British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01691.x
www.brjpharmacol.org

REVIEW

Cyclosporin variably and inconsistently reduces infarct size in experimental models of reperfused myocardial infarction: a systematic review and meta-analysis

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Subject categories
review article; cardiovascular pharmacology; methods and techniques

Keywords
myocardial infarction; cyclosporin; reperfusion injury; pre-clinical; animal studies

Received
19 March 2011
Revised
23 June 2011
Accepted
21 July 2011

Cyclosporin is an immunosuppressant that has recently been proposed as a treatment to prevent reperfusion injury in acute myocardial infarction (MI). We aimed to determine the overall efficacy of cyclosporin in experimental studies of acute reperfused MI. We conducted a systematic review and stratified meta-analysis of published studies describing the efficacy of cyclosporin in experimental models of acute reperfused MI. We included all *in vivo* publications of cyclosporin where infarct size was measured. A literature search identified 29 potential studies of which 20 fulfilled the eligibility criteria. In these studies (involving four species of animals), cyclosporin reduced myocardial infarct size by a standardized mean (95% confidence interval) difference of –1.60 (–2.17, –1.03) compared with controls. Cyclosporin failed to demonstrate a convincing benefit in studies involving pigs. Despite this observation, the overall efficacy of cyclosporin did not differ across species (P = 0.358). The dose of cyclosporin given did not affect final infarct size (P = 0.203). Funnel plots of these data suggested heterogeneity among the studies. Cyclosporin had variable effects on infarct size compared with placebo. Cyclosporin had no effect on myocardial infarct size in swine, raising a question over the potential cardioprotective effects of cyclosporin in man.

Abbreviations

AAR, area at risk; EM, electron microscopy; IV, intravenous; LVA, left ventricular area; LVW, left ventricular weight; MI, myocardial infarction; mPTP, mitochondrial permeability transition pore; PO, per oral; SMD, standardized mean differences; SPECT, single photon emission computed tomography

Introduction

Cyclosporin is a cyclic decapeptide metabolite of soil fungus and a potent immunosuppressant drug (Martindale, 1993). Cyclosporin inhibits immunocompetent T-cell activation by binding the cytosolic protein cyclophilin leading to inhibition of calcineurin, which is required for activation of IL-2 transcription. In fact, cyclosporin has pleiotropic effects not

only on immune cell activation, but also on the mitochondria where it inhibits mitochondrial permeability transition pore opening leading to inhibition of cytochrome c release and reduced apoptosis (Duchen *et al.*, 1993).

Beyond its established use as an anti-rejection drug after allogeneic organ transplantation, cyclosporin has recently been proposed as a treatment to prevent reperfusion injury in the heart (Piot *et al.*, 2008). During acute myocardial infarction (MI), anaerobic glycolysis increases the abundance of cytosolic Ca^{2+}. At the onset of reperfusion, the release of oxygen-derived free radicals coupled with cytosolic Ca^{2+} triggers the opening of the mitochondrial permeability transition pores (mPTP). The opening of these channels results in a sudden change in osmotic forces leading to rupture of the outer mitochondrial membrane and the release of molecules that promote apoptosis into the cytosol leading in turn to cell death (Duchen *et al.*, 1993; Di Lisa *et al.*, 2003; Gomez *et al.*, 2009). Cyclosporin is postulated to prevent reperfusion injury in the heart and other tissues through inhibition of mPTPs and so enhancing cell survival.

Based on findings from studies in experimental acute MI, clinical trials are currently underway to determine the cardioprotective effects of cyclosporin in acute MI patients (Hausenloy and Yellon, 2008). Consequently, the results of the experimental studies with cyclosporin in experimental MI become very important, both in terms of the overall effect of cyclosporin in these studies and also their strengths and potential weaknesses, and their applicability to patients with acute MI is critically important.

However, some but not all (Dow and Kloner, 2007; Leshnower *et al.*, 2008; Pagel and Krolikowski, 2009; Boengler and Hilfiker-Kleiner, 2010; Karlsson *et al.*, 2010; Lie *et al.*, 2010; Matsubara *et al.*, 2010; Skychally *et al.*, 2010) studies have described a cardioprotective effect for cyclosporin, giving rise to uncertainty with regard to the overall effect of this drug in acute MI. Furthermore, the quality of these studies has not been described. Therefore, our purpose was to systematically detect and review publications in which cyclosporin has been studied as a cardioprotective agent in experimental models of acute reperfused MI. The rationale for our study is in line with the recent guidelines for reporting *in vivo* experiments published by the *British Journal of Pharmacology* (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010).

Our first aim was to determine the overall efficacy (if any) of cyclosporin to reduce infarct size compared with placebo. Our second aim was to critically appraise the quality of these studies in order to determine any relationships between study quality and the efficacy of cyclosporin.

Methods

Literature search

We searched for published abstracts and full papers in which cyclosporin or placebo was used *in vivo* to limit reperfusion injury in an *in vivo* animal model of acute MI. The inclusion criterion for outcome was infarct size measured *in vivo* [e.g. by a biochemical method (such as serial troponin) or by imaging (MRI or single photon emission computed tomography)] or *ex vivo* with histological methods. Data from *in vitro* studies with cyclosporin and studies with other mPTP inhibitors other than cyclosporin were not included. All of these criteria were pre-determined before the search was carried out.

Two electronic searches on the Web of Knowledge comprising Medline, Web of Science with conference proceeding, BIOSIS and CABI were carried out. The first search was performed between 14 November 2009 and 10 December 2009. An updated search using the same terms were performed on 4 January 2011. The search terms used were: (myocardial reperfusion OR MI) AND (cyclosporin OR mitochondrial permeability transition pore) AND (animals or animal) NOT (cerebral OR stroke OR hepatic*). No language constraints were applied in the search, but full papers required an English language version. All 'Reviews', 'Editorials', 'Books', 'Case reports' and 'Letters' were excluded.

In order to determine the quality of these studies, we used the ARRIVE guidelines (Table 1; Kilkenny *et al.*, 2010; McGrath *et al.*, 2010) and a validated 10-item quality score (Table 2; Macleod *et al.*, 2005, 2008).

We aimed to quantify the effect of cyclosporin on infarct size compared with placebo and also explored the relationships between measures of study quality and the overall effect of cyclosporin on infarct size.

Statistics

As the outcomes were reported on different scales, unbiased standardized mean differences [SMD, no units (Table 3)] had to be used to compare the results of the different studies in a meta-analysis.

The overall effect and the effect of different moderator variables (species, dose, reperfusion time and quality score) were analysed in random effects meta-regression models.

For one experiment, the observed SDs (σ) in Groups 1 and 2 (intervention and control), respectively, are

$$\hat{\sigma}_1 = \sqrt{\frac{1}{n_1 - 1}\sum_{i=1}^{n_1}(x_{i1} - \bar{x}_1)^2}$$

and

$$\hat{\sigma}_2 = \sqrt{\frac{1}{n_2 - 1}\sum_{i=1}^{n_2}(x_{i2} - \bar{x}_2)^2}$$

The unit for this is the original unit, which was reduction of infarct size as percentage of area at risk. The pooled SD of both groups (intervention and control) is

$$\hat{\sigma} = \sqrt{\frac{(n_1 - 1)\hat{\sigma}_1^2 + (n_2 - 1)\hat{\sigma}_2^2}{n_1 + n_2 - 2}}.$$

The unit for this is still the original unit and the SMD is

$$SMD = \frac{\bar{x}_1 - \bar{x}_2}{\hat{\sigma}};$$

therefore, the numerator and the denominator are both in the original unit, which means that the SMD has no unit.

Table 1

Study quality base on ARRIVE guidelines

Author (year)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Quality score
Karlsson *et al.* (2010)	+	+	+	+	+	+	−	−	−	−	+	+	−	+	+	+	−	+	+	−	13
Lie *et al.* (2010)	+	+	+	+	+	+	−	+	−	+	+	+	−	−	+	+	−	+	+	−	14
Boengler and Hilfiker–Kleiner (2010)	−	−	+	−	+	−	−	−	−	−	−	−	−	−	+	+	−	+	+	−	6
Skychally *et al.* (2010)	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	4
Matsubara *et al.* (2010)	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	+	−	−	+	+	8
Dow *et al.* (2009)	+	−	+	+	−	−	−	−	−	+	−	+	−	−	−	+	−	−	+	−	7
Pagel and Krolikowski (2009)	+	+	+	+	+	−	−	−	−	−	−	+	−	−	+	−	−	+	+	−	9
Gomez *et al.* (2008)	+	+	+	+	+	+	−	+	−	−	+	+	−	+	+	+	−	−	+	−	13
Fang *et al.* (2008)	+	+	+	+	−	+	−	−	−	−	+	+	−	−	−	−	−	+	+	−	9
Leshnower *et al.* (2008)	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	+	−	−	+	+	9
Huhn *et al.* (2008)	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	+	+	8
Lim *et al.* (2007)	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	+	−	−	+	−	8
Xie and Yu (2007)	+	+	+	+	+	−	−	−	−	−	+	+	−	−	−	−	−	−	+	+	9
Ikeda *et al.* (2006)	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	+	−	+	+	+	10
Wang *et al.* (2006)	+	+	+	+	+	+	−	−	−	−	+	+	−	+	−	−	−	+	+	−	11
Argaud *et al.* (2005a)	+	+	+	+	+	+	−	−	−	−	+	+	−	−	−	−	−	−	+	−	9
Krolikowski *et al.* (2005)	+	+	+	+	+	+	−	−	−	−	+	+	−	+	−	−	−	+	+	−	11
Argaud *et al.* (2004)	+	+	+	+	+	+	−	−	−	−	+	+	−	−	−	+	−	−	+	−	10
Niemann *et al.* (2002)	+	+	+	+	+	+	+	+	−	−	+	+	−	−	−	−	−	−	+	−	11
Squadrito *et al.* (1999)	+	+	+	+	+	+	−	−	−	−	+	+	−	−	−	−	−	−	+	−	9

Study quality items are: (1) Title; (2) Abstract; (3) Background; (4) Objectives; (5) Ethical statement; (6) Study design; (7) Experimental procedures; (8) Experimental animals; (9) Housing and husbandry; (10) Sample size; (11) Allocating animals to experimental group; (12) Experimental outcomes; (13) Statistical methods; (14) Baseline data; (15) Numbers analysed; (16) Outcomes and estimation; (17) Adverse events; (18) Interpretation/scientific implications; (19) Generalizability/translation; and (20) Funding.

All analyses have been carried out in R version 2.11.0. The MAd package version 0.8 (http://rwiki.sciviews.org/doku.php?id=packages:cran:ma_meta-analysis) was used for the meta-regression. *P*-values are not adjusted for multiple testing and have to be considered as descriptive.

Results

Our search identified 588 'hits' (Medline 241, BIOSIS 301, Web of Science 43, CABI 3). After screening the electronic abstracts, 29 were considered potentially relevant and full publications retrieved. Of these publications, 9 (31.0%) were excluded for the following reasons, two (6.9%) reports were meeting abstracts that were subsequently published in full (Niemann *et al.*, 2002; Leshnower *et al.*, 2005), two (6.9%) studies described *in vitro* experiments (Massoudy *et al.*, 1997; Jiao *et al.*, 2001), two (6.9%) studies used different mPTP inhibitors and not cyclosporin (Argaud *et al.*, 2005b; Gomez *et al.*, 2007), one (3.4%) paper was written in Spanish (Edmundo *et al.*, 2007), one (3.4%) study used cyclosporin in combination with another agent (Pagel *et al.*, 2006) and one (3.4%) study reported the effect of cyclosporin on mortality but not infarct size (Laudi *et al.*, 2006). Therefore, 20 papers (69.0%) involving *in vivo* models of experimental MI in four species (mice, rats, rabbits and pigs), were included (Table 4).

Design and quality of papers

All of the papers were published in a peer-reviewed journal (Table 4). Of the 20 papers, 16 (80%) reported a statement of compliance with regulatory requirement, 15 (75%) reported random allocation, 14 (70%) reported control of body temperature and 9 (45%) described a method of confirmation. Three (15%) studies had a statement on the authors' conflict of interest, two (10%) studies reported a blinded assessment of outcome and/or described a sample size calculation and only one study (5%) reported blinded induction of ischaemia. None of the studies reported the use of animals with co-morbidities. The median quality score calculated as the sum of quality items in each study was four (range, 2–8) out of a possible 10.

Cyclosporin and infarct size

Thirty-one groups of experiments involving a total of 417 animals were reported (Table 2). Overall, cyclosporin reduced

Table 2

Study quality report

Author (year)	1	2	3	4	5	6	7	8	9	10	Quality score
Karlsson *et al.* (2010)	+	+	+	–	+	–	+	–	+	+	7
Lie *et al.* (2010)	+	+	+	–	+	+	+	+	–	+	8
Boengler and Hilfiker-Kleiner (2010)	+	–	–	–	+	–	+	–	–	–	3
Skychally *et al.* (2010)	+	+	–	–	–	–	+	–	–	–	3
Matsubara *et al.* (2010)	+	–	–	–	+	+	+	–	–	–	4
Dow and Kloner (2007)	+	+	–	–	–	–	–	+	–	–	3
Pagel and Krolikowski (2009)	+	+	–	–	+	+	–	–	–	–	4
Gomez *et al.* (2008)	+	+	–	–	+	+	+	–	–	+	6
Fang *et al.* (2008)	+	+	–	–	+	–	+	–	–	–	4
Leshnower *et al.* (2008)	+	–	–	–	+	+	+	–	–	–	4
Huhn *et al.* (2008)	+	–	–	–	+	–	+	–	–	–	3
Lim *et al.* (2007)	+	–	–	–	+	+	+	–	–	–	4
Xie and Yu (2007)	+	+	–	–	–	+	+	–	–	–	4
Ikeda *et al.* (2006)	+	+	–	–	+	–	+	–	–	–	4
Wang *et al.* (2006)	+	+	–	–	+	+	–	–	–	–	4
Argaud *et al.* (2005a)	+	+	–	–	+	–	+	–	–	–	4
Krolikowski *et al.* (2005)	+	+	–	–	+	+	–	–	–	–	4
Argaud *et al.* (2004)	+	+	–	–	+	–	+	–	–	–	4
Niemann *et al.* (2002)	+	+	–	–	+	–	–	–	–	–	3
Squadrito *et al.* (1999)	+	+	–	–	–	–	–	–	–	–	2

Study quality items are: (1) Publication in a peer-reviewed journal; (2) Randomization to either treatment with cyclosporin or placebo control; (3) Blinded assessment of outcome; (4) Use of animal models with co-morbidity; (5) Statement of compliance with regulatory requirement; (6) Method of confirmation of ischemia; (7) Statement of control of temperature; (8) Sample size calculation; (9) Blinded induction of ischaemia; and (10) Statement of conflict of interest.

infarct size volume by a standardized mean (95 % confidence interval) difference of –1.60 (–2.17, –1.03) units (Figure 1).

A funnel plot of infarct size (Figure 2) revealed heterogeneity in the effect of cyclosporin on infarct size across the studies. In nine studies involving 11 (36%) sets of experiments, cyclosporin had no statistically significant effect on infarct size (Niemann *et al.*, 2002; Dow and Kloner, 2007; Leshnower *et al.*, 2008; Pagel and Krolikowski, 2009; Boengler and Hilfiker-Kleiner, 2010; Karlsson *et al.*, 2010; Lie *et al.*, 2010; Matsubara *et al.*, 2010; Skychally *et al.*, 2010).

The moderators taken into account in the regression analyses were species, dose, reperfusion time and quality score. Quality items 1 and 4 were omitted as there was no variation among the studies for these criteria (item 1 was fulfilled in all studies, item 4 in none). The results of the regression analyses are shown in Table 5.

Cyclosporin had no effect on infarct size in pigs (*sus scrofa domesticus*) and species type was not associated with cyclosporin efficacy (Table 5). The dose of cyclosporin administered ($P = 0.98$) had no significant association with infarct size. The duration of reperfusion before infarct size assessment (or euthanasia) was inversely related to infarct size ($P = 0.04$).

Relationship between quality score and cyclosporin effect on infarct size

We studied the individual components that made up the Quality Score to determine their relationship with final infarct size. Compared with the magnitude of cyclosporin treatment effect [–1.82 (SMD)] in papers that did not describe a sample size calculation, cyclosporin had a smaller reduction in infarct size [0.57 (SMD)] in papers in which there was no calculation of sample size reported ($P = 0.004$). Overall Quality Score was not related to cyclosporin efficacy or infarct size ($P = 0.16$).

Discussion and conclusions

The main findings of our study are, firstly, the meta-analysis confirmed that cyclosporin reduces infarct size when used in experimental models of acute reperfused MI. Secondly, the efficacy of cyclosporine was unrelated to the species studied and thirdly, the presence of one of the measures of study quality, a sample size calculation, was associated with a smaller effect of cyclosporin on infarct size.

Table 3

Standardized mean differences between intervention and control group

Study	Intervention group			Control group			Standardized mean difference (95% CI)	Unbiased standardized mean difference (95% CI)
	N	Mean	SD	*N*	Mean	SD		
Pagel and Krolikowski (2009)	6	42	5.00	6	46	5.00	−0.80 (−1.98, 0.38)	−0.74 (−1.82, 0.35)
Gomez *et al.* (2008)	8	35	14.14	9	58	15.00	−1.57 (−2.66, −0.49)	−1.49 (−2.53, −0.46)
Gomez *et al.* (2008)	7	36	18.52	9	66	21.00	−1.50 (−2.62, −0.39)	−1.42 (−2.48, −0.36)
Fang *et al.* (2008)	12	24.9	3.60	12	47.5	4.20	−5.78 (−7.60, −3.96)	−5.58 (−7.34, −3.82)
Leshnower *et al.* (2008)	12	39	10.39	15	60	7.75	−2.33 (−3.31, −1.35)	−2.26 (−3.21, −1.31)
Huhn *et al.* (2008)	9	31.8	7.70	9	51.4	5.00	−3.02 (−4.37, −1.67)	−2.88 (−4.16, −1.59)
Lim *et al.* (2007)	7	32	7.94	9	48	12.00	−1.53 (−2.65, −0.41)	−1.45 (−2.51, −0.39)
Xie and Yu (2007)	6	30.3	2.70	6	48.8	5.50	−4.27 (−6.32, −2.22)	−3.94 (−5.83, −2.05)
Ikeda *et al.* (2006)	5	27.4	9.84	8	45	9.90	−1.78 (−3.09, −0.47)	−1.66 (−2.88, −0.44)
Wang *et al.* (2006)	8	24	2.00	8	44	4.00	−6.32 (−8.73, −3.92)	−5.98 (−8.25, −3.71)
Argaud *et al.* (2005a)	10	24	12.65	10	60	18.97	−2.23 (−3.35, −1.12)	−2.14 (−3.21, −1.07)
Argaud *et al.* (2005a)	10	24	12.65	10	60	18.97	−2.23 (−3.35, −1.12)	−2.14 (−3.21, −1.07)
Krolikowski *et al.* (2005)	7	43	6.00	8	42	7.00	0.15 (−0.86, 1.17)	0.14 (−0.81, 1.10)
Krolikowski *et al.* (2005)	7	24	3.00	8	42	7.00	−3.26 (−4.80, −1.71)	−3.07 (−4.52, −1.61)
Argaud *et al.* (2004)	12	24	13.86	12	55	27.71	−1.41 (−2.31, −0.52)	−1.37 (−2.23, −0.50)
Argaud *et al.* (2004)	9	26	18.00	12	55	27.71	−1.20 (−2.14, −0.27)	−1.15 (−2.06, −0.25)
Argaud *et al.* (2004)	7	24	15.87	12	55	27.71	−1.28 (−2.30, −0.26)	−1.22 (−2.20, −0.25)
Argaud *et al.* (2004)	7	25	13.23	12	55	27.71	−1.27 (−2.28, −0.25)	−1.21 (−2.18, −0.24)
Niemann *et al.* (2002)	4	35.08	25.84	4	58.15	11.08	−1.16 (−2.66, 0.34)	−1.01 (−2.31, 0.29)
Niemann *et al.* (2002)	4	23.54	27.24	4	58.15	11.08	−1.66 (−3.27, −0.06)	−1.45 (−2.85, −0.05)
Niemann *et al.* (2002)	4	13.62	11.08	4	58.15	11.08	−4.02 (−6.43, −1.61)	−3.49 (−5.59, −1.40)
Niemann *et al.* (2002)	4	16.62	14.30	4	58.15	11.08	−3.25 (−5.36, −1.14)	−2.82 (−4.66, −0.99)
Squadrito *et al.* (1999)	6	12	4.00	6	57	7.00	−7.89 (−11.25, −4.54)	−7.29 (−10.38, −4.19)
Karlsson *et al.* (2010)	12	49.2	13.89	15	41.14	15.94	0.53 (−0.24, 1.31)	0.52 (−0.23, 1.27)
Lie *et al.* (2010)	19	47.3	15.70	19	51.4	16.50	−0.25 (−0.89, 0.38)	−0.25 (−0.87, 0.38)
Boengler and Hilfiker-Kleiner (2010)	10	25.4	6.96	7	25.5	4.76	−0.02 (−0.98, 0.95)	−0.02 (−0.93, 0.90)
Skychally *et al.* (2010)	4	24.52	6.82	4	35.15	7.34	−1.50 (−3.07, 0.07)	−1.30 (−2.67, 0.06)
Dow and Kloner, 2007	4	42	6.00	10	27	12.65	1.32 (0.06, 2.58)	1.24 (0.06, 2.41)
Dow and Kloner, 2007	8	38	11.31	10	27	12.65	0.91 (−0.07, 1.89)	0.87 (−0.06, 1.80)
Matsubara *et al.* (2010)	6	39.1	4.16	7	53.4	23.81	−0.80 (−1.94, 0.33)	−0.75 (−1.80, 0.31)
Matsubara *et al.* (2010)	4	39.6	3.60	7	53.4	23.81	−0.71 (−1.97, 0.56)	−0.65 (−1.80, 0.51)

CI = confidence interval.

Clinical studies have shown that limiting infarct size translates to improve clinical outcome in the longer term (Burns *et al.*, 2002; Gibbons *et al.*, 2004). In our meta-analysis of pre-clinical studies, we demonstrated that although overall cyclosporin reduced infarct size in animal models of reperfused MI, cyclosporin had no effect on infarct size in just over one-third of the experiments (11 out of 31 experiments). Cyclosporin had mixed effects on infarct size in a swine model of acute reperfused MI, with a reduction in infarct size being observed in one study (Skychally *et al.*, 2010) and no effect in three other investigations (Karlsson *et al.*, 2010, 2011; Lie *et al.*, 2010). Because the hearts of swine and man are similar (e.g. in terms of coronary anatomy, few collaterals, myocardial mass), the lack of a cyclosporin treatment effect in swine raises concern as to whether or not cyclosporin may be cardioprotective in man. Experimental conditions (e.g. duration of ischaemia, sample size, dose of cyclosporin) may be relevant for whether or not cyclosporin might attenuate reperfusion injury in this model. In fact, the plasma concentrations of cyclosporin achieved for similar bolus doses differ between swine and man (Karlsson *et al.*, 2011) and there is a narrow therapeutic window to achieve the target concentration of 0.2 $\mu mol \cdot L^{-1}$ (Griffiths and Halestrap, 1993), limiting the chances of a true beneficial effect of the drug in patients

Table 4

Study characteristics

Reference	Species	*n*	Dose (mg/kg)	Timing of cyclosporin administration	Reperfusion time	Route	Assessment of infarct size	Infarct size
Karlsson *et al.* (2010)	Pigs	27	10	3 min R	120 min	IV	Pathology	%AAR
Lie *et al.* (2010)	Pigs	38	10	5 min R	180 min	IV	Pathology Troponin	%AAR
Boengler and Hilfiker-Kleiner (2010)	Mouse	17	10	5 min R	120 min	IV	Pathology	%AAR
Skychally *et al.* (2010)	Pigs	8	5	5 min R	120 min	IV	Pathology	%AAR
Matsubara *et al.* (2010)	Rabbits	17	25	1 h I 0 min R	180 min	IV	Pathology	
Dow *et al.* (2009)	Rats	22	5 10	3 min R	120 min	IV	Pathology	%AAR
Pagel and Krolikowski (2009)	Rabbit	12	5	5 min R	180 min	IV	Pathology	%AAR
Gomez *et al.* (2008)	Mouse	33	10	5 min R	24 h	IV	Pathology Planimetry	%AAR
Fang *et al.* (2008)	Rats	24	10	5 min R	120 min	IV	Pathology Planimetry	%AAR
Leshnower *et al.* (2008)	Rabbit	27	25	0 I	3 h	IV	Pathology Planimetry	%AAR
Huhn *et al.* (2008)	Rats	9	5	5 min R	120 min	IV	Pathology Planimetry	%AAR
Lim *et al.* (2007)	Mouse	7	10	0 min R	120 min	IV	Pathology Planimetry	%AAR
Xie and Yu (2007)	Rats	12	10	10 min I	180 min	IV	Pathology EM	%LVA
Ikeda *et al.* (2006)	Rats	13	5	15 I	2 h	IV	Pathology	%AAR
Wang *et al.* (2006)	Rabbit	16	10	5 min R	180 min	IV	Pathology	%AAR
Argaud *et al.* (2005a)	Rabbit	30	10	10 min I 1 min R	4 h	IV	Pathology Planimetry	%LVW
Krolikowski *et al.* (2005)	Rabbit	22	5 10	5 min R	3 h	IV	Pathology	%AAR
Argaud *et al.* (2004)	Rabbit	47	10	15 min I	4 h	IV	Pathology Planimetry	%LVW
Niemann *et al.* (2002)	Rat	24	5	3 days I	24 h	PO	Pathology Planimetry	%AAR
Squadrito *et al.* (1999)	Rats	12	0.25 0.5 1	5 min A	48 h	IV	Pathology	%AAR %LVA

Cyclosporin was given either before ischemia (I), during ischemia and before reperfusion (R) or after ischaemia (A).
AAR = myocardial area at risk; EM = electron microscopy; IV = intravenous; LVA = total left ventricular area; LVW = left ventricular weight; PO = per oral.

with acute MI in clinical practice. There is also the possibility that cyclosporin might not just have no beneficial effect on infarct size, but instead, as described by Dow and Kloner (2007), cyclosporin therapy might be associated with an increase in infarct size. This possibility is very important because the purpose of therapeutic evaluations in pre-clinical animal models is to provide information on both safety and efficacy.

We showed that infarct size was inversely related to the reperfusion time where reperfusion over a longer period was associated with a smaller infarct size. This observation is likely independent of cyclosporine and probably reflects the

Table 5

Regression results for mixed models

Variable	Effect estimate	95% confidence interval	*P*-value	*P*-value
Intercept	–1.602	(–2.173, –1.030)	<0.001	–
Intercept	–0.977	(–2.802, 0.847)	=0.294	=0.358
Mouse special	–0.443	(–4.106, 3.221)	=0.813	
Pig	0.673	(–1.900, 3.245)	=0.608	
Rabbit	–0.647	(–2.677, 1.383)	=0.532	
Rat	–1.252	(–3.347, 0.842)	=0.241	
Intercept	–1.596	(–2.786, –0.406)	=0.009	–
Dose	–0.001	(–0.098, 0.095)	=0.982	
Intercept	–1.145	(–1.843, –0.448)	=0.001	–
Reperfusion hours	–0.059	(–0.115, –0.003)	=0.040	
Intercept	–2.943	(–4.892, –0.993)	=0.003	–
Quality score	0.330	(–0.128, 0.787)	=0.158	
Intercept	–1.318	(–2.613, –0.023)	=0.046	–
Quality item 2	–0.364	(–1.815, 1.086)	=0.622	
Intercept	–1.727	(–2.299, –1.155)	<0.001	–
Quality item 3	1.858	(–0.248, 3.964)	=0.084	
Intercept	–1.453	(–2.975, 0.069)	=0.061	–
Quality item 5	–0.180	(–1.827, 1.467)	=0.830	
Intercept	–1.575	(–2.324, –0.826)	<0.001	–
Quality item 6	–0.085	(–1.281, 1.111)	=0.889	
Intercept	–1.740	(–2.763, –0.717)	=0.001	–
Quality item 7	0.195	(–1.052, 1.443)	=0.759	
Intercept	–1.816	(–2.345, –1.286)	<0.001	–
Quality item 8	2.398	(0.783, 4.014)	=0.004	
Intercept	–1.675	(–2.246, –1.105)	<0.001	–
Quality item 9	2.194	(–0.799, 5.187)	=0.151	
Intercept	–1.758	(–2.368, –1.147)	<0.001	–
Quality item 10	1.121	(–0.500, 2.743)	=0.175	
Intercept	–2.129	(–3.708, –0.551)	=0.008	–
Total N	0.032	(–0.058, 0.121)	=0.485	

The models are based on the assumption that the correct error type was provided.

cardioprotective effect of repair responses, such as collateral artery recruitment (Berry *et al.*, 2007) and endogenous reperfusion injury salvage kinases (Hausenloy and Yellon, 2007).

Using a previously published quality score (Macleod *et al.*, 2008), we found that the majority [17 (55%)] of the studies had a quality score of 4 out of 10. Only four studies (12.9%) had a score above 5 and around one-third of the studies [10 (32%)] had a score below 4. Most of the studies [26 (84%)] had a quality score of 3 and 4. This observation, coupled with the modest number of animals studied overall, resulted in insufficient power to discount the possibility that a higher quality score might have resulted in a smaller infarct size. One explanation for the similarities in quality score is that similar methods and reporting styles were adopted by different investigators. The only study quality index that showed a significant result was 'calculation of sample size' ($P = 0.004$). Sample size calculation is standard practice in clinical trials and it helps to ensure that the study is sufficiently powered to investigate the question asked (i.e. avoid a false negative result). One potentially contentious reason for why studies without an initial sample size calculation might be associated with a small effect size is that a study may be allowed to continue until such times as an effect, albeit small, might be observed. Some of the other indices might have been available or could have been adopted to enhance the strength of the study. We also hope the inclusion of both 'negative' and 'positive' papers in our analysis should provide some reassurance against reporting bias. However, we cannot exclude the

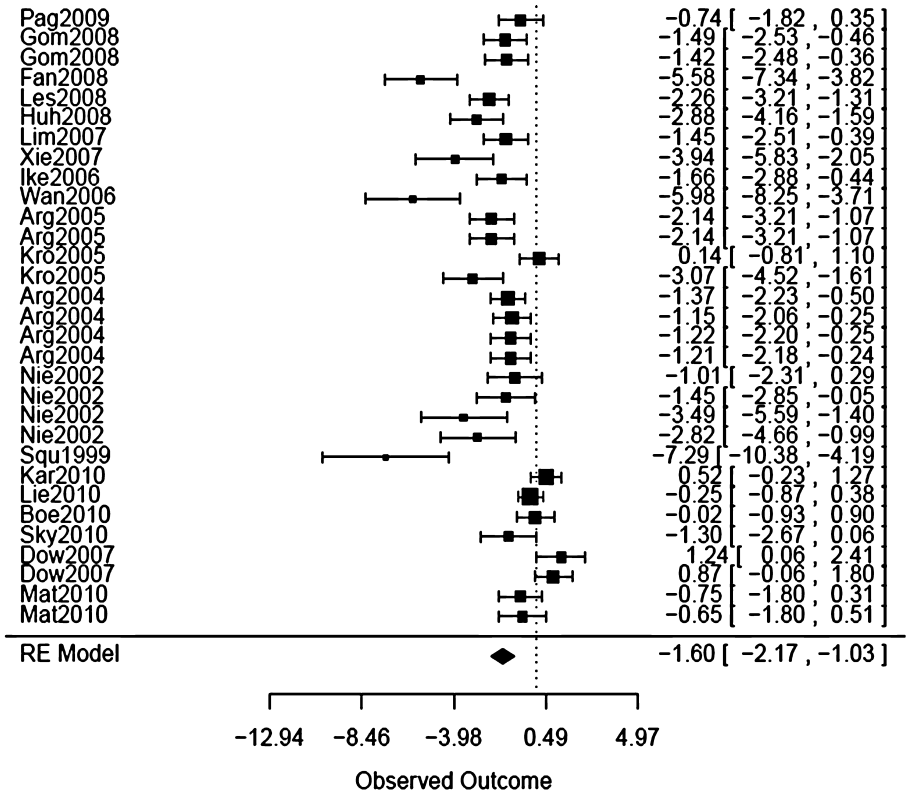

Figure 1

Forest plot of the size of effect of cyclosporin on infarct size. A forest plot illustrates the relative strength of treatment effects in individual scientific studies that address the same question and so graphically represents a 'meta-analysis' of a group of these studies.

possibility that some 'negative' studies may either have been rejected by peer review or may not have ever even submitted.

Study quality is important because the findings of pre-clinical drug development studies may be used to support the transition of candidate drugs, such as cyclosporin, into clinical studies in man. A study with false positive results may lead to an overestimation of the efficacy of a drug potentially leading to inappropriate testing in humans. We wondered whether some of the error terms that were reported as SDs were actually SEMs. There was a clear separation in SDs for the control groups of all experiments (calculated from the SEMs in studies that report SEMs), with SDs in studies that claim to report SEMs being considerably larger than SDs in the studies that claimed to report SDs. Additionally, there was a wide variation in study quality meaning an overestimation or underestimation of the treatment effect cannot be discounted.

Cyclosporin has a propensity to cause adverse effects including infection and cancer related to immunosuppression. However, these effects are mainly related to chronic therapy. A single intravenous dose of cyclosporin, as administered by Piot *et al*. (2008), is less likely to cause safety concerns. We did not include studies of cyclosporin in isolated perfused hearts because our focus was on *in vivo* studies in animal models. While isolated perfused heart studies can provide invaluable mechanistic information on pathways involved in ischaemia and reperfusion, our focus was on *in vivo* models that most closely mimic human MI.

Limitations

We cannot discount the possibility of a negative publication bias against studies that had negative results. However, not all of the studies that we have identified had positive results and importantly, the large animal studies in swine (Karlsson *et al*., 2010; Lie *et al*., 2010) had negative results that were considered in our overall analysis.

Conclusions

In conclusion, in our meta-analysis of 20 *in vivo* experimental studies in animal models (involving four species) reperfused MI, we found that, overall, cyclosporin reduced infarct size but there was considerable heterogeneity of effect across studies. However, the negative studies in porcine hearts raise a concern about the potential cardioprotective effects of cyclosporin in man. We did not show an association between study quality and infarct size, which may have been due to the similar reporting styles adopted in these investigations. Given the critical importance of *in vivo* experimental studies for therapeutic drug development in man, we support the recent ARRIVE guidelines and Gold Standard Publication Checklist (Hooijmans *et al*., 2011) for experimental research recently published in the *British Journal of Pharmacology*.

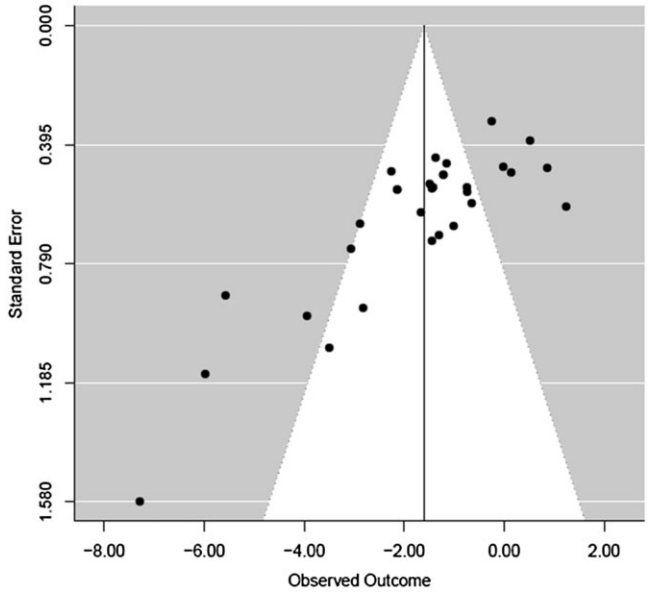

Figure 2

Funnel plot of all experiments. A funnel plot is a scatter plot of treatment effect against a measure of study size. It is used primarily as a visual aid to assess for bias or heterogeneity. An inverted funnel shape arises from a data set in which bias or heterogeneity is unlikely. An asymmetric funnel indicates a relationship between treatment effect and sample size indicating the possibility of bias or a systematic difference between smaller and larger studies ('small study effects'). Asymmetry can also arise from use of an inappropriate effect measure (Egger *et al.*, 1997). The funnel plot in this figure indicates there is heterogeneity in the effect of cyclosporin on infarct size across the studies.

Acknowledgements

This study was funded by the University of Glasgow. Professor Berry is supported by a Senior Fellowship from the Scottish Funding Council and receives grant funding from the British Heart Foundation, Chief Scientist Office, Medical Research Council and Medical Research Scotland.

Conflict of interest

None.

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