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RESEARCH PAPER

Effects of NXY-059 in experimental stroke: an individual animal meta-analysis

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Background and purpose: Disodium 2,4-disulphophenyl-N-tert-butylnitrone (NXY-059) was neuroprotective in experimental stroke models but ineffective in a large clinical trial. This first-ever individual animal meta-analysis was used to assess the preclinical studies.
Experimental approach: Studies were obtained from AstraZeneca and PubMed searches. Data for each animal were obtained from the lead author of each study and/or AstraZeneca. Published summary data were used if individual data were not available. Infarct volume and motor impairment were standardized to reflect different species and scales. Standardized mean difference (SMD), coefficients from multilevel models and 95% confidence intervals (95% CI) are presented.
Key results: Fifteen studies (26 conditions, 12 laboratories) involving rats (544), mice (9) and marmosets (32) were identified (NXY-059: 332, control: 253) with individual data for 442 animals. Four studies were unpublished. Studies variably used randomization (40%), blinding of surgeon (53%) and outcome assessor (67%). NXY-059 reduced total (SMD –1.17, 95% CI –1.50 to –0.84), cortical (SMD –2.17, 95% CI –2.99 to –1.34) and subcortical (–1.43, 95% CI –2.20 to –0.86) lesion volume; efficacy was seen in transient, permanent and thrombotic ischaemia, up to 180 min post occlusion. NXY-059 reduced motor impairment (SMD –1.66, 95% CI –2.18 to –1.14) and neglect. Evidence for performance, attrition and publication bias was present.
Conclusions and implications: NXY-059 was neuroprotective in experimental stroke although bias may have resulted in efficacy being overestimated. Efficacy in young, healthy, male animals is a poor predictor of clinical outcome. We suggest the use of preclinical meta-analysis before initiation of future clinical trials.
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Keywords: stroke; meta-analysis; neuroprotection; animal models; cerebral ischaemia; NXY-059

Abbreviations: AZ, AstraZeneca; CI, confidence intervals; IAD, individual animal data; MCAo, middle cerebral artery occlusion; NXY-059, disodium 2,4-disulphophenyl-N-tert-butylnitrone; SHR, spontaneously hypertensive rats; SMD, standardized mean differences; STAIR, Stroke Therapy Academic Industry Roundtable

Introduction

Disodium 2,4-disulphophenyl-N-tert-butylnitrone (NXY-059) is a nitrone-based compound that traps free radicals (Maples *et al.*, 2001; Williams *et al.*, 2007). Several preclinical studies have reported that NXY-059 reduces infarct volume and motor impairment in experimental models of stroke using rodents, rabbits and primates. These findings have been reported from several laboratories. The beneficial effects have been reported for permanent and transient models of ischaemia, and lesion volume was reduced in both cortex and sub-cortex. Treatment appeared to be effective when given up to 4 h post onset of occlusion. NXY-059 was considered by some (Green, 2008) to fulfil the Stroke Therapy Academic Industry Roundtable (STAIR) criteria for demonstrating neuroprotection (STAIR, 1999).

In view of the positive preclinical data, clinical development was initiated with phase I (Edenius *et al.*, 2002; Strid *et al.*, 2002) and phase II (Lees *et al.*, 2001; 2003) studies, these culminating in two phase III randomized controlled trials

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(Lees *et al*., 2006; Shuaib *et al*., 2007). In the first trial (SAINT 1), NXY-059 reduced combined death and dependency in comparison with placebo (Lees *et al*., 2006). Although the trial was positive using its pre-specified statistical approach (Cochran–Mantel–Haenszel test, $P = 0.038$), *post hoc* analyses using more conventional binary or ordinal (OAST Collaboration, 2007) approaches were neutral (Koziol and Feng, 2006). In a second and larger trial (SAINT 2), NXY-059 had no significant treatment effect (Shuaib *et al*., 2007) and the results from the combined trials were similarly negative (Diener *et al*., 2008).

The conflicting results obtained in the two SAINT trials are most easily explained if SAINT I is considered to have had a false positive finding, a hypothesis supported by its weak statistical significance. More difficult to explain is the difference between the apparent positive preclinical studies and neutral clinical development. One possible explanation is that the preclinical studies may have varied in their findings with undue weight being given to positive rather than neutral ones. To assess this further, we performed a meta-analysis of the preclinical studies using individual data from each animal. Although meta-analyses of preclinical studies based on published summary data are becoming more common (Willmot *et al*., 2005a; Gibson *et al*., 2007), analyses based on individual subjects are considered the gold standard (Stewart and Parmar, 1993), but have never been performed previously in animal studies.

Methods

Study identification

Completed studies that investigated the effect of NXY-059 in experimental models of stroke were identified primarily from AstraZeneca (AZ), the developer of NXY-059, and electronic searches of PubMed (last on 29 March 2008) using the term 'NXY-059'. Studies were included if they had been reported by the end of 2007. Reference lists of earlier reviews and identified trial publications were also checked for additional trials. Publications could be in any language.

Study selection

Completed controlled studies in animals, whether randomized or not, were included. Animals had to have been treated with NXY-059 versus control/placebo.

Data extraction

The following information on the study design was extracted from study reports (provided by AZ), publications and the lead author: species, experimental model (transient, permanent, global thromboembolic), design, whether the study was randomized or pseudo-randomized, whether surgeons were blinded to treatment and whether outcomes were assessed blinded to treatment. Individual data were obtained for each animal, these including: sex, weight, lesion volume, motor impairment (and scale), temperature during treatment and vital status. Information on treatment was also obtained: time to treatment from occlusion, loading and maintenance doses of NXY-059, duration of treatment and plasma concentration.

Where individual animal data (IAD) were not present, published summary (aggregate or group) data were used. In some cases, it was necessary to estimate summary data from published graphs (e.g. lesion volume in Yoshimoto *et al*., 2002a,b). Analyses involving dose assessment used the total dose [loading dose + (maintenance dose × treatment length]. Deaths were included whether spontaneous or resulting from early culling because of poor health. Studies were considered randomized if animals were numbered before study commencement and a randomization code was used to choose which animal would be the next experimental subject; if animals were 'picked at random' from a cage, studies were considered as pseudo-randomized as this approach is open to bias.

The methodological quality of each study was assessed using an 9-point score based on the STAIR (1999) rating as described previously (Macleod *et al*., 2005; Willmot *et al*., 2005a,b; Gibson *et al*., 2006). One point was given for written evidence of each of the following criteria: presence of randomization (0.5 given for pseudo-randomization), monitoring of physiological parameters, assessment of dose–response relationship, assessment of optimal time window, masked outcome measurement, assessment of outcome at days 1–3, assessment of outcome at days 1–30, combined measurement of lesion volume and functional outcome and additionally to the STAIR rating – masked surgery.

Data analysis

Data analysis comprised two stages. First, IAD and summary data were analysed together and, second, IAD were analysed separately. The analysis of summary data and IAD used random effects models to produce standardized mean differences (SMD, for continuous or ordinal data, http://www.cochrane-net.org/openlearning/html/modA1-4.htm) and 95% confidence intervals. Random effects models were used as biological heterogeneity was expected due to the varied nature of studies involving different species, models of ischaemia, time to treatment and doses of NXY-059. Statistical heterogeneity was calculated using the I^2 statistic. The presence of publication bias was assessed using a funnel plot and Egger's test (Egger *et al*., 1997).

For the IAD analysis, data were entered into an Excel spreadsheet with each row containing data for an individual animal. Multilevel models were built to compare NXY-059 with control taking into account the differences between trials. Infarct volume was standardized (score-mean/standard deviation) to account for differences between the brain and infarct sizes in different species. Similarly, motor impairment was standardized as different scales were used, although all were based on that from Bederson *et al*. (1986). Coefficients for dose are given per 100 $mg \cdot kg^{-1}$. For analyses of dose/concentration and concentration/response relationships, NXY-059 concentrations were identified reflecting steady-state levels, typically at 24 h. All analyses were carried out in Stata version 8.

Results

Study identification

Fifteen completed studies fulfilled the inclusion criteria (Figure 1); these included 26 separate experiments involving

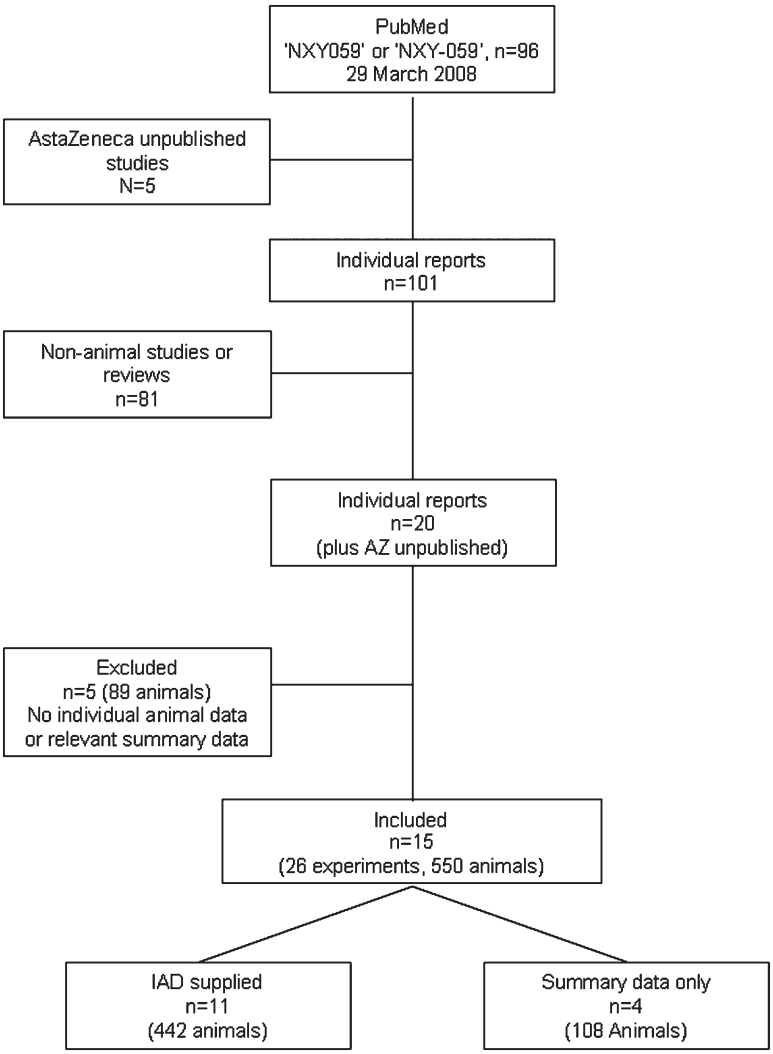

Figure 1 Flow chart of study identification and inclusions and exclusions.

585 animals and came from 12 different laboratories in five countries (Canada, Japan, Sweden, UK, USA). Four of the studies were unpublished (Maples, 1996; Green, 2002; Sydserff, 2002; Zhao *et al.*, 2002). Investigators from five studies (89 animals), all non-commercial, did not share IAD and the publications did not give data in a format suitable for inclusion (Lapchak *et al.*, 2002a,b; 2004); these studies, which involved rabbits, were therefore excluded (Figure 1).

Study design

The studies involved 585 animals (NXY-059 332, control 253) from three species: mice (9 animals), rats (544, from several strains – Long Evans, spontaneously hypertensive, Sprague-Dawley, Wistar) and marmosets (32 animals) (Table 1); individual data were available for 442 (76%) animals. The studies varied in size involving between 9 and 46 (median 12) animals. Seven out of 15 (47%) studies were randomized, three studies were pseudo-randomized and five did not involve randomization. Surgeons were blinded to treatment in 6/15 (40%) studies; outcome assessors were recorded as being blinded to treatment in 8/15 (53%) studies. A variety of anaesthetic agents were used: alphaxolone/alphadolone acetate, chloral hydrate, halothane in N_2O/O_2, isoflurane and pentobarbital; of these, only pentobarbitone may be considered as neuroprotective in its own right. Studies involved different models of ischaemia (temporary, permanent, thrombotic), times from onset of occlusion to treatment (5–480 min; median 90 min), length of treatment (21.75–72 h) and loading (0.3–200 mg·kg^{-1}) and maintenance (0.3–200 mg·kg^{-1} h) doses of NXY-059 (Table 1).

Following onset of occlusion, five studies confirmed that cerebral blood flow was significantly reduced, either using visual inspection of the middle cerebral artery to confirm occlusion post-mortem (Marshall *et al.*, 2001; 2003) or using laser doppler methods (Green, 2002; Zhao *et al.*, 2001; 2002).

Lesion volume

Fifteen studies (26 experiments) had data on total infarct volume (Table 2). Visual and statistical assessment of publication bias using a funnel plot (Figure 2) and Egger's test (Egger

Table 1 Studies included in the analysis

	Laboratory	*Species*	*Sex*	*Anaesthetic*	*Random*	*Surgery blinded*	*Outcome blinded*	*Temperature control*	*Model*	*Time after occlusion onset (min)*	*NXY-059 load ($mg \cdot kg^{-1}$)*	*NXY-059 maintenance ($mg \cdot kg^{-1}$ h)*
Maples (1996)	Sunnyvale, CA, USA	Rat (Sprague-Dawley)	Male	Pentobarbital 40 $mg \cdot kg^{-1}$	Yes	NR	NR	Yes	MCAo-p (cautery)	30	100 i.v.	4.12 i.v. for 23 h
Kuroda *et al.* (1999) (3 studies)	Lund Sweden	Rat (Wistar)	Male	Halothane (1–3%) in N_2O/O_2 (70/30)	No	NR	NR	Yes	MCAo-t (2 h, time–response)	180, 300, 480	0.3, 3, 30	0.3, 3, 30 for 24 h
Marshall *et al.* (2001)	Cambridge, UK	Marmoset (Common)	Both	Alphaxolone (13.5 $mg \cdot kg^{-1}$ i.m.)/alphadolone acetate (4.5 $mg \cdot kg^{-1}$ i.m.)	Pseudo	Yes	Yes	Yes	MCAo-p (cautery)	5	28 i.v.	18 s.c. for 48 h
Zhao *et al.* (2001)	Calgary, Canada	Rat (SHR)	Male	Halothane/N_2O/O_2 (2/70/28%)	Yes	Yes	Yes	Yes	MCAo-p (dose–response)	5	30–60 i.v.	30–60 i.v. for 24 h
Aronowski (2002) (see Green, 2002) (4 studies)	Houston, TX, USA	Rat (Long Evans) and Rat (SHR)	Male	Chloral hydrate and Isoflurane	NR	NR	NR	Yes	MCA/CCAo-t (3 h) and MCA/CCAo-p	15	60 i.v.	50 i.v. for 24 h and 60 i.v. for 24 h
Sydserff *et al.* (2002) (3 studies)	Worcester, MA, USA	Rat (Wistar)	Male	Halothane (2–6%)/O_2 (1.0 $L \cdot min^{-1}$)/N_2O (1.5 $L \cdot min^{-1}$)	Pseudo	Yes	Yes	Yes	MCAo-p (time–response) and MCAo-t (2 h, dose–response)	5–240 135	53.8 s.c., 32.5–75.4 s.c.	50 s.c. for 24 h, 30–70 s.c. for 23 h 55 min and 3–30 i.v. for 21 h 45 min
Zhao *et al.* (2002)	Calgary, Canada	Rat (SHR)	Male	Halothane/N_2O/O_2 (2/70/28%)	Yes	Yes	Yes		MCAo-p	5	120 i.v.	120 i.v. for 24 h
Sydserff (2002)	Worcester, MA, USA	Rat (Sprague-Dawley)	Male	Chloral hydrate (400 $mg \cdot kg^{-1}$)	Yes	NR	Yes		MCAo-t (30 min)	30	30 i.v.	30 i.v. for 72 h
Yoshimoto *et al.* (2002a)	Honolulu, USA	Rat (Wistar)	Male	Halothane (1.5–4%)/N_2O (70/30)	NR	NR	NR		MCAo-t (2 h, filament, time–response)	125, 180	30 i.v.	30 i.v. for 24 h
Yoshimoto *et al.* (2002b)	Honolulu, USA	Rat (Wistar)	Male	Halothane/N_2O/O_2 (1.5–4/70/30)	Yes	NR	NR		MCAo-t (2 h, filament)	180	30 i.v.	30 i.v. for 24 h
Han *et al.* (2003)	Honolulu, USA	Rat (Wistar)	Male	Halothane/N_2O/O_2 (70/30)	NR	NR	NR		MCAo-t (2 h)	180	30 i.v.	30 i.v.
Marshall *et al.* (2003)	Cambridge, UK	Marmoset (Common)	Both	Alphaxolone (13.5 $mg \cdot kg^{-1}$ i.m.)/alphadolone acetate (4.5 $mg \cdot kg^{-1}$ i.m.)	Pseudo	Yes	Yes		MCAo-p (cautery)	240	77 i.v. + 154 s.c.	32 for 48 h
Wang and Shuaib (2004)	Edmonton, Canada	Rat (Wistar)	Male	Halothane (1.5–3%)/O_2/N_2O	Yes	Yes	Yes		MCAo-e	90	65, 200 i.v.	65, 200 i.v. for 4 h
Balogh *et al.* (2005)	Budapest, Hungary	Mouse (NMRI)	Male	Chloral hydrate (550 $mg \cdot kg^{-1}$ i.p.)	Yes	No	Yes		MCAo-p	30	10 i.p.	–
Takizawa *et al.* (2007)	Japan	Rat (Sprague-Dawley)	Male	Halothane	No	No	No	Yes	MCAo (photo thrombotic)	0 and 30	3.10 $mg \cdot kg^{-1}$	–

i.m., intramuscular; i.p., intraperitoneal; i.v., intravenous; MCAo, middle cerebral artery occlusion; MCAo-e, embolic occlusion MCAo; MCAo-p, permanent MCAo; MCAo-t, transient MCAo; NMRI, Naval Medical Research Institute; NR, not recorded; s.c., subcutaneous; SHR, spontaneously hypertensive.

Table 2 Experimental results for included studies

	Animals, total (NXY-059 : control)	*Individual animal data*	*Weight (g), NXY-059 Control*	*Temperature max, NXY-059 Control*	*Exclusions from analysis, NXY-059 Control*	*Death, NXY-059 Control*	*Lesion volume timing (h)*	*Lesion total, NXY-059 Control*	*Motor score, NXY-059 Control*	*Quality/8*	*Comment for NXY-059*
Maples (1996)	46 (24:24)	✓	332 (22) 341 (22)	–	0 1	1 1	23	29 (62) 57 (64)	–	3	Reduced infarct volume
Kuroda *et al.* (1999)	38 (28:10)	✓	**310 (11)** **322 (17)**	38.0 (0.2) 37.9 (0.3)	0 0	0 0	7 days	**120 (113)** **296 (82)**	**0.8 (0.9)** **2.4 (0.7)**	6	Dose-dependent reduction in infarct volume
Kuroda *et al.* (1999)	15 (8:7)	✓	326 (14) 327 (8)	37.9 (0.3) 38.0 (0.2)	0 0	0 0	7 days	**74 (107)** **202 (24)**	**0.5 (0.8)** **2.4 (0.8)**	6	Reduced infarct volume
Kuroda *et al.* (1999)	21 (16:5)	✓	328 (11) 327 (7)	38.0 (0.1) 38.1 (0.1)	0 0	0 0	48	**155 (119)** **289 (64)**	2.2 (0.7) 2.4 (0.7)	6	Reduced infarct volume at 3 h
Marshall *et al.* (2001)	12 (6:6)	✓	–	–	0 1	0 0	20 weeks	**340 (155)** **158 (107)**	–	6.5	Reduced motor impairment and neglect
Zhao *et al.* (2001)	31 (21:10)	✓	275 (26) 269 (13)	–	0 0	0 0	24	**40 (14)** 57 (16)	–	6	No effect on infarct volume at 30 mg·kg^{-1}; significant reduction of infarct volume at 60 mg·kg^{-1}
Aronowski 2002 (see Green, 2002)	20 (10:10)	✓	–	–	0 0	0 0	72	123 (49) 109 (53)	–	2	No effect on infarct volume in MCA/CCAo-t.
Aronowski (2002) (see Green, 2002)	10 (6:4)	✓	–	–	0 0	0 0	72	87 (44) 51 (33)		2	No effect on infarct volume in MCA/CCAo-p.
Aronowski (2002) (see Green, 2002)	17 (10:7)	✓	263 (12) 263 (12)	36.7 (0.2) 36.9 (0.1)	0 0	2 0	72	143 (26) 131 (39)	–	2	No effect on infarct volume, BP or temperature.
Aronowski (2002) (see Green, 2002)	17 (9:8)	✓	**250 (15)** **273 (20)**	36.5 (0) 36.5 (0)	0 0	1 0	72	88 (39) 105 (41)	–	2	No effect on infarct volume, BP or temperature. Also, no effect in positive control (caffeine/ethanol)
Sydserff *et al.* (2002)	47 (39:8)	✓	–	–	0 0	0 0	24	**124 (47)** **226 (34)**	–	6.5	Dose-dependent reduction in infarct volume linearly to plasma concentration
Sydserff *et al.* (2002)	37 (28:9)	✓	310 (6) 307 (7)	37.2 (0.5) 36.9 (0.9)	1 1	3 0	24	**92 (66)** **200 (39)**	**4.2 (1.8)** **5.8 (2.1)**	6.5	
Sydserff *et al.* (2002)	56 (28:28)	✓	304 (13) 309 (10)	–	0 0	0 0	48	**77 (49)** **156 (58)**	**3.5 (1.5)** **5.8 (1.2)**	6.5	Dose-dependent decrease in infarct volume and motor impairment
Zhao *et al.* (2002)	20 (10:10)	✓	232 (16) 229 (23)	–	0 0	0 0	24	99 (19) 94 (12)	–	5	
Sydserff (2002)	10 (5:5)	✓	–	–	0 0	0 0	72	**82 (38)** **147 (27)**	–	4	Reduced infarct volume

Table 2 Continued

	Animals, total (NXY-059 : control)	*Individual animal data*	*Weight (g), NXY-059 Control*	*Temperature max, NXY-059 Control*	*Exclusions from analysis, NXY-059 Control*	*Death, NXY-059 Control*	*Lesion volume timing (h)*	*Lesion total, NXY-059 Control*	*Motor score, NXY-059 Control*	*Quality/8*	*Comment for NXY-059*
Yoshimoto *et al.* (2002b)	10 (6:4)	×	[260–310]	–	–	–	2 h 5 min	**12 (10)** **33 (5)**	–	3	Temperature higher in NXY-059 group higher at 4 h. Reduced infarct volume, secondary decline in mitochondrial respiratory function and mitochondrial release of cytochrome c, but no effect on calcium-induced mitochondrial swelling
Yoshimoto *et al.* (2002b)	10 (6:4)	×	[260–310]	–	–	–	3	**10 (4)** **33 (5)**	–	3	
Yoshimoto *et al.* (2002a)	12 (6:6)	×	[260–310]	–	–	–	4	13 (4.9) 14 (4.9)	–	4	Reduced infarct volume and neuronal mitochondrial cytochrome c release, and normal fall in p-Akt
Yoshimoto *et al.* (2002a)	13 (7:6)	×	[260–310]	–	–	–	24	**16 (5.3)** **26 (14.7)**	–	4	
Yoshimoto *et al.* (2002a)	13 (6:7)	×	[260–310]	–	–	–	48	**15 (2.4)** **30 (10.6)**	–	4	
Han *et al.* (2003)	20 (10:10)	×	[300–350]	–	–	–	24	**13 (8)** **37 (5)**	–	1	Reduced cytochrome c release
Marshall *et al.* (2003)	25 (13:12)	✓	389 (28) 407 (44)	–	0 0	2 2	11 weeks	234 (100) 329 (147)	–	6.5	Reduced infarct volume, functional outcome and neglect
Wang and Shuaib (2004)	24 (16:8)	✓	386 (32) 375 (47.5)	–	0 0	4 3	48	**31% (9)** **43% (15)**	2.5 (0.5) 2.8 (0.8)	4	High-dose NXY-059 showed a higher number of deaths than low dose. Low dose produced a greater protective effect
Balogh *et al.* (2005)	9 (4:5)	✓	29 (1) 31 (1)	–	0 0	0 0	48	295 (13) 306 (11)	–	3	No significant effect
Takizawa *et al.* (2007)	20 (10:5)	×	[306 (12)]	–	0 0	0 0	24	223 (19) 244 (10)	–	3	Low dose reduced motor impairment but not infarct volume
Takizawa *et al.* (2007)	20 (10:5)	×	[306 (12)]	–	0 0	0 0	24	**192 (10)** **244 (10)**	–	3	High dose reduced infarct volume and motor impairment

Mean (SD) or median (IQR); statistically significant differences in lesion volume and motor score between the treatment groups are shown in bold (*t*-test). Figures for lesion size are generally infarct volume in mm^3. However, in some cases values are those given for the particular measure made (e.g. MAP2 kinase staining in case of Yoshimoto *et al.*, 2002a).
NXY-059, disodium 2,4-disulphophenyl-N-tert-butylnitrone.

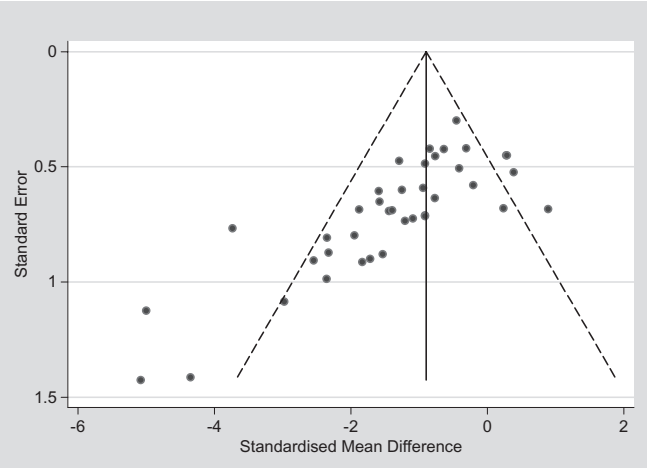

Figure 2 Funnel plot of NXY-059 on total infarct volume incorporating both individual animal data and summary data as assessment of publication bias, Egger *et al.* (1997) test: $P < 0.0001$. NXY-059, disodium 2,4-disulphophenyl-N-tert-butylnitrone.

et al., 1997) showed significant bias ($P < 0.0001$) with the majority of included studies showing beneficial effects for NXY-059.

In the combined IAD and summary analysis (Table 3), NXY-059 reduced total, cortical and subcortical lesion volumes by one to two standard deviations between the means. Although NXY-059 reduced lesion volume in rats and marmosets, no effect was seen in mice; however, the point estimate (SMD –0.91) for mice suggests that this is due to a type II error as only nine animals were studied (Balogh *et al.*, 2005). Comparable results were found when using IAD alone; this analysis shows the coefficient by total dose of NXY-059 with adjustment for time to treatment (Table 4).

In combined IAD and summary analyses, total lesion volume was reduced to a similar extent in models of transient, permanent and thrombotic ischaemia (although the latter finding is based on only one study) (Table 3, Figure 3). In the adjusted IAD analysis, NXY-059 reduced total lesion volume in transient and permanent models (but not in the thrombotic study, probably reflecting the different methods of

Table 3 Effect of NXY-059 on infarct volume by brain regional, stroke model, animal species, time from occlusion to treatment onset, length of treatment, loading dose and maintenance dose

Group	*Studies*	*Experiments*	*Animals*	*SMD*	*95% CI*	P-*value*	*Heterogeneity* P-*value*
Total volume	**15**	**26**	**585**	**–1.17**	**–1.50 to –0.84**	**<0.0001**	**<0.0001**
Cortical	**5**	**6**	**145**	**–2.17**	**–2.99 to –1.34**	**<0.0001**	**<0.0001**
Subcortical	**5**	**6**	**145**	**–1.43**	**–2.00 to –0.86**	**<0.0001**	**0.05**
Species							
Mice	1	1	9	–0.91	–2.31 to 0.49	0.20	–
Rats	**12**	**23**	**544**	**–1.05**	**–1.53 to –0.57**	**<0.0001**	**<0.0001**
Marmosets	**2**	**2**	**32**	**–0.95**	**–1.70 to –0.21**	**0.01**	0.45
Model							
Permanent	**8**	**13**	**302**	**–1.05**	**–1.53 to –0.57**	**<0.0001**	**<0.0001**
Transient	**6**	**12**	**259**	**–1.33**	**1.84 to –0.82**	**<0.0001**	**<0.0001**
Thrombotic	**1**	**1**	**24**	**–1.08**	**–2.00 to –0.16**	**0.02**	0.47
Start time (min)							
–30	**9**	**18**	**282**	**–0.87**	**–1.36 to –0.38**	**0.001**	**<0.0001**
>30 to –60	**1**	**1**	**10**	**–2.36**	**–4.29 to –0.43**	**0.02**	–
>60 to –120	**1**	**2**	**34**	**–1.21**	**–2.03 to –0.40**	**0.003**	0.63
>120 to –180	**4**	**9**	**208**	**–1.47**	**–2.04 to –0.89**	**<0.0001**	**0.001**
240	2	2	30	–2.31	–5.79 to 1.18	0.19	**0.02**
300	1	1	10	–1.53	–3.27 to 0.19	0.08	–
480	1	1	11	–0.91	–2.30 to 0.48	0.20	–
Treatment length (h)							
0[†]	**2**	**3**	**39**	**–2.19**	**–4.21 to –0.16**	**0.03**	**0.006**
>0 to –24	**10**	**19**	**489**	**–1.08**	**–1.44 to –0.71**	**<0.0001**	**<0.0001**
48	**3**	**3**	**47**	**–1.13**	**–1.76 to –0.50**	**<0.0001**	0.50
72	**1**	**1**	**10**	**–1.94**	**–3.51 to –0.38**	**0.02**	–
Loading dose (mg·kg^{-1})							
No load	**1**	**3**	**77**	**–0.60**	**–1.07 to –0.12**	**0.01**	0.66
≤30	**9**	**18**	**231**	**–1.69**	**–2.18 to –1.20**	**<0.0001**	**0.004**
>30 to ≤60	**3**	**12**	**154**	**–0.88**	**–1.58 to –0.18**	**0.01**	**<0.0001**
>60 to ≤120	**5**	**5**	**111**	**–0.74**	**–1.44 to –0.04**	**0.04**	**0.03**
200	**1**	**1**	**12**	**–0.77**	**–2.02 to 0.48**	**0.23**	**–**
Maintenance dose (mg·kg^{-1})							
No maintenance	**2**	**3**	**39**	**–2.19**	**–4.21 to –0.16**	**0.03**	**0.01**
≤30	**9**	**20**	**326**	**–1.23**	**–1.63 to –0.82**	**<0.0001**	**0.001**
>30 to ≤60	**4**	**12**	**164**	**–0.94**	**–1.60 to –0.28**	**0.01**	**<0.0001**
>60 to ≤120	3	3	44	–1.07	–2.69 to 0.56	0.20	**0.01**
200	1	1	12	–0.77	–2.02 to 0.48	0.23	–

Data are standardized mean difference (SMD), 95% confidence intervals (95% CI) and significance for effect and heterogeneity. Results in bold are statistically significant.

[†]Two studies administered one or two loading doses only, without maintenance.

NXY-059, disodium 2,4-disulphophenyl-N-tert-butylnitrone.

Table 4 Effect of NXY-059 on infarct volume by brain region subdivided by animal species and sex, and model of ischaemia

Group	*Experiments*	*Animals*	*Coefficient*	*95% CI*	P-*value*
Species					
Total	**18**	**442**	**–0.03**	**–0.04 to –0.02**	**<0.0001**
Mice	1	9	–8.67	–19.69 to 2.36	0.12
Rats	**15**	**401**	**–0.03**	**–0.04 to –0.02**	**<0.0001**
Marmosets	**2**	**32**	**–0.06**	**–0.10 to –0.01**	**0.01**
Males	2	17	–0.02	–0.06 to 0.01	0.13
Females	**2**	**15**	**–0.05**	**–0.09 to –0.005**	**0.03**
Cortical†	**4**	**111**	**–0.08**	**–0.11 to –0.06**	**<0.0001**
Rats	**2**	**79**	**–0.13**	**–0.16 to –0.10**	**<0.0001**
Marmosets	2	32	–0.04	–0.09 to 0.003	0.07
Males	2	17	–0.03	–0.10 to 0.03	0.32
Females	2	15	–0.04	–0.08 to 0.01	0.13
Subcortical†	**4**	**111**	**–0.10**	**–0.13 to –0.08**	**<0.0001**
Rats	**2**	**79**	**–0.13**	**–0.16 to –0.10**	**<0.0001**
Marmosets	**2**	**32**	**–0.07**	**–0.11 to –0.02**	**0.002**
Males	2	17	–0.06	–0.13 to 0.01	0.11
Females	**2**	**15**	**–0.07**	**–0.11 to –0.03**	**0.001**
Model					
Total					
Transient	**6**	**160**	**–0.05**	**–0.08 to –0.03**	**<0.0001**
Permanent	**11**	**258**	**–0.10**	**–0.13 to –0.08**	**<0.0001**
Thrombotic	1	24	–0.06	–0.15 to 0.03	0.18

No data were available for cortical/subcortical volumes in mice. Analyses based on individual animal data only. Data are coefficients per 100 mg·kg^{-1} of total administered NXY-059 dose with standardization for model and adjustment for time to treatment; 95% confidence intervals (95% CI) and significance. Results in bold are statistically significant.
†Permanent models only.
NXY-059, disodium 2,4-disulphophenyl-N-tert-butylnitrone.

analysis) (Table 4). Both cortical and subcortical lesion volumes were less with NXY-059 in permanent models of ischaemia.

Reductions in total lesion volume were seen with treatment commenced between 5 and 180 min post onset of ischaemia; declining trends to efficacy were seen beyond this out to 480 min, but the limited number of animals prevents further interpretation (Table 3). Treatment with NXY-049 for 48 or 72 h did not appear to increase efficacy over and above that seen with 24 h (similar confidence intervals around the effects). Similarly, there was no evidence for dose–response effects in respect of either loading or maintenance dose. Eighteen out of 255 (7%) animals receiving NXY-059 had no lesion in comparison with 0/163 (0%) receiving control ($P = 0.001$). These findings came from two studies (four experiments); in both cases, surgeons and outcome assessors were unblinded to treatments (Maples, 1996; Kuroda *et al.*, 1999).

When IAD data for lesion volume were assessed by markers of study quality, important observations were present. Total lesion volume was only reduced in studies with pseudo-randomization, but not those with true or no randomization (Table 5). The blinding of surgeons to treatment did not appear to alter treatment effects on total lesion volume. When considering the seven studies involving randomization (pseudo or full), surgeon and outcome-blinding, and monitoring of physiological variables, lesion volume was significantly reduced (–0.04, 95% confidence interval –0.05 to –0.02). Lesion volume was reduced in studies where a non-neuroprotective anaesthetic agent was used. However, efficacy was only seen in normotensive rather than hypertensive animals, and no studies in old animals were identified (Table 5, Figure 4).

Clinical phase III trials commenced in May 2003; total lesion volume was significantly reduced by NXY-059 in the 15 studies completed by this time (–0.03, 95% confidence interval –0.04 to –0.02).

Motor impairment and neglect

Data on motor impairment were available for six studies in rats (Table 6), this being recorded at 24 h post onset of occlusion in all but one study (48 h; Kuroda *et al.*, 1999). In adjusted analyses based on IAD, impairment was reduced in rats (Table 6), this effect being present in transient and permanent but not thrombotic models.

When considering two primate studies in marmosets (Marshall *et al.*, 2001; 2003), motor impairment was similar at baseline and reduced at 3 weeks post treatment in NXY-059-treated animals (Table 7). Similarly, neglect at 3 weeks was less in NXY-059-treated primates, an effect that was significant in females with a trend to reduced neglect being seen in males. Results for data at 10 weeks were not available for individual animals, so no analysis was possible, although both marmoset studies individually were positive at 10 weeks (Marshall *et al.*, 2001; 2003).

Death

No difference in death rates were present between NXY-059 (13/281, 5%) and control (6/176, 4%) animals ($P = 0.4$); however, death was not recorded in four studies.

NXY-059 plasma concentration

The relationship between NXY-059 dose and plasma concentration was assessed in five studies on rats (Zhao *et al.*, 2001;

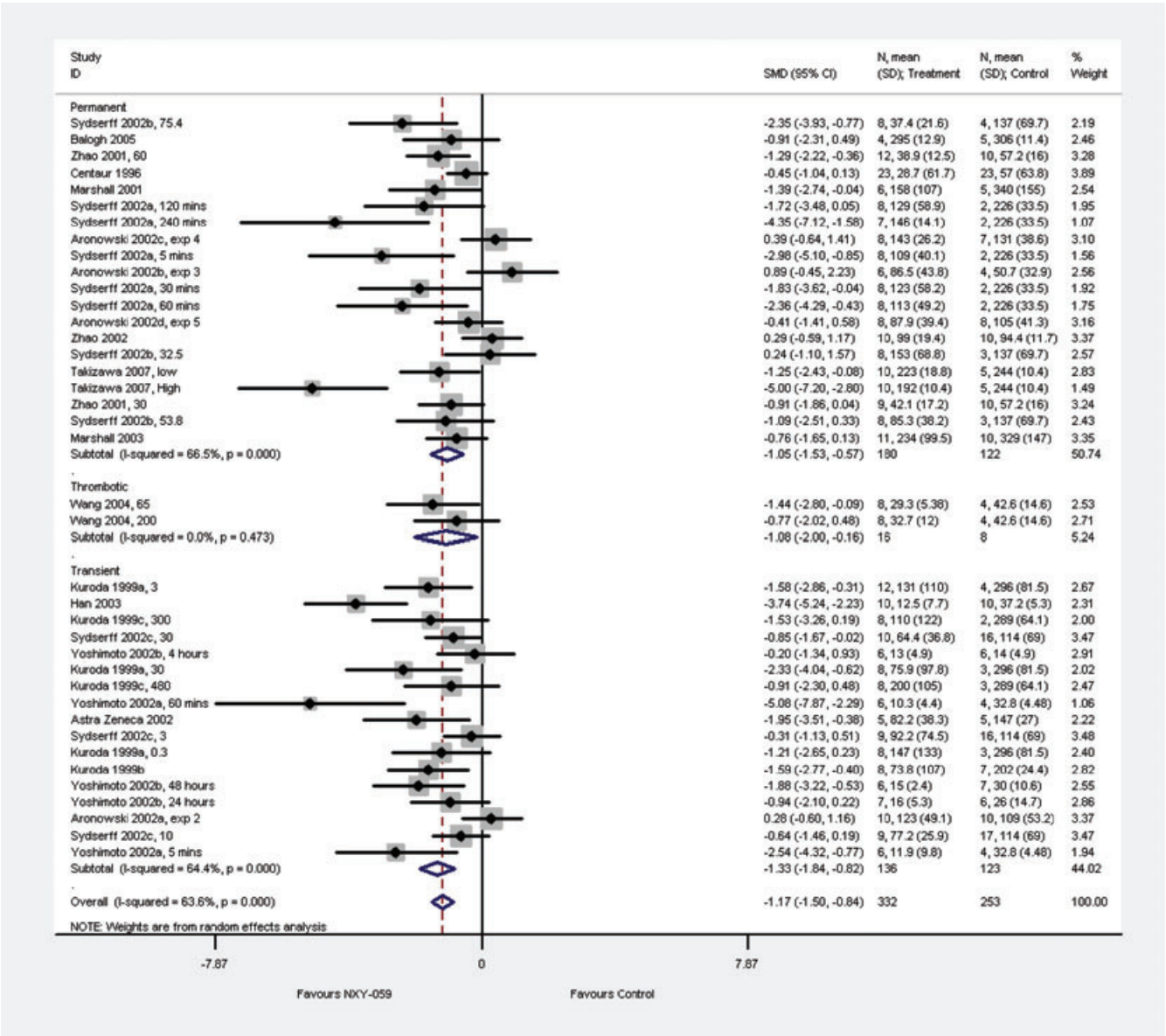

Figure 3 Forest plot of NXY-059 on total infarct volume incorporating both individual animal data and summary data. NXY-059, disodium 2,4-disulphophenyl-N-tert-butylnitrone.

Sydserff *et al.*, 2002; Wang and Shuaib, 2004) and two on marmosets (Marshall *et al.*, 2001; 2003) (Table 8); some studies did not involve stroke models (Maples, 1997). A linear concentration–dose relationship was seen (Figure 5) with $[NXY\text{-}059]_{total\ in\ plasma}$ ($mg{\cdot}L^{-1}$) = 1.57 × maintenance dose ($mg{\cdot}kg^{-1}$ h) ($P < 0.0001$); a comparable relationship based on a sub-set of these studies was used by AZ and Centaur Pharmaceuticals in preparing for human studies, $[NXY\text{-}059]_{total\ in\ plasma}$ ($\mu g{\cdot}mL^{-1}$) = 1.7 × maintenance dose (Maples, 1997). The unbound fraction of NXY-059 in blood varies between species; figures of 30% for rats (Zhao *et al.*, 2001; Sydserff *et al.*, 2002; Wang and Shuaib, 2004) and 70% for marmosets (Marshall *et al.*, 2001; 2003) were used in calculations to assess the relationship between unbound NXY-059 and total stroke lesion size. As a result, lower maintenance doses of NXY-059 could be used in marmosets than rats to achieve the same unbound plasma concentration. Using measured (Marshall *et al.*, 2001) or estimated unbound concentrations, Figure 6 shows the negative correlation between the effect of NXY-059 on total lesion volume and unbound plasma concentration [SMD = –0.13 – 0.02 × unbound plasma concentration ($mg{\cdot}L^{-1}$); $P < 0.0001$].

Discussion

This meta-analysis is the first to use IAD in any area of medicine. The results confirm, on the basis of the available data, that NXY-059 is neuroprotective in preclinical models of stroke. Specifically, treatment with NXY-059 was associated with reduced total, cortical and subcortical lesion volumes. The beneficial effects on lesion volume were seen in three

Table 5 Effect of NXY-059 on infarct volume by markers of study quality

Group	*Experiments*	*Animals*	*Coefficient*	*95% CI*	P-*value*	*Interaction test* P-*value*
Randomization						
Yes	6	140	–0.02	–0.03 to 0.003	0.11	–
Pseudo	**5**	**167**	**–0.10**	**–0.13 to –0.08**	**<0.0001**	**<0.0001***
No	7	135	–0.02	–0.05 to 0.01	0.16	0.99*
Blinded surgery						0.10
Yes	**8**	**242**	**–0.04**	**–0.05 to –0.02**	**<0.0001**	
No	**10**	**200**	**–0.02**	**–0.05 to –0.002**	**0.03**	
Outcome blinded						0.12
Yes	**10**	**261**	**–0.04**	**–0.05 to –0.02**	**<0.0001**	
No	8	181	–0.02	–0.04 to 0.01	0.16	
Anaesthetic						0.19
Neuroprotective	1	46	–0.23	–0.51 to 0.06	0.12	
Not neuroprotective	**17**	**396**	**–0.04**	**–0.05 to –0.02**	**<0.0001**	
Blood pressure						**<0.0001**
Hypertensive	5	92	–0.002	–0.02 to 0.02	0.88	
Normotensive	**13**	**350**	**–0.07**	**–0.09 to –0.05**	**<0.0001**	
Quality score						
2	4	61	0.02	–0.02 to 0.05	0.34	**<0.0001†**
3	2	55	–0.20	–0.47 to 0.06	0.14	0.34†
4	**2**	**34**	**–0.06**	**–0.11 to –0.02**	**0.003**	0.13†
5	1	20	0.01	–0.02 to 0.04	0.50	**<0.0001†**
6	**4**	**105**	**–0.07**	**–0.11 to –0.34**	**<0.0001**	0.62†
6.5	**5**	**167**	**–0.10**	**–0.13 to –0.08**	**<0.0001**	–

Analyses based on individual animal data only. Data are coefficients per 100 mg·kg^{-1} of total administered NXY-059 dose with standardization for model and adjustment for time to treatment; 95% confidence intervals (95% CI) and significance. Results in bold are statistically significant.
*In comparison with randomized studies.
†in comparison with the highest quality studies.
NXY-059, disodium 2,4-disulphophenyl-N-tert-butylnitrone.

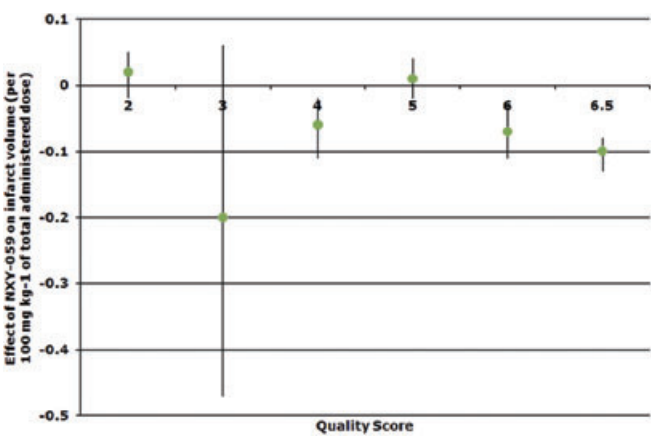

Figure 4 Relationship between study quality and infarct volume, coefficients per 100 mg·kg^{-1} of total administered NXY-059 dose. NXY-059, disodium 2,4-disulphophenyl-N-tert-butylnitrone.

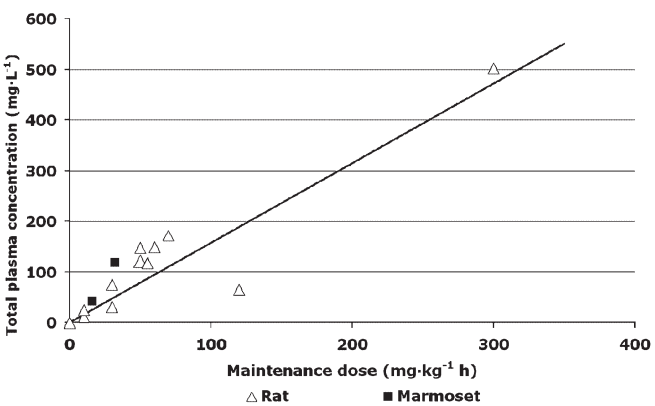

Figure 5 Relationship between total plasma concentration and maintenance dose of NXY-059. Equation of best fit $[NXY\text{-}059]_{total\ in\ plasma}$ (mg·L^{-1}) = 1.57 × maintenance dose (mg·kg^{-1} h). NXY-059, disodium 2,4-disulphophenyl-N-tert-butylnitrone.

species (significant in rats and marmosets, a trend in the few studied mice), and in three models of stroke – transient, permanent and thrombotic. Treatment was effective when started between 5 and 180 min after the onset of ischaemia; interestingly, the length of treatment did not appear to matter. Surprisingly, there was no evidence for a dose–response relationship in respect of both loading and maintenance dose, although such a relationship was observed in an individual study in rats designed to examine this relationship in both transient and permanent ischaemia (Sydserff *et al.*, 2002). Furthermore, a dose relationship was observed using the plasma concentration (Figure 5) and plasma concentration has previously been shown to relate linearly to the administered dose in an individual study in rats (Sydserff *et al.*, 2002).

In addition to effects on lesion volume, NXY-059 treatment was associated with reductions in motor impairment (in two species) and neglect (a higher cortical function) in marmosets. These data come from multiple laboratories, both commercial and academic, and were published in quality peer-reviewed journals. Superficially, these results appear to fulfil the modified STAIR criteria for demonstration of preclinical activity (see Green, 2008). And yet, the positive results in 585 animals did not translate into positive clinical data in 5028 patients with acute ischaemic stroke (Diener *et al.*, 2008).

Table 6 Effect of NXY-059 on motor impairment in rats by model of ischaemia

Group	*Experiments*	*Animals*	*Coefficient*	*95% CI*	P-*value*
Rats	**6**	**180**	**–0.06**	**–0.10 to –0.02**	**0.001**
Transient	**4**	**130**	**–0.10**	**–0.16 to –0.04**	**0.001**
Permanent	**1**	**31**	**–0.05**	**–0.10 to –0.002**	**0.04**
Thrombotic	1	19	–0.02	–0.14 to 0.09	0.67

Analyses based on individual animal data only. Data are coefficients per 100 mg·kg^{-1} of total administered NXY-059 dose with standardization for model and adjustment for time to treatment; 95% confidence intervals (95% CI) and significance. Results in bold are statistically significant.
NXY-059, disodium 2,4-disulphophenyl-N-tert-butylnitrone.

Table 7 Effect of NXY-059 on motor impairment and neglect in marmosets (permanent ischaemia)

Group	n	*Coefficient*	*95% CI*	P-*value*
Motor				
Baseline	32	0.03	–0.43 to 0.49	0.90
3 weeks	**32**	**3.20**	**0.12 to 6.29**	**0.04**
Males	17	2.54	–0.58 to 5.67	0.11
Females	15	3.17	–2.05 to 8.40	0.24
Neglect				
Baseline	32	0.004	–0.003 to 0.01	0.24
3 weeks	**32**	**5.99**	**2.05 to 9.94**	**0.003**
Males	17	5.11	–0.72 to 10.94	0.09
Females	**15**	**6.67**	**1.47 to 11.86**	**0.01**

Analyses based on individual animal data only. Data are coefficients by presence or absence of NXY-059; 95% confidence intervals (95% CI) and significance. Results in bold are statistically significant.
NXY-059, disodium 2,4-disulphophenyl-N-tert-butylnitrone.

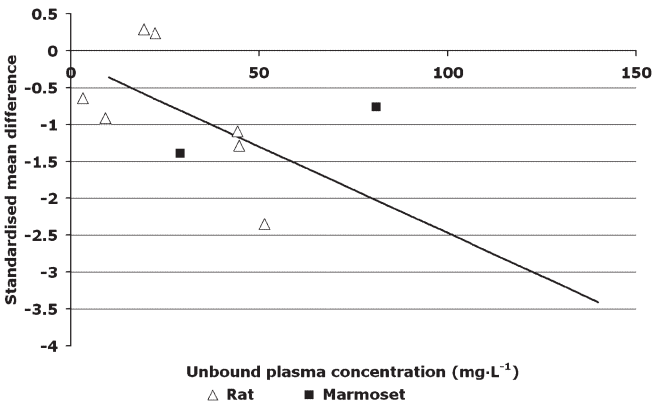

Figure 6 Relationship between unbound plasma concentration (mg·L^{-1}) and standardized mean difference in infarct volume. Equation of best fit standardized mean difference= –0.13 – 0.02 × unbound plasma concentration (mg·L^{-1}).

The data show some concerns when outcomes are judged by animal sex and blood pressure status. The interaction between sex and response was only available to study in marmosets; while females showed significant reductions in total and subcortical lesion volumes and neglect (but only a trend in cortical lesion volume and motor impairment), NXY-059 did not significantly reduce any of these parameters in males (although trends were present for many of these) (Tables 4 and 7). Nevertheless, it is likely that these findings reflect limited statistical power rather than real differences in response by sex as male rats did respond to NXY-059; point estimates for effect sizes were similar for male and female marmosets, and no differences in efficacy were seen between men and women in the SAINT-I and -II trials (Lees *et al.*, 2006; Shuaib *et al.*, 2007). Of more concern is the observation that only normotensive rats responded to NXY-059 (Table 5); no effect was apparent in hypertensive rats with a point estimate approximating to 0. Although this finding could also reflect a type II error, the finding was present in 92 rats across five experiments. Whether spontaneously hypertensive rats are a good model of experimental stroke is probably a moot point as the heterogeneity present in human stroke demands that interventions should be effective in a wide range of species, subspecies and co-morbidities.

It is important to assess the validity of this meta-analysis, based in the main on data from individual animals. Individual subject meta-analyses are considered the gold standard (Stewart and Parmar, 1993), partly because they allow analyses to be performed at the level of individuals rather than studies. The data on NXY-059 allowed subgroups to be studied (e.g. effects by species, sex, stroke model, treatment time, treatment length and dose) as well as interactions between variables. As individual data were not available for all animals we also used summary approaches to meta-analysis. The findings for the subset of studies with IAD were compatible with the overall summary results with any differences between these two likely to reflect the small number of studies and animals in certain analyses.

The integrity of meta-analyses depends on identifying all relevant studies, whether published or not, and then obtaining data from each study. Publication bias can lead effect size to be overestimated (if neutral or negative studies are not included) or underestimated if positive data relating to unpatented or newly patented interventions are not published. In the case of NXY-059, it is unlikely that positive data were missed as AZ shared all data known to them, including that from four unpublished studies (of which two were neutral). Importantly, AZ encouraged publication when supplying NXY-059 to independent laboratories [A.R. Green, pers. comm.], so the failure to publish may lie with either investigators, or journal referees or editors. However, data from other neutral or negative studies may have been absent as publication bias was detected using standard statistical techniques (Egger *et al.*, 1997). Indeed, IAD from several studies were not made available by the authors (Lapchak *et al.*, 2002a,b; 2004) and the published data were not presented in a form suitable for inclusion in this meta-analysis. Whether these data could have contributed to the meta-analysis is unclear as it was

Table 8 Plasma concentration of NXY-059

	Species	*Model*	n	*Average weight (g)*	*NXY-059 dose, maintenance mg·kg^{-1} h*	*Administration (h)*	*Total (NXY-059) in plasma, mean (SD) mg·L^{-1}*	*SMD*
Marshall *et al.* (2001)	Marmoset (common)	Permanent	2	~12 months	16	24	41.6 (4.3)	–1.39
Marshall *et al.* (2003)	Marmoset (common)	Permanent	2	397	32	24	117.5 (7.0)	–0.76
Zhao *et al.* (2001)	Rat (SHR)	Permanent	10	269	0	24	0	–
		Permanent	9	275	30	24	30.6 (19.9)	–0.91
		Permanent	10	275	60	24	149.1 (78.9)	–1.29
Sydserff *et al.* (2002)	Rat (Wistar)	Permanent	2	313	50	24	122.4 (–)	–
		Transient	8	304	10	21.75	10.9 (3.2)	–0.64
		Transient	10	309	0	21.75	0	–
		Permanent	8	307	30	24	74.7 (37.8)	0.24
		Permanent	8	311	50	24	147.6 (56.1)	–1.09
		Permanent	8	311	70	24	171.6 (73.3)	–2.35
Zhao *et al.* (2002)	Rat (SHR)	Permanent	10	229	0	24	0	–
		Permanent	10	232	120	24	64.5 (30.8)	0.29
Wang and Shuaib (2004)[‡]	Rat (Wistar)	Thrombotic	4	383	65	4	100.7 (18.3)	–1.44
		Thrombotic	4	383	200	4	399.6 (78.6)	–0.77
Maples (1997)[†]	Rat (Sprague-Dawley)	†	4	–	10	672	23.5 (5.4)	†
		†	4	–	55	672	116.7 (23.0)	†
		†	4	–	300	672	502.0 (75.0)	†

Unbound fractions are calculated as a proportion of total concentration: marmosets 70%, rats 30%.
[†]Maples: safety study in non-stroked animals.
[‡]Wang studies are excluded from further plasma analyses as the measurement was carried out much earlier than the others.
NXY-059, disodium 2,4-disulphophenyl-N-tert-butylnitrone; SHR, spontaneously hypertensive rats; SMD, standardized mean differences.

based on a very different methodological approach, namely determining the dose of blood clot required to cause a particular lesion in the presence or absence of NXY-059. Nevertheless, it is possible that other unpublished studies exist, perhaps where NXY-059 was used as a 'positive control', as occurred in two included studies (Balogh *et al.*, 2005; Takizawa *et al.*, 2007) where one study was neutral and the other positive.

The validity of a meta-analysis is also dependent on the quality of included studies. Key factors in study quality include randomization, and blinding of the surgeon/experimenter and outcome assessor. Absence of these measures leads to selection, performance and observer bias respectively. In the case of NXY-059, there was no direct evidence that the absence of randomization and blinding led to biased results (Table 5), why studies involving pseudo-randomization were the only type to be positive is unclear, but critically there was no evidence for a gradient in efficiency from randomized to unrandomized. Nevertheless, the absence of any lesions in some animals given NXY-059 could reflect such experimental bias so that investigators were not 'blinded' to treatment assignment, either at the stage of surgery [so that occlusion of the middle cerebral artery (MCAo) might have been incomplete], or when outcomes were assessed. Exclusion of performance bias can be estimated experimentally by confirming that MCAo has occurred, either by direct visualization (Marshall *et al.*, 2001; 2003) or through assessment of blood flow using laser Doppler techniques, as done in three studies (Zhao *et al.*, 2001; 2002; Green, 2002).

Overall study quality can be summarized as a composite score encompassing randomization and blinding factors as well as others; previous meta-analyses have found that efficacy may only be present in low-quality studies (Crossley *et al.*, 2008). Importantly, the beneficial effects of NXY-059 on lesion volume were present across the range of study quality (Table 5). However, other forms of potential bias were present, including the exclusion from analysis of some enrolled animals ('attrition bias'), as occurred in one study (Sydserff *et al.*, 2002). Additionally, enrolled animals that died spontaneously or were culled for welfare reasons were not always included in analyses (Maples, 1996; Marshall *et al.*, 2001; 2003; Green, 2002; Sydserff *et al.*, 2002; Wang and Shuaib, 2004), thereby preventing analysis by intention-to-treat. This issue raises the question of whether all outcomes should include death, a practice that is standard in clinical trials, for example, with the modified Rankin Scale; in this respect, death is usually assigned a 'worst' value. A further deficit in all studies was the absence of a sample size calculation.

In summary, this meta-analysis of preclinical studies and based on data from individual animals finds that NXY-059 does have neuroprotectant properties; in particular, lesion volume, motor impairment and neglect were all reduced, although differential efficacy by sex and blood pressure status introduces some uncertainty into the robustness of these findings. The discrepancy between this conclusion and the overall neutral findings of large clinical trials (Diener *et al.*, 2008) needs explaining. Several possibilities exist, including: (i) the results of preclinical studies are simply not relevant to humans; (ii) effective concentrations of NXY-059 in rats and marmosets were not predictive of the required concentrations

needed in man; and (iii) NXY-059 has restricted access to brain tissue (Kuroda *et al.*, 1999; Green *et al.*, 2006). Nevertheless, the presence of several potential sources of bias in the preclinical work, especially performance, attrition and publication bias, is worrying, as is the absence of sample size calculations for most studies. Such deficits may have led to overestimation of the preclinical efficacy of NXY-059.

It has to be remembered that several of the NXY-059 preclinical studies are from the last decade with two antedating the STAIR criteria of 1999. As such, it might be harsh to judge these studies by today's standards. Nevertheless, a number of recommendations arise for the future conduct of preclinical stroke studies. First, they must embody the general principles by which clinical trials avoid bias, these resting on proper concealment of allocation (Schulz and Grimes, 2002), randomization, 'blinding' of the surgeon and outcome assessor to treatment assignment. Drugs should always be compared with a matching placebo, and the investigators should remain ignorant of treatment assignment until all experiments have been completed, outcomes assessed and the database locked. Studies should be designed on the basis of sample size calculations, so that they are of a sufficient size to exclude false negative findings at the 80–90% level. Occlusion of cerebral arteries should be confirmed visually or using approaches such as laser doppler. Journal editors and reviewers need to ensure that information on the above factors is always given in publications. The type and design of studies also needs to be reviewed. For example, the majority of NXY-059 experiments (18 of 34; 282 of 585 animals) tested short delays (5–30 min) between onset of ischaemia and treatment, a situation that does not mirror human stroke. Future developments should focus on studies with longer delays to treatment. Finally, sufficient numbers of female and older animals, and those with co-morbidites (such as hypertension) should be studied.

Following the initial submission of this paper, a meta-analysis of the quality of the preclinical NXY-059 data was published (Macleod *et al.*, 2008). While several of the conclusions were similar to those published here, there are several major differences in the two studies. First, as pointed out earlier, this is the first study performed using IAD; the Macleod study used only measures of outcome lesion results from published studies. The Macleod *et al.* (2008) study was primarily concerned with study quality and its relationship to the outcome measures. No attempt was made to analyse neuroprotection data on the basis of time of drug administration, type of ischaemia model or dose administered. Furthermore, only the current investigation analysed both published and crucially the unpublished studies, both positive and neutral. While Macleod *et al.* (2008) assessed the 'quality score' of the study, their decision to not contact the authors for information, but rather relying on the publication, sometimes led to errors in the score given (although we would concur that all experimental details should always be included in publications, however obvious the investigators may deem them to be). For example, the Marshall *et al.* (2001; 2003) studies are suggested to be unblinded whereas these were fully blinded for both allocation and outcome measures. Surprisingly, there are also other errors in the quality scores given by Macleod *et al.* (2008). The Marshall *et al.* (2001; 2003) studies are marked down for not stating that there was temperature control of the animals, when this was clearly stated to have taken place in those publications, and points were awarded for blinded induction of ischaemia in the Yoshimoto *et al.* (2002a) study when there is no published evidence that this procedure was followed. These errors weaken the accuracy of the graphs presented by Macleod *et al.* (2008) that relate study quality to size of neuroprotection. Interestingly, we failed to find a close association between some aspects of quality, such as blinding and outcome measures.

In conclusion, we suggest that at the completion of the preclinical studies, a formal systematic review with quantitative meta-analysis (ideally using IAD) of all known studies should be performed to assess the overall effect of treatment and potential modifying factors. This meta-analysis should inform the decision to proceed to clinical studies. Researchers should be willing to share data with such meta-analyses, especially as most source studies will have been funded from either the developing pharmaceutical company or from public or charity sources (of note, the NXY-059 studies where data were not shared were funded by several organizations, including the US National Institutes of Health/National Institutes of Neurological Disorders and Stroke, US Christopher Reeve Paralysis Foundation, and US Veterans Affairs). In summary, the comprehensive reporting of results, sharing of data and a continuous improvement of experimental standards are essential to reduce the risk of having further neutral clinical trials.

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Conflicts of interest

A.R.G. was formerly employed by AZ and was the preclinical leader in the NXY-059 development team. P.M.W.B. chaired the Data Monitoring Committee of one of the phase II trials of NXY-059 (Lees *et al.*, 2003) and served on the Data Monitoring Committees of the SAINT I, II and CHANT trials.(Lees *et al.*, 2006; Lyden *et al.*, 2007; Shuaib *et al.*, 2007). L.J.G. is funded, in part, by the Medical Research Council (G0501797, G0501997). P.M.W.B. is Stroke Association Professor of Stroke Medicine.

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