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Kidney regeneration in vivo

Imaging technologies for monitoring the safety, efficacy and mechanisms of action of cell-based regenerative medicine therapies in models of kidney disease

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ABSTRACT

The incidence of end stage kidney disease is rising annually and it is now a global public health problem. Current treatment options are dialysis or renal transplantation, which apart from their significant drawbacks in terms of increased morbidity and mortality, are placing an increasing economic burden on society. Cell-based Regenerative Medicine Therapies (RMTs) have shown great promise in rodent models of kidney disease, but clinical translation is hampered due to the lack of adequate safety and efficacy data. Furthermore, the mechanisms whereby the cell-based RMTs ameliorate injury are ill-defined. For instance, it is not always clear if the cells directly replace damaged renal tissue, or whether paracrine effects are more important. Knowledge of the mechanisms responsible for the beneficial effects of cell therapies is crucial because it could lead to the development of safer and more effective RMTs in the future. To address these questions, novel in vivo imaging strategies are needed to monitor the biodistribution of cell-based RMTs and evaluate their beneficial effects on host tissues and organs, as well as any potential adverse effects. In this review we will discuss how state-of-the-art imaging modalities, including bioluminescence, magnetic resonance, nuclear imaging, ultrasound and an emerging imaging technology called multispectral optoacoustic tomography, can be used in combination with various imaging probes to track the fate and biodistribution of cell-based RMTs in rodent models of kidney disease, and evaluate their effect on renal function.

1. Introduction

Cell-based regenerative medicine therapies (RMTs) are showing great promise in rodent models of kidney disease (Bussolati and Camussi, 2015; Murray and Woolf, 2014) but clinical translation of these novel therapies is currently hampered because accurate safety and efficacy data from the rodent studies are lacking. These data are essential for determining the risk:benefit ratio of the RMTs in order to judge whether they would be appropriate for use in man. A difficulty in assessing cell-based RMTs is that the standard 'absorption, distribution, metabolism and excretion' (ADME) and pharmacokinetic (PK) testing that are used to assess the disposition of pharmacological compounds are not directly applicable. This is mainly because, unlike pharmacological compounds, cellular therapeutics can persist and even proliferate in the recipient over the long-term, and also have the potential to migrate to other tissues where they could cause adverse effects (Heslop et al., 2015). Nevertheless, the general scientific principles in the fields of pharmacology and toxicology should be considered and applied where possible. The application of these principles is facilitated by recent progress in the field of in vivo imaging, which is making it possible to visualise administered stem cells, track their fate and 'see' the effects they have on host tissues and organs (James and Gambhir, 2012; Meleshina et al., 2015; Wang and Yan, 2008), thus enabling the behaviour of administered cells to be evaluated with a degree of accuracy that until now, has only been possible for drugs. For instance, using the appropriate imaging agent/imaging modality combination, it is possible to determine how an administered cell population is distributed within each body compartment, thus defining the maximum tissue distribution (equivalent to 'Cmax' for administered drugs). Then by

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measuring the distribution kinetics of the cells, it is possible to define the complete spatiotemporal profile of distribution (equivalent to 'pharmacokinetics' (PK) for administered drugs) and the rate of accumulation and elimination from target and non-target tissues. Simultaneously, it is also possible to monitor the biological effects on host tissues and organs, thus defining the complete spatiotemporal profile of responses (equivalent to 'pharmacodynamics' (PD) for administered drugs). By co-registering and correlating the kinetics and dynamics, it should be possible to define the efficacy and safety for each cell therapy. In this review, we will discuss how in vivo imaging technologies can be used to evaluate cell-based RMTs in rodent models of kidney disease, with particular focus on the biodistribution of cell-based RMTs and their effect on renal function.

2. Rodent models of kidney disease

Most studies investigating the potential of cell-based RMTs to treat kidney disease have used rodent models of ischaemia reperfusion injury (IRI) (Donizetti-Oliveira et al., 2012; Feng et al., 2016) or various drug-induced injury models, such as cisplatin, adriamycin, aristolochic acid (Bruno et al., 2012; Li et al., 2012; Qi and Wu, 2013; Ronconi et al., 2009) and the glycerol model of induced rhabdomyolysis (Angelotti et al., 2012; Geng et al., 2014). All of these models are clinically relevant. For instance, IRI, which has been proposed to be the optimal model for evaluating cell-based RMTs (Wang et al., 2012), represents the type of tubular injury incurred by renal allografts during transplantation (Asderakis et al., 2001), and by the kidneys of patients undergoing cardiopulmonary bypass surgery (Okusa et al., 2009). Clinical trials have already been undertaken to assess the potential of mesenchymal stem/stromal cells (MSCs) to ameliorate kidney disease in cardiac surgery patients, with both positive and negative outcomes being reported (NCT00733876; NCT01602328)(Gooch and Westenfelder, 2016). A clinical trial is also currently underway to establish the safety and feasibility of administering MSCs to cancer patients receiving cisplatin (NCT01275612), an anti-cancer drug that causes acute tubular injury, which in 20% of patients, progresses to chronic kidney disease (Inai et al., 2013). Likewise, the safety and efficacy of bone marrow-derived mononuclear cells are being assessed in patients with focal segmental glomerulosclerosis (NCT02693366), a disease that resembles adriamycin-induced nephropathy in rodents (Scarfe et al., 2015). Cell-based therapies for treating aristolochic acid and rhabdomyolysis-induced nephropathy have only been tested in rodent models so far, but both models are good representations of the tubulo-interstitial injury that can occur in human patients following ingestion of aristolochic acid (Yang et al., 2014) or crush injury (Gibney et al., 2014), respectively.

A common problem with all rodent kidney injury models is that the extent of injury incurred can vary considerably between individuals within the same cohort, making it difficult to accurately assess the efficacy of the cell therapies. Some studies address this by using large numbers of animals in the treatment and control groups, and culling animals at various time points (Angelotti et al., 2012; Ronconi et al., 2009). However, an alternative approach is to use methodologies that enable the same animal to be evaluated over time, so that the extent of injury and therapeutic response can be monitored in each individual animal. The key advantage of undertaking such longitudinal assessments is that correlated data are generated, thus increasing the power of the statistical tests, which in compliance with the principles of 'Replacement, Refinement and Reduction' (the '3Rs'), enables the number of animals in these type of experiments to be reduced.

3. Cell-based regenerative medicine therapies

The most common cell types used as RMTs include MSCs from bone marrow (Qi and Wu, 2013) and adipose tissue (Donizetti-Oliveira et al., 2012), kidney-derived progenitor cells (Ronconi et al., 2009), renal progenitors derived from embryonic stem cells or induced pluripotent stem cells (iPSCs) (Toyohara et al., 2015), or heterogeneous populations such as adipose-derived regenerative cells (Feng et al., 2010) or bone marrow-derived mononuclear cells (Semedo et al., 2010). MSCs, adipose-derived regenerative cells and bone marrow-derived mononuclear cells ameliorate renal injury via paracrine factors, whereas kidney-derived progenitor cells have been reported to engraft in the kidney and generate specialised renal cells (Angelotti et al., 2012; Bussolati et al., 2005; Ronconi et al., 2009). iPSC-derived renal progenitors can also engraft in the kidney and generate renal cells (Imberti et al., 2015; Toyohara et al., 2015), though their therapeutic effects appear to be mediated by paracrine mechanisms (Toyohara et al., 2015). As an alternative to administering cells, several studies have investigated the therapeutic potential of cell-derived extracellular vesicles, which in many cases, have been shown to be as efficacious as the cells themselves (Bruno et al., 2009). It is anticipated that extracellular vesicles would be less hazardous than cells as they would not form tumours and would present a low risk of forming emboli. As we will discuss in Section 5, it is crucial to monitor the in vivo biodistribution of cellular therapeutics in order to assess their safety, efficacy and mechanisms of action. There are two broad methods for labelling cells so that they can be tracked following their administration:introducing a genetic reporter, or labelling the cells with a nanoprobe or small molecules, such as near infrared (NIR) dyes or fluorescent proteins. For adipose-derived regenerative cells and bone marrow-derived mononuclear cells, which are heterogeneous populations of autologous cells that are used at the point-of-care, it is not possible to introduce genetic reporters, because this would require culturing the cells in vitro, a process which would be expected to alter their composition and phenotype. MSCs, iPSCs and kidney-derived progenitor cells on the other hand, are routinely expanded in vitro, and so for these cell types, there is the opportunity to introduce reporters. The biodistribution of extracellular vesicles can be monitored using both genetic reporters and NIR dyes (Grange et al., 2014b; Lai et al., 2014).

4. Imaging agents and technologies

4.1. Imaging agents for cell tracking

Genetic reporters are excellent tools for tracking cell fate and biodistribution in small animals. When expressed under the control of a constitutive promoter, reporter genes can be used for long-term biodistribution analysis, as the signal is not depleted when the cells proliferate. Constitutively expressed reporters also indicate whether the cells are viable, because expression is rapidly lost if the cells die. When expressed under the control of a cell-type specific promoter, reporters can be used to monitor cell fate and/or function by indicating the differentiation status of administered cells. The most commonly used reporter for cell tracking studies is firefly luciferase, an enzyme that emits light in the presence of D-luciferin, oxygen and ATP and can be detected using bioluminescence imaging. Other luciferases include the sea pansy (*Renilla reniformis*) and marine cope pod luciferases (*Gaussia princeps*), but compared to firefly luciferase, the *Renilla* is less intense, and the *Gaussia* has a very short emission half-life (James and Gambhir, 2012). In addition to bioluminescence imaging, genetic reporters can also be used for imaging with other modalities;