Published in final edited form as:

Eur J Pharmacol. 2014 September 5; 738: 368–373. doi:10.1016/j.ejphar.2014.05.052.

Combination treatment of r- tPA and an optimized human apyrase reduces mortality rate and hemorrhagic transformation 6h after ischemic stroke in aged female rats

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Abstract

Recombinant tissue plasminogen activator (r-tPA) is the only FDA-approved drug treatment for ischemic stroke and must be used within 4.5 hours. Thrombolytic treatment with r-tPA has deleterious effects on the neurovascular unit that substantially increases the risk of intracerebral hemorrhage if administered too late. These therapeutic shortcomings necessitate additional investigation into agents that can extend the therapeutic window for safe use of thrombolytics. In this study, combination of r-tPA and APT102, a novel form of human apyrase/ADPase, was investigated in a clinically-relevant aged-female rat embolic ischemic stroke model. We propose that successfully extending the therapeutic window of r-tPA administration would represent a significant advance in the treatment of ischemic stroke due to a significant increase in the number of patients eligible for treatment. Results of our study showed significantly reduced mortality from 47% with r-tPA alone to 16% with co-administration of APT102 and r-tPA. Co-administration decreased cortical (47±5% vs 29±5%), striatal (50±2%, vs 40±3%) and total (48±3% vs 33±4%) hemispheric infarct volume compared to r-tPA alone. APT102 improved neurological outcome (8.9±0.6, vs 6.8±0.8) and decreased hemoglobin extravasation in cortical tissue (1.9±0.1 mg/dl vs 1.4±0.1 mg/dl) striatal tissue (2.1±0.3 mg/dl vs 1.4±0.1 mg/dl) and whole brain tissue (2.0±0.2 mg/dl vs 1.4±0.1 mg/dl). These data suggest that APT102 can safely extend the therapeutic window for r-tPA mediated reperfusion to 6 h following experimental stroke without increased

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Competing interests: R.C. is the founder of APT Therapeutics, Inc. and the shareholder of the company.

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hemorrhagic transformation. APT102 offers to be a viable adjunct therapeutic option to increase the number of clinical patients eligible for thrombolytic treatment after ischemic stroke.

Keywords

Acute ischemic stroke; APT102/ apyrase; hemorrhagic transformation; infarction size; recombinant tissue plasminogen activator

1. Introduction

Recombinant tissue plasminogen activator (r-tPA) is the only FDA approved drug for treatment of focal ischemic stroke. Unfortunately, less than 5% of people suffering an ischemic stroke receive r-tPA due to increased risk of secondary cerebral hemorrhage and edema formation. Thus, an unmet clinical need exists to develop novel therapeutics that work in combination with r-tPA to improve stroke outcome, reduce secondary complications, and extend the time window for administering tPA.

Reasons for the failed translation of therapeutics from bench-to-bedside are unclear but notable contrasts exist between preclinical models of ischemic stroke and the clinical population having ischemic strokes. Specifically, stroke is a disease of the elderly with approximately 72% of stroke patients being over the age of 65 (Goldstein et al., 2006). In 2009, the Stroke Therapy Academic Industry Roundtable (STAIR) committee recommended that following evaluation of proposed therapeutics in young, healthy male animals, further studies should be performed in aged female animals and animals with comorbid conditions (Fisher et al., 2009; Sacco et al., 2006).

Apyrase is a calcium-activated enzyme that catalyzes the hydrolysis of ATP and ADP to yield AMP and inorganic phosphate. Apyrase has found clinical application as a novel pathway for inhibition of platelet aggregation through the increased hydrolysis of extracellular ADP. ATP 102 is an experimental ayrase formulation that has shown both antithrombotic and anti-inflammatory activity with minimal risk of increased bleeding. Previous studies have demonstrated that APT102 alone or combination with r-tPA prevents ischemia-reperfusion injury following lung infarction (Eckle et al., 2007; Sugimoto et al., 2009), myocardial infarction (Kohler et al., 2007; Moeckel Douglas, 2011) and acute ischemic stroke in young male rats (Belayev et al., 2003; Sun et al., 2011; Wang et al., 2010). Thus, in this study, we used a combination therapy of r-tPA and APT102 in a clinically relevant embolic stroke model in aged female rats to evaluate whether the combination could safely extend the therapeutic time window for r-tPA out to 6 h following ischemic stroke. By using aged female rodents, tissue plasminogen activator mediated reperfusion, and functional/neurological assessment; we successfully address numerous STAIR recommendations for improving translation of therapeutics from bench-to-bedside. Furthermore, we attempt to increase the number of patients potentially eligible for rTPA-mediated reperfusion by extending the therapeutic window to 6 hours post-ischemia, a significant finding with regards to reducing ischemic stroke morbidity and mortality.

2. Materials and methods

2.1 Animals

Seventy-two female Sprague-Dawley rats (18-20 months old) were purchased from Hilltop Laboratories (Scottsdale, PA) and housed under 12-h:12-h light-dark conditions with food and water ad libitum. All studies involving rats were approved by the West Virginia University Animal Care and Use Committee.

2.2 Drug Treatment

Rats were randomly divided into three treatment groups: r-tPA group (N=30), saline group (N=23), and r-tPA + APT102 group (N=19). The r-tPA group received r-tPA (5mg/kg, Genentech; South San Francisco, CA, USA) as an intravenous infusion (30% bolus and 70% infused over 30 min) at 6 h after MCAO. The saline group received an intravenous infusion of saline at 6 h after MCAO. The r-tPA+APT102 group received r-tPA (5 mg/kg) plus APT102 (2.25 mg/kg, APT Therapeutics Inc, St Louis, Missouri, 63108, USA) as an intravenous infusion (30% bolus and 70% infused over 30 min) at 6 h after MCAO. Standard physiologic parameters were monitored before, during, and after MCAO in a subset of rats (n=3 per group) to ensure uniformity and consistency with prior studies. All parameters were consistent with the ranges we have previously published (DiNapoli et al., 2006), indicating that APT102 did not induce obvious physiologic changes in blood pressure, blood pH, and blood gas composition. Rats from all three groups were evaluated at 24 h following MCAO.

2.3 Surgical procedure for MCAO

Rats were given a reversible embolic MCAO as previously described (Dinapoli et al., 2006; DiNapoli et al., 2008; Tan et al., 2009). Briefly, rats were anesthetized with 2% isoflurane in a mixture of 30% oxygen and 70% nitrous oxide. A servo-controlled homeothermic heating blanket, equipped with a rectal thermometer, was used to maintain body temperature at 37°C. Cerebral blood flow was monitored with a laser Doppler probe precisely positioned over the area supplied by the middle cerebral artery (MCA). A modified PE 50 microcatheter was inserted into the external carotid artery stump and advanced into the MCA. Placement in the proximal MCA was verified by a sudden decrease in cerebral blood flow as measured by laser Doppler. The microcatheter was withdrawn ~1 mm, allowing cerebral blood flow to return to baseline, and then a 25 mm fibrin-enriched clot was injected. Successful MCA occlusion was confirmed by a drop in perfusion greater than 80% of baseline flow. Recanalization was achieved when cerebral blood flow increased to at least 75% of initial baseline. Rats not achieving these standards were excluded from the study.

2.4 Modified Neurological Severity Score (mNSS)

Neurological function was assessed at 24 h post-MCAO by an investigator blinded to the treatment group using the modified Neurological Severity Score (mNSS). The modified Neurological Severity Score (mNSS) evaluated motor, sensory, balance and reflex measures. Scores range from 0 to 17 with higher scores indicating greater neurologic injury (Seyfried et al., 2004).

2.5 TTC staining for determination of infarction volume

At 24 h following MCAO, rats were sacrificed and infarct size and volume were measured by 2,3,5-triphenyltetrazolium chloride (TTC; 2%) staining. 2 mm coronal brain slices were stained for 15 min at 37°C. Hemispheres were scanned on a flatbed scanner and analyzed using Image J software. On each slice, the non-stained area (ischemic brain) was outlined, and the infarct volume was calculated. The corrected infarction volume (CIV) was calculated using the following equation: $CIV = (CHV - [IHV - IHI]) \times d$, where CHV was the area of the contralateral cortex or striatum in mm², IHV was the area of the ipsilateral cortex or striatum in mm², IHI was the infarct area (cortical or striatal) in mm², and d was slice thickness (2 mm). Edema index was calculated as follows: $Edema\ index = ([volume\ of\ ipsilateral\ hemisphere - volume\ of\ contralateral\ hemisphere] / volume\ of\ contralateral\ hemisphere) \times 100\%$ (Yang et al., 1998).

2.6 Spectrophotometric assay of hemoglobin

Hemorrhagic transformation was quantified using a spectrophotometric assay of brain hemoglobin content (QuantiChrom™ Hemoglobin Assay Kit). At 24 h after MCAO, the rats were anesthetized with ketamine (90 mg/kg; i.p.; Webster Veterinary; Sterling, MA) and xylazine (5 mg/kg; Webster Veterinary), and then transcardially perfused with 0.1 mol/L phosphate buffered saline until the outflow fluid from the right atrium was clear. MCA areas in left and right hemisphere were dissected out, homogenized, and centrifuged at $13,000 \times g$ for 30 min. Supernatant (50 µl) from each sample was aliquoted into a 96 well plate and 200 µl of assay reagent was added. After 15 min incubation, optical density was measured at 400 nm with a spectrophotometer. Hemoglobin content was expressed as mg/dl tissue (Christoforidis et al., 2007).

2.7 Statistical analysis

Data were presented as mean ± S.E.M. Differences in infarct volume, mNSS, edema index and hemoglobin content between treatment groups were compared using Students t-test. Differences in mortality rate between treatment groups were compared using Fisher's exact test. Level of significance was set at $P < 0.05$.

3. Results

3.1. APT102 and r-tPA in combination significantly decreased mortality rate at 24 h following middle cerebral artery occlusion (MCAO)

No rats were excluded from the study for not meeting the ischemic brain injury criteria and no differences in reperfusion were seen amongst groups receiving r-tPA. Mortality rate was evaluated at 24 h following MCAO. Combination therapy of APT102 and r-tPA significantly reduced the mortality rate from 47% in the r-tPA group to 16% in the r-tPA + APT102 group ($P = 0.027$). No significant difference ($P = 0.163$) in mortality rate was found between saline and r-tPA + APT102 group rats. There was no significant difference ($P = 0.384$) in mortality rate between saline and r-tPA rats (Fig.1). Some rats dying prior to 24 h were autopsied with typical findings including larger hemispheric infarctions, extensive brain edema, and macroscopic hemorrhage. Rats dying prior to 24 h were not included in the

histological, hemoglobin assay, and performance studies. Many of the rats that died prior to 24 h, particularly within the r-tPA group, exhibited extensive hemorrhagic transformation and enlarged infarcts. The higher mortality rate in the r-tPA group compared to the r-tPA + APT102 group, introduces a survival bias in that the most severely infarcted and impaired animals died and were excluded from analysis. Therefore, the difference in parameters between r-tPA and r-tPA+APT102 is likely even larger than reported herein due to this bias.

3.2. APT102 and r-tPA in combination reduced cortical, striatal, and total hemispheric infarction size at 24 h following MCAO

Stroke volume of the cortex, striatum, and total cerebral hemisphere was measured at 24 h following MCAO (Fig. 2). Cortical ($P=0.011$), striatal ($P=0.013$), and total hemispheric ($P=0.004$) infarction volume were significantly reduced by r-tPA + APT102 combination treatment compared with r-tPA alone. Cortical ($P=0.033$), striatal ($P=0.010$), and total hemispheric ($P=0.011$) infarction volume were significantly reduced by r-tPA + APT102 combination treatment compared with saline treated rats. No significant difference in cortical ($P=0.698$), striatal ($P=0.409$), and total hemispheric ($P=0.844$) infarction volume was observed between saline and r-tPA treated rats.

3.3. APT102 and r-tPA in combination improved neurological function at 24 h post-MCAO

Before MCAO, neurological score was normal (score=0) in all animals. Neurological function using the modified neurological severity score (mNSS) was assessed at 24 h following MCAO. Higher numbers indicate more severe deficits. APT102 and r-tPA in combination significantly ($P=0.045$) improved the neurological score compared with r-tPA alone. No significant ($P=0.331$) difference in mNSS was noted between saline and r-tPA + APT102 treated rats. No significant ($P=0.138$) difference in neurological scores was observed between saline and r-tPA treated rats (Fig. 3).

3.4 APT102 and r-tPA in combination significantly decreased hemorrhage transformation in cortex, striatum and total hemisphere at 24 following MCAO

Hemoglobin levels in cortex, striatum, and total cortical hemisphere were measured by hemoglobin assay at 24 h following MCAO. r-tPA and APT102 combination significantly decreased hemoglobin levels in cortex ($P=0.009$), striatum ($P=0.045$), and total cerebral hemisphere ($P=0.018$) compared to r-tPA treatment alone. Results also showed a significant decrease of hemoglobin levels in the cortex ($P=0.017$) and total hemisphere ($P=0.008$) in r-tPA+ APT102 group compared with saline group. No significant difference ($P=0.056$) in hemoglobin levels in striatum was found in r-tPA+ APT102 group compared with saline group. No significant difference in hemoglobin levels in cortex ($P=0.893$), striatum ($P=0.234$), and total cerebral hemisphere ($P=0.420$) was observed comparing saline group with r-tPA group rats (Fig. 4 A). Representative coronal sections showed there were different levels of hemorrhage with saline (moderate), r-tPA (severe) and r-tPA+APT102 treated rats (mild) following MCAO at 24 h (Fig. 4 B).

3.5. APT102 and r-tPA in combination improved edema formation in saline group rats but not in r-tPA group rats at 24 h following MCAO

Edema formation (brain swelling) was measured at 24 h following MCAO (Fig. 5). Results showed a significant decrease ($P=0.038$) in edema formation between r-tPA+APT102 and saline treated rats. No significant difference ($P=0.366$) in edema formation was observed between r-tPA and r-tPA +APT102 treated rats. No significant difference ($P=0.408$) in edema formation was found between saline and r-tPA treated rats

4. Discussion

This is the first report of the evaluation of a soluble apyrase in a reproducible and clinically relevant embolic stroke model of aged female rats. The protective effect of r-tPA over placebo (saline) was lost at 6 h following ischemic stroke. The main finding of this study, taking into account prior findings demonstrating a protective effect of r-tPA when administered at 2 hours after ischemic stroke (DiNapoli *et al.*, 2008; Tan *et al.*, 2009), was that r-tPA in combination with the recombinant human apyrase (APT102) safely extended the time window for r-tPA out to 6 h after ischemic stroke in aged female rats. This study showed that co-administration of APT102 with r-tPA significantly reduced mortality rate, decreased infarct volume and cerebral hemorrhage, and improved neurological functional outcome at 24 h following MCAO as compared to subjects treated with r-tPA alone. These findings have significant relevance in that this work can potentially translate to the clinical environment. The management of ischemic stroke would thereby change radically. Specifically, r-tPA could potentially be administered safely and efficaciously until 6 hours post-ischemia, significantly reducing morbidity and mortality.

Consistent with our previous studies, higher mortality and larger infarct volumes were observed in aged female rats at 24 h following MCAO and r-tPA reperfusion (Dinapoli *et al.*, 2006; DiNapoli *et al.*, 2008; Tan *et al.*, 2009). Prior studies in our lab also show that experimental therapeutics (EEMMID and apocynin) combined with r-tPA were neuroprotective following ischemic stroke in young animals but not in aged animals (Tan *et al.*, 2009; Kelly *et al.*, 2009). As a thrombolytic agent, r-tPA has direct and deleterious effects on the neurovascular unit that substantially increases death rate and infarct volume and causes severe hemorrhage when administered intravenously following an MCAO in a delayed fashion. Recent studies indicate that aged rats were more severely impaired following ischemic stroke than younger rats with diminished functional recovery (Popa-Wagner *et al.*, 2006a; Popa-Wagner *et al.*, 2006b; Popa-Wagner *et al.*, 2007). Clinically, increased age negatively affects spontaneous recovery of stroke patients and is a primary risk factor for fragility of the blood brain barrier and hemorrhagic transformation following thrombolytic therapy (Fisher *et al.*, 2009). In the largest clinical trial of rtPA use in stroke, the International Stroke Trial 3 (IST-3), r-tPA treatment increased symptomatic intracranial hemorrhage within 7 d from 1% to 7%. This increase was then associated with a mortality of 11% in the r-tPA group versus 7% in the control group at 7 d following ischemic stroke (Davalos *et al.*, 2012). Much of the mortality following ischemic stroke is due to increased intracranial pressure produced by edema formation.

Vascular endothelium and platelets maintain vascular integrity and promote primary and secondary hemostasis following interruption of vessel continuity. Cerebral artery occlusion and reperfusion is associated with endothelial damage and activation of platelets (Marcus et al., 2001; Marcus et al., 2005; Sugimoto et al., 2009). ATP and ADP are released in high concentrations by activated endothelial cells and platelets following aggregation on endothelium. In turn, ATP and ADP further increase additional platelet aggregation, leading to microthrombus formation and the eventual worsening of endothelial injury. Platelet and fibrin deposition downstream of an occlusive lesion contribute significantly to the postischemic hypoperfusion and tissue injury; thus, complicating stroke injury (Belayev et al., 2003; Bours et al., 2006; Dwyer et al., 2004; Imai et al., 2000; Kohler et al., 2007; Wang and Guidotti, 1996).

The beneficial effects of co-administration of APT102 with r-tPA at 6h following MCAO may likely be explained by the effect of apyrase. APT102, a novel form of the naturally occurring human apyrase/ADPase (CD39, recombinant soluble human CD39), promotes the breakdown of extracellular ATP and ADP by catalyzing the removal of the gamma phosphate from ATP and the beta phosphate from ADP (Imai et al., 2000; Marcus et al., 1991; Pinsky et al., 2002; Robson, 2006; Robson et al., 2005). APT102 does not act on platelets per se but rather on prothrombotic mediators (ATP and ADP). Increased MMP9 activity along the basement membrane of the blood brain barrier is believed to cause hemorrhagic transformation due to increased release and extravasation of neutrophils. Moreover, APT102 most likely prevents hemorrhagic transformation by inhibiting ATP-induced endothelial cell activation and neutrophil infiltration and re-sensitizing platelets (Belayev et al., 2003; Guckelberger et al., 2004; Kohler et al., 2007; Marcus et al., 2005; Moeckel Douglas, 2011; Robson, 2006). As such, the protective effect of APT102 likely requires reperfusion to be observed, a finding consistent with prior work in a thrombo-embolic model of ischemic stroke with r-tPA mediated reperfusion (Sun et al., 2011). For this reason, APT102 was not studied in isolation in this study and consequently, we cannot comment on any protective effect of APT102 in isolation within our clinically-relevant model of ischemic stroke in aged female rats.

The effects of exogenously administered apyrase are consistent with a prior study, in which CD39 knockout mice demonstrated increased cerebral infarction volumes compared with control CD39 wild type mice following experimental stroke. Additionally, the study showed that endogenous CD39 was protective after stroke, and administration of pharmacological doses of soluble CD39 was effective in inhibiting thrombosis and tissue injury following ischemic stroke (Pinsky et al., 2002). Since soluble CD39 does not interfere with primary GPIIb-mediated platelet adhesion at the site of vessel damage, soluble CD39 administration should not prevent a layer of platelets from forming at the site of injury or interfere with hemostatic mechanisms that prevent intracerebral hemorrhage (Belayev et al., 2003; Marcus et al., 2005). Compared with aspirin, soluble CD39 may induce more potent inhibition of platelet aggregation by blocking ADP-induced platelet recruitment. Soluble CD39 was shown to be more efficient in inhibiting platelet recruitment than aspirin via blockade of the arachidonate/thromboxane pathway. Soluble CD39 disaggregates platelets under recruitment, but it does not have a deleterious effect on primary hemostasis. These actions may explain how soluble CD39 can inhibit microvascular thrombosis and confer

cerebroprotection in ischemic stroke without worsening intracerebral hemorrhage (Kohler et al., 2007; Marcus et al., 1991; Marcus et al., 2005; Moeckel Douglas, 2011).

5. Conclusion

In conclusion, combination of r-tPA and APT102 reduces mortality rate, decreases cerebral infarct volume, improves neurological deficit scores, and prevents r-tPA-induced hemorrhage transformation in our clinical relevant ischemic stroke model in aged female rats. APT102 can extend the therapeutic window out to at least 6 h by reducing r-tPA-induced intracerebral hemorrhage following MCAO.

Acknowledgments

This work was supported by NIH grant NS060175 (to R.C.,C.R) and NIH Training Grant 5T32GM08174 (to R.T.).

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Fig. 1.

Mortality rate was evaluated at 24 h following MCAO. Results showed a 47% mortality rate at 24 h when r-tPA was administered alone at 6 h following MCAO (N=30). Combination of APT102 and r-tPA administration (N=19) at 6 h after MCAO reduced mortality at 24 h from 47% to 16% ($P<0.05$). No significant difference ($P>0.05$) in mortality rate was observed between saline group (35%, $n=23$) and r-tPA+TPA102 (16%, $n=19$). There was no significant difference ($P>0.05$) in mortality rate between saline group (35%, $n=23$) and r-tPA group (47%, $n=30$). * = $P<0.05$.

Fig. 2.

Measurement of stroke volume of cortex, striatum, and total cerebral hemisphere at 24 h following MCAO. Results showed a significant decrease ($P<0.05$ in cortex and striatum, $P<0.01$ in total hemisphere) for stroke volume in cortex ($29\pm4\%$ vs $47\pm5\%$), striatum ($40\pm3\%$ vs $50\pm2\%$), and total hemisphere ($33\pm4\%$ vs $48\pm3\%$) in r-tPA+APT102 group ($n=14$) compared with r-tPA group ($n=14$). Results also showed a significant decrease ($P<0.05$) in stroke volume in cortex ($29\pm4\%$ vs $44\pm5\%$), striatum ($40\pm3\%$ vs $54\pm4\%$), and total hemisphere ($33\pm4\%$ vs $47\pm4\%$) in r-tPA+APT102 group ($n=14$) compared with saline group ($n=13$). No significant difference ($P>0.05$) in stroke volume in cortex ($44\pm5\%$ vs $47\pm5\%$), striatum ($54\pm4\%$ vs $50\pm2\%$), and total hemisphere ($47\pm4\%$ vs $48\pm3\%$) was observed in saline group ($n=13$) compared with r-tPA group ($n=14$). Bars represent mean $\pm$ S.E.M. * = $P<0.05$. **, * = $P<0.01$.

Fig. 3.

Assessment of neurological function using a 17 point mNSS following MCAO. Results showed that combination of r-tPA with APT102 had significantly improved mNSS ($P < 0.05$) from r-tPA group (8.9 ± 0.6 , $n=14$) to r-tPA+ APT102 group (6.8 ± 0.8 , $n=16$). No significant difference ($P > 0.05$) in mNSS scores was demonstrated between saline group (7.3 ± 0.5 , $n=15$) and r-tPA+ APT102 group (6.8 ± 0.8 , $n=16$). No significant difference ($P > 0.05$) was observed between saline group (7.3 ± 0.5 , $n=15$) and r-tPA group (8.9 ± 0.6 , $n=14$). Bars represent mean $\pm$ S.E.M. * = $P < 0.05$.

Fig. 4.

(A) Hemoglobin levels were measured in cortex, striatum and total hemisphere by hemoglobin assay at 24 h following MCAO. Results showed a significant decrease ($P < 0.05$ in striatum and total hemisphere, $P < 0.01$ in cortex) in hemoglobin levels in cortex (1.39 ± 0.08 vs 1.88 ± 0.14 mg/dl), striatum (1.37 ± 0.10 vs 2.04 ± 0.29 mg/dl), and total hemisphere (1.37 ± 0.08 vs 1.95 ± 0.20 mg/dl) in r-tPA+ APT102 group ($n=13$) compared with r-tPA group ($n=12$). Results also showed a significant decrease ($P < 0.05$ in cortex, $P < 0.01$ in total hemisphere) in hemoglobin levels in cortex (1.39 ± 0.08 vs 1.85 ± 0.14 mg/dl) and total hemisphere (1.37 ± 0.08 vs 1.76 ± 0.10 mg/dl) in r-tPA+ APT102 group ($n=13$) compared with saline group ($n=15$). No significant difference ($P > 0.05$) in hemoglobin levels was observed in striatum (1.37 ± 0.10 vs 1.66 ± 0.10) in r-tPA+ APT102 group ($n=13$) compared with saline group ($n=15$). No significant difference ($P > 0.05$) in hemoglobin levels in cortex (1.85 ± 0.14 vs 1.88 ± 0.14 mg/dl), striatum (1.66 ± 0.10 vs 2.04 ± 0.29 mg/dl), and total hemisphere (1.76 ± 0.10 vs 1.95 ± 0.20 mg/dl) was demonstrated in saline group ($n=15$) compared with r-tPA group ($n=12$). Bars represent mean $\pm$ S.E.M. * = $P < 0.05$, * * = $P < 0.01$. **(B)** Representative coronal sections through the infarct regions of brains in saline (moderate hemorrhage), r-tPA (severe hemorrhage) and r-tPA+APT102 (mild hemorrhage) rats following MCAO at 24 h.

Fig. 5.

Measurement of edema formation in the infarct hemisphere following MCAO. Results showed a significant decrease ($P < 0.05$) in edema formation between r-tPA+APT102 group ($22.4 \pm 2.3\%$, $n=14$) and saline group ($28.4 \pm 1.5\%$, $n=13$). No significant ($P > 0.05$) difference in edema formation was observed between r-tPA+APT102 group ($22.4 \pm 2.3\%$, $n=14$) and r-tPA group ($25.7 \pm 2.8\%$, $n=14$). No significant ($P > 0.05$) difference in edema formation was shown between saline group ($28.4 \pm 1.5\%$, $n=13$) and r-tPA group ($25.7 \pm 2.8\%$, $n=14$). Bars represent mean $\pm$ S.E.M. * = $P < 0.05$.