Cyclosporin in Cell Therapy for Cardiac Regeneration

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Abstract

Stem cell therapy is a promising strategy in promoting cardiac repair in the setting of ischemic heart disease. Clinical and preclinical studies have shown that cell therapy improves cardiac function. Whether autologous or allogeneic cells should be used, and the need for immunosuppression in non-autologous settings, is a matter of debate. Cyclosporin A (CsA) is frequently used in preclinical trials to reduce cell rejection after non-autologous cell therapy. The direct effect of CsA on the function and survival of stem cells is unclear. Furthermore, the appropriate daily dosage of CsA in animal models has not been established. In this review, we discuss the pros and cons of the use of CsA on an array of stem cells both in vitro and in vivo. Furthermore, we present a small collection of data put forth by our group supporting the efficacy and safety of a specific daily CsA dosage in a pig model.

Keywords

Immunosuppression; Allogeneic cell therapy; Autologous cell therapy; Cardiac regeneration; Cyclosporine

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Cardiac Regenerative Therapy

Stem Cell Therapy for Cardiac Repair

Stem cell therapy (or progenitor- or precursor cell therapy) has emerged as a promising therapy for cardiac repair. Despite the presence of endogenous cardiac stem cells [1, 2], the heart's ability to self-renew is inadequate for compensating the extensive ischemic injury [3]. In the acute setting, delivery of stem cells may modulate the post-inflammatory response, while regeneration and prevention of further cardiac remodelling may be achieved in a more chronic phase.

Apart from differentiation of stem cells into cardiomyocytes, a more likely mechanism of action is through paracrine signalling [2–6]. Paracrine signalling may reduce the inflammatory response, promote vasculogenesis, and stimulate endogenous (cardiac) stem cells [7].

Stem cell therapy has successfully been investigated for the recovery of cardiac function in ischemic heart disease in clinical and preclinical setting [8–10]. Although these results are promising, low delivery efficiency and engraftment rates (10 %) should be emphasized [5, 11–14].

Mechanical washout and/or loss, cell death [15], and redistribution to other organs [12] play a role. Additionally, in non-autologous therapy, cell rejection may cause even lower engraftment, due to decreased survival of transplanted cells in the hostile environment.

Allogeneic Versus Autologous Stem Cells

Allogeneic cell therapy enables prior preparation of the right cell type and immediate "off-the-shelf" therapy but may require immune suppression to avoid cell rejection. Autologous cell therapy lacks immunologic concerns but is associated with low cost-effectiveness, logistic concerns and lifelong exposure of cells to ageing, comorbidity, and risk factors [3, 4, 16]. A meta-analysis of preclinical trials showed no difference in effect size between autologous and allogeneic cell therapy for cardiac repair, irrespective of immunosuppressive therapy [17]. This underscores the potential paracrine working mechanism of cell therapy and might even imply that immunosuppression is not necessary.

The use of mesenchymal stem cells (MSCs) for allogeneic cell therapy may obviate the need for immune suppression due to the MSC's proposed immunomodulatory effect and apparent immune-privileged state [18–20]. The immunosuppressive capability of MSCs can even be enhanced by pharmacological agents like cyclosporine (CsA) [21, 22]. Conflicting studies, however, have shown that MSCs are indeed immunogenic and provoke an immune response [23, 24]. Thus, the potential role of immunosuppressive drugs cannot be ignored for MSCs as well.

The need of immunosuppression in clinical application of allogeneic cells for cardiac regeneration is unknown, as is the role of CsA in this setting. An overview of preclinical data might be elucidating and guiding for future clinical studies.

Alloreactivity

Alloreactivity depends on foreign peptide presentation by major histocompatibility complex (MHC) on antigen presenting cells and detection by T cells [25]. Immunomodulation for prevention of alloreactivity should therefore act on T cell suppression. T cell suppressors include calcineurin inhibitors, corticosteroids, antimetabolites, and target-of-rapamycin inhibitors. As CsA, a calcineurin inhibitor, is most often used in preclinical trials of allogeneic cell therapy, it will be the focus of this review. Little information exists on the pharmacokinetics and subsequent correct dosage of CsA in large animals.

Cyclosporin

Mechanism of Action of CsA

CsA suppresses T cell activity by forming a complex with the intracellular receptor cyclophilin. This CsA-cyclophilin complex subsequently binds to calcineurin A, inhibiting its phosphatase activity [26–30]. Inhibition of calcineurin A blocks activity of nuclear factor of activated T cells (NFAT). The inhibition of the calcineurin/NFAT pathway reduces IL-2, IL-4, and interferon-γ production [31–35], leading to cell cycle arrest, DNA and RNA inhibition, and inhibition of protein synthesis and growth factor production of T cells [36].

In clinical practice, CsA is used as an immunosuppressor in organ transplantation, bone marrow transplantation, and inflammatory diseases like rheumatoid arthritis and psoriasis. CsA is known for its interaction with several other pharmacological agents and its serious adverse effects. The most often described adverse effect is nephrotoxicity [37].

CsA in Clinical Practice

In clinical application of CsA, dosages differ based on indication [38]. Defining the correct daily CsA dosage and therapeutic serum levels in organ transplantation proves to be challenging as a fine balance must be found between organ rejection (underdose) and toxicity (overdose) [39–41]. Large intra- and interindividual differences in absorption, distribution, metabolism, and elimination are observed, and no true definitions of cell rejection and toxicity exist [42]. Nevertheless, serum concentrations exceeding 300 ng/mL are associated with toxicity, nephrotoxicity in particular, while lower levels were linked to organ rejection [39].

In renal transplant patients, an initial serum level of 250–400 ng/mL and a maintenance level of 100–250 ng/mL are achieved with dosages of 4–6 and 2–6 mg/kg/day, respectively [43, 44].

In a survey carried out among 87 "European Group for Blood and Marrow Transplantation" centers, the median daily oral CsA maintenance dose was 10 mg/kg/day, with a range of 2–12.5 mg/kg/d [45]. Most target serum concentration ranges were 100–500 ng/mL.

CsA in Cardiology

In the field of cardiology, CsA has been investigated for reduction of infarct size after myocardial ischemia. In a meta-analysis of animal models of myocardial ischemia/

reperfusion, cyclosporine was associated with a smaller infarct size compared to control [46]. The proposed mechanism is prevention of opening of the mitochondrial permeability transition pores, which is triggered by the oxygen-derived free radicals coupled with Ca^{2+} during reperfusion. However, in a third of the included studies, there is no effect of CsA, and there was evidence for publication bias, based on funnel plotting. The direct effect of CsA on myocardial infarct size, on top of the effect of CsA on stem cells, must be emphasized, in a setting of stem cells therapy for acute myocardial ischemia.

In order to identify studies of the effect of CsA on stem cells in vitro and in vivo in the setting of myocardial infarction, we conducted a search in the PubMed database on August 1, 2013, using the search terms (((((((((ciclosporin[Title/Abstract]) OR cyclosporin[Title/Abstract]) OR ciclosporine [Title/Abstract]) OR cyclosporine [Title/Abstract]) OR Neoral[Title/Abstract]) OR Sandimmune [Title/Abstract]) OR CsA[Title/Abstract])) AND ((((((("stem cells"[Title/Abstract]) OR "stem cell"[Title/Abstract]) OR progenitor*[Title/Abstract]) OR "bone marrow cells"[Title/Abstract]) OR "bone marrow derived cells"[Title/Abstract]) OR pluripotent [Title/Abstract]).

In Vitro Experiments

Aside from both a (nephro) toxic and immunosuppressive effect, CsA may have a direct effect on stem cells. In several experiments, stem cells were incubated with varying concentrations of CsA and proliferation [47–57], survival [49], apoptosis [58, 59], differentiation [53, 55, 57], migration [53], and angiogenesis [59] were analysed. Table 1 summarizes the studies that revealed a positive effect (enhanced differentiation, proliferation, survival, or decreased apoptosis) or negative impact (reduced differentiation, proliferation, survival, or increased apoptosis) of CsA on different cell types, compared to control.

As can be seen in Table 1, numerous studies have shown a pro-proliferative effect of CsA on stem cells. A calcineurin/NFAT pathway-independent effect has been proposed [47, 49, 56], because of the absence of a significant effect when using other calcineurin/NFAT inhibitors such as tacrolimus and 11R-VIVIT [56].

Apoptosis

Reduced apoptosis and increased survival, as seen in Chen et al. and Hunt et al, are explained by prevention of mitochondrial dysfunction [49, 58] and promotion of bcl-2, an antiapoptotic factor [58]. However, Hunt et al. showed decreased cell viability and decreased cell proliferation at higher concentrations (5 μg/mL) [49]. Yang et al. reported increased apoptosis of endothelial progenitor cells by all concentrations tested (0.1–10 μg/ml) and attributed this to decreased NO generation, which promotes EPC apoptosis [59].

A dose dependent effect is endorsed by Perry et al. showing enhanced proliferation in dosages until 1 μg/mL and decreased proliferation in higher dosages.

Proliferation

Different studies showed both anti- and pro-proliferative effects of CsA. The mechanism behind the anti-proliferative effect remains unclear. Disruption of the calcineurin pathway may influence the fate of stem cells [57]. Other studies showed or stated the reduced proliferation to be caused through alteration in the NOS/NO pathway by CsA, where a decrease in NO production causes decrease in proliferation and differentiation [55, 59, 60].

The pro-proliferative effect is ascribed to inhibition of IFN-γ [50], decreased cell-adhesion by decreased cyclophilin, and subsequently decreased matrix-metalloproteinase production [49].

Cell Types

Incubation of CsA with MSCs seems to negatively influence stem cell growth. Two studies showed reduced proliferation [54, 55], one study showed no effect on proliferation [52], and only one study showed a positive effect in the form of reduced MSC apoptosis [58]. Two studies used embryonic stem cells (ESCs) [51, 56], and both revealed a positive pro-proliferative effect of CsA, suggesting ESCs may benefit from the presence of CsA.

In Vivo Experiments

We identified only four studies investigating the role of CsA in cell therapy in an animal model of ischemic heart disease. Stem cells were transplanted into the ischemic myocardium following ligation of the left anterior descending artery, with or without CsA [61–64]. Immune cell infiltration, cell survival, contractile performance, and/or ejection fraction (EF) were monitored. Table 2 summarizes the positive and negative effects (positive: enhanced survival or engraftment, reduced immune reaction, improved cardiac function; negative: decreased survival or engraftment, immune reaction, less improved cardiac function) of CsA on non-autologous stem cells in vivo.

Guo et al. investigated immune cell kinetics, myoblast survival, and cardiac function after myocardial injection of human myoblasts, human myoblast + CsA, rat myoblasts and rat myoblasts + CsA, in rats 1 week after MI. CsA caused less infiltration of immune cells, more myoblast survival, and improved cardiac function, compared to cells alone. The observed improvement in cardiac function was largely attributed to the reduced infiltration of macrophages and CD4+/CD8+ cells, causing prolonged myoblast survival [61]. However, 10 min after injection, stated as baseline, two-thirds of the injected cells were already lost. After 1 day, only 10 % of the numbers of cells at baseline were still present in all groups.

Westrich et al. compared intramyocardial syngeneic and allogeneic MSCs injections in rats, both with and without CsA, 5 days after MI. Cell survival of syngeneic cells was higher than allogeneic, regardless of use of CsA. However, cell survival per cell source was higher in CsA-treated groups compared to cells only, explained by the anti-inflammatory and anti-apoptotic effect of CsA [62].

In contrast, Chiavegato et al. showed the activity of macrophages and dendritic cells remains unaffected by CsA and graft cell rejection still occurs [63]. In this study, human amniotic

fluid-derived stem cells (AFS) were injected in the myocardium of three groups of rats: healthy animals; 20 min after MI; and athymic animals. The authors were surprised by the results because of their previous experience with engraftment and differentiation of human amniotic MSCs in immune competent rats after MI and engraftment of human "amnion cells" in neonatal swine and rats. Because of the rejection in athymic rats and rats treated with CsA, a T cell independent mechanism is proposed, where natural antibodies, complement factors, NK cells, or macrophages may play a role [63].

Zeng et al. demonstrated that injection of allogeneic pMultistem cells (porcine derived multipotent adult progenitor cells) in pigs immediately after MI, led to improvement in cardiac function, irrespective of CsA treatment. Engraftment rate was also not affected by CsA, and only 0.35% of the cells could be detected after 4 weeks. Differentiation of present cells into cardiomyocytes was limited, and infarct size was not affected. Therefore, paracrine signalling was proposed as the main mechanism of effect [64].

Endogenous Recruitment

Hunt et al., showing increased proliferation and survival in vitro (Table 1), also investigated the effects of CsA in vivo in healthy mice [49]. Authors observed an increased number of neurospheres from endogenous neural progenitors in CsA-treated animals compared to control animals. Furthermore, an increased number of neural stem cells was observed. This recruitment of endogenous progenitor cells is confirmed by Wang et al. [65] In this study, authors show endothelial progenitor cell mobilization by CsA in a hind limb ischemia mouse model. Proposed mechanism is by decreased activity of CD26, which causes an increase in the level of chemo attractants for progenitor cells (like stroma-derived factor-1α). In contrast to this data, Davies et al. showed in a porcine cardiac transplant model that endothelial and smooth muscle outgrowth colony numbers decreased after treatment with CsA in sham-operated as well as transplanted animals [53]. In the sham-operated + CsA-treated animals, the number of colonies increased to above baseline levels at 4 weeks. In sham alone, without CsA, the endothelial colony numbers remained stable. This detrimental effect in vivo is in accordance with the effects shown by Davies et al. in vitro (Table 1).

Target CsA Levels in Large Animals

The therapeutic range and the subsequent recommended daily dosage of CsA in large laboratory animals are largely unknown. The pig serves as an excellent model for cardiac and pharmacological studies due to its similar physiology and anatomy compared to men [66]. Nevertheless, Frey et al. demonstrated that pigs require two times higher intravenous doses and up to four to six times higher oral doses of CsA in order to reach the same serum levels as in humans [67]. In accordance with Frey et al., Cibulskyte et al. demonstrated that an oral dosage of 30 kg/mg/day in pigs is needed to reach blood concentration levels of 475 ng/ml. However, at such high dosages acute nephrotoxicity was observed within 4 weeks. A dosage of 15 mg/kg/day was stated to be safe and caused a trough concentration of 338 ng/ml [68].

Dosing and Safety in a Pig Model of Myocardial Infarction

Rationale

In order to address the problem of the inconsistency in recommended CsA dosages in the setting of cardiac repair, we performed a small study investigating serum levels and animal safety after administration of CsA in a myocardial ischemia/reperfusion pig model.

Methods

Open chest LAD occlusion was performed for 90 min in pigs (n=5, mean weight 32.5±2.1 kg at start of study). CsA was orally administered twice daily (15 mg/kg/day), and venous blood was collected at different time points through a peripherally inserted central line, tunneled to the back. The mean follow-up was 44.6 days. We determined the target range to be between 100–300 ng/mL, based on levels for safety and efficacy suggested by others [39, 43, 53].

Results

CsA serum levels were within the therapeutic range in 81.7 % of all cases (49/60) (Fig. 1). The mean CsA levels (and the upper and lower limit) per pig were as follows: pig 1, 182 ng/mL (280–85); pig 2, 177 ng/mL (344–108); pig 3, 182 ng/mL (261–81), pig 4, 167 ng/mL (287–50); pig 5, 153 ng/mL (319–30).

To investigate the safety of the given CsA dosage and the significance of the determined therapeutic range, serum values were analyzed and compared to existing reference values (Table 3).

None of the serum levels exceeded the reference values. In conclusion, our data suggest that the administration of 15 mg/kg/day twice a day in a pig model of myocardial infarction is feasible and represents a safe dosage that ensures adequate CsA serum concentration values with 82 % of the values within the determined therapeutic range.

Conclusion

This essay summarizes current knowledge on the role of CsA in non-autologous stem cell therapy. CsA has been reported to exert inconsistent effects on stem cells both in vitro and in vivo. In vitro, a positive effect was observed through enhanced proliferation, enhanced survival, or reduced cell apoptosis. At the same time, different studies using a similar methodology reported negative effects, namely reduced proliferation, angiogenesis, differentiation and migration, and increased apoptosis. In both the positive and negative studies, approximately the same range of CsA concentration was used.

Interestingly, enhanced proliferation was reported, while cell viability was drastically reduced at higher concentrations [48–50], suggesting CsA may only have a beneficial effect up to a certain concentration.

In vivo, similar conflicting results were observed. In both the positive and negative studies, CsA dosage was fairly equal. Studies focused on both cell engraftment and cardiac function, but little is known on the link between engraftment rate and cardiac function.

The conflicting results in both in vivo and in vitro data, maybe explained by heterogeneity in included studies. Methodology of included studies is quite similar, but cell type and cell source are different. From these data, the exact role of CsA in animal models cannot be fully elucidated.

Non-autologous cells will eventually be rejected, with or without immune suppression [4]. A large proportion of the observed beneficial effect of stem cell therapy is thought to be through indirect pathways. Cell rejection may not be a matter of concern as long as enough time has passed for these paracrine effects to occur [4]. At the same time, avoiding cell rejection and hence prolonging graft survival may contribute to overall improvement in cardiac function.

We have also presented data showing that oral administration of CsA twice a day in a pig model of myocardial infarction is feasible and safe and blood concentrations remain within the therapeutic range. As others have suggested, the daily dose in our pig study is higher than in humans.

In conclusion, there is no evidence to clearly demonstrate whether CsA, or immune suppression in general, is essential and beneficial in non-autologous cell therapy. Regardless of this limited knowledge, the issue of immune suppression is one of great importance and thus should be further elucidated.

Limitations

First, the effect of CsA on endogenous stem cell properties was not discussed. Since one of the suggested mechanisms of effect of cell therapy is paracrine signaling, this might be of importance. Second, we focused on animal models of myocardial infarction. The effect of, and need for CsA, in other disease models might be different. Finally, no reliable meta-analysis could be performed, because of the large variability of cell type, CsA dosages, and measures of effect size.

Future Directions

Although CsA is commonly used as an immunosuppressant in organ transplantation, its contribution to cell therapy for cardiac regeneration remains unclear. If immune suppression is deemed redundant, future studies should focus on the impact of cell rejection (and low engraftment) on cardiac function following cell transplantation.

On the other hand, if immune suppression is a requirement for cell engraftment in non-autologous stem cell therapy, future experiments should focus on the effect of CsA on cardiac function after cell therapy in a myocardial ischemia large animal model. Furthermore, a dose-response experiment could shed light on the fine balance between successful cell engraftment and animal safety.

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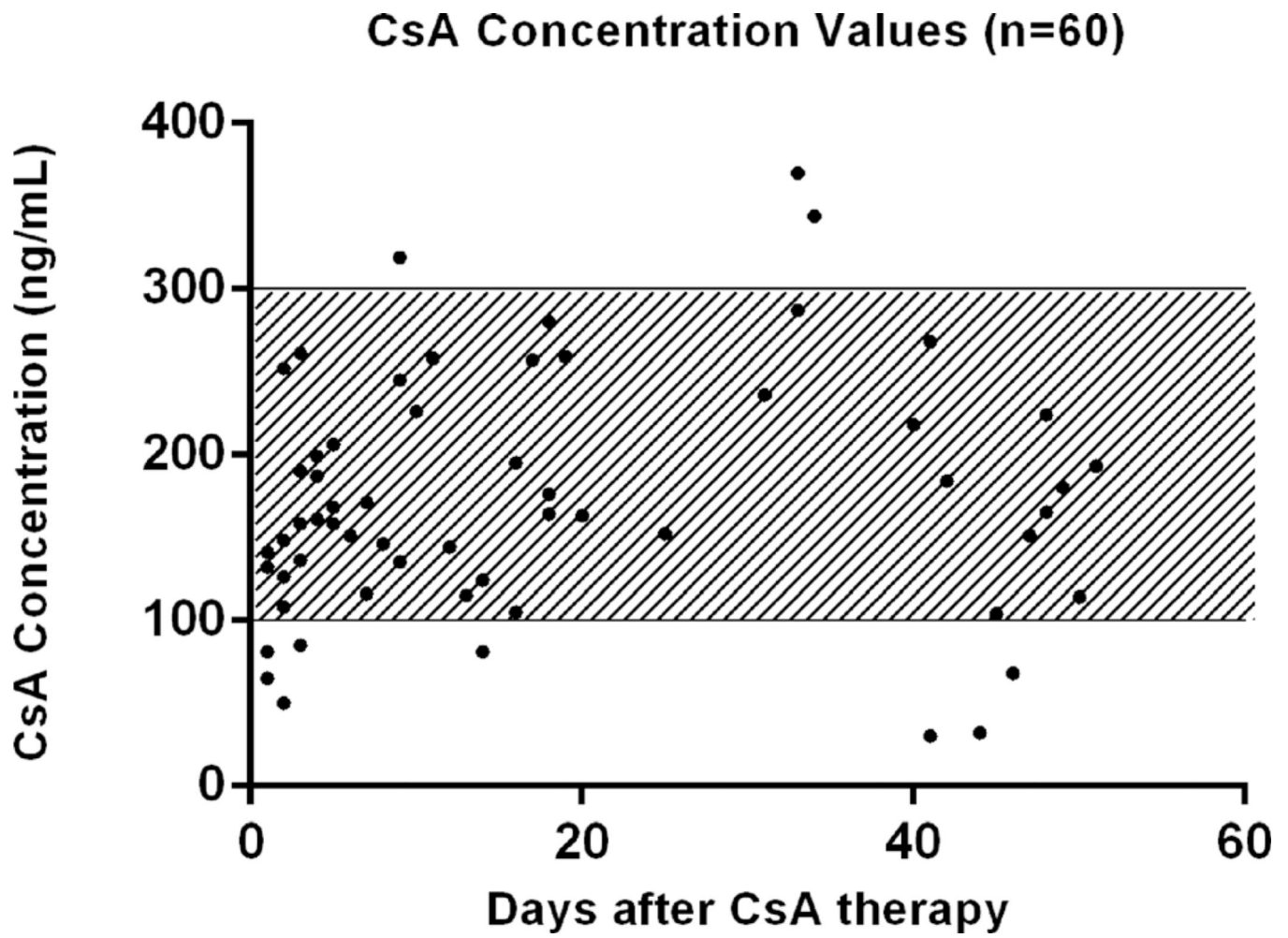
CsA Concentration Values (n=60)

Fig. 1.
CsA concentration values of all five pigs (*n*=60). Therapeutic range determined at 100–300 ng/mL

Table 1

In vitro effects of CsA on different cell types

Reference	Cell type	CsA concentration	Conclusion
Positive effect			
Chen at al. [58]	Rat MSC	0.5–5 μM (0.6–6 μg/mL)	Reduced apoptosis after hypoxia/reoxygenation
Fujiwara et al. [47]	Mouse/Human iPSC	1–3 μg/mL	Enhanced cardiac differentiation
Hunt et al. [49]	Mouse NPC	0.1–0.5 μg/mL	Enhanced proliferation, enhanced survival
Perry et al. [50]	Mouse HSPC/BMC	0.1–100 μg/mL	Enhanced proliferation in low dose, inhibition in high dose
Sachinidis et al. [51]	Mouse ESCs	1 μM (1.2 μg/mL)	Enhanced cardiac differentiation
Yan et al. [56]	Mouse ESC	1–3 μg/mL	Enhanced cardiac differentiation
Negative effect			
Byun et al. [52]	Rat MSCs	50–500 nM (0.06–0.6 μg/mL)	No effect on proliferation
Davies et al. [53]	Porcine MNCs	0.001–0.2 μg/mL	Decreased proliferation, differentiation, migration of smooth muscle and endothelial outgrowth cells
Guo et al. [57]	Rat NSC	0.5–5 μg/mL	Decreased proliferation
Poncelet et al. [54]	Swine MSC	0.2 μg/mL	Reduced proliferation
Song et al. [55]	Mouse MSC	1–10 μM (1.2–12 μg/mL)	Reduced proliferation
Yang et al. [59]	Human EPC	0.01–10 μg/mL	Reduced proliferation, increased apoptosis, reduced angiogenesis

iPSC induced pluripotent stem cell, *HSPC* hematopoietic stem/progenitor cell, *BMC* Bone marrow cells, *EB* embryonic bodies, *ESC* embryonic stem cell, *NPC* neural precursor cell, *MSC* mesenchymal stem cell, *MNC* mononuclear cell, *EPC* endothelial progenitor cell, *NSC* neural stem cell

Table 2

In vivo effects of CsA on different non-autologous cell types, compared to control

Reference	Cell type	Animal model	N	CsA dosage (mg/kg/day)	Administration	Conclusion
Positive effect						
Guo et al. [61]	Human SkM	Rat	208	10	Intraperitoneal	Reduced immunocyte infiltration, enhanced cell survival, improved EF
Westrich et al. [62]	Rat MSC	Rat	12	10	NA	Enhanced cell survival
Negative or neutral effect						
Chiavegato et al. [63]	Human AFS	Rat	42	5	Intramuscular	Unsuccessful inhibition of cell rejection
Zeng et al. [64]	Swine pMultistem	Swine	30	15	Oral	No effect on engraftment and cardiac function

SkM skeletal myoblasts, *MSC* mesenchymal stem cells, *AFS* amniotic fluid-derived stem cells, *MNC* mononuclear cell, *pMultistem* bone marrow-derived adherent stem cells, *EF* ejection fraction

Table 3

Mean serum value per pig throughout the follow-up period

	Reference		Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Mean
Creatinine	1.2–2.0	mg/dL	1.7	1.4	1.1	1.5	1.6	**1.5±0.23**
AST	14–56	IU/L	26.1	46.2	52.6	43.2	44.0	**42.4±9.8**
ALT	5–78	IU/L	37.6	59.8	51.9	50.2	51.2	**50.1±8.0**
Hb	9.0–16.2	g/dL	9.0	11.1	10.2	10.4	11.2	**10.4±0.9**
WBC	6.3–21.1	10^3/mL	14.2	15.4	19.6	13.9	18.5	**16.3±2.6**
BUN	8–24	mg/dL	11.1	10.1	7.6	10.0	9.4	**9.6±1.3**
Sodium	133–153	meq/dL	NA	141.3	135.4	139.3	143.6	**139.9±3.5**
Potassium	3.1–6.2	meq/dL	NA	4.3	4.4	4.4	4.4	**4.4±0.1**

Number of measurements per pig: pig 1 n=19; pig 2 n=10; pig 3 n=9; pig 4 n=8; pig 5 n=5. The items in bold represent the mean values of the 5 pigs together

AST aspartate transaminase, *ALT* alanine aminotransferase, *Hb* hemoglobin, *WBC* white blood cells, *BUN* blood urea nitrogen