Abstract. In this study, we report a model of spontaneous cyst formation in vitro and a procedure to obtain large quantities of cysts from polycystic rat kidney cells. Furthermore, we assess the effects of epidermal growth factor, a modulator of morphogenesis, and of taxol, a stabilizer of microtubules, which has recently been proposed as a useful treatment of human polycystic kidney disease (PKD). It is anticipated that data generated from in vitro studies using cysts from PKD-affected rat kidneys may yield further insights to the pathophysiological and cellular basis of fatal renal cyst formation processes, and may lead to specific therapeutic strategies directed at controlling the growth of cysts, thereby reducing the number of animal tests.

Key words: cell culture; confocal microscopy; epidermal growth factor; polycystic kidney disease; taxol

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) constitutes one of the most frequent hereditary disorders in humans and is a major cause of end-stage renal insufficiency. The disease is characterized by the bilateral formation and enlargement of enormous fluid-filled cystic expansions in all segments of the kidney and similar pathologic manifestations in several other organs [1,2]. It has been proposed that quantitative and qualitative alterations in the extracellular matrix composition, enhanced epithelial cell proliferation and dedifferentiation as well as abnormal transepithelial transport and fluid accumulation are involved in the mechanisms of pathogenic cyst formation and expansion [3–6]. Recently it has been predicted that the product of the PKD1 gene is a transmembrane protein, suggesting that cystogenesis is a complex epigenetic problem probably involving several secondary consequences of deregulated cell functions such as unbalanced cell death or abnormal targeting of membrane proteins (e.g. Na⁺-K⁺-ATPase) [7]. Various animal and in vitro models of PKD have been developed and proved to be particularly suitable to study the cell pathology of cystogenesis. Although no causative treatment preventing formation and enlargement of renal cysts is available at present, those bioassays have opened up the possibility of defining new therapeutic avenues [8–11]. Among the various cell culture approaches the MDCK cell line (Madin-Darby canine kidney) has been extensively used as an analogue model of pathological cyst formation in studies investigating the establishment of cell polarity [12], cell–matrix interactions [13] and transepithelial transport [14]. Nevertheless, results obtained with such an in vitro model are difficult to extrapolate to the complex in vivo situation of PKD due to the absence of homologous animals for comparative studies. Although several spontaneous hereditary murine models, such as the cpk [15–17], jcpk [18], pcy [19], jck [20] and bpk [21] mice, reflecting several important features of PKD, have been described, they resembled the human disease only partially. Furthermore, the small size of the mouse renders accompanying biochemical investigations more difficult. Eventually this led to the search for additional animal models of PKD. A recently described mutant strain of Sprague–Dawley rats exhibiting autosomal dominant PKD (Han:SPRD-PKD) most closely resembled the human disease [22]. This animal model proved to be particularly suitable to study interactions between tubular epithelia and extracellular matrix at the beginning of tubular cystic transformation in vivo [23, 24]. Furthermore, cystic dilatations developed in all segments of the nephron and animals died due to uraemia [25–27]. However, due to the complex physiological and biochemical environment that exists in vivo and the difficulties surrounding manipulations in.
In vitro formation of cysts

In situ, the development of an in vitro model has valuable consequences for biological and medical investigations. While a cell culture model can never be expected to respond exactly as similar tissue would in its natural setting, it does enable experimental inquiries into the relative influences of specific alterations in environmental conditions and physiological stimuli. This report presents the results of our attempts in the isolation and cultivation of cysts from PKD-affected Han:SPRD rats to form a representative in vitro model of PKD. The intermediate size of the rat provides adequate kidney tissue from one individual for sustainable culture, which will facilitate future studies which will integrate experiments with live PKD rats and their cultured cysts.

Cell culture methods including the formation of cysts in vitro from disaggregated renal cells of different origins have been used to study the mechanisms of cystogenesis and the factors affecting the initiation and progression of this pathological process. Primary human renal cells and MDCK cells develop spherical monolayered cysts, which are fluid-filled structures, when cultivated in collagen type I matrix [28,29]. Cysts in vitro can also be obtained from the cpk mouse but not from its normal counterpart, when disaggregated cells are prevented from adhering to the substratum by a solid agarose coat.

We obtained cysts in cultures from the rat model of PKD using the following procedure: kidneys, obtained from normal or heterozygously affected male rats of the Han:SPRD strain, were minced in Iscove’s modified Dulbecco’s medium (IMDM, Gibco). The pieces were incubated for 1–2 h at 37°C in IMDM containing collagenase type IV (200 U/ml; Sigma). After a few minutes, and avoiding the pieces of tissue, the upper 4 ml were diluted in 4 ml of IMDM–FCS (IMDM supplemented with 10% heat-inactivated fetal calf serum). A final suspension containing $2 \times 10^5$ living cells/ml was propagated. Culture plastic dishes (35 mm diameter; Greiner), with a solid coat of 2 ml of agarose (1% in PBS), containing 1.5 ml of the final cell suspension, were placed in an incubator (5% CO$_2$ in air, 37°C). In some experiments the culture medium contained 2 ng/ml of epidermal growth factor (EGF; Boehringer Mannheim) or taxol (Bristol-Myers) at a concentration of 25 μM. After 24 h cysts were quantified under an inverted microscope using a x20 objective as the average of cysts counted in 24 fields along the diameter of the dish multiplied by 527 (the number of fields contained in the dish area).

Isolated kidney cells from PKD-affected rats (PKD-cells), when transferred directly to the plastic surface of a Petri dish, developed confluent monolayers and grew as compact colonies of polygonal cells which may appear from above as a dome-like structure (not shown). Typical for these cultures is the presence of fibroblasts, but no cyst formation was detected during the

Fig. 1. In vitro formation of cysts from autosomal dominant polycystic rat kidney cells. (a–d) Phase contrast, dispersed polycystic kidney cells formed spherical cysts within 6 h (a and c) in agarose-coated Petri dishes. Cysts are fully developed after 24 h (b and d). The size of the cysts varies (average diameter: 45 μm) and either 1 or 2 (rarely 3) cavities are formed. (e) Total number of cysts/dish derived from ADPKD rats (PKD) and unaffected animals (CON) after 24 h. Values are means ± SEM, *P < 0.05 versus control.

Fig. 2. Effect of EGF on cyst formation in vitro. Total number of cysts formed after 24 h in agarose-coated dishes from polycystic rat kidney cells in the absence (vehicle) or presence of EGF (2 ng/ml). Values are means ± SEM. *P < 0.05 versus control.
period of observation (up to 15 days). On the contrary, when cultured immediately on agarose-coated dishes, primary PKD cells started to aggregate within a few hours and eventually developed into geometrically stable cystic spheres. Although a few small cysts were detected as early as 6 h after the beginning of the culture, in vitro cysts are clearly developed at 18–24 h (Figure 1a–d) and comprised up to 50 cells/cyst. Whether the increase in cell number initiates from one or several cells has not been determined in detail. However, after periodic observations of individual cultures in hanging drops we suggest that the formation of cysts in vitro starts either from individual cells or from small aggregates of <5 cells. In comparison with control cells derived from unaffected healthy rats, cells from the mutant strain showed an increase in cyst formation over the observed period of 1 week. Thus, the ability to form numerous cysts on agarose-coated dishes appears as an intrinsic property of the PKD cells which developed a significantly greater number of spherical structures than do cultures of their normal counterparts from unaffected control animals (Figure 1e).

Effect of EGF and taxol on cyst formation

In another set of experiments we were interested to further enhance the number of cysts in culture and to investigate their responsiveness to growth factors. Among the various growth factors identified, EGF has been demonstrated to exert a potent proliferative action in ADPKD epithelial cells, probably due to the increased expression of EGF receptor mRNA. Figure 2 shows that the number of cysts obtained is significantly increased by adding EGF (0.1–2 ng/ml) to the medium (Figure 2). The mean EC50 for EGF-mediated cyst formation in three experiments was 0.5 ng/ml.

In vivo the renal cysts are lined by a monolayered epithelium exhibiting the apical face to the lumen. In contrast, the topology of the in vitro cysts is the opposite of the in vivo situation as judged by immunofluorescence localization of a laminin layer facing the fluid-filled cavity (Figure 3). We therefore investigated the validity of the described cell cultures as a system for pre-screening in vitro by studying the effect of taxol, an enhancer of microtubule assembly and inhibitor of the depolymerization of tubulin, which has recently been proposed as a useful treatment of human PKD [6,30,31]. Figure 4a shows that the in vitro formation of cysts was strongly reduced by taxol (25 μM). This result is in accordance with the antineoplastic activity of taxol, which arrests dividing cells in mitosis. Interestingly, however, taxol also induces regression of cysts already formed (Figure 4b), suggesting that microtubules are necessary not only for the formation of the cysts but also for maintenance of structural cyst organization.

![Fig. 3. Immunofluorescence localization of laminin by confocal laser scanning microscopy.](https://academic.oup.com/ndt/article-abstract/11/supp6/58/1810420/1760x1760)

**Fig. 3.** Immunofluorescence localization of laminin by confocal laser scanning microscopy: (a) Laminin was detected by indirect immunofluorescence using a rabbit anti-laminin antibody and a second anti-rabbit fluorescein isothiocyanate-conjugated antibody (shown in yellow). The cysts were counterstained for actin with phalloidin–rhodamin (shown in red); bar = 25 μm. (b) The density histogram of laminin distribution clearly demonstrates that the staining is concentrated at the luminal site of the cyst.

![Fig. 4. Effect of taxol on cyst formation and regression in vitro.](https://academic.oup.com/ndt/article-abstract/11/supp6/58/1810420/1760x1760)

**Fig. 4.** Effect of taxol on cyst formation and regression in vitro. (a) Total number of cysts formed after 24 h in agarose-coated dishes from polycystic rat kidney cells in the absence (24 h + V) or presence of taxol (24 h + T, 25 μM). (b) Effect of taxol on cyst regression. Total number of cysts formed after 48 h in agarose-coated dishes from polycystic rat kidney cells after 24 h of culture in the absence (V) and presence of taxol (T, 25 μM). Values are means ± SEM. *P < 0.05 versus control.
Conclusions

Today, experiments in pharmacology are usually dependent on the use of animals, which is of limited public acceptance, expensive and accompanied by ethical problems. In addition, the results obtained in vivo are difficult to interpret due to the fact that they deal with a complex system consisting of different organs and their interactions. A probable solution can be offered by suitable cell culture systems, which allow the monitoring of pharmaceutical effects in an animal-saving and organ-specific manner. Another advantage of in vitro tests is their ability to determine and control the experimental parameters in a wider range than in whole animals. In summary, the described simple cell culture technique of spontaneous cyst formation in vitro is advocated as a pre-screening device to assess the presence or absence of therapeutic or adverse effects of potential drugs. This will allow an informed judgement on the need or desirability to proceed to the live animal stage with a given test compound. It is anticipated that data generated from in vitro studies using this model may yield further insights into the pathophysiological and cellular basis of fatal renal cyst formation processes, and may lead to specific therapeutic strategies directed at controlling the growth of cysts, thereby reducing the number of animal tests.

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References