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REVIEW ARTICLE

RENAL CELL LINES FOR STUDY OF NEPHROTOXICITY *IN VITRO*

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Summary

The kidneys are one of the organ that can be commonly damaged by a number of toxic compounds (heavy metals, xenobiotics, drugs, etc.). To characterize the mechanism of toxicity, a variety of methods have been developed. The *in vitro* methods belong among the mostly used. Especially, the use of cell lines seems to be the leading approach to test and to characterize the toxicity mechanisms. At present, several cell lines of animal (from rat, dog, pig) or human origin are available. A detailed evaluation must go before any selection of a suitable cell line for experiments. Therefore, the aim of this review was to describe and to evaluate the mostly used renal cell lines.

Key words: Kidney; nephrotoxicity; cell lines; human kidney cells

LIST OF ABBREVIATIONS

BCRP – Breast Cancer Resistance Protein;
HEK293 – Human Embryonic Kidney cell line;
HK-2 – Human Kidney 2 cell line;
JTC-12 – monkey kidney cell line;
LLC-PK1 – pig kidney cell line;
MDCK – Madin Darby Canine Kidney cell line;
MRP – Multidrug Resistance Protein;
NRK-52E – Normal Rat Kidney-52 Epithelial Cells;
OAT – Organic Anion Transporter;
OATP – Organic Anion-Transporting Polypeptide;
OCT – Organic Cation Transporter;
OK – Opossum Kidney cell line.

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INTRODUCTION

A number of renal cell lines have been introduced for nephrotoxicity testing *in vitro*. They differ in origin (animal or human) and also in kidney localization (proximal/distal tubules or other parts of nephron). Although a great attention have been given to develop a standard cell line for *in vitro* testing, each of cell lines possesses some limitations for their use. The overview of mostly used kidney cell lines (table 1) and their description are noted in the text bellow.

NRK-52E cell line

The mostly used rat cell line, NRK-52E *(Normal Rat Kidney-52E Epithelial Cells)* possesses characteristics similar to proximal tubules. These cells have been described as a suitable model for study of effects of a variety of xenobiotics, metals, and cell regeneration after nephrotoxic injury [8]. NRK-52E

Table 1. Overview of selected renal cell lines

Cell line	Origin	Reference
LLC-PK1	pig Hampshire *(Sus scrofa)*	[1]
HEK293	human *(Homo sapiens)*	[2]
HK-2	human *(Homo sapiens)*	[3]
MDCK	dog *(Canis familiaris)*	[4]
JTC-12	monkey *(Macaca fascicularis)*	[5]
NRK-52	rat *(Rattus species)*	[6]
OK	opossum *(Didelphis marsupialis virginiana)*	[7]

is a stable immortalized cell line originated from rat kidney tubule [6], the cells mostly exhibit characteristics of proximal tubules. Although some problems have been identified based on dedifferentiation of NRK-52E cells to fibroblasts, these concerns have been solved after addition of D-valine and L-ornithine into cultivation medium. NRK-52E cells show a typical structure of epithelial cells since they attach to the bottom of incubation wells creating the monolayer. The apical membrane contains microvilli [9].

The NRK-52E cells synthesize a number of kidney specific enzymes. Most of them are present in brush border (alkaline phosphatase; gamma-glumyl transpeptidase; etc.), lysosomes (*N*-acetyl-beta-glukosaminooxidase), and also in cytosol (lactate dehydrogenase; beta-lyase; *N*-acylase) [8;10]. The basolateral membrane contains protein laminin, a number of organic anion transporters and Na^+/K^+-ATPase [9]. In contrast, the activities of glutathione reductase and glutathione-*S*-transferase are lower in comparison to *in vivo* conditions [10]. All these properties must be considered for eventual use of NRK-52E cells for studied carried out *in vitro*. At present, NRK-52E cell line has been used in both acute kidney injury and mechanistic toxicity studies [11].

OK cell line

The OK *(Opossum Kidney)* cell line has been derived from the kidney of opossum [12]. The cell line has been developed for study of X-chromosomes, but consequently it has been used also for nephrotoxicity study [7]. OK cells exhibit proximal tubular origin because they grow in a monolayer of polarized cells with desmosomes and microvilli at apical membrane [13].

The OK cells can transport neutral and acidic amino acids with/without Na^+ presence, glucose with/without Na^+, Na^+/H^+ and phosphate [14; 15]. The cells produce a number of enzymes (alkaline phosphatase, amino peptidase, gamma-glumyl transpeptidase, lactate dehydrogenase, hexokinase, succinate dehydrogenase, *N*-acetyl-beta-glukosaminooxidase, etc.) [16]. These characteristics are very similar to other commonly used cell line, LLC-PK1. In addition, OK cells produce enzymes specific for dopamine metabolism and that is why they have been used in studies on dopamine receptors and amine metabolism [17]. OK cells have been widely used in recent studies at similar extent as NRK-52E cells, mostly to study membrane transport [18; 19] and parathyroid hormone [20; 21].

MDCK cell line

The MDCK cell line was derived from kidney of an apparently normal adult female cocker spaniel in 1958 [14]. MDCK *(Madin-Darby Canine Kidney)* cells were developed as a distal tubular cell line. At present, however, these cells have been considered as a heterogeneous population with dissimilar properties regarding the number of passages and other conditions [22]. The use of these cells has been often focused on study of viral infection and cytopathologic effects [23; 24; 25], membrane transport [26; 27; 28; 29].

The MDCK cells exhibit characteristics similar to distal tubular cells. They create a monolayer of polarized cells with brush border and tight junctions. The cells contain a number of mitochondria, polyribosomes and abundant Golgi complex [30; 31; 32]. The function of MDCK cells is regulated by some distal tubule specific mechanisms because they increase production of cAMP in response to vasopressin, glucagon and adrenaline presence. In addition, proximal tubular specific hormones like parathyroid hormone and calcitonin possess no effects on MDCK cells [30]. The identified membrane transport systems of MDCK cells are: Na^+/H^+ antiport, Na^+/K^+-ATPase, Na^+-dependent transporter of neutral amino acids [30; 33]. The uptake transporters show stable expression of P-glycoprotein, BCRP, MRP2, OCT1, OCT2, OAT1, OAT3 in MDCK cells [27; 29]. The cultivation medium for MDCK cells must contain a number of hormones (insulin, glucagon, and hydrocortisone), growth factors and other compounds (transferrin, prostaglandin E2). According to literature, the MDCK cells have been used more than 10-times more often

than OK and NRK-52E cells in *in vitro* studies on kidney cells.

JTC-12 cell line

Epithelial-cell line derived from monkey kidney, JTC-12, was established in 1962 [5]. It is a homoge-homogenous cell line that is able to respond to parathyroid hormone and prostaglandin E1. According to proximal tubular origin, brush border and desmosomes occur at the membrane of JTC-12 cells. In addition, the production of enzymes (alkaline phosphatase, gamma-glumyl transpeptidase) and capacity to transport of hexoses and amino acids was proved in these cells. Although these cells exhibit similar properties to MDCK and LLC-PK1 cells, they have not been used routinely due to often dedifferentiation of cells after multiple passages.

LLC-PK1 cell line

The LLC-PK1 cell line is of animal origin since the cells were isolated from male Hampshire pig. LLC-PK1 *(Lilly Laboratories Cell-Porcine Kidney)* cells have some unique morphological characteristics including 3D-growth and aggregates forming [1; 34]. These immortalized cells form 3D spheroids with monolayer of polarized cells on the surface and produce brush border on the apical membrane. The basal membrane can absorb water from lumen. Although the LLC-PK1 cell line is considered as proximal tubular cell line, some characteristics seem to be of non-proximal tubular origin [35], i.e. presence and synthesis of vasopressin receptors and the shape and localization of mitochondria in the cell.

The cells produce a number of proximal tubule specific enzymes (alkaline phosphatase, gamma-glumyl transpeptidase), membrane transporters (Na^+/H^+ antiport, Na^+/K^+-ATPase, transporters of amino acids, hexoses and phosphate). On the other hand, the LLC-PK1 cells do not synthesize the en-zymes for gluconeogenesis [12; 14] and the activity of brush bolder enzymes can be variable regarding cultivation conditions [35]. The LLC-PK1 cell line is a suitable model for physiology, membrane transport and biochemical studies due its 3D growth and structure [34]. Therefore, the cells have been recently used for a variety of studies on membrane transport [36; 37; 38; 39], aquaporin [40; 41] and nephrotoxicity *in vitro* [42; 43].

HEK293 cell line

One of two mostly used human kidney cell lines was established from embryonic kidney cells transfected by an adenovirus. The HEK293 *(Human Embryonic Kidney)* cell line was the second cell line at all that was successfully formed by a virus transfection [2; 44]. The embryonic cells exhibit a typical structure of adeno-transfected cells. The HEK293 cell line shows an epithelial morphology but the cells do not show similar growth.

HEK293 cells produce a number of cytoskeletal fibers (vimentin, ceratine 8, and neurofilaments) and neurogranine that are specific for neural tissue. In addition, the tests confirming presence of mRNA for neural enolase 2 were also performed [45]. Therefore, the renal origin of the HEK293 cell line and their use in kidney specific experiments can raise some concerns.

HK-2 cell line

Due the necessity of performing nephrotoxicity experiments in human cells, a recent cell line was established. The HK-2 *(Human Kidney)* cells were prepared from proximal tubular kidney cells immortalized by transduction with human papilloma virus 16 (HPV-16) E6/E7 genes. These genes regulate DNA replication and cellular proliferation. A cellular clone of population was isolated and was named HK-2 [3].

The HK-2 cells sustain morphological and biochemical properties of proximal tubules and that is why they have been recently used as a standard model in study of nephrotoxicity *in vitro*. HK-2 cells grow as monolayer of cells with tight junctions and microvilli on the apical membrane. Brush border contains enzymes typical for proximal tubule, i.e. alkaline phosphatase, gamma-glumyl transpeptidase, leucinaminopeptidase and acidic phosphatase [46]. The cells produce vimentin, cytokeratine and integrins [47]. Another provement of proximal tubular origin is that they can respond to parathyroid hormone and cannot respond to vasopressin [3]. The gluconeogenesis capacity and hexokinase pro-duction occur in these cells [48].

The transport and metabolic capacity have been also studied in detail but the HK-2 cells have been used only in limited amount of experiments, mostly on mechanisms of nephrotoxicity [49; 50; 51; 52; 53]

and glucose transport [54; 55]. The HK-2 cells possess capacity of Na^+-dependent glucose transport, H^+-dependent transport of lactate and fatty acids [3; 46]. In addition to a number of organic anion transporters, the membrane consists of P-glycoprotein, a non-specific membrane transporter of xenobiotics and their metabolites [56].

CONCLUSION

A number of cell lines have been used to study nephrotoxicity *in vitro*. These cell lines originate from animals or human and differ also in the origin of kidney localization. Therefore, all known characteristics of a cell line ought to be considered for a proper use in laboratory testing. The MDCK cells are the mostly used cell line due maintenance of functional membrane transporters and cellular cytochromes. Another cell line suitable for membrane transport experiments is the LLC-PK1. Its disadvantage can be particularly seen in porcine origin. The use of the most recent cell line, HK-2 cells, has been growing but some studies remain to be performed to characterize its suitability for experiments, especially in drug metabolism research.

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