

Neuroprotection in Combat Casualty Care: Partnering with Industry

Combat Casualty Care and Chemical/Biological Defense

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Brain Trauma and Neuroprotection Research Program

BIOMEDICAL TECH AREAS:

- **Penetrating Head Injuries (Ballistic Wounding) (RADII)**
- **Chemical/Biological Exposures (RADIV)**

APPROACH:

- **Multidiscipline neuroscience based effort integrated as a basic and applied sciences program.**
- **Utilize molecular, cellular, neurophysiological, behavioral and pharmacological disciplines to study mechanisms of brain injury and repair, and functional recovery.**
- **STUDY NOVEL NEUROPROTECTION STRATEGIES**
- **IMPROVE TRIAGE AND TREATMENT DOCTRINE**



Performers and Partnerships

PERFORMERS

- **Division of Neuroscience, Walter Reed Army Institute of Research**
- **Drug Assessment Division, USARICD**
- **Henry M. Jackson Foundation (USUHS)**

PARTNERSHIPS

- **Several CRADA Partnerships (@ \$2,000K since 1998)**
 - » **McKnight Foundation, University of Florida**
 - » **Millennium Pharmaceuticals, Cogentix Inc., Guilford Pharmaceuticals, Roche Biosciences**
- **MTAs: Hollis-Eden, J&J, Paion**



Current Research Program

- I. Molecular and cellular mechanisms of neuronal injury and neuroprotection. (Drs. J. Dave)**
- II. Non-convulsant seizures and spreading depression in experimental brain injury.**
- III. Pre-clinical neuroprotection: Cherry Picking for advanced neuroprotection drug studies in rat models of brain injury.**
- IV. Establishing a triage algorithm defining limits and thresholds of critical physiological parameters in head injury.**
- V. Published Accomplishments (2000-2003)**
 - **# Journal articles: 20 published; 6 “in press”**
 - **# Communications: 35+ abstracts; 3 ATACCC Conferences; 2 BioScience Conferences**
 - **# General public media: 3 Press Releases**



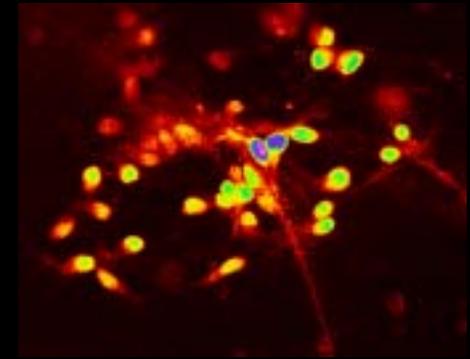
Brain Trauma and Neuroprotection

- **Pharmacology of brain injury (ex. MLN519)**
- **Pathophysiology of brain injury**
- **Molecular Biology of brain injury (Dr. Jitendra Dave)**
- **Neurological and Behavioral Consequences of brain injury**
 1. **Clinical Neurological Exams**
 2. **Simple Motor Exams (paw placement; grasping; beam walking)**
 3. **Cognitive/Learning Tasks (morris water maze; visual discrimination tasks)**
- **Electrophysiology of brain injury**
 1. **High Resolution qEEG Analysis**
 2. **Topographic Mapping**
 3. **Cortical Spreading Depression**

Pre-clinical Models

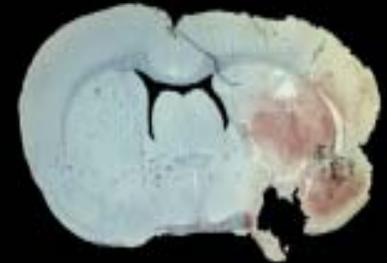
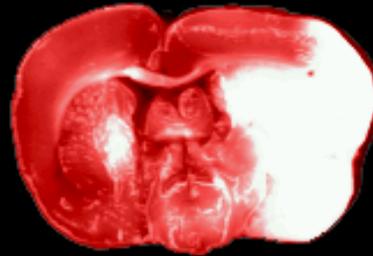
Primary Cultured Brain Neurons

- Neuroprotection Screening
- Mechanisms



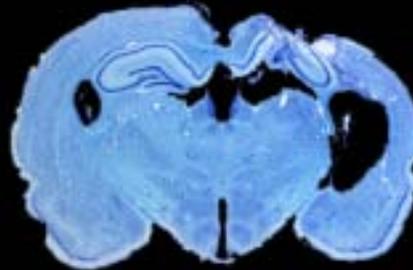
Ischemic Brain Injury (IBI)

- Secondary Cell Death Processes
- Leveraging CRADA Partners



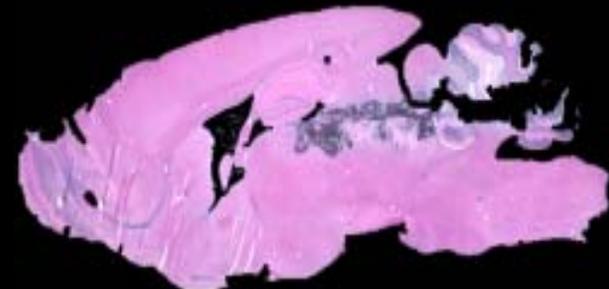
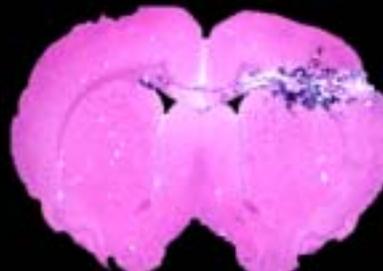
Traumatic Brain Injury (TBI)

- Cortical Impact/Concussive
- FPI = Gold Standard in TBI



Penetrating Brain Injury (PTBI)

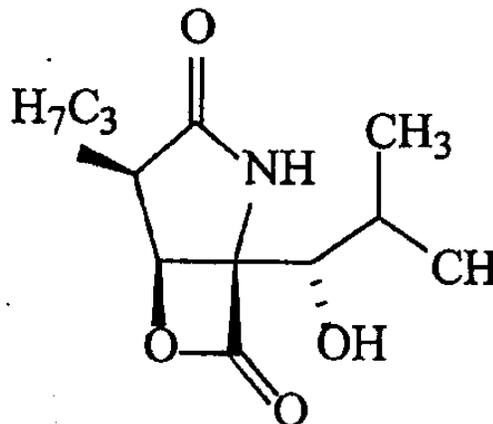
- Models a Ballistic Injury
- Revolutionary



Pre-clinical neuroprotection: **Cherry Picking** for advanced neuroprotection drug studies in rat models of brain injury

MLN519

- Millennium Pharmaceuticals, Cambridge, MA
- CRADA Partner since 1999 (ProScript = LeukoSite = Millennium)
- Lactacystin analogs (substituted β -Lactones) developed as safe, non-toxic inhibitors of the 20S Proteasome, IKK2 and NF- κ B activation



Proteasome Inhibitor PS519 Reduces Infarction and Attenuates Leukocyte Infiltration in a Rat Model of Focal Cerebral Ischemia

James B. Phillips, PhD; Anthony J. Williams, BS; Julian Adams, PhD;
Peter J. Elliott, PhD; Frank C. Tortella, PhD

(Stroke. 2000;31:1686-1693.)

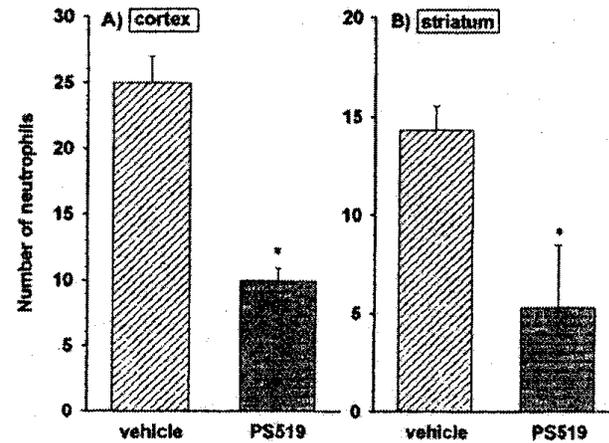
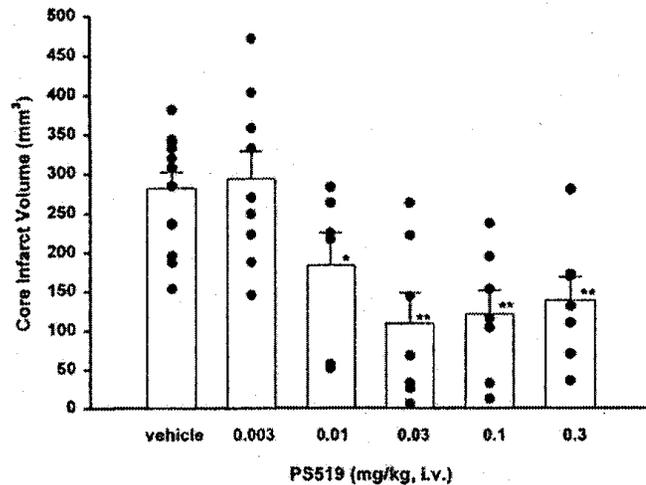


TABLE 2. Effect of PS519 (0.1 mg/kg) on Total Core Infarct Volume, EEG Recovery, and Neurological Function at 72 Hours After 2-Hour MCAo and Reperfusion

Treatment	n	Infarct Volume, mm ³	Percent Neuroprotection	Percent EEG Recovery	Neurological Score
Vehicle	7	231±20	0.0±6	6.3±5.5	2.4±0.9
PS519	5	138±25*	40±11*	61±20*	1.0±0.0*

Values are mean±SEM.

*P<0.05 compared with vehicle controls (Student's *t* test).

Delayed Treatment With MLN519 Reduces Infarction and Associated Neurologic Deficit Caused by Focal Ischemic Brain Injury in Rats via Antiinflammatory Mechanisms Involving Nuclear Factor- κ B Activation, Gliosis, and Leukocyte Infiltration

*Anthony J. Williams, †Sarah L. Hale, *John R. Moffett, *Jitendra R. Dave, ‡Peter J. Elliott, §Julian Adams, and *Frank C. Tortella

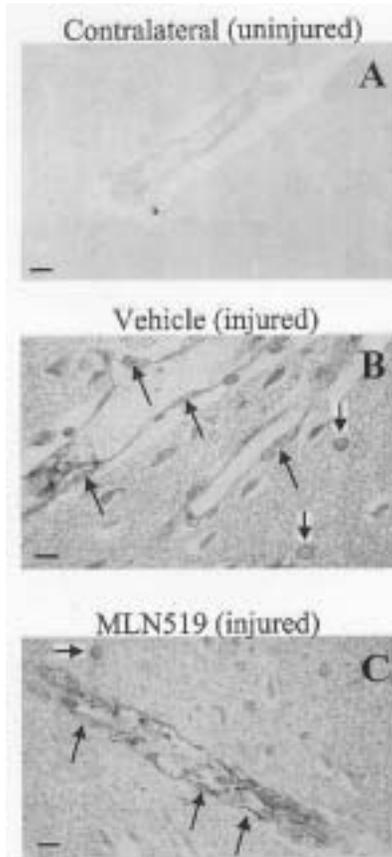
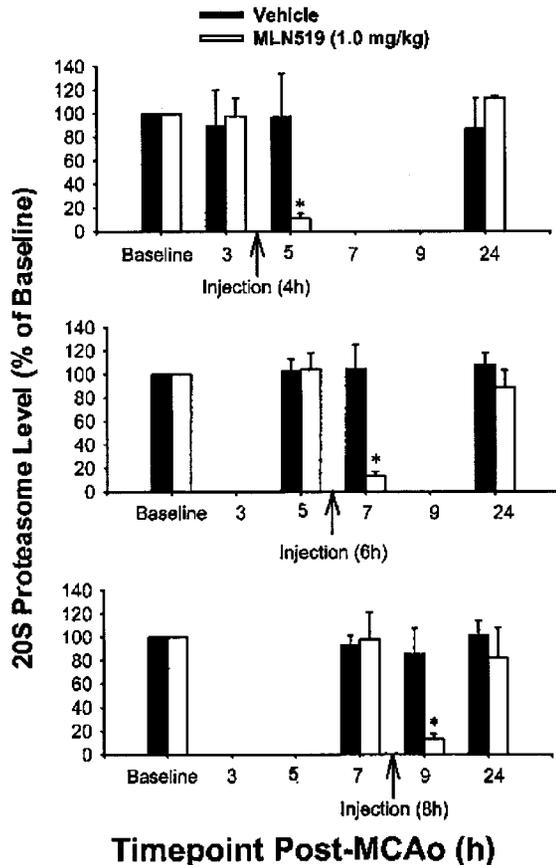


TABLE 3. Optical density measurements from nuclear factor- κ B-stained brain sections, 24 hours after middle cerebral artery occlusion

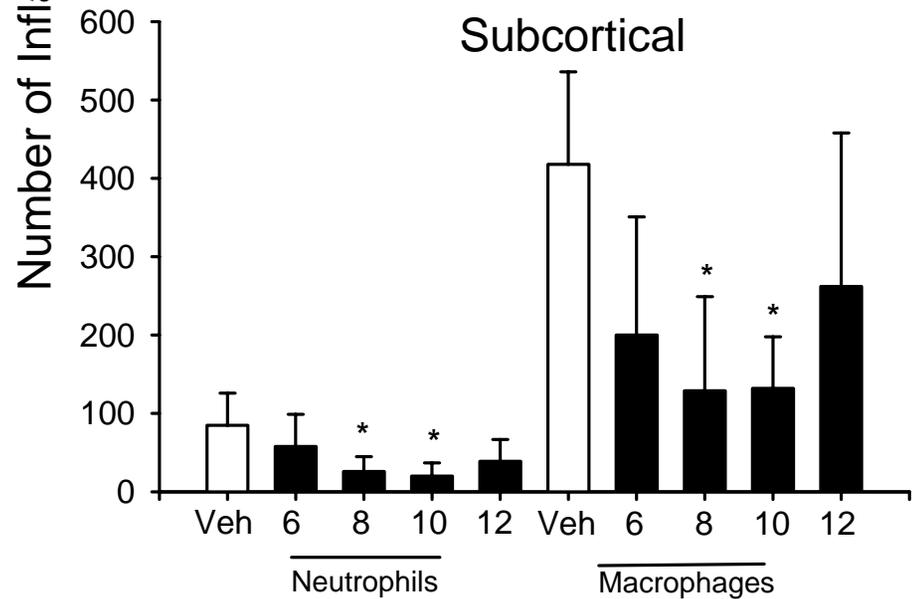
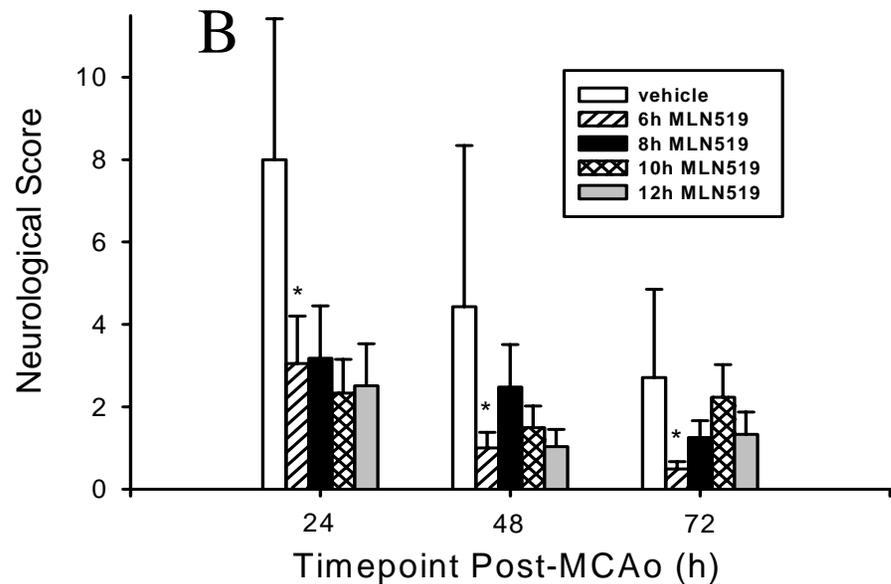
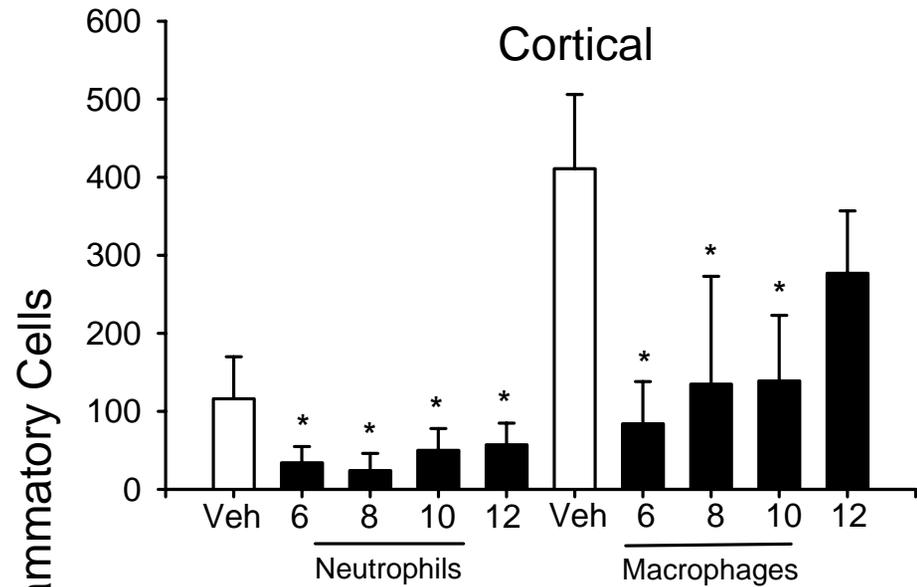
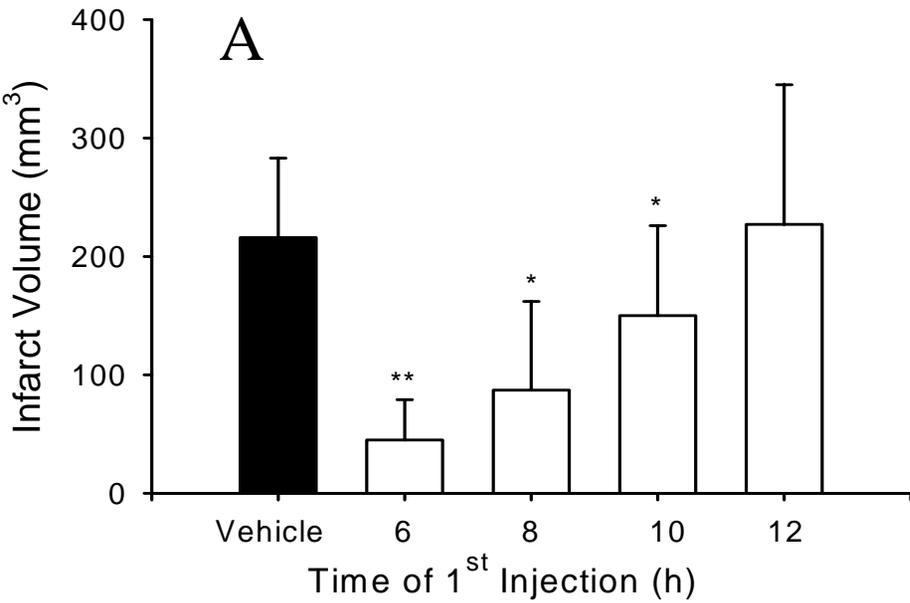
Cell types	Vehicle	MLN519	Percent reduction of OD (%)
Endothelial	0.209 ± 0.069	0.111 ± 0.068*	47
Leukocytes	0.266 ± 0.120	0.147 ± 0.090*	45
Neuronal and glial	0.096 ± 0.060	0.063 ± 0.044*	34

Values are given as mean ± SD.

* $P < 0.001$, independent t -test between MLN519 and vehicle group (intravenous injection delivered 6 hours after middle cerebral artery occlusion).

OD, optical density.

Chronic (3 days) IBI and MLN519 Neuroprotection



Time of 1st Injection

MLN-519: Phase I Clinical Trial

Background Information

- Healthy normal male volunteers**
- Double-blind, placebo randomized trial**
- Two Studies: Ascending single & multiple doses to examine safety & PK**
- End-points = toxicity or 20S inhibition**

Early clinical experience with the novel proteasome inhibitor PS-519

I. M. Shah, K. R. Lees, C. P. Pien¹ & P. J. Elliott¹

University Department of Medicine & Therapeutics, Western Infirmary, Glasgow, UK., G11 6NT and ¹Millennium Pharmaceuticals Inc, Cambridge, MA, USA

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Table 1 Dose escalation and groups.

Group	Number of subjects	Dose of PS-519
1	4	0.012 mg m ⁻² single dose or placebo
2	4	0.04 mg m ⁻² single dose or placebo
3	4	0.12 mg m ⁻² single dose or placebo
4	4	0.25 mg m ⁻² single dose or placebo
5	4	0.5 mg m ⁻² single dose or placebo
6	4	1 mg m ⁻² single dose or placebo
7	3	1.4 mg m ⁻² single dose or placebo
8	4	1.4 mg m ⁻² single dose or placebo
9	4	1.6 mg m ⁻² single dose or placebo
10	4	1.6 mg m ⁻² single dose or placebo
11	4	0.5 mg m ⁻² triple dose or placebo
12	4	1 mg m ⁻² triple dose or placebo
13	4	1 mg m ⁻² triple dose or placebo
14	4	1.3 mg m ⁻² triple dose or placebo
15	4	1.6 mg m ⁻² triple dose or placebo
16	4	1.6 mg m ⁻² triple dose or placebo
17	4	1.6 mg m ⁻² triple dose or placebo

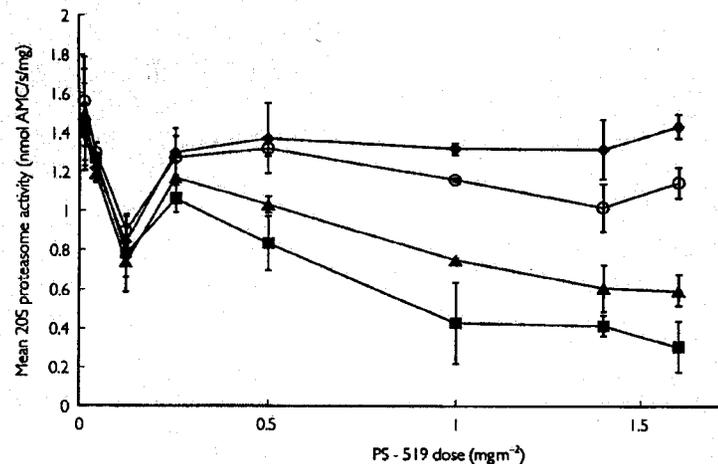


Figure 2 Single dose PS-519:20S proteasome activity.

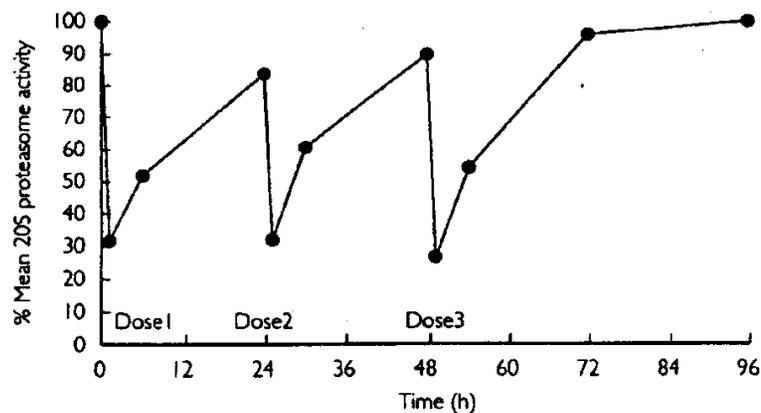


Figure 3 Triple dose PS-519 (1.6 mg m⁻²): 20S proteasome activity.

Table 3 Adverse events.

<i>Adverse effect</i>	<i>Number of subjects affected</i>	<i>Dose group (Placebo or drug)</i>	<i>Onset time</i>	<i>Duration</i>	<i>Severity</i>
Altered taste sensation	11	0.5 mg m ⁻² (2 drug) 1 mg m ⁻² (1 placebo, 4 drug) 1.4 mg m ⁻² (1 placebo, 1 drug) 1.6 mg m ⁻² (1 placebo, 1 drug)	Immediately after giving bolus	2-3 min	Mild
Discomfort in injection arm	13	0.5 mg m ⁻² (4 drug) 1.3 mg m ⁻² (3 drug) 1.6 mg m ⁻² (5 placebo, 1 drug)	During drug administration	10-20 s	Mild
Headache	5	0.5 mg m ⁻² (1 placebo, 1 drug) 1.3 mg m ⁻² (1 drug) 1.4 mg m ⁻² (1 drug) 1.6 mg m ⁻² (1 drug)	> 8 h after giving drug	1-2 h	Mild
Flu-like symptoms	3	1 mg m ⁻² (1 placebo, 1 drug) 1.6 mg m ⁻² (1 drug)	One before study Two: 2 days after study.	1-3 days	Mild