7 ± 1.26 days survival ($n = 10$, $p < .001$).

3.2. Histology of the graft

The appearance of the intestinal grafts on POD6 from the PQA-group were clearly different from those in the allogeneic control group, in that they were in much better condition with less ischemia (Fig. 1B).

Intestinal graft tissue for histological analysis was also obtained on POD6. The intestinal graft in the allogeneic control group showed severe acute rejection. Extensive mucosal destruction was observed, along with decreased numbers of crypts. In addition, the residual crypts exhibited marked epithelial injuries. On the other hand, in the graft from the PQA-18 group the villi were not shortened and the crypt epithelium showed less evidence of mucin depletion. The symptoms relate to rejection measured as less pronounced in the PQA-18 group (Fig. 1C).

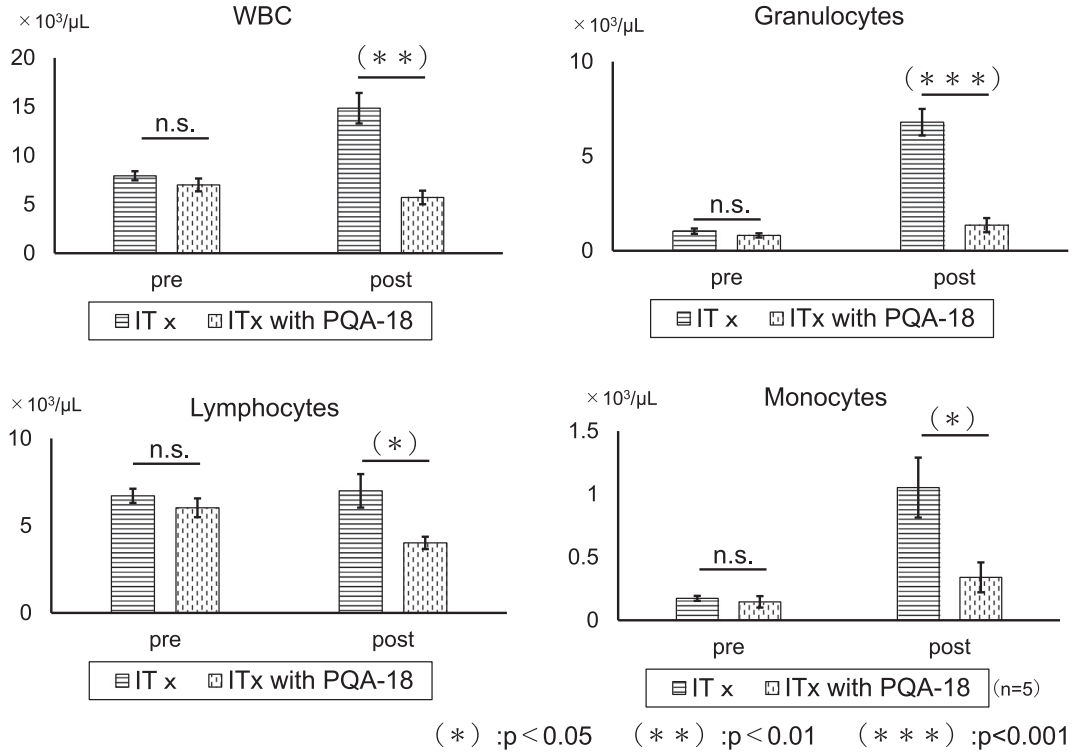


Fig. 2. Changes in the number of the blood cells in recipient rats. The number of White blood cells (WBC), Lymphocytes, Granulocytes and Monocytes on POD6 were counted in both the control and the PQA-18 groups, and the percent of the each cell was calculated, compared with those before transplantation. The percent of Lymphocytes in the control group was not changed between pre and post transplantation, but the others were significantly changed. Moreover, significant differences were found for all types of cells in post transplantation between the control and the PQA-18 groups.

3.3. Changes in blood cells in recipient rats

Concerning the assessment of blood cells, the number of blood cells, i.e., white blood cells, lymphocytes, granulocytes and monocytes were prepared and the numbers determined (Fig. 2). The difference in the % cell number between pre and post transplantation was significant in WBC, monocytes and granulocytes, and the % of each cell was significantly different between the control and the PQA-18 groups in the post transplant.

3.4. Assessment of toxicity

Body weight and kidney function were evaluated to determine the extent of toxicity due to PQA18 on the POD7 and POD14. No obvious renal dysfunction was observed at POD7 and POD14 in the PQA-18 group compared to the DMSO group (Fig. 3B, C). Although a slight decrease in body weight on day 14 was observed compared to the control group, this decrease was not significant. (Fig. 3A).

3.5. MLR assay

To examine the immunological reaction of T cells, MLR assays were performed. Compared with the allogeneic control group (9.44 ± 1.21), the MLR was significantly reduced in the PQA-18 group on POD6 (4.71 ± 0.55, $p < .05$, $n = 4$). Based on the results from the MLR assay, the donor specific immune response is lower in recipients treated with PQA-18 (Fig. 4).

3.6. Suppression of rat macrophage differentiation by PQA-18 in vitro

To evaluate the in vitro effect of PQA-18 in the differentiation of rat macrophages, bone marrow stem cells were isolated and cultured in the absence or presence of PQA-18 for 4 days. The % of both MHC class II and CD11b/c positive cells, estimated as differentiated macrophages, in bone marrow cells was determined by FACS analysis. PQA-18 induced a significant decrease in macrophage differentiation and this decrease was dose dependent (Fig. 5A, B).

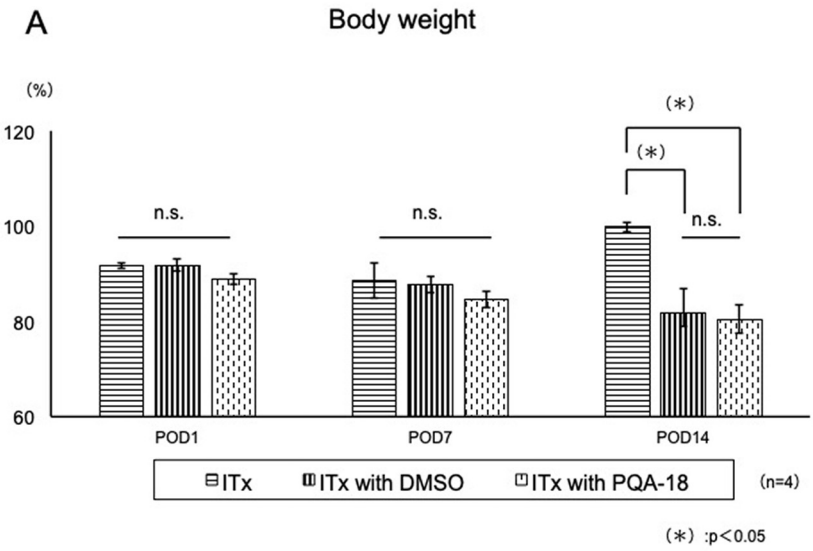
3.7. PQA-18 suppresses the accumulation of macrophages in graft intestinal MLN

To determine the in vivo effect of PQA-18 against rat macrophages, we measured the % of both MHC class II and CD11b/c positive cells, estimated as differentiated/polarized macrophages, in MLN and PP from recipient LEW rats by flowcytometry, on POD 6 (Fig. 6). The number of infiltrated macrophages was also significantly reduced to 45.20 ± 3.20% in the MLN of the PQA-18 group, while the number was 60.55 ± 2.18% ($p < .01$) in the control group. In addition, the number of the macrophages in the PP was 42.42 ± 3.61% in the control group, whereas the corresponding value for the PQA-18 group was 29.8 ± 3.21% ($p < .05$).

4. Discussion

After organ transplantation, the long-term use of high-dose immunosuppressants, such as CNI, may result in increased adverse effects [12,13]. Therefore, the objective of this study was to identify a new immunosuppressant for use in conjunction with organ transplantation, especially intestinal transplantation, with a different suppressive function from CNI and the mammalian target of rapamycin (mTOR), with less side effects.

In our previous series of studies, using an identical rat intestinal transplantation model, we analyzed the effects of several drugs, including FK506 (CNI), a major immunosuppression drug that is used in the case of clinical intestinal transplantation. In the clinic, FK506 caused a significant attenuation in the proliferation of allogeneic CD4+



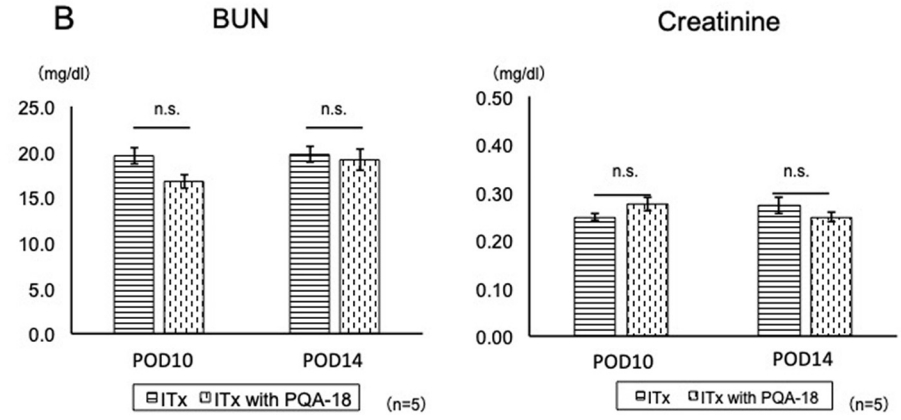


Fig. 3. Body weight loss and the failure of renal function after transplantation.
(A) Postoperative weight change after intestinal transplantation was shown. Changes in body weight in three groups are indicated as compared with those of pre transplantation. In the PQA group, PQA-18 (4 mg/kg) dissolved in 200 μl of DMSO was intraperitoneally administrated starting on POD1. On the other hand, 200 μl of DMSO, a solvent for PQA-18, was daily intraperitoneally administrated in the DMSO group (ITx with DMSO). There is no significant difference between the DMSO and PQA-18 groups. (B) Renal function of the recipient rats was evaluated by measuring creatinine and BUN levels in the serum. PQA-18 does not affect the renal function in recipient rats.

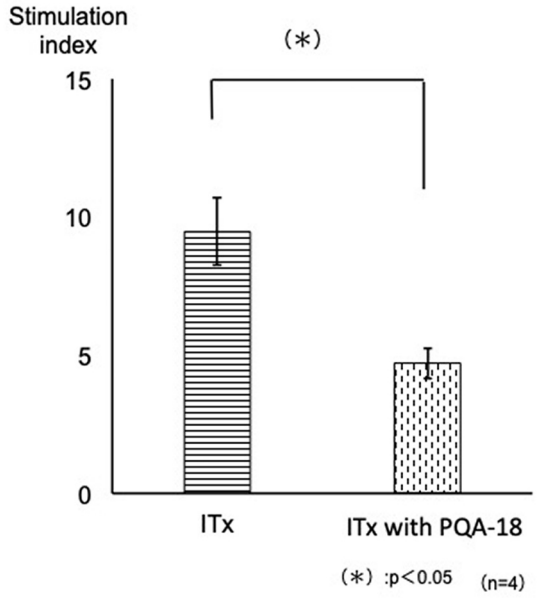


Fig. 4. Mixed lymphocyte reaction (MLR) functional assays.
The T cells from recipient LEW of both the control and the PQA-18 groups on POD6 were cultured with MMC-treated DA PBMC for 5 days. The ordinate shows the % corporation of WST-8. The stimulation index significantly reduced in the PQA-18 group.

T cells and IFN-γ secreting effector functions [11]. On the other hand, FYT720 resulted in a marked reduction in the numbers of lymphocytes in the graft, associated with a reduction of T cell recruitment. However, because of side effects, this drug is not in common use in transplantation field [8]. Concerning TAK779, a nonpeptide, this synthetic CCR5 and CXCR3 antagonist diminished the number of graft-infiltrating cells by suppressing the expression of CCR5 and CXCR3 in the graft. As a result, it reduced the total number of recipient T cells that are involved in graft rejection [10], suggesting that it has the potential for serving as a new immunosuppressant. In addition, an Anti-MadCAM-antibody, significantly down-regulated CD4+ T cell infiltration in the intestinal grafts by blocking the adhesion molecule, and could be useful as an induction therapy for preventing acute rejection [9].

A number of other reports by other groups using the same rat intestinal transplant model have appeared. These groups studied the effects of drugs, such as Clodronate (CL_2MDP) [14–16], FK506 [17], Rapamycine [18], and an anti-TNF antibody [19,20], mainly using a histological approach. Among them, Clodronate was found to induce the depletion of resident macrophages in a graft, and alleviated the infiltration & activation of leukocytes. On the other hand, FK506 also reduced the infiltration of ED1 & ED2-positive monocytes(Mo)/macrophages(Ma) to a greater extent than Rapamycin (mTOR) [18].

As the next step, we focused on a new drug, PQA-18, which has been reported to function as a novel immunosuppressant that attenuates the production of various cytokines, such as IL-2 & TNF-α, function on T cells, and the differentiation of macrophages by inhibiting PAK2. We demonstrated (using human cells) that it suppresses M1 macrophage differentiation and macrophage-mediated cytotoxicity in both the

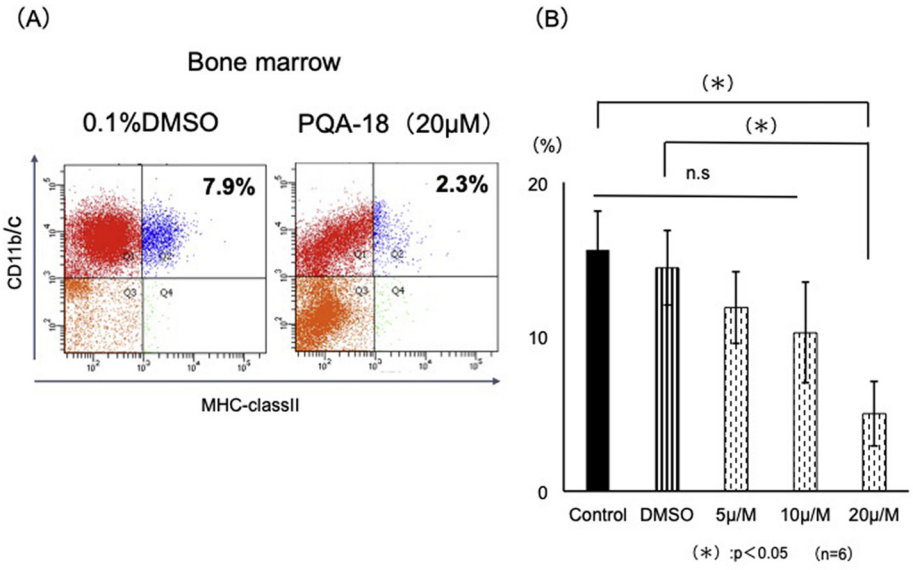


Fig. 5. Effect of PQA-18 on rat macrophage differentiation.
Bone marrow stem cells were isolated from naive LEW rats. After culturing for 4 days with L929 conditioned media and several doses of PQA-18, the cells were stained with anti-CD11b/c and MHC-II antibodies. Both CD11b/c and MHC-II positive cell was estimated as differentiated/polarized macrophages. (A) The FACS histogram in the group with 20 μM of PQA-18 indicated the suppression on macrophage differentiation by PQA-18. Data are representative of five different experiments. (B) Suppression of macrophage differentiation by each dose of PQA-18 was observed as compared with the control group and the DMSO group. In the DMSO group, DMSO, as the solvent for PQA-18, was added to the medium to adjust the condition of the cells to those of the PQA-18 group. The % differentiation was reduced in the dose dependent manner. Data represent the Mean ± SEM, $n = 6$, $^{*}p < .05$.

differentiation and effector phases, by suppressing the expression of CD11b, HLA-DR, CCR7 and CD40, as evidenced by MLR assays [5,6]. Furthermore, our experiments confirmed that PQA-18 is non-toxic to swine endothelial cells (SECs) and human monocytes [6].

On the other hand, in vitro assays using BMDM revealed that the PAK2 signaling pathway contributes to macrophage differentiation. It was previously reported that macrophages from $p21^{Waf1}$ KO mice are not protected from apoptosis that is induced by the withdrawal of growth factor, indicating that $p21^{Waf1}$ is a critical molecule in macrophage survival [21]. Another report also demonstrated that PAK2 activation occurs in murine BMDM stimulated with RANTES and that PAK2 activation is an essential step in macrophage proliferation [22]. These reports are in agreement with our findings in rat macrophages.

Based on these results, we focused on the in vivo influence of this drug on the rat Mo/Ma system using an intestinal transplantation model, and the findings indicated that graft survival was significantly improved by PQA-18 with no significant nephrotoxicity (Fig. 3). Concerning rat dendric cells (DC), we are not able to distinguish them from Mo/Ma at this time, because class II and CD11b/c are also expressed in established DC, and DC also is affected by this drug. One reasonable mechanism for the extended graft survival is that PQA-18 can affect the antigen presentation function of Mo/Ma/DC by suppressing their differentiation/polarization. Therefore, the lymphocytes are not able to receive sufficient stimulation to reject the graft. On the other hand,

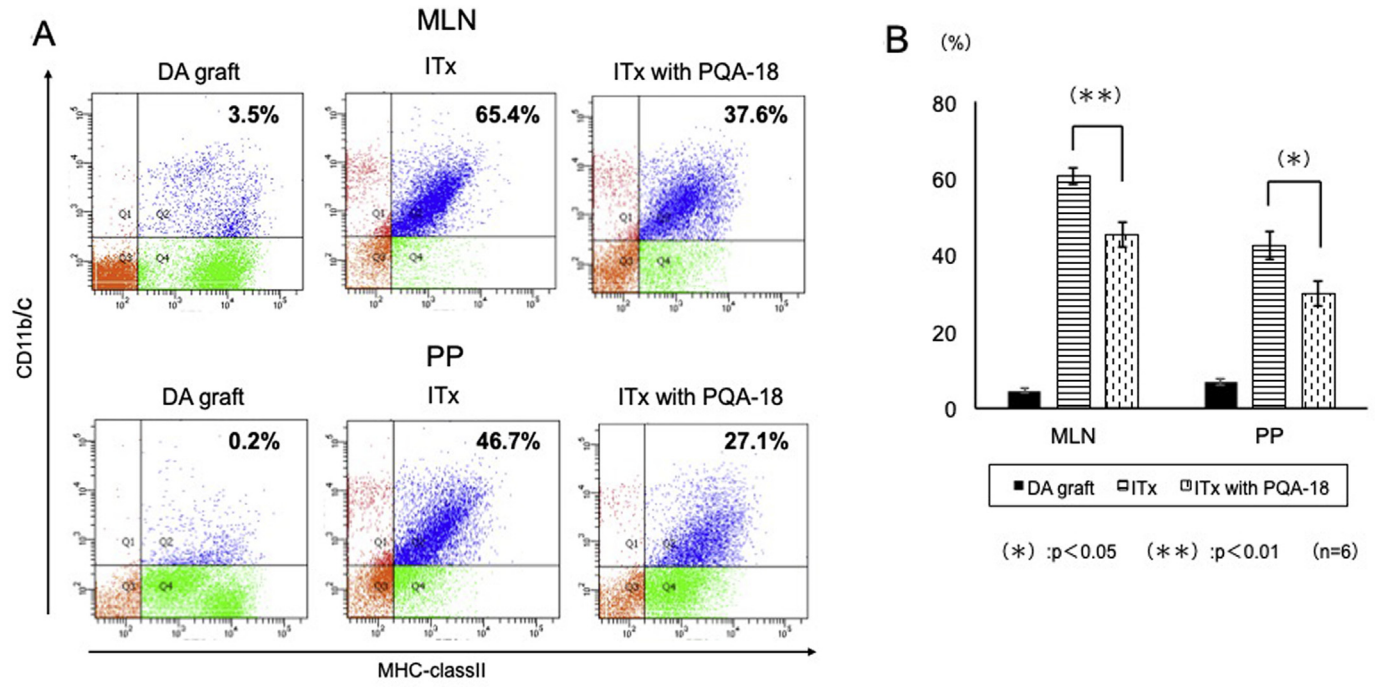


Fig. 6. Percent differentiation of the macrophage in graft.
MLN and PP were obtained from the grafted intestine in the control and the PQA-18 groups on POD 6. (A) The percent occupancy of both MHC class II and CD11b/c positive macrophages in graft MLN was examined by FACS. The MLN & PP from DA rats were used as control. Data are representative of five different experiments. (B) The percent occupancy of both MHC class II and CD11b/c positive macrophages in graft MLN and PP was represented by a FACS histogram. The % of the macrophages significantly reduced in the PQA-18 group. Data represent the Mean ± SEM, $n = 6$, $^{*}p < .05$, $^{**}p < .01$.

concerning the macrophage-mediated graft injury, there is little possibility that this injury in the case of an allograft [23,24], is not analogous to a xenograft [25]. In addition, PQA-18 has a direct function on T cells, even after presentation by DC & Mo/Ma. Therefore, in the MLR assay (Fig. 6), the difference was relatively clear between recipient rats with or without PQA-18.

Moreover, it was recently reported that PAK2 regulates the development of myeloid derived suppressor cells (MDSC) [26]. In their study, MDSC obtained from PAK2 KO mice are more proliferative than those from naïve mice, suggesting that a PAK2 inhibitor may be an effective strategy for inducing MDSC. The expansion of MDSC has been widely reported in various animal allogeneic graft models, and monocytic MDSCs have also been reported to be responsible for the induction of tolerance in a solid organ transplantation model [27]. Taking these findings into consideration, MDSC may be induced in our animal model as the result of PQA-18 injection. However, while the mouse MDSC is well identified as a CD11b + Gr-1 + subset, the rat MDSC has not been well recognized because of the absence of a Gr-1 homolog. Therefore, in this study we were not able to evaluate the effect of PQA-18 on MDSC accumulation into intestinal graft tissue [28–31].

In summary, we report herein that PQA-18 clearly prolongs the graft survival in a rat intestinal transplant system with no significant side effects. PQA-18 could function efficiently in rat immune cells by virtue of its ability to suppress not only rat mixed lymphocyte reactions but macrophage differentiations/polarizations as well. These results suggest that PQA-18 is a viable candidate for use as a new immunosuppressant for intestinal transplantation.

Authors' contribution

TK performed the research and participated in writing the article AM, and PL participated in performing the research. YN, CT, YT, TU, YT, HE, KM and HO participated in analysing the data, and AM and SM participated in creating the research design, and in writing the article.

Declaration of competing interest

The authors declare that they have no competing financial interests.

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References

[1] A. Rao, C. Luo, P.G. Hogan, Transcription factors of the NFAT family: regulation and function, Annu. Rev. Immunol. 15 (1997) 707–747.
[2] S.L. Schreiber, G.R. Crabtree, The mechanism of action of ciclosporin A and FK506, Immunol. Today 13 (1992) 136–142.
[3] K.E. Lamb, S. Lodhi, H.U. Meier-Kriesche, Long-term renal allograft survival in the United States: a critical reappraisal, Am. J. Transplant. 11 (2011) 450–462.
[4] D. Grant, K. Abu-Elmagd, G. Mazareigos, R. Vianna, A. Langnas, R. Mangus, et al., Intestinal transplant registry report: global activity and trends, Am. J. Transplant. 15 (2015) 210–219.
[5] M. Ogura, H. Kikuchi, T. Suzuki, J. Yamaki, M.K. Homma, Y. Oshima, et al., Prenylated quinolinecarboxylic acid derivative suppresses immune response through inhibition of PAK2, Biochem. Pharmacol. 105 (2016) 55–65.
[6] P.C. Lo, A. Maeda, T. Kodama, C. Takakura, T. Yoneyama, R. Sakai, et al., The novel immunosuppressant prenylated quinolinecarboxylic acid-18 (PQA-18) suppresses macrophage differentiation and cytotoxicity in xenotransplantation, Immunobiology (2019), https://doi.org/10.1016/j.imbio.2019.04.003.
[7] J. Weischenfeldt, B. Porse, Bone Marrow-Derived Macrophages (BMM): isolation and applications, Cold Spring Harb. Protoc. 3 (2008) 1–7.
[8] T. Kimura, T. Hasegawa, H. Nakai, T. Azuma, N. Usui, T. Sasaki, et al., FTY720 reduces T-cell recruitment into murine intestinal allograft and prevents activation of graft-infiltrating cells, Transplantation 75 (2003) 1469–1474.
[9] Y. Ihara, S. Miyagawa, T. Hasegawa, T. Kimura, H. Xu, M. Fukuzawa, Effect of blocking the mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in a rat small intestinal transplantation model, Transpl. Immunol. 17 (2007) 271–277.
[10] H. Xu, S. Firdawes, A. Yamamoto, Y. Zhao, Y. Ihara, S. Uehara, et al., Effects of blocking the chemokine receptors, CCR5 and CXCR3, with TAK-779 in a rat small intestinal transplantation model, Transplantation 86 (2008) 1810–1817.
[11] Y. Takama, S. Miyagawa, A. Yamamoto, S. Firdawes, T. Ueno, Y. Ihara, et al., Effects of a calcineurin inhibitor, FK506, and a CCR5/CXCR3 antagonist, TAK-779, in a rat small intestinal transplantation model, Transpl. Immunol. 25 (2011) 49–55.
[12] M. Wijkstrom, N. Kirchhof, M. Graham, E. Ingulli, R.B. Colvin, U. Christians, et al., Cyclosporine toxicityin immunosuppressed streptozotocin-diabetic nonhuman primates, Toxicology 207 (2005) 117–127.
[13] X. Cai, J. Harnaha, P.N. Rao, J. Flowers, R. Venkataramanan, V. Warty, et al., Low-dose of FK 506 and associated blood levels in allotransplantation of rat liver, heart, and skin, Transplant. Proc. 24 (1992) 1403–1405.
[14] J. Fryer, D. Grant, J. Jiang, P. Metrakos, N. Ozcay, C. Ford, et al., Influence of macrophage depletion on bacterial translocation and rejection in small bowel transplantation, Transplantation 62 (1996) 553–559.
[15] N. Schaefer, K. Tahara, J. Schmidt, S. Wehner, J.C. Kalff, K. Abu-Elmagd, et al., Resident macrophages are involved in intestinal transplantation-associated inflammation and motoric dysfunction of the graft muscularis, Am. J. Transplant. 7 (2007) 1062–1070.
[16] N. Schaefer, K. Tahara, M. von Websky, S. Wehner, T. Pech, et al., Role of resident macrophages in the immunologic response and smooth muscle dysfunction during acute allograft rejection after intestinal transplantation, Transpl. Int. 21 (2008) 778–791.
[17] T. Pech, J. Fujishiro, T. Finger, I. Ohsawa, M. Praktiknjo, K. Abu-Elmagd, et al., Combination therapy of tacrolimus and infliximab reduces inflammatory response and dysmotility in experimental small bowel transplantation in rats, Transplantation 93 (2012) 249–256.
[18] T. Pech, J. Fujishiro, T. Finger, M. von Websky, B. Stoffels, S. Wehner, et al., Effects of immunosuppressive therapy after experimental small bowel transplantation in rats, Transpl. Immunol. 25 (2011) 112–118.
[19] T. Pech, T. Finger, J. Fujishiro, M. Praktiknjo, I. Ohsawa, K. Abu-Elmagd, et al., Perioperative infliximab application ameliorates acute rejection associated inflammation after intestinal transplantation, Am. J. Transplant. 10 (2010) 2431–2441.
[20] T. Pech, M. von Websky, I. Ohsawa, K. Kitamura, M. Praktiknjo, A. Jafari, et al., Intestinal regeneration, residual function and immunological priming following rescue therapy after rat small bowel transplantation, Am. J. Transplant. 12 (2012) S9–17.
[21] M. Comalada, J. Xaus, E. Sanchez, A.F. Velledor, A. Celada, Macrophage colony stimulating factor-, granulocyte-macrophage colony-stimulating factor-, or IL-3 dependent survival of macrophages, but not proliferation, requires the expression of p21(Waf1) through the phosphatidylinositor 3-kinase/Akt pathway, Eur. J. Immunol. 34 (2004) 2257–2267.
[22] C. Weiss-Haljiti, C. Pasquali, H. Ji, C. Gilleron, C. Chabert, M.L. Curchod, et al., Involvement of phosphoinositide 3-kinase gamma, Rac,and PAK signaling in chemokine-induced macrophage migration, J. Biol. Chem. 279 (2004) 43273–43284.
[23] C. Ashokkumar, M. Ningappa, S. Ranganathan, B.W. Higgs, Q. Sun, L. Schmitt, et al., Increased expression of peripheral blood leukocyte genes implicate CD14 + tissue macrophages in cellular intestine allograft rejection, Am. J. Pathol. 179 (2011) 1929–1938.
[24] K.R. Wyburn, M.D. Jose, H. Wu, R.C. Atkins, S.J. Chadban, The role of macrophages in allograft rejection, Transplantation 80 (2005) 1641–1647.
[25] A. Maeda, T. Kawamura, T. Ueno, N. Usui, H. Eguchi, S. Miyagawa, The suppression of inflammatory macrophage-mediated cytotoxicity and proinflammatory cytokine production by transgenic expression of HLA-E, Transpl. Immunol. 29 (2013) 76–81.
[26] Y. Zeng, S. Hahn, J. Stokes, E.A. Hoffman, M. Schmelz, M. Proytcheva, et al., Pak2 regulates myeloid-derived suppressor cell development in mice, Blood Adv. (22) (2017) 1923–1933.
[27] M.R. Garcia, L. Ledgerwood, Y. Yang, J. Xu, G. Lal, B. Burrell, et al., Monocytic suppressive cells mediate cardiovascular transplantation tolerance in mice, J. Clin. Invest. 120 (2010) 2486–2496.
[28] A.S. Dugast, T. Haudebourg, F. Coulon, M. Heslan, F. Haspot, N. Poirier, et al., Myeloid-derived suppressor cells accumulate in kidney allograft tolerance and specifically suppress effector T cell expansion, J. Immunol. 180 (2008) 7898–7906.
[29] A. Mazzoni, V. Bronte, A. Visintin, J.H. Spitzer, E. Apolloni, P. Serafini, et al., Myeloid suppressor lines inhibit T cell responses by an NO- dependent mechanism, J. Immunol. 168 (2002) 689–695.
[30] N. Dilek, N. Poirier, C. Usal, B. Martinet, G. Blancho, B. Vanhove, Control of transplant tolerance and intragraft regulatory T cell localization by myeloid-derived suppressor cells and CCL5, J. Immunol. 188 (2012) 4209–4216.
[31] A. Maeda, H. Eguchi, K. Nakahata, P.C. Lo, K. Yamanaka, T. Kawamura, et al., Monocytic MDSCs regulate macrophage-mediated xenogenic cytotoxicity, Transpl. Immunol. 33 (2015) 140–145.