Effects of the new immunosuppressive agent AEB071 on human immune cells

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Abstract

Background. The novel immunosuppressive agent AEB071 is currently being evaluated for its capability to prevent rejection after kidney transplantation as a potential adjunct to calcineurin inhibitor-based regimen. AEB071 is a selective protein kinase C inhibitor and has been shown to be well tolerated in humans. We here present extensive in vitro studies that contribute to the understanding of AEB071 effects on human lymphocyte, natural killer (NK) cell and dendritic cell (DC) action.

Methods. The impact of AEB071 on several T-cell activation and costimulatory markers was assessed. Furthermore, assays were performed to study the effect on T-cell proliferation and intracellular cytokine production. Additionally, the effect of AEB071 on DC maturation and their capacity to stimulate allogeneic T-cells was examined. Also, an evaluation of AEB071 effects on the lytic activity of human NK cells was performed.

Results. We were able to show that T-cell proliferation and cytokine production rates are significantly reduced after AEB071 administration. Also, mitogen-induced T-cell activation characterized by expression levels of surface markers could be significantly inhibited. In contrast, the T-cell stimulatory capacity of AEB071-treated mature monocyte-derived DC (Mo-DC) is not reduced, and AEB071 administration does not prevent lipopolysaccharide (LPS)-induced Mo-DC maturation. It could be demonstrated that AEB071 significantly inhibited the cytotoxic activity of NK cells.

Conclusions. The promising immunosuppressive agent AEB071 has a strong impact on T-cell activation, proliferation and cytokine production as well as NK cell activity, but not DC maturation in vitro, and therefore, seems to function T-cell and NK cell specific via protein kinase C (PKC) inhibition.

Keywords: AEB071; immunosuppression; kidney; transplantation

Introduction

Renal transplantation is an established therapy method for organ failure, and 1-year success rates are high. Immunosuppressive therapy has improved in recent years, but acute rejection remains a serious and frequent complication after renal transplantation. Since the infiltration of transplanted organs with immunocompetent cells may lead to tissue damage and an impaired long-term function, a lifelong administration of immunosuppressants is required. The majority of renal transplant recipients are currently being treated with inhibitors of the calcium-dependent serine/threonine phosphatase calcineurin, like cyclosporin A and tacrolimus. As a consequence of calcineurin inhibition, the activity of the transcription factor nuclear factor of activated T-cell (NFAT) is decreased, which is essential for the signal cascade leading to allograft rejection. Although effective, the use of calcineurin inhibitors can cause severe side effects, most notably nephrotoxicity [1,2]. The development of new immunosuppressive substances that lack toxicity of current agents but show high efficiency in preventing rejection processes is highly important for transplant medicine, but no new immunosuppressive agent has been approved in recent years.

One potential target for new immunosuppressive agents is protein kinases C (PKC). These enzymes, which were isolated for the first time in 1977 [3], are a family of serine- and threonine-specific protein kinases that hold a central role in diverse cell-signalling pathways due to their regulatory functions. The PKC family consists of at least 12 currently known isoenzymes with different amino acid sequences and regulatory mechanisms and is divided into three subfamilies due to their structure and type of activation [4]: classical/conventional (cPKC), novel (nPKC) and atypical (aPKC) PKC. The members of the PKC family not only serve as major receptors for phorbol esters, a class of tumour promoters [5,6], but activation also triggers a translocation of calmodulin that precedes one of PKC's major substrates myristoylated alanine-rich C-kinase substrate.
stimulate allogeneic T-cells was studied. AEB071 on human DC maturation and their capacity to activate, proliferation, cytokine production and NK cell activity against K562 cells were performed. Additionally, the effect of AEB071 on human lymphocyte activation, proliferation and especially T-cells and DCs as the two key players in mediating processes like development, differentiation, activation and survival of lymphocytes [13,14], macrophage and dendritic cell (DC) activation [15,16] and B-cell survival [17,18].

The development of PKC-specific inhibitors and the understanding of PKC isoforms mode of action could lead to the establishment of new treatment options for disease. Several modulators of PKC activity have been used in animal models and also in clinical trials, concerning the treatment of cancer [19–22], leukemia [23], heart failure [24] and diabetes-associated complications [25,26]. The relevance of PKCs for graft rejection has also been demonstrated in animal transplantation models [27,28]. AEB071 (molecular weight of acetate salt 498.55) is a novel therapeutic agent [29] displaying highly potent and selective inhibitory properties of the cPKC and nPKC isoforms [30]. It has been shown to prolong islet graft survival in a rodent allogeneic transplant model [31] and has been proven to safely prolong rat and monkey organ allograft survival times (World Transplant Congress 2006; Abstracts number 57, 546, 550, 741). This first orally administered PKC inhibitor is also well tolerated in humans [32], and its efficacy, safety and tolerability are currently being investigated in an open label, randomized multi-centre study with de novo renal transplanted patients. For the initiation of T-cell-mediated immune responses and subsequently acute and chronic allograft rejection, DCs as antigen-presenting cell (APC) play a pivotal role. We therefore present here the first detailed evaluation of the in vitro immunomodulatory effects of the new PKC inhibitory compound AEB071 on natural killer (NK) cells and especially T-cells and DCs as the two key players in rejection processes. Investigations concerning the impact of AEB071 on human lymphocyte activation, proliferation, cytokine production and NK cell activity against K562 cells were performed. Additionally, the effect of AEB071 on human DC maturation and their capacity to stimulate allogeneic T-cells was studied.

Material and methods

**T lymphocyte proliferation—mitogenic stimulation**

Isolation of peripheral blood mononuclear cells (PBMC) from six healthy volunteers was performed by density gradient centrifugation over Ficoll-Paque (GE Healthcare, Uppsala, Sweden). The cells were incubated in complete medium [RPMI 1640 with 2 mM l-glutamine (Biochrome AG, Berlin, Germany) and 1% penicillin/streptomycin (Biochrome AG, Berlin, Germany)], supplemented with 10% FCS (Biochrome AG, Berlin, Germany), in BD Primaria™ 24-well Multitray™ plates for 1.5 hours. Adherent cells were used for monocyte isolation, whereas the cells in the supernatant were washed and labelled with 5 μM carboxyfluorescin diacetate succinimidylester (CFSE) (Invitrogen, Karlsruhe, Germany) in PBS for 4 min at room temperature. CFSE-labelled cells were washed three times, incubated in complete medium/10% FCS/50 μM 5-Mercaptoethanol (Strategene, LaJolla, USA) with or without 7.5 μg/mL ConA (Sigma, Munich, Germany) stimulation and either treated with 5, 50, 250, 500 nM or 5 μM AEB071 (gift from Novartis Pharma Basel; dissolved in pure DMSO), respectively, or left untreated with DMSO (Sigma, Munich, Germany) as solvent control in 96-well plates. After 96 hours, cells were harvested, stained with labelled a-CD4 mAb (BD Biosciences, Heidelberg, Germany), and cell division was analysed by measuring CFSE intensity using flow cytometry.

**T lymphocyte proliferation—allogenic stimulation**

Untouched CD4+ T-cells were isolated from PBMC after gradient centrifugation with the CD4+ T Cell Isolation Kit II (Miltenyi, Bergisch Gladbach, Germany). They were labelled with CFSE as described above and incubated with CD3-depleted allogeneic PBMC as APC (Human CD3 Depletion Cocktail, StemCell Technologies, Köln, Germany) or APC as syngeneic controls in complete medium supplemented with autologous serum. The cells were treated with various AEB071 concentrations and harvested, stained and measured as described above on Day 5.

**Expression of mitogen-stimulated T lymphocyte surface markers**

PBMC were isolated by density gradient centrifugation and stimulated with 7.5 μg/mL ConA in complete medium supplemented with 10% FCS, or left unstimulated in complete medium/FCS only. The cells were either treated with 30, 250 or 500 nM AEB071, respectively, or remained untreated with DMSO as solvent controls for 72 hours at 37°C in 5% CO2. After incubation the cells were harvested, washed three times and stained with a-CD3 mAb and the relevant labelled mAb (a-ICOS, a-CTLA-4, a-CD69, a-CD71, a-CD25, a-PD-1, a-Ox40, a-CXCR3; BD Biosciences, Heidelberg, Germany) according to the manufacturer's protocol. Samples were analysed by flow cytometry, and percentage of marker positive CD3+ cells were determined.

**Expression of intracellular cytokines**

After PBMC isolation, cells were stimulated for 5 hours with ionomycin (MP Biomedicals, Eschwege, Germany) / PMA (Sigma) as an optimal stimulus [33] for cytokine production, and secretion was blocked with breflidin A (BD Biosciences, Heidelberg, Germany). AEB071 in three different concentrations or DMSO as solvent control was added. After harvesting, the cells were labelled with a-CD3 mAb, and fixation and permeabilization were carried out with the BD Fixation/Permeabilization Solution kit according to the manufacturer's instructions. Antibodies directed against interleukin 2 (IL-2) and interferon-gamma (IFNγ) (BD Biosciences, Heidelberg, Germany) were used to label intracellular cytokines for fluorescence-activated cell sorting (FACS) analysis.

**Generation of mature human Mo-DC/allogeneic T-cell proliferation**

Monocytes were purified from peripheral blood by density gradient centrifugation and by 1.5-hour plastic adherence in BD Primaria™ 24-well Multitray™ plates followed by extensive washing of plates. For monocyte-derived DC (Mo-DC) differentiation, cells were incubated in complete medium containing 1% heat-inactivated (56°C, 30 min) autologous serum, supplemented with 800 U/mL rGM-CSF (Cellgenix, Freiburg, Germany) and 500 U/mL rIL-4 (Cellgenix, Freiburg, Germany). Fresh medium containing 400 U/mL GM-CSF and 500 U/mL IL-4 was applied after 48 hours. Two days later, Mo-DC maturation was induced by adding 500 ng/mL lipopolysaccharide (LPS) (Axoxa, Loerrach, Germany), 40 U/mL GM-CSF and 500 U/mL IL-4 to the cells, whereas controls were cultured without LPS. AEB071 at 250 or 500 nM, respectively, or

**AEB071 on human DC maturation and their capacity to...**
DMSO as solvent control was administered during the maturation period, and cells were incubated at 37°C in 5% CO₂ for 48 h. For assessment of the T-cell stimulatory capacity of AEB071-treated Mo-DC, irradiated (15 min, 3000 rad, 137Cs source) Mo-DC as stimulator cells were added to CFSE-labelled allogeneic PBMC (isolation and staining procedure as described above) in a 1:10 ratio and cultured in complete medium supplemented with 10% FCS/50 μM B-Mercaptoethanol. After a 96-hour culture period in V bottom 96-well microtiter plates, cells were harvested and stained with labelled α-CD4 mAb (BD Biosciences, Heidelberg, Germany), and cell division was analysed by measuring CFSE intensity using flow cytometry.

Mo-DC surface marker expression
Human PBMC were isolated, and monocytes were obtained as described above. Differentiation of Mo-DC was performed for 4 days, and cells were subsequently cultured without LPS or matured as described above, either with DMSO as solvent control or in the presence of 30, 250 or 500 nM AEB071, respectively. The DC phenotype was analysed on CD11c+ cells by flow cytometry using the following labelled mAb according to the manufacturer's instruction: α-CD40, α-CD80, α-CD83, α-CD86, α-HLA-DR (BD Biosciences, Heidelberg, Germany) and α-B7-DC (eBioscience, San Diego, USA).

Calcein–AM cytotoxicity assay
Cytotoxicity was quantified by a modified version of the calcein–acetoxymethyl (AM) release assay described by Neri et al. [34]. Cells of the NK-susceptible erythroleukaemia line K562 were used as targets. They were cultured in suspension in complete medium supplemented with 10% FCS. K562 cells were kept at log growth phase before use, harvested and incubated in serum-free culture medium containing 20 μM calcein–AM (MoBiTec, Goettingen, Germany) for 30 min at 37°C with occasional shaking. Afterwards, cells were washed three times in cold medium and plated in V bottom 96-well microtiter plates. PBMC from six healthy donors were used as effector cells. After separating the cells from the peripheral blood by density gradient centrifugation, they were activated with 7.5 μg/mL ConA (Sigma, Munich, Germany) for 44 hours to mimic an inflammatory environment and either treated with 500 nM AEB071 or left untreated with DMSO as solvent controls. After incubation, the lymphocytes were washed three times and added to the target cells in a final volume of 150 μL with effector:target (E:T) ratios ranging from 50:1 to 0.5:1 in triplicate. For maximum calcein–AM release, target cells only in at least six replicate wells were incubated in medium containing 2% Triton-X 100 (Sigma, Munich, Germany). The spontaneous release was determined in at least six replicate wells by cells incubated in complete medium/FCS. After 4-hour incubation at 37°C in 5% CO₂, the cells were pelleted by centrifugation, and 75 μL of each supernatant was harvested and transferred into Black ViewPlate-96 plates (PerkinElmer, Rodgau-Juegesheim, Germany). Samples were measured using a fluorimeter by reading arbitrary fluorescent units (485 nm excitation/535 nm emission; Tecan Spectrafluor, Tecan Deutschland GmbH, Crailtheim, Germany). Specific killing (%) was calculated with the formula [(test release − spontaneous release) / (maximum release − spontaneous release)] × 100.

Statistical analysis
The statistical significance of the results (*P < 0.05) was calculated with the non-parametric Wilcoxon matched pairs test applying SPSS 12.0 for Windows.

Results
AEB071 effect on T-lymphocyte proliferation after mitogenic/allogeneic stimulation
The influence of AEB071 administration on human T-lymphocyte proliferation was explored by measuring the proliferation rates of CFSE-labelled CD4+ T-cells that were stimulated with either ConA or allogeneic APC in the absence or presence of AEB071. We detected a clear suppression of proliferative responses of CD4+ T-cells (Figure 1B) that was dose-dependent after ConA induction (Figure 1A) as well as allogeneic stimulation (Figure 1C).
To analyse the impact of AEB071 on T-cell surface markers, CD3+ T-cells from six healthy individuals were examined by flow cytometry after treatment with or without AEB071 and stimulation with the lectin ConA, which preferentially activates T-cells. The expression of activation and costimulatory markers inducible co-stimulator (ICOS), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), which binds with high avidity to CD80 and CD86 present on APC, CD69, CD71, CD25, Ox40, PD-1 and CXCR3, which interacts with its ligands to mediate T-cell chemotaxis, was examined. The ConA-stimulated control CD3+ T-cells showed high expression levels of all analysed markers compared to unstimulated controls. AEB071 administration during activation caused a significant downregulation of ICOS, CTLA-4, CD71, CD25, CXCR3, Ox40, PD-1 and PD-1 expression, but not of CD69 expression (Figure 2, Table 1).

**AEB071 effect on the mitogen-stimulated expression of T-cell surface markers**

To analyse the impact of AEB071 on T-cell surface markers, CD3+ T-cells from six healthy individuals were examined by flow cytometry after treatment with or without AEB071 and stimulation with the lectin ConA, which preferentially activates T-cells. The expression of activation and costimulatory markers inducible co-stimulator (ICOS), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), which binds with high avidity to CD80 and CD86 present on APC, CD69, CD71, CD25, Ox40, PD-1 and CXCR3, which interacts with its ligands to mediate T-cell chemotaxis, was examined. The ConA-stimulated control CD3+ T-cells showed high expression levels of all analysed markers compared to unstimulated controls. AEB071 administration during activation caused a significant downregulation of ICOS, CTLA-4, CD71, CD25, CXCR3, Ox40, CXCR3 and PD-1 expression, but not of CD69 expression (Figure 2, Table 1).

**AEB071 influence on intracellular cytokine production of PMA/ionomycin-stimulated T-lymphocytes**

The impact of AEB071 on cytokine production of T-cells induced by PMA/ionomycin stimulation was analysed by flow cytometry after cell treatment with the potent PKC inhibitor. PKC isoforms are crucial for IL-2 production and therefore, T-cell proliferation as well as IFN-γ production [35,36]. PMA/ionomycin-stimulated control cells displayed high cytokine expression levels. The administration of high AEB071 concentrations caused significantly suppressed expression levels of IL-2 and IFN-γ in CD3+ T-cells (Figure 3A and B).

**AEB071 effect on Mo-DC-mediated T-lymphocyte proliferation**

The T-cell stimulatory capacity of Mo-DC was assessed in mixed cultures of allogeneic PBMC and irradiated Mo-DC that were LPS-matured or LPS-unstimulated in the presence or absence of AEB071. CFSE was used to examine proliferation rates of responder CD4+ T-cells after 96 hours of co-culture. LPS-induced maturation of stimulator

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**Table 1. Effect of AEB071 treatment on mitogen-stimulated surface marker expression on CD3+ T-cells**

<table>
<thead>
<tr>
<th>Surface molecule</th>
<th>30 nM AEB071</th>
<th>250 nM AEB071</th>
<th>500 nM AEB071</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICOS</td>
<td>43.5* (±4.93)</td>
<td>65.3* (±2.16)</td>
<td>77.4* (±3.13)</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>35* (±7.98)</td>
<td>29.4* (±12.77)</td>
<td>n.i.</td>
</tr>
<tr>
<td>CD69</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>CD71</td>
<td>7.7* (±6.61)</td>
<td>20.3* (±3.69)</td>
<td>24.0* (±4.96)</td>
</tr>
<tr>
<td>CD25</td>
<td>15.0* (±4.46)</td>
<td>36.8* (±3.88)</td>
<td>46.5* (±5.53)</td>
</tr>
<tr>
<td>Ox40</td>
<td>29.4* (±5.94)</td>
<td>54.6* (±4.55)</td>
<td>65.7* (±3.44)</td>
</tr>
<tr>
<td>PD-1</td>
<td>n.i.</td>
<td>n.i.</td>
<td>28.5* (±9.35)</td>
</tr>
<tr>
<td>CXCR3</td>
<td>11.4* (±5.12)</td>
<td>32.6* (±6.29)</td>
<td>43.0* (±5.46)</td>
</tr>
</tbody>
</table>

Expression of surface markers on T-cells after ConA stimulation was assessed by flow cytometry. The percentage of CD3+ cells positive for ICOS, CTLA-4, CD69, CD71, CD25, Ox40, PD-1 and CXCR3 was determined, and results are displayed as median percent inhibition caused by AEB071 administration relative to untreated control. 95% CI is shown in parentheses (**P < 0.05; n.i. no inhibition).
Mo-DC caused a significant upregulation of Mo-DC-mediated CD4+ T-cell proliferation after 96 hours, but the administration of different AEB071 concentrations during Mo-DC maturation did not affect T-cell proliferation rates (Figure 4).

**Phenotypic characterization of mature Mo-DC after AEB071 treatment**

Human immature Mo-DC were generated in vitro under the influence of GM-CSF and IL-4 and matured with LPS upon which they upregulate the expression of adhesion and co-stimulatory molecules as well as both major histocompatibility complex (MHC) classes and molecules essential for migration [37]. The effects of AEB071 treatment on their phenotype was analysed on CD11c+ Mo-DC. The LPS-matured control Mo-DC showed high expression levels of the typical DC marker CD83, the co-stimulatory markers CD40, CD80 and CD86, and the new DC marker B7-DC as well as human leucocyte antigen-DR (HLA-DR). The administration of AEB071 had no effects on the expression of surface and maturation markers CD83, CD40, CD80, CD86 and HLA-DR and only a minor effect with the lowest dose of 30 nM AEB071 on B7-DC (Figure 5).

**AEB071 influence on lytic activity of NK cells against K562**

To evaluate the AEB071 influence on cell-mediated cytotoxicity, AEB071-treated or untreated PBMC were used as effectors in a calcein–AM cytotoxicity assay. To provide an inflammatory environment, cells were stimulated during AEB071 administration. K562 cells do not express MHC class I antigens and cannot be lysed by cytotoxic T-lymphocytes. They were labelled with calcein and used as target cells. The immunosuppressive agent caused a significant reduction in cell lysis at all E:T ratios (Figure 6).

**Discussion**

Since the survival and long-term function of renal allografts depend on the suppression of alloreactive immune responses, we have investigated the impact of the new immunosuppressive substance AEB071 on human effector cells of alloresponses leading to rejection. The results of this detailed in vitro study with human primary cells (approved by the local ethics committee) show that the novel PKC inhibitor seems to be highly potent in suppressing immune functions of specifically T-cells and NK cells.

Concerning T-cells as one of the primary mediators of rejection processes, we investigated if AEB071 is indeed a PKC inhibitor that interferes with T-cell activation and function. The concentration range was chosen according to the known trough level of 400–1000 ng/mL (Budde et al. 2010). The cell surface markers measured on lectin-activated CD3+ T-cells all play pivotal roles in either initiating downstream signalling for rejection processes by binding of cytokines, or in T-cell-APC interactions and therefore, in providing necessary costimulatory signals for immune responses to donor antigen [38–45]. Our experiments revealed that AEB071 potently inhibits activation of CD3+ T-cells as assessed by the surface expression of activation and co-stimulatory markers ICOS, CTLA-4, CD71, CD25, Ox40, CXCR3 and PD-1 (Figure 2, Table 1). CD69 is expressed early during T-cell activation, but reports about a correlation of the CD69 expression levels on peripheral blood lymphocytes and ongoing renal allograft rejection seem to be controversial [46,47]. In our in vitro study, we did not observe an impact of AEB071 on CD69 expression, which indicates that the AEB071-mediated PKC inhibition is differentially regulated and might not function unspecifically. Since T-cell activation and proliferation are major hallmarks of graft rejection, we additionally investigated the effects of AEB071 on APC-independent/Con-A-stimulated as well as APC-stimulated proliferative processes and observed a dose-dependent inhibition of CD4+ T-cell proliferation caused by AEB071 treatment (Figure 1A–C). The production of IL-2 as the crucial adaptive immunity cytokine and of the prototypic Th1 cell cytokine IFNγ was also significantly downregulated (Figure 3A and B). Taken together, the significant impairment of IL-2 production (Figure 3A) and also the subunit of the IL-2 receptor CD25 expression (Figure 2E) would indicate a limitation of T-cell clonal expansion which corresponds with the observation that AEB071 treatment also blocked T-cell proliferation (Figure 1A–C). In summary, this new immunosuppressant suppressed T-cell activation, proliferation and cytokine production, which are mechanisms causally linked to graft rejection.

DCs are the other major mediators of rejection by initiating T-cell responses. Various costimulatory molecules on APC such as members of the B7-family are involved. Maturation is the essential step for DC in becoming potent APC, and its pharmacological inhibition could suppress unwanted immune responses subsequently leading to T-cell activation and allograft rejection. We therefore addressed the question if AEB071 had effects on this stage.
in DC development. The *in vitro* administration of AEB071 had no significant impact on the maturation of human Mo-DC defined by an upregulation of DC surface markers and co-stimulatory molecules HLA-DR, CD80, CD86, CD83, B7-DC and CD40 (Figure 5) [48–53]. Also, the T-cell stimulatory function of AEB071-treated, mature DC was not impaired as shown in mixed cultures with allogeneic PBMC (Figure 4).

Especially, T-cell and DC function in rejection has been extensively studied, but the role of other cell types like NK cells in allograft rejection is still under investigation [54]. It has been known that NK cells are prevalent within allografts during early stages of rejection and that NK cells are activated after human renal transplantation [55]. The field of recipient NK cell cytotoxicity against the allograft and therefore, their involvement in promoting acute and chron-
ic rejection is not well understood yet [56]. For in vitro studies, PBMC can be examined for NK cell activity using the K562 cell line as targets [34]. We here evaluated the effect of AEB071 on the lytic activity of human NK cells and were able to show that the new immunosuppressive agent significantly inhibited their cytotoxic activity in vitro (Figure 6).

The in vitro data presented herein concerning the inhibitory effect of AEB071 on T-cell activation and other immune cell functions complement recently published studies: AEB071 affects early T-cell activation in murine models [57] and prolongs rat cardiac allograft survival and renal allograft survival in non-human primates (for review, see [29]). Additionally, the immunosuppressive compound together with cyclosporine (CsA) prevents islet rejection in rats [31] and also inhibits acute allergic contact dermatitis in rats [32]. In this context, it has been suggested that AEB071 might not only be a therapeutic option for renal transplanted patients but also for type 1 diabetes mellitus and psoriasis patients.

Several isoforms of PKC play a pivotal role in immune cell activation and are therefore involved in processes mediating allograft rejection, such as cytokine production, transcription factor activation, promotion of cell cycle progression and regulation of apoptosis [11,16,58]. PKC are consequently promising targets for immunosuppression after transplantation and in other immune disorders. The precise mode of action and downstream signalling in rejection processes are not fully described for all PKC isoforms, but we here present an extensive in vitro study concerning the effects of the new PKC inhibitor AEB071 on different types of human immune cells as major key mediators of human renal allograft rejection. Concluding from the presented data, AEB071 has a clear and strong impact on T-cell activation, proliferation, cytokine production and NK cell activity, but not DC maturation. These findings suggest that AEB071 inhibits PKC isoforms and the correspondent downstream pathways that are involved in T-cell and NK cell activation but not the pathways leading to DC maturation. AEB071 might therefore function T-cell and NK cell specific via PKC inhibition, and is thus a promising immunosuppressive agent.

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Conflict of interest statement. The authors hereby declare that the results presented in this paper have not been published previously in whole or part, except in abstract format.

References


Fig. 6. AEB071 administration reduces NK cell activity. To measure cytotoxicity, calcein–AM release assays were performed with the K562 cell line and activated PBMC that were either AEB071 treated or untreated. AEB071 treatment inhibited the specific lysis of target cells significantly at all effector:target ratios. Results are shown as box plots with median and SD.
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