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Acute anti-inflammatory approaches to ischemic stroke

Gregory J. del Zoppo

Departments of Medicine (Division of Hematology) and Neurology, University of Washington School of Medicine, Seattle, Washington

Abstract

In preparation for designing and undertaking trials of strategies that can modulate “innate inflammation” to improve outcomes of ischemic injury, consideration of approaches that have managed cellular inflammation in ischemic stroke are instructive. Robust experimental work has demonstrated the efficacy (and apparent safety) of targeting PMN leukocyte–endothelial cell interactions in the early moments following focal ischemia onset in model systems. Four clinical trial programs were undertaken to assess the safety and efficacy of inhibitors to PMN leukocyte interactions with the endothelial cell during ischemic stroke. Experiences in those clinical trial programs indicate specific limitations that halted progress in this line of investigation before an adequate hypothesis test could be achieved. Although innate inflammation is a central part of injury evolution following focal ischemia, great care in the translation from experimental studies to Phase I/II clinical safety assessments and to the design and conduct of Phase III trials is needed.

Keywords

PMN leukocytes; ischemic stroke; inflammation; clinical studies; treatment

Introduction

One component of “innate inflammation” is the contribution that cellular inflammation from systemic leukocyte subpopulations makes to the development of cerebral injury following focal ischemia. Based broadly on strong experimental work, Phase II and III studies to test the impact of activated leukocytes on brain tissue in the setting of ischemic stroke were undertaken.¹ The general hypothesis to be tested stated that inhibition of polymorphonuclear (PMN) leukocyte adhesion, activation, or transmigration is likely to significantly reduce the ultimate volume and neurological impact of focal ischemic injury, by inhibiting the development of infarction. Important for the design of clinical trials to determine the benefit-risk relationships of interventions in “innate inflammation” are concerns arising from the clinical studies that have already tested various components of this hypothesis.

Correspondence: Prof. Gregory J. del Zoppo, Division of Hematology, Department of Medicine, Department of Neurology, University of Washington School of Medicine, Box 359756 at Harborview Medical Center, 325 Ninth Avenue, Seattle, WA 98104, grgdlzop@u.washington.edu.

Conflict of interest

The author declares no conflict of interest.

The microvessel as interface

The exquisite sensitivity of neurons to ischemic injury is paralleled by events that happen rapidly following the onset of focal ischemia in the microvessels. The endothelial cell layers of cerebral microvessels provide the brain tissue interface for cellular inflammation.² Responses of cerebral microvessels to ischemia include i) loss of the permeability barrier with the extravasation of plasma constituents, ii) leukocyte adhesion to endothelial cell-leukocyte receptors leading to transmigration of leukocytes, and/or obstruction of the microvasculature (the focal “no-reflow” phenomenon), and iii) processes that lead to degradation of the basal lamina matrix.^{3–7} These changes in the microvasculature vary with the location of the injury in the arterial territory-at-risk, and in the time from injury onset. Of these responses, the adhesion of leukocytes to the activated endothelial cell surface has provided several targets for intervention.

Expression of endothelial cell-leukocyte adhesion receptors on endothelial cells in microvessels activated in the ischemic territory is relatively sequential (Fig. 1). P-selectin appears within 60–90 minutes, ICAM-1 appears within 4 hours, and E-selectin appears 7 hours after the onset of focal ischemia (by middle cerebral artery occlusion, MCA:O).^{5,6} The maximum transit of human leukocytes is 6–8 hours following MCA:O in the non-human primate. The activation of human leukocytes within the vasculature can, together with activated platelets, cause microvessel obstruction that has been termed focal “no-reflow.” Blockade of the β_2 -integrin CD18 early during reperfusion of the occluded MCA significantly increases the patency of capillaries and post-capillary venules in the ischemic territory. This implies that disrupting adhesion between PMN leukocytes and the endothelial cell surface early following ischemia onset, during reperfusion, can allow microvascular patency. Endothelial adhesion and PMN leukocyte migration are a complex processes.

Monocytes follow PMN leukocytes in their incursion into the ischemic territory. Activation of resident microglia and of monocyte-macrophages can contribute to long-term consequences of the inflammatory response.^{8,9} In the Wistar rat, Garcia *et al* noted significant invasion of ischemic brain tissue at 24 hours after the onset of focal ischemia.¹⁰

Hence, it has been postulated that PMN leukocytes can contribute to cerebral injury following focal ischemia by impairing local blood flow via microvessel obstruction, injuring endothelial cells and extracellular matrix (ECM) by hydrolytic enzymes and free radicals that are generated in the interaction, promoting intravascular thrombus formation together with platelet activation, and expressing cytokines and chemotactic factors that promote expansion of the inflammatory response.

Evidence for the roles of leukocytes in focal cerebral ischemic injury

Early studies in both animal models and patients failed to appreciate the contributions of innate inflammation and cellular inflammation to the evolution of focal ischemic injury and ischemic stroke. Hallenbeck *et al* first described the potential involvement of leukocyte activation in a limited form of injury to the central nervous system (CNS).¹¹ Subsequently, del Zoppo *et al* demonstrated the early appearance of PMN leukocytes within hours of the onset the focal ischemia (Fig. 1).³ A number of studies followed, which confirmed the

participants in leukocyte-vascular adherence and “no-reflow,” leukocyte transmigration, and activation and receptor exposure on both endothelial cells and PMN leukocytes. Early studies, involving mostly strategies for inhibition of receptor-receptor interactions, and cell reduction techniques, variably confirmed the ability in small animal model systems for inhibition to reduce injury volumes.

Most importantly, knockout preparations with deletions of P-selectin/E-selectin, intercellular adhesion molecule-1 (ICAM-1), or NADPH oxidase demonstrated significant decreases in infarction volume in those murine preparations.^{12–16} Of those, deletion of NADPH oxidase, an enzyme involved in the PMN leukocyte respiratory burst, reduced infarction volume significantly. Studies from several laboratories examined the impact of the blockade of P-selectin/E-selection, ICAM-1, or ICAM-1 in the presence of rt-PA on infarction development. The majority of studies demonstrated significant reduction in injury volume in small animal models. Those observations, and the known role of PMN leukocytes in secondary injury in the myocardium and skeletal muscle, suggested targets for clinical intervention in ischemic stroke.

Clinical strategies

Acute application of anti-inflammatory strategies intended to block cell-mediated inflammation, based on experimental work, have so far addressed three targets in evolving ischemic stroke patients using four strategies (Table 1). These include a humanized monoclonal antibody (MoAb) against ICAM-1 expressed on activated endothelial cells, a humanized MoAb against PMN leukocyte CD18, the recombinant neutrophil inhibitory factor (rNIF) that prevents neutrophil adhesion, and an inhibitor of the action of the interleukin-1 β (IL-1 β), the interleukin-1 receptor antagonist (IL-1ra). Trials were designed at various stages, either Phase II or Phase III. So far, no Phase III acute interventional trial has been successfully completed that demonstrated improvement with the intervention.

Trials

Enlimomab study—This study tested the hypothesis that PMN leukocyte adherence to endothelium and transmigration could be successfully blocked with the endothelial cell adhesion receptor ICAM-1 inhibitor RR 1.1 within 6 hours of stroke symptom onset.¹⁷ Improvement in 3-month disability outcome and in mortality at one year were the intended efficacy outcome events. Stable RR1.1 plasma concentrations were obtained throughout the infusion period. However, the enlimomab preparation had a significantly negative effect on survival in treated patients, compared with placebo. Indeed, a significant worsening of outcome at 3 months by the modified Rankin Score (mRS) was observed ($2p = 0.004$).¹⁸ An excess of fevers and mortality were seen in the enlimomab group, which were related to the RR1.1 preparation. However, the cause of deterioration of these patients was not the fever *per se*. Hence, those who received enlimomab displayed reduced functional recovery by mRS, Barthel index and NIHSS at day 90, increased mortality at day 90, increased incidence of serious adverse events, and more frequent infections.

Contributors to CNS injury that might explain these observations included the development of human anti-mouse antibodies, the activation of intracellular signaling of the endothelium

through ICAM-1, complement activation by the enlimomab preparation, and activation of non-vascular cells in the brain. A subsequent study, “bedside-to-bench-to-bedside,” demonstrated that sequential infusion of heterologous antibodies in a rodent focal ischemia model could significantly increase cerebral injury volume.¹⁹ This suggested that augmentation of *in situ* cerebral inflammatory processes was probably at play.

The HALT stroke study—The hypothesis of this study was that inhibition of PMN leukocyte adherence and transmigration by blocking the adhesive function of PMN leukocyte β_2 integrin CD18 with the humanized MoAb Hu23F2G, within 12 hours of stroke symptom onset, could improve 3-month disability and outcome at one year. A dose-escalation study against placebo was performed in Phase II format to determine the safety of this approach and potential dose efficacy. A concentration of 1.5 mg/kg Hu23F2G in single dose was found to be safe, with a modicum of increased fever. However, a twice-daily infusion of Hu23F2G at this dose appeared to produce an improvement in mRS score. Based on this Phase II data, a Phase III study was performed. However, the sponsor terminated the study at first-interim analysis, when a “futility analysis” suggested that no benefit of the treatment would occur if the study was completed. No public information regarding the database, outcomes, safety issues, or relative number of SAEs has appeared.²⁰

rNIF stroke study (ASTIN)—The hypothesis tested in this study was that inhibition of PMN leukocyte adherence and transmigration by blocking PMN leukocyte β_2 integrin CD18 with recombinant NIF within 6 hours of stroke symptom onset could improve 3-month disability outcome, measured by mRS. rNIF is a 41 kDa CD18 antagonist from hookworm species that interacts with domain I of CD11b/CD18.²¹ A Phase II study was performed to examine the effects of rNIF in patients, based upon a limited experimental data set in rodent MCA:O models.^{22,23} The Phase II clinical trial design was based on a Bayesian approach, wherein patients were placed in open dose-rate cohorts, depending upon previous dosing experience in the series.^{24,25} In the trial, 966 patients were enrolled in 16 dose tiers, but no dose-rate effect was observed compared to placebo. This result differed from a theoretical model upon which the study design was based. However, the results at week 10 did not differ from the results at the end of the study (week 66). The project did not continue after completion of this trial.

IL-1ra stroke study—Here, the hypothesis stated that inhibition of IL-1 β , generated in the setting of ischemic stroke from CNS sources, would be safe and reduce cerebral injury assayed by clinical outcomes. Significant experimental background suggested that in rodents, the receptor antagonist IL-1ra, already available for clinical use in arthritis, would be safe and beneficial.^{26,27} The agent was applied within 12 hours of stroke symptom onset and was intended to improve disability outcome at 3 months. In a small pilot study, 34 patients were randomized to 2 mg/kg/hr over 72 hours or matched placebo.²⁸ No adverse events were attributed to the IL-1ra treatment among 34 patients randomized, and SAEs constituted 5 patients in each group. There was a non-significant increase in patients with mRS 0–1. A second dose-ranging study was recently reported.²⁹ To date, no Phase III study has been completed.

Limitations in the anti-inflammatory approaches to stroke

Based upon a strong experimental background for the early clinical work, the hypothesis that inhibition of PMN leukocyte adhesion, activation, and/or transmigration could be beneficial in the development of focal ischemia in patients has received a very limited test. Failing a remarkable improvement in outcome, these studies together have been taken to suggest that the hypothesis has failed. In actuality, the theoretical hypothesis based upon pre-clinical data has not been fully tested in the clinical setting.

A number of limitations have been exposed variously by this prospective clinical experience that owe to the application of pre-clinical data to the clinical setting, design issues, suitability of the agent for testing, and outcome interpretation. Among these (Table 2), failure to translate observations from animal models into the clinic and from Phase II studies into Phase III studies stand out. Perhaps more central to the problem of translation has been the failure to understand how cellular inflammation causes injury in the CNS, and the probability that endogenous mechanisms of inflammation (“innate inflammation”) are set into motion, which can contribute to the injury evolution beginning with the initial ischemic event.

Those studies exposed a number of weaknesses that must be addressed in future trials of interventions to modulate “innate inflammation.” These include the appropriate application of basic principles of immunology, a more thorough understanding of brain injury mechanisms, the need for an adequate pre-clinical data set to translate experiments to clinical trial design, the translation of observations from Phase II studies into Phase III studies, avoidance of “novel” trial design elements not previously or adequately tested, the need to properly validate the outcome measures used, elimination of conflict of interest, and full consideration of alternative hypotheses.

Beyond cellular inflammation

Nearly ten years on from the initial experience with cellular inflammation, new insights into the nature of cell-cell interactions and receptor mediated processes have led to further understanding of PMN leukocyte activation and transmigration. The vascular cell responses to ischemia include not only the endothelium, but also the contributions of astrocytes, pericytes, and microglial cells, which themselves each have a specific response to ischemia that involve elements of cellular inflammation. Furthermore, each of the cells within the “neurovascular unit” have a response to ischemia that involves inflammation.² An appreciation of these elements and their role in innate inflammation will be necessary for future success in clinical trials.

These considerations indicate that the fundamental hypothesis that reducing or ablating the impact of leukocyte-mediated injury on the evolution of the infarction following the onset of focal CNS ischemia has not been fully or adequately tested, and therefore remains open. Appreciation for the way in which the components of the “neurovascular unit” can interact with each other may be very important in understanding both the endogenous inflammatory responses to ischemia and how events within the cerebral microvasculature can affect processes within the neuropil.

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4 hours post-MCA:O

maximum transit at 6-8 hours post-MCA:O

P-selectin	60-90	min
ICAM-1	4+	hr
E-selectin	7+	hr

Figure 1. Interaction of PMN leukocytes with activated endothelium within the ischemic territory (clockwise from upper left). Adherence of PMN leukocytes to the endothelial lining of a post-capillary venule 4 hours after MCA:O. Sequence of events involving rolling, firm adhesion, and transmigration of PMN leukocytes through the wall of the post-capillary venule following the onset of focal ischemia. The appearance of endothelial cell-leukocyte adhesion receptors after ischemia onset. Occlusion of cerebral microvasculature (4.0–30.0 μm diameter) observed in the territory undergoing ischemia/reperfusion (I/R) compared with the normoxic territory.³⁻⁶

TABLE 1

Strategies to inhibit impact of cellular inflammation acutely in ischemic stroke.

agent	design	phase	status
anti-ICAM-1 MoAb	trial of efficacy and safety of enlimomab (RR 1.1) vs placebo *	III	complete
anti-CD 18 MoAb	trial of Hu23F2G anti-adhesion to limit cytotoxic injury un acute ischemic stroke (HALT stroke) *	pilot III	complete aborted
rNIF	trial of recombinant neutrophil inhibitory factor in acute stroke *	II	complete
IL-1ra	trials of IL-1 β inhibition in acute ischemic stroke	II	complete

* multi-centre

TABLE 2

Limitations of clinical trials undertaken to block cellular inflammation acutely in ischemic stroke.

intervention exacerbated evolving CNS injury
failure to apply basic principles of immunology
inadequate understanding of brain injury mechanisms
exacerbation of systemic effects
exacerbation of pre-existing infection or inflammation
failure to require an adequate pre-clinical dataset to translate experiment to clinical trial design
failure to translate observations from phase II studies to phase III
use of “novel” trial design elements not previously or adequately tested
failure to adequately validate outcome measures to be used
failure to appreciate alternative hypotheses
conflict of interest
failure of the fundamental hypothesis