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Arginine Vasopressin Release Inhibitor RU51599 Attenuates Brian Oedema Following Transient Forebrain Ischaemia in Rats

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Summary

RU51599 is an arginine vasopressin (AVP) release inhibitor and a selective kappa opioid agonist which has a pure water diuresis effect without associated electrolyte excretion. The effect of RU51599 on brain oedema following transient forebrain ischaemia in rats was examined. Under microscopy, the visible vertebral arteries at the second vertebra could be easily electrocauterized and completely cut by microscissors to yield complete cessation of circulation of both vertebral arteries. Transient forebrain ischaemia was induced by this improved highly reproducible technique of four-vessel occlusion model. Forty-three male Wistar rats were separated into six groups; saline-treated (1 ml/kg) normal rats (n = 10), RU51599-treated (1 mg/kg) normal rats (n = 4), saline-treated (1 ml/kg) rats with complete occlusion of both vertebral arteries (n = 5), RU51599-treated (1 mg/kg) rats with complete occlusion of both vertebral arteries (n = 5), saline-treated (1 ml/kg) rats with both complete occlusion of both vertebral arteries and carotid occlusion bilaterally during 45 minutes followed by 60 minutes of reperfusion (n = 11), RU51599-treated (1 mg/kg) rats with both complete occlusion of both vertebral arteries and carotid occlusion bilaterally during 45 minutes followed by 60 minutes of reperfusion (n = 8). The brain water content was determined by the dry-wet weight method. Cerebral blood flow was monitored during ischaemia and reperfusion was performed by laser Doppler flowmetry to make sure to obtain reversible forebrain ischaemia. Effects of RU51599 on concentration of glutamate released from the hippocampal CA1 of rats subjected to 5 minutes four-vessel occlusion and 60 minutes of reperfusion were also investigated by the microdialysis method. This modified four-vessel occlusion method produced reversible forebrain ischaemia with a high level of success. Bilateral carotid occlusion followed by 60 minutes reperfusion caused a significant increase in brain water content ($P < 0.01$), which was significantly attenuated by RU51599 ($P < 0.01$). These findings indicate that the AVP-release inhibitor RU51599 reduced brain oedema following transient forebrain ischaemia in rats.

Keywords: Arginine vasopressin; brain oedema; four-vessel occlusion; kappa opioids; microdialysis; rat.

Introduction

Humoral mechanisms, regulated by arginine vasopressin (AVP) and atrial natriuretic peptide, play an important role in the regulation of water and electrolytes in the central nervous system. AVP is elevated in the cerebrospinal fluid of patients with ischaemic and traumatic brain injuries [20, 23, 35]. Recent experimental studies have demonstrated that intraventricular injection of AVP increased brain water contents and aggravated cryogenically induced oedema and AVP receptor antagonists reduced vasogenic brain oedema [5, 32]. Recently we have demonstrated that reduction or inhibition of AVP release significantly reduced cryogenic-induced brain oedema in rats [19]. The agent RU51599 (Roussel Uclaf, France, [N-methyl-2-(3-nitrophenyl)-N-[(1S,2S)-2-(1-pyrrolidinyl)-1-indanyl] acetamide monohydrochloride; niravoline]) is a AVP release inhibitor [15]. RU51599 is a selective kappa opioid agonist which has been shown in animals to have potent aquaretic activity, characterized by a pure water diuresis without associated electrolyte excretion (Fig. 1). Selective kappa opioid agonists have been observed to antagonize the

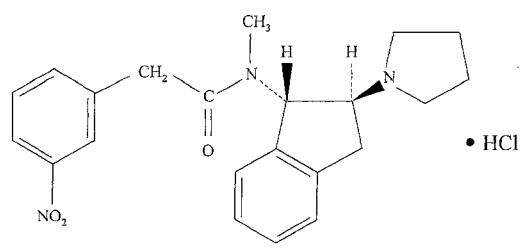

Fig 1. Chemical structure of RU51599

actions of excitatory neurotransmitters and afford neuroprotection against N-methyl-D-aspartate-induced neuronal injury [17]. The implication of these reports stimulate the postulation that AVP-release inhibitor RU51599 can be used to treat ischaemic brain oedema. The four-vessel occlusion method introduced by Pulsinelli *et al.* [29, 30] is widely used as an experimental model for reversible forebrain ischaemia in rats. However, this model has a major problem, which is the difficulty in producing complete occlusion of the vertebral arteries by electrocauterization through the alar foramina of the C_1 vertebra. The anatomical course of the vertebral artery suggested by Sugio *et al.* [36] indicates that the vertebral artery at the level of the C_2 vertebra is visible. We further developed a highly reproducible model of reversible forebrain ischaemia [38]. In the present study, we investigated the protective effect of AVP release inhibitor RU51599 on brain oedema following this modified four-vessel occlusion in rats and also investigated the inhibitory effect of RU51599 on the amounts of glutamate released in the hippocampal CA1 region by the microdialysis method.

Materials and Methods

The procedures followed during the present experiments were in accordance with institutional guidelines.

Surgical Preparation

Male Wistar rats, weighing 250 to 300 gr. each, were anaesthetized intraperitoneally with chloral hydrate (360 mg/kg). Forebrain ischaemia was induced by four-vessel occlusion. Under microscopy the visible vertebral arteries at the second vertebra could be easily electrocauterized and completely cut by microscissors to yield complete cessation of circulation in both vertebral arteries without failure (Fig. 2). After 24 hours, animals were subjected to 45 minutes of forebrain ischaemia by occluding both common carotid arteries with Sugita's temporary clips. Rectal temperature was maintained at close to 37.0 °C with a heating pad during and after ischaemia.

Measurement of Brain Water Contents

The brain water content was determined by the dry-wet weight method. Animals were killed by decapitation. The brain was removed immediately and both hemispheres were immediately weighed to obtain the wet weight. The tissue was then dried in a 100 °C oven for 24 hours and reweighed to obtain the dry weight. The brain water contents, expressed as a percentage of the wet weight, was calculated as (wet weight – dry weight) / wet weight × 100.

Monitoring of Cerebral Cortical Blood Flow

Each animal's head was placed in a stereotactic frame. The skull was exposed by a midline sagittal incision and a craniectomy (diameter 3 mm) was performed at 5 mm from the sagittal suture and 3 mm anterior to the coronal suture. The dura was incised and removed to expose the cortex. A needle-type probe was placed on the brain surface. Monitoring of cerebral cortical blood flow during ischaemia and reperfusion was performed by laser Doppler flowmeter [LBF-III F, Biomedical Science Inc., Japan] to make sure to produce reversible forebrain ischaemia.

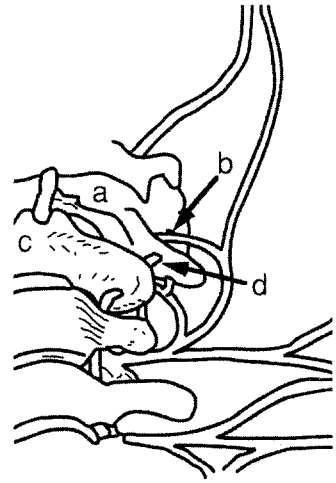

Fig. 2. Schematic figure of I to IV cervical vertebrae (right side). *a* first cervical vertebra, *b* right alar foramen, *c* second cervical vertebra, *d* right vertebra artery at the part of electrocauterized cut by microscissors

Drug Administration

Forty-three of the animals were separated into six groups; saline-treated (1 ml/kg) normal rats (n = 10), RU51599-treated (1 mg/kg × 2) normal rats (n = 4), saline treated (1 ml/kg) rats with complete occlusion of both vertebral arteries (n = 5), RU51599-treated (1 mg/kg × 2) rats with complete occlusion of both vertebral arteries (n = 5), saline-treated (1 ml/kg) rats with both complete occlusion of both vertebral arteries and bilateral carotid occlusion during 45 minutes followed by 60 minutes of reperfusion (n = 11), RU51599-treated (1 mg/kg × 2) rats with both complete occlusion of both vertebral arteries and bilateral carotid occlusion during 45 minutes followed by 60 minutes of reperfusion (n = 8). In the latter two groups, RU51599 (1 mg/kg) was administered intravenously before and after bilateral carotid occlusion. The vertebral arteries were electrocauterized and completely cut by microscissors at 24 hours before the drug administration.

Intracerebral Microdialysis

One burr hole was drilled on the exposed skull. A microdialysis probe (3 mm length of dialysis membrane, CAM-10, BAS, Carnegie Medicine, Sweden) was inserted into the right CA1 region (3.6 mm posterior and 2 mm lateral to the bregma and 3.5 mm ventral to the dura). It was perfused with Ringer's solution (Na 147 mM, K 4 mM, Ca 2.3 mM, Cl 153 mM) at a flow rate of 5 µl/min by a microinjection pump (CMA/100, BAS, Sweden). The dialysates were collected for each 5-min perfusion and analysed for glutamate [18, 40]. The concentrations of glutamate were determined by the HPLC with fluorescence detection (CMA/280, BAS, Carnegie Medicine, Sweden). Seventeen of the animals were separated into two groups; saline-treated (1 ml/kg)

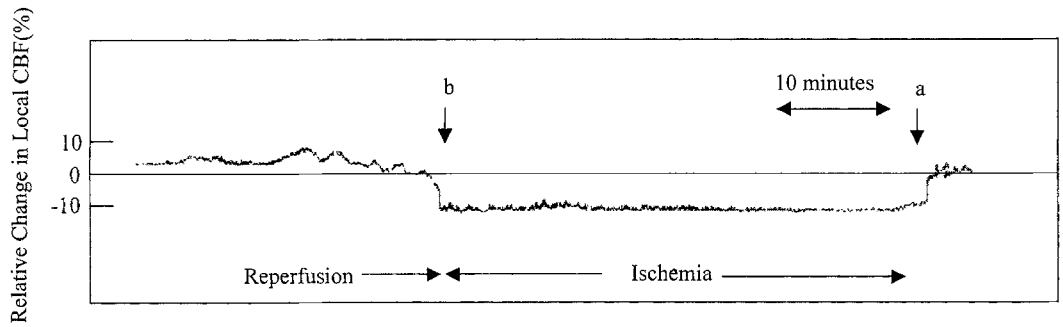

Fig. 3. Cerebral blood flow measured by laser Doppler flowmetry. *a* occlusion of carotid arteries, *b* reperfusion of both carotid arteries

rats with both complete occlusion of both vertebral arteries and bilateral carotid occlusion during 5 minutes followed by 60 minutes of reperfusion (n = 10), RU51599-treated (1 mg/kg) rats with both complete occlusion of vertebral arteries bilaterally and bilateral carotid occlusion during 5 minutes followed by 60 minutes of reperfusion (n = 7). In both groups, saline or RU51599 was administered intravenously at 30 minutes before bilateral carotid occlusion.

Statistical Analysis

Data are presented as mean values ± standard error. Non-paired t-test was used to assess significance, with $p < 0.05$ considered to indicate statistical significance.

Results

A definite reperfusion confirmed by the laser Doppler flowmetry demonstrated that a rapid reduction to approximately 90% of baseline values was seen with bilateral carotid occlusion and during carotid occlusion the cerebral blood flow remained at this level and recirculation resulted in temporary hyperaemia (approximately 110% of base values) about 20 to 30 minutes and then returned to baseline values (Fig. 3).

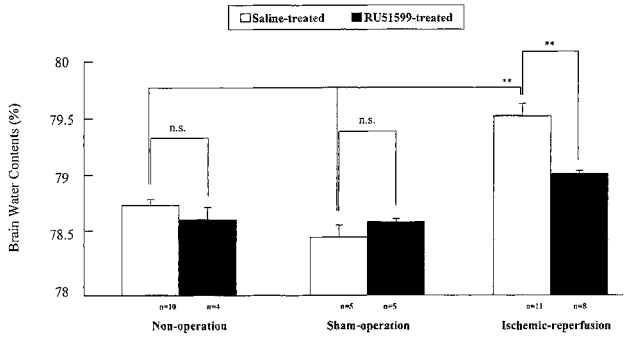

Fig. 4. Effects of RU51599 on brain water contents following transient forebrain ischaemia in rats. The brain water contents, expressed as a percentage of the wet weight, was calculated (wet weight – dry weight) / wet weight × 100. The data represents the mean ± S.E. *n.s.* not significant, ** $p < 0.01$ (t-test)

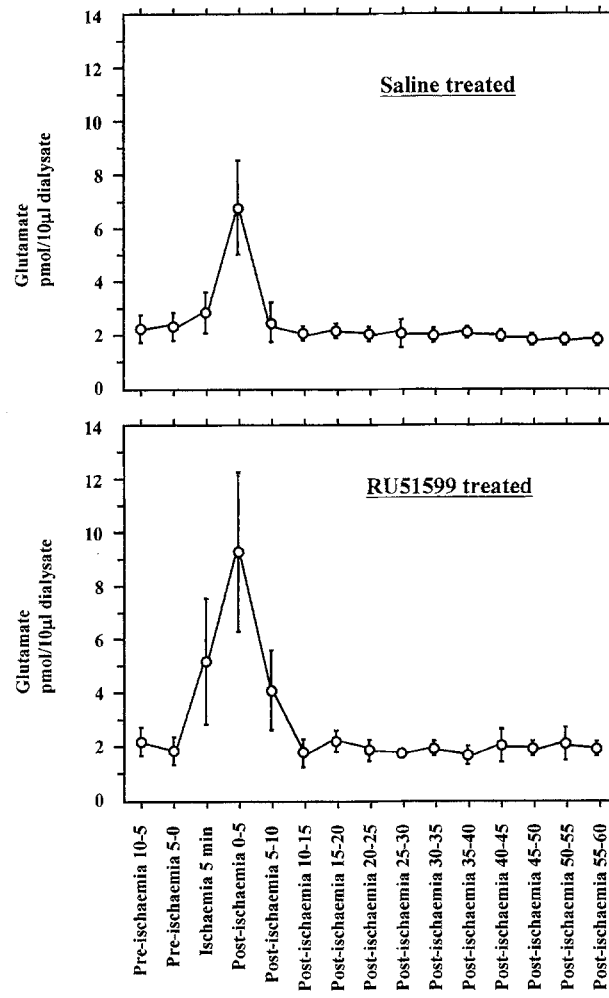

Fig. 5. Effects of RU51599 on concentration of glutamate in microdialysates from the hippocampal CA-1 of rats subjected to 5 minutes of four-vessel occlusion and 60 minutes of reperfusion. The data represents the mean ± S.E.

Brain water content in saline-treated normal rats was (78.77 ± 0.45), that in RU51599-treated normal rats 78.64 ± 0.08, that in saline-treated rats with complete occlusion of both vertebral arteries (78.48 ± 0.07), and that in RU51599-treated rats with

complete occlusion of vertebral arteries bilaterally 78.61 ± 0.03%.

The RU51599 treatment did not significantly change the brain water content in any of these groups. Bilateral carotid occlusion followed by 60 minutes of reperfusion caused a significant increase in brain water content compared to both that in saline-treated normal rats (non-operation group) and that in saline-treated rats with complete occlusion of both vertebral arteries (sham-operation group) ($p < 0.01$). Bilateral carotid occlusion followed by 60 minutes of reperfusion caused a significant increase in brain water content, which was significantly attenuated by RU51599 (79.52 ± 0.08 vs. 79.03 ± 0.09, $p < 0.01$) (Fig. 4).

The concentrations of glutamate in the dialysed perfusate during the 5-minute microdialysis during 5 minute occlusion of carotid arteries bilaterally were increased, which were not changed by the RU51599 treatment (Fig. 5).

Discussion

Brain Oedema and AVP

Centrally released AVP has been implicated in the regulation of intracranial pressure and brain water content [4]. AVP is elevated in the cerebrospinal fluid of some patients with ischaemic stroke, subarachnoid haemorrhage and traumatic brain injuries [20]. Recent studies have demonstrated that AVP enters the CSF directly from the brain and not from the systemic circulation [8]. Physiological evidence for a role of AVP in brain extracellular fluid regulation was obtained by Raichle and Grubb [31], who showed an increase in brain capillary permeability following intraventricular AVP injection in monkeys. In the rat, intraventricular AVP administration increased the brain capillary permeability, whereas the intravenous administration of AVP had no such effect. Dóczi *et al.* [5] showed that intraventricular AVP administration increased the brain water content in rats. Reeder *et al.* [32] also demonstrated that cryogenic brain oedema is accelerated following intraventricular AVP administration. László *et al.* [23] reported that the increase in AVP levels in the early stage of subarachnoid haemorrhage is of pathogenetic significance in the development of brain oedema.

These findings suggest that AVP receptor antagonists reduce brain oedema. There are two primary types of AVP receptor, namely, V_1 receptor found in vascular smooth muscles and hepatocytes that modulate the pressor effect and V_2 receptor found in the renal medulla links to generation of cyclic AMP and the antidiuretic effect. The V_1 receptor is the primary receptor type in the brain. Isolated cerebral microvessels have the V_1 receptor [26]. Nagao *et al.* [26] demonstrated that the centrally administered AVP V_1 receptor antagonist reduced cold-induced brain oedema and the AVP V_1 receptor antagonist proved to be more effective than the AVP V_2 receptor antagonist. Rosenberg *et al.* [33] also reported that AVP V_1 receptor antagonists and the atrial natriuretic peptide both significantly reduce haemorrhagic brain oedema. These reports indicate that centrally administered AVP V_1 receptor antagonists may be useful for the treatment of brain oedema. The only minor feature of AVP V_1 receptor antagonists is that they can be administered intraventricularly or orally, but not intravenously. The AVP-release inhibitor RU51599 has been shown to have selective action in binding in vitro to the kappa opioid receptor, an interaction which in pharmacological studies in vivo has been shown to be agonistic. RU51599 has an aquaretic effect, including a pure water diuresis effect without associated electrolyte excretion. RU51599 reduced circulation AVP levels in water deprived rats and dogs and the action of RU51599 was mediated through its effects on AVP secretion rather than any effect on the interaction of AVP with its receptors. Pharmacokinetic studies in rats have shown RU51599 to be rapidly and well distributed after intravenous administration and to have a short half-life of about 0.5 to 1.5 hours. RU51599 had no effect on blood pressure or heart rate [15]. Our recent experimental study [19], demonstrated that RU51599 significantly reduced cryogenically induced brain oedema in rats. The Brattleboro strain, originally derived from the Long-Evans strain, has diabetes insipidus as the result of an autosomal recessive defect AVP gene regulation. Dickinson and Bety [4] has reported that in the AVP-deficient Brattleboro rat using a middle cerebral artery occlusion, ischaemic brain oedema and sodium accumulation were attenuated compared to the control Long-Evans rats and they have suggested a role for central AVP in the development of ischaemic brain oedema. Reduction or inhibition of AVP release may indicate the beneficial and potential therapeutic strategy for all types of brain oedema.

Cerebral Ischaemia and Kappa Opioids

Endogenous opioid systems have been proposed as secondary or delayed brain injury factors, largely on the basis of the therapeutic efficacy of opioid receptor

antagonists in models of cerebral ischaemia [1, 7, 39]. The opioid receptor antagonist naloxone has been shown to improve ischaemia-induced neurological deficits [1]. However, some investigators [39] did not find naloxone effective in cerebral ischaemia. Recent experimental studies have demonstrated the existence of at least three receptor subtypes for the endogenous opioid peptides and among them, kappa-opioid receptor binding was increased after ischaemic injury and activation of a kappa-opioid receptor system contributed to exacerbate cerebral ischaemia, while more recent studies [10, 21, 34] have suggested that pharmacological stimulation of the kappa-opioid receptor subtype can attenuate ischaemic injuries and neurological recovery. Significant reduction of cerebral ischaemia has been demonstrated using a number of kappa-receptor agonists, including U-50488, PD117302 and CI-977 [39]. Fried *et al.* [9] have observed that hippocampal dynorphin concentration was reduced after transient cerebral ischaemia. Tang and his colleagues [37] have demonstrated an anti-ischaemic effect of the selective kappa-opioid receptor agonist U-50488H and this agent reduced postischaemic brain oedema. Hall *et al.* [14] have reported that the kappa agonist served to restore protection. Baskin and his colleagues [2] evaluated the therapeutic efficacy of variable kappa opioid agonists for delayed treatment of experimental focal cerebral ischaemia. The mechanism by which kappa-opioid agonists reduce brain oedema has been suggested to be via an inhibition of AVP [27]. Kappa-opioid agonists have been reported to reduce brain damage not only by inhibiting brain oedema after ischaemia but also by suppressing the release of glutamate [11–13, 16, 22, 24, 25, 28] due to restriction of Ca^{2+} into presynaptic terminals [3, 41, 42, 43]. In our present study, RU51599 did not inhibit the release of glutamate probably because of differences in the animal model used, dosing conditions, and brain site of glutamate measurement.

In conclusion, our present findings indicate that the AVP release inhibitor has a potential protective effect on brain oedema following transient forebrain ischaemia.

Acknowledgements

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Comments

Comment 1

This is a well-planned experiment in rats to estimate the effect of the AVP-release inhibitor RU51599 on ischaemic brain oedema, as the authors have previously conducted on cryogenic brain oedema. In the Methods section, they have not clearly described, whether the microdialysis experiment, which might conceivably influence water content by impairing the blood-brain barrier, has been conducted on the same groups of animals they have used to evaluate the effect of RU51599, or that the microdialysis experiment has been conducted on a separate group of (how many?) animals. In the Discussion section the authors have referred to other similar studies, but what do they think of the possible mechanism of action on tentative water channels [Harris HW *et al.*, Pediatr Nephrol 7, 680 (1993)], as the nephron and other epithelial tissues possess?

B. Go

Answer from the authors:

1) The microdialysis experiment has been conducted on a separate group of animals (seventeen animals).
2) The basic pharmacological study has demonstrated that the action of RU51599 is mediated through its effects on AVP secretion rather than any effect on the interaction of AVP with V-1 and V-2 receptors. We have to investigate the possible mechanism of action on tentative water channels as you suggested, but at present no data is available for us regarding this matter.

Y. Ikeda

Comment 2

The authors have performed a series of experiments with the aim of assessing whether RU51599, an arginine vasopressin (AVP) release inhibitor, which can be administered intravenously, significantly decreases the formation of brain oedema in rats exposed to transient forebrain ischaemia (45 minutes four vessel occlusion followed by 60 minutes reperfusion). Since RU51599 also acts as a selective kappa opioid agonist and thus may antagonize the action of excitatory neurotransmitters they also assessed the effect of this

agent administered 30 minutes before producing reversible forebrain ischaemia (5 minutes four vessel occlusion followed by 60 minutes reperfusion) on the amounts of glutamate released in the hippocampus.

They found that RU51599 (1 mg/kg) administered both before and after inducing global forebrain ischaemia significantly attenuated the abnormal increase in brain water content (determined by the dry-wet weight method) but did not modify the increased amounts of glutamate (assessed by a microdialysis method) measured in rats exposed to reversible brain ischaemia. The authors conclude that RU51599 may play an important role in the early phase of brain oedema following brain ischaemia in this animal model.

This is a well written paper but there are some questions.

1. In the present experiments they have not demonstrated that the mechanism of brain water reduction observed in rats pretreated with RU 51599 is an inhibition of AVP release as they did not measure AVP concentrations in their animals, neither in basal conditions nor following the ischaemic insult. The authors did not investigate the possible mechanisms underlying the apparent therapeutic effect they observed. In fact, the sentence in the authors main conclusion "that centrally released AVP plays an important role in the early phase of brain odema following transient forebrain ischaemia" is not supported by the experiments they are presenting in the paper. By contrast, the sentence of the conclusion i.e. that "the AVP release inhibitor has a potential protective effect on ischaemic brain oedema" is reasonably supported by the present experiments.
2. The authors did not consider that anaesthetics influence AVP levels and that there is a AVP diurnal rhythm. On the other hand, they do not explain why they used this particular dose (1 mg/kg) of RU51599, and why they did not try to determine if the effect of this pharmacological agent on the relative water content in ischaemic brains is dose-dependent.

R. D. Lobato

Answer from the authors:

1) I agree with your comment regarding the measurement of AVP concentrations. We have already tried to measure AVP concentrations in brain tissues, but AVP levels in these brain tissues were too low to measure. We also tried to measure AVP levels in brain tissues by using intracerebral microdialysis method, but we could not get enough data. We need to investigate the change of AVP levels to clarify the possible mechanism of RU51599.

2) We did not consider influence of the anaesthetics on AVP levels and on AVP diurnal rhythm. Chloral hydrate is most widely used in experimental ischaemic and traumatic brain injury in rats. No data is available in the literature, but chloral hydrate is well known to have less effect on vital signs and other metabolic changes.

Dose-dependent study is very important. We have done the dose-dependent study of RU51599 (0.1–3 mg/kg) in cryogenically induced brain oedema, which was documented in the paper entitled "Attenuation of Cryogenic Induced Brain Oedema by Arginine Vasopressin Release Inhibitor RU51599". Based on this experiment, we decided to use the particular dose (1 mg/kg) as the optimal dosage in this study.

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