Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Temple University of the Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 01/01/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine Calicat, MLA

4. **Grant Contact Person's Telephone Number:** 215.204.7655

5. **Grant SAP Number:** 4100047651

6. **Project Number and Title of Research Project:** 5 – CBI Agonists as Neuroprotective Drugs

7. **Start and End Date of Research Project:** 7/1/2009 – 6/30/2010

8. **Name of Principal Investigator for the Research Project:** Ronald F. Tuma, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 25,000

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Zachary Reichenbach	Graduate Student	100	$20,000

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Ronald F. Tuma	Professor	5
Ming Zhang	Post Doctoral Fellow	5

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____x_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____x____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E. Do not include funding from your own institution or from CURE (tobacco settlement funds).

Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Selective CB2R agonists modulate inflammatory responses in cerebral ischemia	xNIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	01-Sep-2012	$1,904,550	$0
CB2 receptor activation attenuates damage following stroke	☐NIH ☐ Other federal (specify:_____) x Nonfederal source (specify: __AHA_____)	1/24/13	$74,000	$ PENDING

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

We plan to submit a new application to NIH related to this project.

12. Future of Research Project. What are the future plans for this research project?

We will continue to seek extramural funding to be able to continue the project.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	1
Female				
Unknown				
Total			**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	1
Unknown				
Total			**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	
Black				
Asian				1
Other				
Unknown				
Total			**1**	**1**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No_____x___

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes___x_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

It involved other investigators in cannabinoid research and additional grant funding for the university.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No_____x_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____x____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____x____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

The hypothesis that was to be investigated in this project is that the drug SR141716A, originally developed as a selective CB_1 antagonist, has activity at receptors other than the CB_1 receptor that contribute to its neuroprotective activity and that the mechanisms responsible for its neuroprotective effect are different from those responsible for the protective effect of CB2 agonists. Preliminary results from our laboratory have provided evidence that the CB_1 antagonist SR141716A provides protection for the CNS (central nervous system) during ischemia and trauma (Zhang, Martin et al. 2007). We have also shown that the combination of a CB1 antagonist and a CB2 agonist has a synergistic effect in protecting the nervous system. These finding are novel in that it is speculated that activation of the CB_1 receptor should be neuroprotective. An explanation for the discrepancy between our findings (that a CB1 antagonist is neuroprotective) and the prediction that CB_1 activation would be neuroprotective is that SR141716A functions as an agonist at receptors other than CB1. Elucidation of the mechanism through which SR141716A has a neuroprotective effect could lead to the development of more effective therapies for the treatment of CNS diseases such as stroke and trauma. The therapeutic measures currently available for treatment of these diseases are very limited. In specific aim 1 SR141716A was administered to genetically altered animals that lack the CB1 receptor to determine whether the drug still has a neuroprotective effect following cerebral ischemia and reperfusion. Dose response studies with both SR141716A and a CB2 agonist was conducted to provide data to evaluate receptor interactions using mathematical modeling techniques. In the second specific aim the drug will be administered to animals that have been treated with antagonists for the other receptors (vanilloid and serotonin) that may be activated by SR141716A and contribute to the neuroprotective effect following stroke.

It is expected that the results obtained from this project will significantly enhance our understanding of how activation specific molecules (receptors) on cells within the central nervous system may provide protection from injury. Currently there are no effective treatments to attenuate the damage to the central nervous system following trauma or an interruption in blood flow. The development of more effective drugs that can provide protection to the central nervous system would significantly reduce the damage that occurs following trauma or stroke, and improve the quality of life for individuals suffering from these problems.

Summary of Research Completed

The primary goal of this investigation was to determine if the drug rimonabant (SR141716A), originally developed as a selective CB_1 receptor antagonist, has activity at receptors other than the CB_1 receptor that contribute to its neuroprotective activity and that the mechanisms

responsible for its neuroportective effect are different from those responsible for the protective effect of CB_2 agonists.

Methods

To accomplish this goal, the effect of rimonabant on a number of outcome parameters following stroke were examined in the presence of selective antagonists for the vanilloid and serotonin receptors and in CB_1 and CB_2 knockout mice. Included among the outcome parameters evaluated were white cell rolling and adhesion, infarct size and neurologic score. White cell rolling and adhesion are a prerequisite for white cell invasion, a factor clearly demonstrated to exacerbate damage in ischemia/reperfusion injury. All studies were carried out using C57/Bl6 mice as the animal model.

Cranial Windows were implanted to allow for direct visualization and measurement of the pial microcirculation in the region of ischemia/reperfusion. On the day of cranial window implantation, the animals were anesthetized with an intraperitoneal injection of Ketamine (100mg/ml) - Xylazine (20mg/kg) mixture (1:1) at a dose of 1ml/kg. The head was shaved and positioned in a stereotactic head holder. A 1cm area of skin on the dorsal surface of the skull over the right cortical hemisphere was excised and the periosteum was removed. A 4mm diameter circular craniotomy was performed using a high speed drill (Champ-Air Dental Drill Benco Dental) over the right parietal cortex extending from attachment of temporal muscle to midpoint of sagittal suture in the coronal direction and aligned to middle of sagittal suture, so that the window contained some terminal branches of middle cerebral artery. Normal saline (37 $^{\circ}$C) was dripped over the cranium to avoid thermal injury of the cortex. The dura was removed and exposed brain was kept moist with 37 $^{\circ}$C artificial cerebrospinal fluid (CSF) solution. A 5mm diameter coverglass was then placed over the exposed brain, andan airtight seal was produced using Nexaband Quick seal. The coverglass provided adequate mechanical protection from infection or contamination. A recovery period of four days was allowed between implantation of the cranial window and the induction of transient focal ischemia.

Ischemia/reperfusion injury was induced by transient occlusion of the middle cerebral artery. The animals were anesthetized with an intraperitoneal injection of Ketamine (100mg/ml) - Xylazine (20mg/kg) mixture (1:1) at a dose of 1ml/kg. Body temperature was maintained at 37±5°C by a heating lamp and heating pad. Middle cerebral artery occlusion was achieved by the intraluminal filament methods.(Hata, Mies et al. 1998) Briefly, a midline neck incision was made under the operation microscope, the right common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA) were isolated. The ECA was ligated with 6-0 silk suture distal from the ICA-ECA branch and then cut distally from the ligation point. Another 6-0 silk suture was tied loosely around ECA at close to the origin at the CCA. A blunted 5-0 monofilament nylon suture coated with poly-L-lysine (0.1% in deionized water, Sigma) was introduced through a small incision on ECA and then advanced into the circle of Willis, and finally to the origin of the middle cerebral artery. The silk suture around the ECA stump was tied tightly to prevent bleeding and secure the nylon suture. The nylon suture was removed after 60 minutes occlusion, and ECA was permanently tied. Reperfusion was confirmed when pulsations were again observed in ICA.

A laserPro Blood Perfusion Monitor (TSI Inc) was used to monitor regional cerebral blood flow (rCBF) prior to ischemia, during MCAO and during reperfusion. A 1mm diameter microfiber laser-Doppler probe was attached to the skull 4mm lateral and 2mm posterior of bregma. The MCAO was considered adequate if rCBF showed a sharp drop to 25% of the baseline (pre-ischemia) level, otherwise, animals were excluded.

Leukocyte/endothelial interactions were evaluated before MCAO, 1 hour after MCAO and 24 hours after MCAO. Each vessel was exposed to the light of the microscope for 30 seconds at each viewing to minimizing phototoxicity. Three venules (with diameter 30-50 μm) and three arterioles (with diameter 20-40 μm) in each animal were assessed. The number of rolling leukocyte was considered to be the total number of leukocytes moving along the endothelial cells at substantially slower velocity compared with the midstream blood cell velocity. They were counted when they passed an arbitrary line perpendicular to the longitudinal axis of the vessel over a period of 30 seconds. Adhering leukocytes were defined as the total number of the leukocytes firmly attached to the microvascular endothelium that did not change their location during the entire 30 seconds of observation period. Adhering leukocytes were scored as the number of cells per mm^2 of the vascular surface area, calculated from the diameter and standardized length (100 μm) of the vessel segment under investigation.

For assessment of infarct volume, the animals were euthanized with an overdose of pentobarbital (200mg/kg i.p) 24 hours after MCAO, and then the brains were removed. The brains were chilled in ice for 10 minutes to slightly harden the tissue. Five 2mm coronal sections were cut using a mouse brain matrix (Zivic lab). The brain sections were placed in 2% triphenyltetrazolium chloride (TTC) (Sigma, Inc) dissolved in saline and stained for 20 minutes at 37°C in the dark. The brain sections were then fixed in 4% paraformaldehyde at 4°C for 24 hours, and the anterior and caudal face of each section was scanned by a flatbed color scanner (Microtek Inc). The resulting images were captured as JPEG files and analyzed with NIH image software. The infarct volumes were expressed as mm^3 as well as the percentage of the ipsilateral hemisphere.

The severity of neurological deficits was evaluated twenty four hours after ischemic insult using a five-point deficit score(0=normal motor function; 1=flexion of torso and of contralateral forelimb upon lifting of the animal by tail; 2=circling to the contralateral side but normal posture at rest; 3=leaning to contralateral side at rest; and 4=no spontaneous motor activity).

Statistical analysis

Numbers of leukocytes rolling on and adhering to venules or arterioles were analyzed by two-way (treatments, times) analysis of variance (ANOVA) with repeated measurements followed by Bonferroni test. One-way ANOVA was used for analyzing differences in either infarct volume or neurological score. Data were presented as means±SEM. A statistically significant difference was assumed at $P<0.05$.

Results

The results of our investigation demonstrated that when rimonabant and a selective CB_2 agonist (O-1966) were co-administered, cerebral blood flow during occlusion increased above the levels measured in untreated animals. (Figure 1). Neither the selective CB1 antagonist nor CB2 agonist given in isolation were able to increase flow during occlusion. This increase in flow was

accompanied by an increase in the diameter of pial arterioles (Figure 2).This is an important finding since this drug combination may have potential to enhance collateral blood flow during stroke and perhaps myocardial infarction. We were also able to demonstrate that this effect was not influenced when administered in the presence of an antagonist for the vanilloid receptor (capsazapine), but was significantly attenuated when administered in the presence of an antagonist for the serotonin receptor (WAY100135). This was the first evidence that rimonabant may have activity at the serotonin receptor.

We were also able to demonstrate that the synergistic effect of the selective CB2 agonist and CB1 antagonist in reducing infarct size was not influenced by capsazepine, but was significantly attenuated when a serotonin antagonist was administered (Figure 3). In addition the synergistic effect of the combined CB1 antagonist and CB2 agonist were maintained when given to CB1, but not CB2 deficient animals. This provided evidence that the protective effect of the combined therapy did not require CB1 activation.

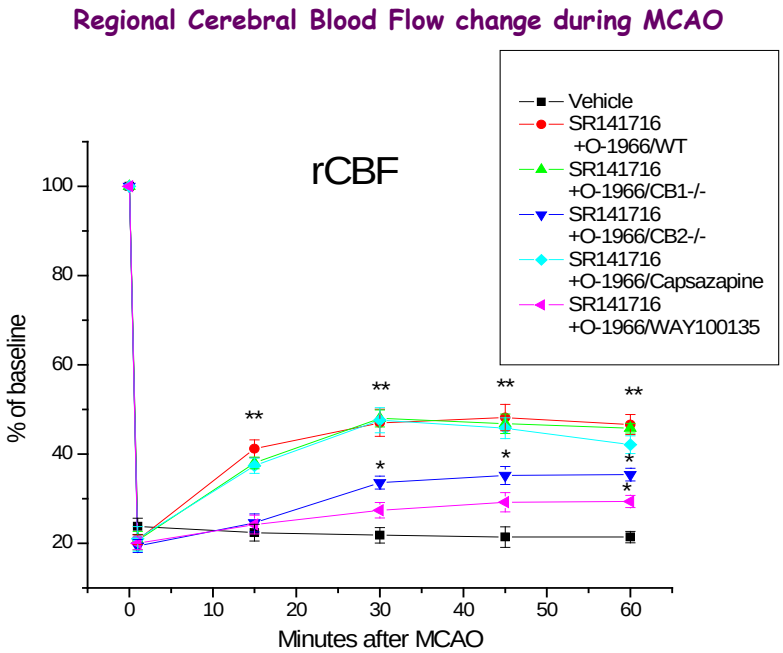
Although the synergistic effect of the CB1 antagonist and CB2 agonist involve the serotonin receptor, we were also able to demonstrate that rimonabant, when given alone is still neuroprotective, but this mechanism of neuroprotection is independent of the serotonin receptor (Figure 4). The results from this component of the study correct a number of misconceptions in the literature. Although CB1 receptor activation was reported to be neuroprotective though inhibition of excitotoxicity, this was later shown to be the effect of CB1 induced hypothermia. The results of our investigation showed that the CB1 receptor antagonist reduced infarct size and improved neurologic score when given alone. It also showed that blocking the serotonin receptor had no influence on this protective effect.

It is usually reported in the literature that the CB2 receptor predominantly influences inflammatory cells while the CB2 receptor only influence synaptic transmission. The results of our investigation also provided evidence that this is not the case, since administration of rimonabant significantly attenuated white blood cell rolling and adhesion (Figure 5). The results of this component of the study indicate a direct effect of the CB1 antogonist on endothelial and/or white blood cells. These studies provide evidence for a proinflammatory effect of the CB1 receptor.

Summary

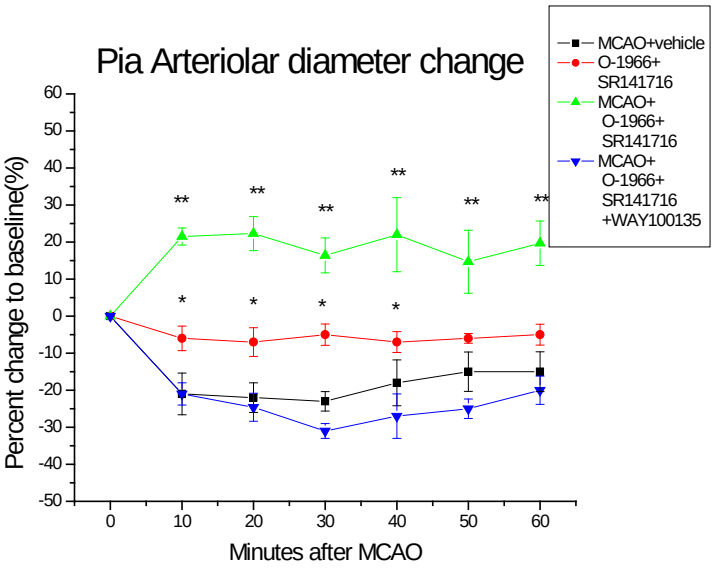
The results of our investigation provide new information about the actions of rimonabant and the CB1 receptor. These results demonstrate that the combination of a CB2 agonist and CB1 antagonists produces unique effects, including: enhanced blood flow, reduced infarct size and improved neurologic function, and that these effects involve activation of the serotonin receptor. The vanilloid receptor does not appear to be involved in these responses. In addition the results of this project demonstrate a proinflammatory effect for the CB1 receptor, and that this effect involves endothelial cells and/or white blood cells.

FIGURE 1



N=5 in each group, * p<0.05 vs MCAO+ vehicle; ** p<0.01vs MCAO+vehicle

FIGURE 2



N=4 in each group, * p<0.05 vs MCAO+ vehicle; ** p<0.01vs MCAO+vehicle

FIGURE 3

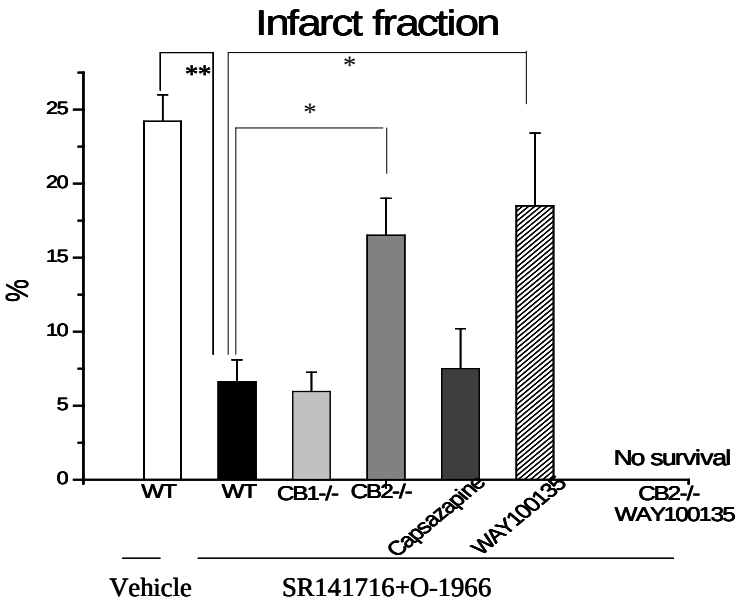
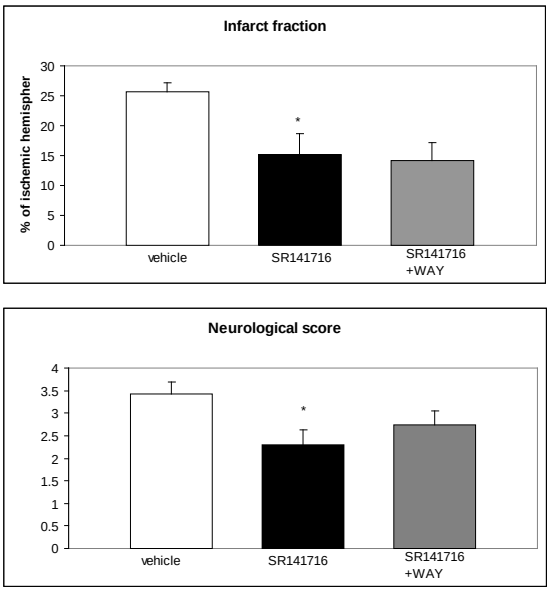
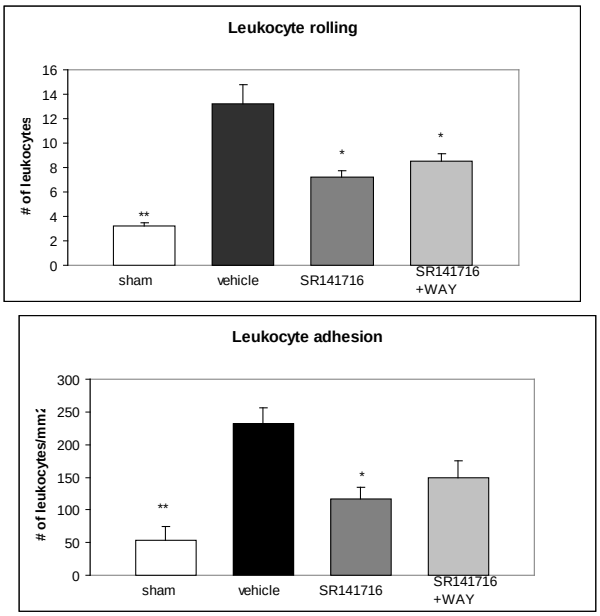


FIGURE 4



SR141716 and WAY were given at 1 hour after ischemia, at both 5mg/kg by i.p.

FIGURE 5



SR141716 and WAY were given at 1 hour after ischemia, at both 5mg/kg by i.p.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
x_____No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
x_____No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__x___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

_____Yes

_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____ No__x_____

If yes, please describe your plans:

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___x_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____x_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant*

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Ronald F. Tuma, Ph.D.	Professor of Physiology
eRA COMMONS USER NAME tumarf	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Muhlenberg College	B.S.	1966-70	Biology
Temple University School of Medicine	Ph.D.	1970-75	Physiology
Temple University School of Medicine	Postdoctoral	1975-76	Physiology

A. Personal Statement

For the past 25 years, research in my laboratory has focused on investigations of leukocyte/endothelial interactions in the central nervous system and how modulation of these interactions can influence CNS damage in a number of disease states including: ischemia reperfusion injury, spinal cord injury, EAE and traumatic brain injury. We have a considerable number of publications that have employed intravital microscopy for a significant component of the work done. The studies of stroke and EAE have all employed cranial windows for direct visualization of the pial microcirculation. In addition to allowing us to study white cell rolling and adhesion, use of the cranial window has also allow us to make direct measurement of vascular diameter and permeability changes in the living animal. My experience in the study of the cerebral microcirculation is reflected in my selection as one of the editors of the Handbook of Physiology-Microcirculation published through the American Physiological Society and my authorship of the chapter on “Cerebral Blood Flow”. The research efforts in my laboratory have focused on modulating the endocannabinoid system as a means of attenuating CNS damage following stroke, spinal cord injury and autoimmune disease for the past seven years. Our laboratory was the first to demonstrate the CB2 selective agonists were protective in mouse models of multiple sclerosis, stroke and spinal cord injuries. Our laboratory was also the first to demonstrate a synergistic effect of a CB2 agonist and a CB1 antagonist when given in combination. We were also the first to demonstrate a neuroprotective effect of hypertonic saline following spinal cord injury. We have been involved in evaluations of the neuroptrotective properties of hypertonic saline for over fifteen years. I therefore feel that my expertise in the areas described above will allow me to make significant contributions to the current proposal. The collaborative efforts between Dr. Doina Ganea’s laboratory and my laboratory have allowed us to combine both inravital microscopic applications and extensive neuroimmunologic examination to study the effects of cannabinoid receptor 2 (CB2R) signaling in various models of neuroinflammation. Our research project related to the effects of CB2R agonists in EAE has been funded by NIAID in 2009.

B. Positions and Honors.

Positions and Employment

1977 Assistant Professor of Physiology, Temple University School of Medicine

1982 Associate Professor of Physiology, Temple University School of Medicine
1984 Guest Professor, Department of Experimental Surgery,
Heidelberg University School of Medicine, Heidelberg Germany
1986- Associate Professor of Neurosurgery, Joint Appointment
1994-2006 Professor and Chairman of Physiology, Temple University School of Medicine
1997-2002 Senior Associate Dean for Research, Temple University School of Medicine
2006-2008 Senior Associate Dean for Education
1990-present G.H. Professor of Physiology, Temple University School of Medicine

Other Experience and Professional Membership
Grant and Peer Review

1999 President of the Microcirculatory Society
1997, 2001 Reverse Site Visit Chair (NIA) Growth Hormone & IGF in CNS and Cerebrovascular Aging,
2002 Reverse Site Visitor (NIA) Growth Hormone & IGF in CNS and Cerebrovascular Aging
2002 NHLBI Special Emphasis Panel, Effects of whole body ischemia and subsequent blood flow restoration on cardiovascular and neurological function.
2003 Advanced Technology Panel -Congressionally-Directed Peer Reviewed Medical Research
2009-2010 Nielsen Foundation Grant Reviewer
2010 Panel Member, Department of Defense Congressionally Directed Medical Research Programs, Spinal Cord Injury Research Program, Exploratory Hypothesis Development Award

Professional Membership and Honors

1975-present	Member, American Physiological Society
1975-present	Member Microcirculatory Society
1980, 1993, 1995	Golden Apple Awards for Teaching
1989	George E. Sowell Award for Excellence in Basic Science Teaching
1990	Lindback Award for Distinguished Teaching
1996	Alpha Omega Alpha The National Honor Medical Society
2004	Editorial Board, Microvascular Research
2006- present	Member Society on NeuroImmune Pharmacology
2007- present	Member International Cannabinoid Research Society
2004	Charter Member Academy of Distinguished Educators in Medicine Temple University School of Medicine

C. Selected peer-reviewed publications (Total 95 peer-reviewed publications)
<u>Most relevant to the current application</u>

1. Mao YY, Zhang M, Tuma RF and Kunapuli SP Deficiency of PAR4 attenuates cerebral ischemia/reperfusion injury in mice. Journal of Cerebral Blood Flow and Metabolism (In Press)
2. Zhang M, Martin BR, Adler MW, Razdan RK, Jallo JI, Tuma RF, Cannabinoid CB(2) receptor activation decreases cerebral infarction in a mouse focal ischemia/reperfusion model. J Cereb Blood Flow Metab 27:7(1387-96)2007 Jul PMCID 17245417.
3. Zhang M, Martin BR, Adler MW, Razdan RJ, Kong W, Ganea D, Tuma RF, Modulation of cannabinoid receptor activation as a neuroprotective strategy for EAE and stroke. J Neuroimmune Pharmacol 4:2(249-59)2009 Jun 1 PMCID 9255856.
4. Zhang M, Martin BR, Adler MW, Razdan RK, Ganea D, Tuma RF, Modulation of the balance between cannabinoid CB(1) and CB(2) receptor activation during cerebral ischemic/reperfusion injury. Neuroscience 152:3(753-60)2008 Mar 27 PMCID 18304750

5. Zhang M, Adler MW, Abood ME, Ganea D, Jallo J, Tuma RF, CB2 receptor activation attenuates microcirculatory dysfunction during cerebral ischemic/reperfusion injury. Microvasc Res 78:1(86-94)2009 Jun PMCID 19332079.