Title Page

Title: Circadian Rhythms in the Efficacy of Intravenous Alteplase in Patients with Acute Ischemic Stroke and Middle Cerebral Artery Occlusion

Running Title: Circadian Rhythms in the Efficacy of Thrombolysis in Stroke

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Abstract

Circadian rhythm interactions of haemostatic factors can modify tissue plasminogen activator (tPA) effects. We assess the relationship of the timeframe of intravenous tPA administration with the outcome of patients with acute ischemic stroke (AIS). We studied 135 consecutive patients with AIS and transcranial Duplex documented middle cerebral artery (MCA) occlusion treated with intravenous tPA. Complete recanalization was defined as total improvement on Thrombolysis in Brain Ischemia (TIBI) grades 2-hours after tPA infusion. Clinical response was evaluated by the modified Rankin scale at 90 days. We determined plasminogen activator inhibitor-1 (PAI-1) levels in 33 patients with available plasma samples before treatment. Our results are: Ninety-two (68.1%) patients were treated in the diurnal (9am-9pm) and forty-three (31.8%) in the nocturnal period (9pm-9am). Complete recanalization was recorded in 52/135 (38.5%) patients. Both the rate of complete recanalization (45.6% versus 23.2%; p=.01) and good clinical outcome (64.1% versus 44.2%, p=.02) were significantly higher in the group of diurnal tPA administration compared to those treated in the nocturnal period. The adjusted OR of diurnal-tPA treatment for complete MCA recanalization was 2.37 (95%CI, 1.02-5.52; p= .045). Diurnal-tPA infusion significantly improved the overall distribution of scores on the modified Rankin scale, as compared with nocturnal treatment (OR, 2.07; 95%CI, 1.16 to 4.64 by ordinal regression analysis). Low PAI-1 levels were associated with complete recanalization but did not significantly differ between the two timeframes. As conclusions, diurnal administration of tPA is independently associated with early MCA recanalization and better functional outcome at 90 days in patients with AIS.

Key words: circadian rhythms, acute stroke, thrombolysis, tPA.

Background

Circadian rhythm has been described for the time of onset of acute vascular events, such as myocardial infarction, pulmonary embolism and stroke, with a major morning peak and secondary early evening peak (Casetta, Granieri et al. 2002; Manfredini, Boari et al. 2005). Some endogenous factors as sympathetic activity, blood pressure, blood coagulation and fibrinolysis may influence the clock time pattern of stroke onset (Stergiou, Vemmos et al. 2002).

Concerning the circadian rhythm of fibrinolysis, it is to a large extent determined by the interaction of the tissue plasminogen activator (t-PA) and its potent inhibitor, the tissue plasminogen inhibitor-1 (PAI-1) (Andreotti, Davies et al. 1988; Andreotti and Kluft 1991). PAI-1 activity shows the lowest levels during the day and, consequently, t-PA activity shows a peak concentration in the 9:00am to 9:00pm timeframe when thromboembolic events are more frequent (Andreotti and Kluft 1991).

Our aim was to assess the relationship of the circadian rhythms with arterial recanalization and outcome of patients with acute ischemic stroke and middle cerebral artery (MCA) occlusion treated with intravenous tPA.

Methods

From June 2003 to March 2011, 571 consecutive patients with acute ischemic stroke were admitted to our acute stroke unit and treated with reperfusion therapies. One hundred and fifty three out of 571 patients were excluded because they were treated with primary or rescue endovascular therapy; so 418 patients received intravenous alteplase (0.9 mg/Kg, 10 % in bolus, 1 hour infusion). Prior or immediately after the tPA bolus, a transcranial duplex (TCDx) was performed to evaluate the patency of cerebral arteries. TCDx monitoring was maintained during the one-hour infusion and repeated 2-hours after the infusion and between 24 and 72 hours. The flow pattern was graded according to the Thrombolysis in Brain Ischemia (TIBI) classification(Demchuk, Burgin et al. 2001) by experienced neurologists certified by the Spanish Neurological Society. TIBI grades 0-I and II-III were considered indicative of proximal and distal middle cerebral artery (MCA) occlusion. We excluded 63 patients because had no arterial occlusion, 40 cases with occlusions out of MCA (24 vertebrobasilar, 1 anterior cerebral artery, 1 posterior cerebral artery, 12 terminus internal carotid artery (ICA) and 2 tandem ICA-MCA occlusions), 6 patients due to an inappropriate temporal bone window, 64 patients because of non-available transcranial duplex during and after the tPA infusion or at two hours, and 110 patients that were treated beyond 4.5 hours or

in whom the time of the stroke onset was unknown. Therefore, 135 patients with MCA occlusion were eligible for this study. Patients were classified in two groups according to the time at which tPA was administered: diurnal group (from 9:00 am to 9:00 pm; n=92) and nocturnal group (from 9:00 pm to 9:00 am; n=43).

Complete recanalization was defined as a TIBI pattern IV or V at 2-hours after the tPA infusion. Reocclusion was evaluated at 24-72 hours as a worsening of 2 or more points in the TIBI grade. Baseline stroke severity was assessed by the National Institutes Health Stroke Scale score and clinical outcome at 3 months was evaluated by the modified Rankin Scale (mRS). PAI-1 levels were determined by enzyme-linked immunosorbent assay (AssayMax Human PAI-1 ELISA Kit, ASSAYPRO, St. Charles, MO, USA) in 33 patients with available plasma samples drawn at admission. Determinations were performed in an independent laboratory blind to clinical and neuroimaging data.

The information used in the study was retrospectively collected from the clinical protocols and databank approved by the Ethics board of our hospital; therefore patients signed no specific informed consent for this study.

Statistical Analysis

We tabulated the clinical characteristics of patients who showed or not early recanalization and good or poor clinical outcome categorized as a mRS ≤2 or >2. T-test or Mann-Whithney U test were used to test differences in continuous variables, and the chi-square test was used to compare proportions between groups. The independent relationship of the time frame of tPA administration with the distribution of the mRS scores was analyzed by shift analysis in order to preserve as much information as possible in the outcome. Shift analysis was performed through ordinal logistic cumulative regression fitted with function *polr* in R package MASS. We studied the underlying assumption of equal relationship for different cutpoints with the help of forest plot (result not shown), that suggested pooling together mRS values of 0, 1 and 2. In order to avoid sparse data, continuous predictors were divided in quartiles and treated as numerical in the model. Similar results were obtained when we treated them as continuous. Odds ratios were adjusted for age, baseline NIHSS, and serum glucose. The association of the time frame of tPA administration with early recanalization was analyzed by binary logistic regression analysis controlling for covariates related in the univariate analyses.

Results

A total of 92 patients were treated in the diurnal and 43 in the nocturnal timeframe. These two groups showed similar baseline characteristics, except for the presence of atrial fibrillation, which was more frequent in the nocturnal group (*see appendix 1*).

Complete recanalization 2-hours after tPA infusion was recorded in 52/135 (38.5%) patients and good functional outcome at 3 months in 78/135 (57.8%). Complete recanalization was significantly associated with good outcome (78.8% versus 44.6%, p<.001). Both the rate of complete recanalization (45.6% versus 23.2%; p=.01) and good clinical outcome (64.1% versus 44.2%, p=.02) were significantly higher in the group of patients with diurnal tPA administration compared to those treated in the nocturnal period. Other prognostic variables of poor clinical outcome were age, atrial fibrillation, stroke severity and serum glucose. No other factors were associated with early recanalization (**Table 1**).. Reocclusion was found in 3 patients of the diurnal group.

Figure 1 shows the distribution of modified Rankin Scale scores at three months by the tPA administration time frame. The raw odds of diurnal tPA infusion with the overall distribution of scores on mRS obtained through ordinal regression was 2.07 (95%CI, 1.16 to 4.64). **Figure 2** shows very similar results after adjustment for age, NIHSS and serum glucose -although the later slightly lost statistical significance. Day-tPA treatment was also associated with arterial recanalization (OR, 2.37 95%CI, 1.02-5.52) in binary logistic regression after adjustment for covariates. In sensitivity analysis we found that day-tPA treatment was also associated with favorable distribution of mRS scores (crude OR, 1.86 95%CI, 1.06-3.25) when patients with absence of bone temporal window or lack of TCDx examination during tPA infusion or at 2 hours (n=70) were also included in the ordinal regression model.

PAI-1 levels on admission were determined in 33 patients, 20 patients treated in the diurnal period and 13 in the nocturnal period. PAI-1 levels were significantly lower in patients with subsequent early complete recanalization than in those without (mean [SD], 16.0 [3.8] versus 20.9 [6.0] mg/dL; p=.038). However, PAI-1 levels did not significantly differ between the two timeframes (17.8 [4.4] vs 21.1 [7.3] mg/dL; p=NS).

Discussion

This study shows that the diurnal administration (from 9am to 9pm) of intravenous tPA in patients with an acute ischemic stroke within the first 4.5 hours from symptoms onset is independently associated with MCA complete recanalization and favorable distribution of scores on the modified Rankin scale, as compared with nocturnal treatment. Although discordant p-values over the .05 cutpoint may suggest different conclusions for the raw and the adjusted estimators of the relationship between time frame and outcome, the estimated OR (figure 2) showed concordant results for most of the analysis, suggesting that patient evolution after tPA may differ depending upon time-frame. Delayed reocclusion in a few patients could also prevent a stronger clinical effect. These findings are reinforced by the positive results also found in the sensitivity analysis including those patients without TCDx monitoring study.

Several reports have highlighted the importance of circadian rhythms in the efficacy of thrombolysis (Kurnik 1995; Kono, Morita et al. 1996; Casetta, Granieri et al. 2002; Stergiou, Vemmos et al. 2002) Resistance to thrombolysis in the early morning has been associated with the elevation of plasminogen activator inhibitor-1 whereas the underlying mechanisms for late evening resistance peaks are not clear. Since this resistance is independent of the type of thrombolytic agents (urokinase or t-PA), other mechanisms apart of PAI-1 such as circadian variations of platelet activity (Tofler, Brezinski et al. 1987; Haus, Cusulos et al. 1990) and diurnal variation in thrombus composition might be involved in the efficacy of the thrombolytic treatment. Despite we found a relationship between lower PAI-1 levels and early recanalization, a PAI-1 circadian rhythm was not found. This lack of association could be explained in part by the small sample size of patients with PAI-1 determinations. Then, the mechanism of the resistance to thrombolysis was not clearly elucidated in the present study.

Patients treated with IV tPA during day hours (8am-6pm) did not show better clinical outcome in a large multicenter registry, but stroke severity and the rate of MCA occlusions were not reported (Jauss, Schutz et al. 2010). The present study has included a large homogeneous sample of IV t-PA treated patients within 4.5 hours from symptoms onset with MCA occlusion studied with sequential transcranial Duplex,

but is limited by its retrospective design. However our findings remained significant after controlling for potential confounders and may be generalized since we found similar results when patients in whom TCDx was not performed were included in the analysis. Quality standards of treatment such as time from stroke onset to tPA infusion, rate of symptomatic ICH, and protocol violations were similar in the two time frames (Appendix 1), as it was the neurologic team that assisted patients during diurnal and nocturnal periods. So, we think that the association of diurnal tPA administration and favorable response

was not confounded by a distinct technical expertise between the two time frames.

If other studies replicate our findings, they may have potential therapeutic implications based on the time interval at which stroke symptoms start. Further research should clarify whether the dose of thrombolytic agents should be adjusted on the basis of the time of stroke onset, the putative advantages of lytic agents without a circadian pattern of efficacy, and the need of more aggressive reperfusion therapies in the nocturnal time frame.

In summary, diurnal administration of tPA was independently associated with early recanalization and better functional outcome at 90 days in patients with AIS and MCA occlusion. Despite we found a relationship between lower PAI-1 levels and complete recanalization, a PAI-1 circadian rhythm was not observed.

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Disclosures:

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The authors report no conflicts of interest. All the co-authors have seen and approved the final version of the manuscript. All co-authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 1. Baseline characteristics by early MCA recanalization and good functional outcome.

	COMPLETE RECANALIZATION			FUNCTIONAL OUTCOME		
	YES (n=52)	NO (n=83)	*p*	GOOD (n=78)	POOR (n=57)	*p*
Age, mean±SD	68.23±11.3	68.77±12.9	.80	66.8±12.7	70.96±11.29	.05
Men (%)	34 (65.4)	48 (57.8)	.24	48 (61.5)	34 (59.6)	.48
Tobacco (%)	21 (40.4)	28 (33.7)	.27	32 (41)	17 (29.8)	.12
Hypertension (%)	27 (51.9)	50 (60.2)	.22	42 (53.8)	35 (61.4)	.24
Diabetes Mellitus (%)	11 (21.2)	19 (22.9)	.49	17 (21.8)	13 (22.8)	.52
Dyslipimia (%)	20 (38.5)	44 (53.7)	.06	38 (48.7)	26 (46.4)	.46
Peripheral arteriopathy (%)	7 (13.5)	6 (7.2)	.18	7 (9)	6 (10.5)	.49
Atrial fibrillation (%)	14 (26.9)	32 (38.6)	.11	21 (26.9)	25 (43.9)	.03
Ischemic heart disease (%)	7 (13.5)	15 (18.1)	.32	13(16.7)	9 (15.8)	.54
Duplex pattern, TIBI 0,1, (%)	17 (32.7)	31 (37.3)	.35	25 (52.1)	23 (47.9)	.36
Early signs in CT	16 (33.3)	25 (31.3)	.47	21 (29.2)	20 (35.7)	.27
Systolic blood pressure, mmHg	147.6±21.2	146.2±22.48	.70	145.41±22.54	148.65±21.17	.39
Diastolic blood pressure, mmHg	73.3±13.9	77.2±12.2	.77	74.63±12.82	77.04±13.31	.29
Fibrinogen, mg/dl	453.26±105.2	464.42±163.1	.66	449.79±147.45	474.17±136.39	.33
Glycemia, mg/dl	116.9±32.9	132.8±44.5	.06	118.4±37.3	138±43.4	.006
Time from onset, min	147.7±51.6	148.9±51.3	.89	148.15±48.72	148.96±54.94	.92
NIHSS (median [min-max]	14 [2-22]	11[2-23]	.16	10 [2-22]	15 [2-23]	.001

Values represent mean ±SD, n (%) and median [IQR]. MCA: middle cerebral artery; CT: computed tomography; TIBI: thrombolysis in brain infarction score

Legends.-

Figure 1.- Distribution of modified Rankin Scale scores at three months by the tPA administration time frame.

Figure 2.- Forest plot showing unadjusted and adjusted odds ratios of the relationship between time-frame of tPA administration and favorable shift of the mRS scores.

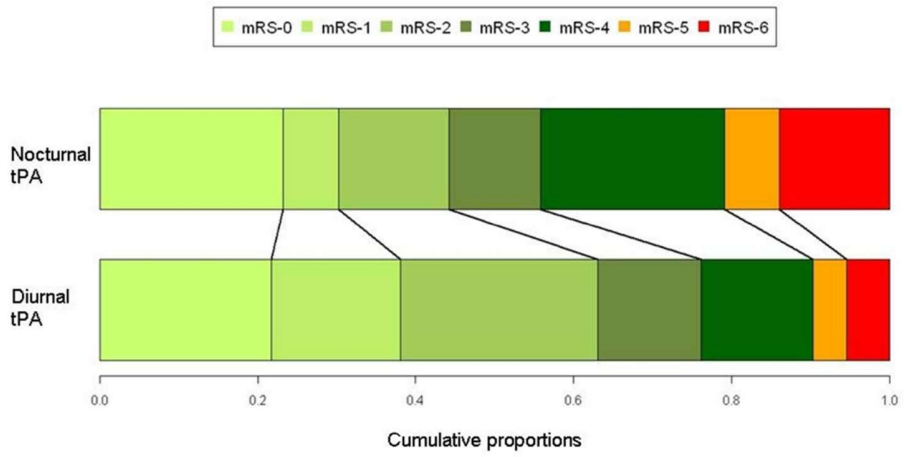

Figure 1.- Distribution of modified Rankin Scale scores at three months by the tPA administration time frame.
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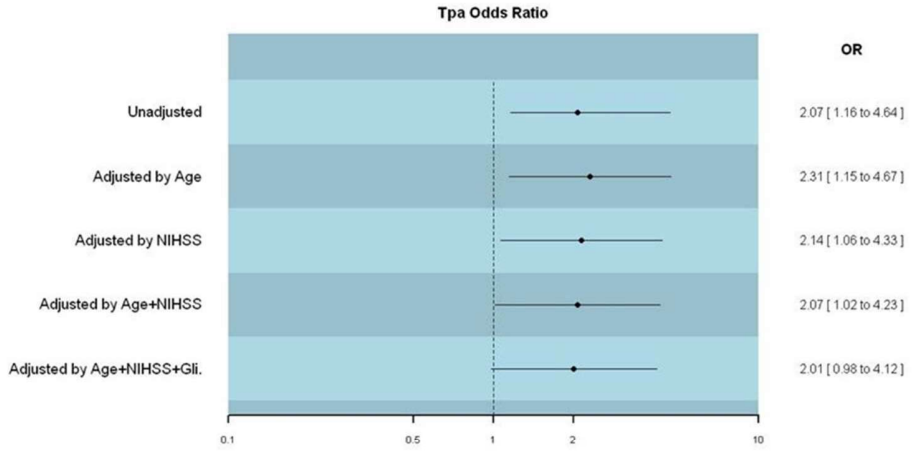

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