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Systematic Review and Meta-Analysis of the Efficacy of Tirilazad in Experimental Stroke

Emily Sena, BSc; Philippa Wheble, BSc; Peter Sandercock, MD; Malcolm Macleod, PhD

Background and Purpose—Tirilazad is a candidate neuroprotective drug with reported efficacy in animal models of stroke that was, however, without benefit in clinical trials. This apparent contradiction might be explained if the animal studies were falsely positive, if the clinical trials were falsely negative, or if tirilazad was not tested under the same conditions in animal and clinical studies. Here we use systematic review and meta-analysis to describe the characteristics and limits to the neuroprotective action of tirilazad in animal models of stroke.

Methods—Systematic review and meta-analysis of studies describing the efficacy of tirilazad in animal models of focal ischemia, in which outcome was measured as infarct volume and/or neurological score. Weighted mean difference random effects meta-analysis was used to measure overall efficacy in prespecified subgroups.

Results—Eighteen studies describing outcome in 544 animals were identified. Study quality (median score, 5/10; interquartile range, 4 to 6) was similar to that seen in systematic reviews of other candidate neuroprotective drugs. Tirilazad reduced infarct volume by 29.2% (95% confidence interval 21.1% to 37.2%) and improved neurobehavioral score by 48.1% (95% confidence interval 29.3% to 66.9%).

Conclusion—Tirilazad may have substantial efficacy in animal models of stroke, but this conclusion must be qualified because of the presence of potential sources of bias. (*Stroke*. 2007;38:000-000.)

Key Words: meta-analysis ■ neuroprotection ■ stroke ■ systematic review ■ tirilazad

At least 883 candidate thrombolytic and neuroprotective drugs have been tested in animal models of stroke¹ and show at least some evidence for efficacy; 97 of these drugs have been tested in human ischemic stroke. To date, there is unequivocal evidence for efficacy for only 2 drugs, aspirin² and tPa.³ Clinical trials of NXY-059⁴ are ongoing.

Explaining the discrepancy between efficacy in animal studies and lack of efficacy in clinical trials might lead to important insights and guide the future design of studies of both animal and human strokes.⁵ There are a number of potential reasons for such differences, including systematic errors in the ways in which data from promising animal studies are used to inform the design of clinical trials. Specifically, the animal data may be falsely positive, reporting a protective effect in which no biological efficacy exists; or the clinical trials may be falsely neutral, reporting no effect when clinical efficacy does in fact exist.

Systematic review of all the available animal evidence reduces selection bias and random error in the assessment of drug efficacy. Useful information can still be extracted even when identified animal studies are too heterogeneous (because of differences in dose, time, species) to provide a reliable estimate of “average” efficacy. Stratified meta-analysis (in which studies are analyzed by dose or time or species) can describe dose-response relationships, time dependence of

efficacy, and the impact of study quality and study design characteristics.

These techniques have proved useful in the analysis of data from clinical trials and have been advocated for the analysis of data from animal experiments.^{5,6} Animal models of stroke lend themselves to this approach, as evidenced by the publication of a growing number of such analyses in recent years.⁷⁻¹³

During ischemia and reperfusion, free radicals play an important role in inducing cerebral injury through effects on DNA, on mitochondria, and through the effects of lipid peroxidation.¹⁴ Tirilazad is a synthetic lipid-soluble 21-aminosteroid with antioxidant effects that is proposed to interact with the lipid peroxidation cascade at various stages including: (1) scavenging of hydroxyl and lipid peroxyl radicals; (2) maintenance of endogenous antioxidant levels; and (3) prevention of propagation of lipid peroxidation by membrane stabilization.¹⁵

Experimental animal data had suggested tirilazad as a treatment for ischemic stroke, but it was subsequently demonstrated to increase death and dependency in a meta-analysis of clinical studies.¹⁶ Here we present a systematic review and meta-analysis of the efficacy of tirilazad in experimental stroke. We have set out to establish the quality of the animal studies; the limits (dose response, time dependence) to

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TABLE 1. Study Characteristics

Author	Year	Drug	Species	Sex	N (C)	N (Rx)	Dose Range	Time of Admin, min	Anaesthetic	Type of Ischemia	Route of Delivery	Outcome Measure(s)
Alessandri	2000	Tirilazad	Rat	Male	6	6	29 mg/kg	15	Halothane	Permanent	IV	Infarct Volume
Beck	1991	Tirilazad	Rat	Unknown	12	10	4–40 mg/kg	–30	Halothane	Permanent	IP	Infarct Volume
Gross	1997	Tirilazad	Rabbit	Both	8	8	3 mg/kg	210	Ketamine	Thrombotic	IV	Infarct Volume
Hellström	1994	Tirilazad	Rat	Male	8	10	6 mg/kg	10	Halothane	Permanent	IV	Infarct Volume Neurological Score
Lythgoe	1990	Tirilazad	Rat	Male	7	7	26 mg/kg	10	Pentobarbital	Permanent	IV	Infarct Volume
Öktem	2000	Tirilazad	Rabbit	Unknown	6	6	6 mg/kg	15	Ketamine	Permanent	IV	Infarct Volume Neurological Score
Orozco	1995	Tirilazad	Rabbit	Unknown	10	10	3 mg/kg	120	Acepromazine	Thrombotic		Infarct Volume
Park	1994	Tirilazad	Rat	Male	7	7	1.89–18.9 mg/kg	15	Halothane	Permanent	IV	Infarct Volume Neurological Score
Schmid-Elaesser	1998	Tirilazad	Rat	Male	10	10	6 mg/kg	–15	Halothane	Reversible	IV	Infarct Volume
Schmid-Elaesser	1999b	Tirilazad	Rat	Male	10	10	6 mg/kg	–20	Halothane	Reversible	IV	Infarct Volume Neurological Score
Schmid-Elaesser	1999a	Tirilazad	Rat	Male	10	10	6 mg/kg	–15	Halothane	Reversible	IV	Infarct Volume Neurological Score
Schöller	2004	Tirilazad	Rat	Male	12	17	6 mg/kg	–30	Halothane	Permanent	IV	Infarct Volume Neurological Score
Takehima	1993	Tirilazad	Cat	Female	7	9	1.5 mg/kg	0–70	Halothane	Reversible	IV	Infarct Volume
Umemura	1994	Tirilazad	Rat	Male	5	5	1 mg/kg	4	Pentobarbital	Thrombotic	IV	Infarct Volume
Wilson	1992	Tirilazad	Rabbit	Both	10	8	6 mg/kg	–30	Ketamine	Thrombotic	IV	Infarct Volume
Xue	1991	Tirilazad	Rat	Male	19	19	20–30 mg/kg	360	Halothane	Reversible	IP	Infarct Volume
Zausinger	2003a	Tirilazad	Rat	Male	10	10	6 mg/kg	–20	Halothane	Reversible	IV	Infarct Volume Neurological Score
Zausinger	2003b	Tirilazad	Rat	Male	12	10	6 mg/kg	0–300	Halothane	Reversible	IV	Infarct Volume Neurological Score

IP indicates intraperitoneal; IV, intravenous.

TABLE 2. Study Quality Score

Author	Year	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	Quality Score
Alessandri	2000	X	X				X	X		X		5
Beck	1991	X	X				X					3
Gross	1997	X	X							X		3
Hellström	1994	X	X	X		X	X			X		6
Lythgoe	1990						X					1
Öktem	2000	X	X	X		X				X		5
Orozco	1995	X	X	X			X			X		5
Park	1994	X	X	X	X	X	X					6
Schmid-Elaesser	1998	X	X	X		X	X			X		6
Schmid-Elaesser	1999b	X	X	X		X	X			X		6
Schmid-Elaesser	1999a	X	X	X		X	X			X		6
Schöller	2004	X	X	X		X	X			X		6
Takeshima	1993	X	X	X		X	X					5
Umemura	1994	X	X	X		X	X					5
Wilson	1992	X	X	X		X						4
Xue	1991	X	X			X	X					5
Zausinger	2003a	X	X			X	X			X		5
Zausinger	2003b	X	X	X		X	X			X		6

efficacy; the impact of study characteristics on efficacy; and to establish the fidelity with which clinical trial design reflected the conditions under which efficacy was seen in animals.

Methods

Identification of Relevant Studies

We used: (1) electronic search of Pubmed, EMBASE, BIOSIS using search terms tirilazad OR U-74006F OR Freedox OR 21-aminosteroid AND stroke OR ischemia OR cerebrovascular OR middle cerebral artery AND Animals (Mesh:noexp) OR MCA AND Animals (Mesh:noexp) OR ACA AND Animals (Mesh:noexp) OR anterior cerebral artery AND Animals (Mesh:noexp) OR MCAO AND Animals (Mesh:noexp) AND Animals (Mesh:noexp) NOT coronary OR myocardia; (2) hand searching of abstracts of 3rd to 5th World Stroke Congresses, European Stroke Conference (from 2nd meeting/1992 onwards), International Stroke Conference (from 25th meeting/2000 onwards; previously in BIOSIS), conferences of the International Society for Cerebral Blood and Metabolism (from 16th meeting/1993 onwards); and (3) requests to authors of publications identified above for other published or unpublished data.

Criteria for Inclusion

Two investigators (E.S., P.W.) independently extracted those publications identified here that described controlled studies of tirilazad given in models (whole live animals excluding humans) of focal cerebral ischemia induced by occlusion of the middle or anterior cerebral artery or their branches, where tirilazad was administered by any route and outcome compared with animals receiving placebo or no tirilazad. Disagreements were resolved in discussion with a third investigator (M.M.).

End Points Considered

The primary outcome measure was infarct area or volume (determined histologically or by cross-sectional imaging), with secondary outcome measures of death and of neurobehavioral score.

Methods of the Review

Quality Assessment

There was no quality threshold for inclusion. Study quality was assessed against our published ten item checklist⁹ comprising: (1)

publication in peer reviewed journal; (2) statement of control of temperature; (3) randomization to treatment or control; (4) blinded induction of ischemia; (5) blinded assessment of outcome; (6) avoidance of anesthetics with marked intrinsic neuroprotective properties; (7) use of animals with hypertension or diabetes; (8) sample size calculation; (9) statement of compliance with regulatory requirements; and (10) statement regarding possible conflicts of interest.

Data Extraction

From each source we identified individual comparisons in which outcome was measured in a group of animals receiving a specified dose(s) of drug at a specified time(s) and compared with outcome in a control group. When the treatment group received more than one intervention, this was recorded. For each comparison and for each of treatment and control group, we extracted data for number per group, mean outcome, and its standard deviation. When an outcome was measured serially, only the last measure was used. When data were given graphically, we contacted authors seeking data; when this was not available we estimated values by measurement from publications. Data were extracted onto a data extraction form by 2 reviewers independently, and differences were resolved by discussion.

We also collected other relevant data including anesthetic used, time of outcome measurement, and method of induction of ischemia, as well as the individual component items of the quality checklist.

Analysis

For continuous variables (infarct volume, neurobehavioral score), we calculated an overall weighted mean difference with a random effects model. When a single control group served multiple treatment groups, the size of the control group entered to the meta-analysis was adjusted by division by the number of treatment groups served. Meta-analyses were conducted stratified according to: drug dose; time of administration; study quality; components of study quality checklist; presence of cotreatments; duration of ischemia; method of induction of ischemia; anesthetic used; use of mechanical ventilation; species and gender of animal used; outcome measure used; and interval to quantification of outcome. The significance of differences between n groups was assessed by partitioning heterogeneity and by using the χ^2 distribution with $n-1$ degrees of freedom (df). To allow for multiple comparisons we set our significance level at $P<0.001$. Publication bias was assessed with a funnel plot and with the Egger method.⁷

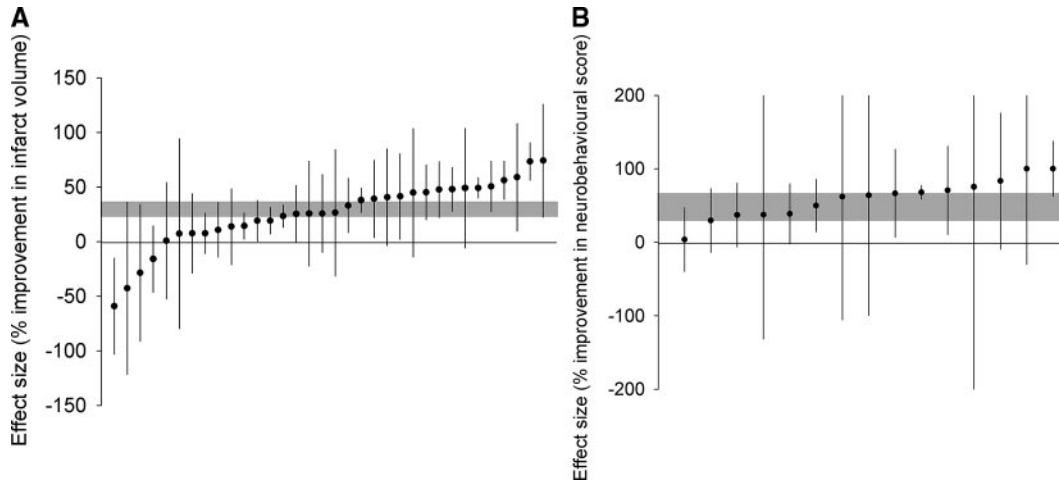


Figure 1. Individual comparison ranked according to their effect on (a) infarct volume and (b) neurobehavioral score. The shaded gray bar represents the 95% confidence limits of the global estimate. The vertical error bars represent the 95% confidence intervals for the individual estimates.

Results

Electronic searching identified 19 publications describing the effect of tirilazad in focal cerebral ischemia; hand-searching did not identify any further relevant data. One of the identified studies reported infarct volume as median and interquartile range, but the author was unable to provide us with the raw data. This analysis is therefore based on 18 publications, 17 full articles,^{18–34} and 1 abstract³⁵ (Table 1). Within these 18 studies, 34 comparisons were identified describing outcome in 544 animals. All 18 publications reported infarct volume, 8 also reported a neurobehavioral outcome (242 animals), and 1 reported infarct volume, neurobehavioral score, and mortality (24 animals).

Study Quality and Publication Bias

No study described a sample size calculation or contained a statement of potential conflict of interest, although 2 of the 18 publications were directly funded by and a further 2 studies describe some form of assistance from the manufacturers of

tirilazad. The median quality score was 5 (interquartile range, 4 to 6), and whereas this compares favorably with similar reviews for other candidate neuroprotective drugs, this still represents an important potential source of bias (Table 2).

Efficacy

Tirilazad reduced infarct volume by 29.2% (95% confidence interval, 21.1% to 37.2%; 34 comparisons; Figure 1a), and improved neurobehavioral score by 48.1% (95% confidence interval, 29.3% to 66.9%; 16 comparisons; Figure 1b). There was substantial heterogeneity for both outcome measures (infarct volume, $\chi^2=120$, $df=33$, $P<10^{-12}$; neurobehavioral score, $\chi^2=35.8$, $df=15$, $P=0.002$). Only one study reported mortality data and was not analyzed further.

Data for infarct volume were used for prespecified stratified meta-analyses. There was a narrow window of therapeutic effect, with doses between 3 and 9.9 mg/kg giving maximum efficacy and doses only slightly lower (1 to 2.9 mg/kg) or higher (10 to 29 mg/kg) being only half as effective

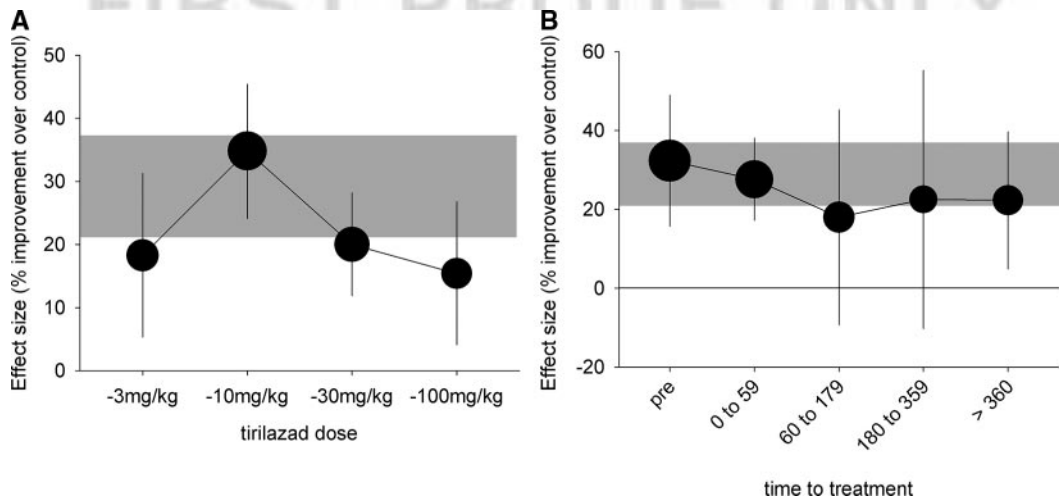


Figure 2. Effect of (a) tirilazad dose and (b) delay to treatment on the estimate of efficacy. The shaded gray bar represents the 95% confidence limits of the global estimate. The vertical error bars represent the 95% confidence intervals for the individual estimates. The size of each point reflects the log of the number of animals contributing to that comparison. Stratification by dose, but not by time to treatment, accounts for a significant proportion of the heterogeneity observed between studies ($P<0.001$).

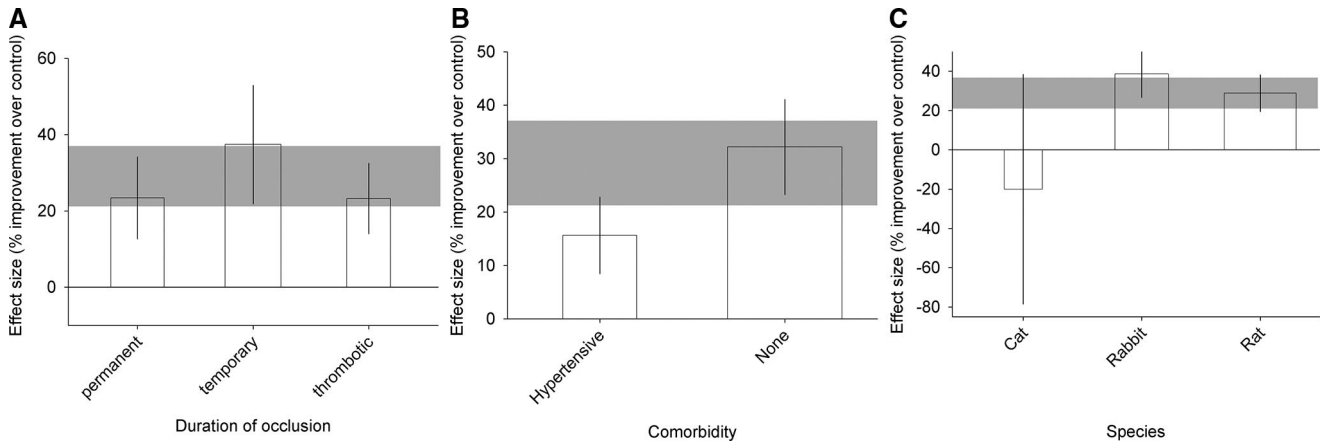


Figure 3. Effect of (a) duration of occlusion, (b) comorbidity, and (c) species on the estimate of efficacy. The shaded gray bar represents the 95% confidence limits of the global estimate. The vertical error bars represent the 95% confidence intervals for the individual estimates. The width of each vertical bar reflects the log of the number of animals contributing to that comparison. Each stratification accounts for a significant proportion of the heterogeneity observed between studies ($P < 0.001$).

($P < 0.001$; Figure 2a). Although there was no significant relationship between delay to treatment and efficacy it is interesting to note that maximum efficacy was seen when treatment was given before the onset of ischemia, with a trend for efficacy to fall thereafter with time (Figure 2b). Although the longest interval between stroke onset and initiation of treatment was 6 hours, the median was only 10 minutes. There was no relationship between study quality and treatment effect. Efficacy was higher in temporary occlusion than in either permanent or thrombotic occlusion models ($\chi^2 = 16.5$, $df = 2$, $P < 0.001$; Figure 3a), and was lower in the presence of comorbidity (4 comparisons undertaken among spontaneously hypertensive rats; no other comorbidities were tested) than in healthy animals ($\chi^2 = 26.6$, $df = 1$, $P < 0.001$; Figure 3b). Tirilazad nonsignificantly increased infarct volume in cats, whereas it improved outcome in both rats and rabbits ($\chi^2 = 14.9$, $df = 2$, P for difference between species < 0.001 ; Figure 3c). There was no significant effect of the anesthetic used; the use of cotreatments; single or multiple dosages; the route of drug administration; the sex of animal used; the method of quantification of infarct volume; or the use of mechanical ventilation (not shown).

Discussion

Effects of Tirilazad in Animal Models

Tirilazad demonstrates substantial neuroprotective efficacy in data from 18 publications in animal models of stroke. However, this evidence must be interpreted with some caution. First, no study scored on > 6 of 10 items on our quality checklist. We have previously shown that low study quality is associated with higher estimates of efficacy,³⁵ and of particular concern are the use of ketamine anesthesia, unblinded assessment of outcome, and the small proportion of studies using animals with a comorbidity. Some of the efficacy of tirilazad may be caused by such bias. We did not find evidence for significant publication confounded by such biases.

Lack of Concordance With Human Studies

This review was conducted in the context of a project examining concordance between animal and human studies

for six interventions. Clinical trials in ischemic stroke showed tirilazad increased death and disability.¹⁶ While one explanation for this lack of concordance might indeed be that the animal experiments were falsely positive as described, comparison with tPA, a drug with efficacy in both animal and human strokes, suggests 2 further plausible explanations. First, the animal data suggest that tirilazad is effective over a narrow dose range (3 to 10 mg/kg). Other aspects of study design may confound this finding, and a single experiment of adequate power would be required to confirm it; however, these data are consistent with the narrow effective dose range reported for another free radical scavenger, PEG-SOD.³⁶ A narrow dose range for tirilazad would contrast with tPA, for which efficacy in animals was seen across a broad range of doses (Sena E and Macleod M, unpublished observations, 2006). Clinical trials of tirilazad used a broad range of doses (0.15 to 15 mg/kg), with no difference in outcome when patients were dichotomized to those receiving more than or less than 6 mg/kg/d; if the narrow effective dose range suggested by our analysis also exists in humans, this might explain why the clinical studies were negative.

Second, the interval between stroke onset and the initiation of treatment was substantially longer in clinical studies (median, ≈ 5 hours) than in the animal studies (median, 10 minutes). This contrasts with tPA, for which the median delay to treatment in the animal studies, 90 minutes, is broadly similar to the time window in which clinical efficacy has been shown (within 180 minutes).

Because of concerns that findings in small laboratory animals may not generalize to much larger human brains, the STAIR criteria includes the demonstration of efficacy in larger animals. The only gyrencephalic species in which tirilazad was tested was in cats, in which no efficacy was seen. Whereas this may reflect lack of efficacy in larger animals, this was also the only experiment using female animals; and partitioning heterogeneity by sex was of borderline significance ($P = 0.006$). Establishing the source of this lack of efficacy would require direct head-to-head comparison.

Robustness of These Conclusions

Although we prespecified our choice of stratification variables and set a very stringent significance level, some of our results may have been attributable to the play of chance; therefore, our observations should be interpreted with caution. This meta-analysis has other weaknesses. First, meta-analysis can only include available data, and publication bias may result in our analyses overestimating the efficacy of tirilazad. Furthermore, although we consider that our search strategy is likely to have ascertained most of the relevant publications, it has yet to be validated.

We elected to use weighted mean difference meta-analysis. We consider that standardized mean difference meta-analysis, although appropriate for clinical studies that individually have large numbers of participants, is less suited to animal studies in which the number of subjects is substantially lower. This is because the observed (sample) standard deviation used in standardization will be a poorer estimate of the population standard deviation when sample size is smaller. However, direct comparisons of the strengths and weaknesses of each statistical approach in this context have not yet been performed.

Third, our analysis is observational and should only be considered as hypothesis-generating. There were insufficient data to allow multiple linear regression analysis that could help disentangle the impact of different factors. However, by pooling such data from reviews of different neuroprotectants, it should be possible to identify those aspects of study quality for instance most closely associated with overstatement of efficacy. This work is underway.

This post-hoc approach allows potential sources of bias to be taken into account in the interpretation of published evidence for efficacy for candidate neuroprotective drugs. However, we believe that a better solution would be for the design, conduct, and reporting of animal studies to be refined to minimize the impact of these sources of bias.

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Disclosures

None.

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