

neurological diseases such as ischemic stroke and spinal cord injury (SCI). Barriers to successful neuroprotective drug development include a high rate of drug metabolism, rapid central nervous system clearance, and difficulty penetrating the blood-brain and blood-spinal cord barriers. By enhancing pharmacokinetic profiles and improving neurovascular access, nanotechnology has been shown both to improve drug efficacy and to promote brain delivery.<sup>1</sup> One known neuroprotective agent, adenosine, exerts pleotropic protective functions by decreasing excitatory amino acid release, limiting calcium influx and free radical formation, promoting ATP formation, modulating astrocytic and microglial cells, and minimizing *N*-methyl-D-aspartate receptors.<sup>2</sup> Unfortunately, adenosine is unable to penetrate the blood-brain or blood-spinal cord barrier, has a short half-life, and promotes cardiovascular depression at high doses.

In a recent issue of *Nature Nanotechnology*, Gaudin et al<sup>3</sup> generate an intravenous nanoparticle through the bioconjugation of adenosine with squalene (a biocompatible lipid). This squalenoylation technology creates an amphiphilic prodrug that spontaneously forms nanoparticles that are 120 nm in size, allowing prolonged drug interaction with the neurovascular unit while minimizing toxic side effects. In a middle cerebral artery occlusion model, mice were subjected to 2 hours of occlusion followed by 22 hours of reperfusion. Squalenoyl-adenosine (SQAd) nanoassemblies (7.5 or 15 mg/kg) were delivered intravenously before ischemia. Infarct volumes were reduced to  $31 \pm 2 \text{ mm}^3$  in the 7.5-mg/kg group and  $17 \pm 1 \text{ mm}^3$  in the 15-mg/kg arm. These volumes were significantly decreased compared with the control arms: 5% dextrose ( $49 \pm 1 \text{ mm}^3$ ), free adenosine (5.5 mg/kg,  $55 \pm 3 \text{ mm}^3$ ), or unconjugated squalenoyl nanoassemblies (9.45 mg/kg,  $44 \pm 2 \text{ mm}^3$ ; Figure). The authors went on to show that the timing (2 hours after occlusion or 24 hours after permanent occlusion in the absence of reperfusion) of SQAd delivery did not significantly alter infarct size ( $23 \pm 2$  vs  $24 \pm 4 \text{ mm}^3$ , respectively). Interestingly, systemic administration of both adenosine-1 (CCPA) and adenosine-2 (CGS21680) receptor agonists resulted in high mortality owing to adverse cardiac events and did not confer neuroprotection, respectively.<sup>3</sup> These results suggest that increased drug bioavailability rather than the timing of drug delivery or direct adenosine receptor activation accounts for the neuroprotective effect observed with the SQAd nanoassemblies.

The pathogenesis of secondary SCI shares many of the same injury mechanisms known to play a role

in cerebral ischemia. To assess the effect of SQAd on SCI, behavioral and ultrastructural studies were performed on Sprague-Dawley rats subjected to a T9 contusion injury.<sup>3</sup> To reproduce an SCI, a 4-g weight was dropped from a height of 12.5 mm after a thoracic laminectomy. SQAd nanoassemblies (32 mg/kg), SQ nanoassemblies (20.5 mg/kg), free adenosine (11.5 mg/kg), or 5% dextrose was administered 5 minutes after injury. Functional evaluations were performed in a blinded fashion at 24, 48, and 72 hours and 28 days, followed by ultrastructural analysis of a sample of contused tissue 48 hours and 28 days after injury. Animals receiving dextrose or free adenosine were unable to move their hind limbs after injury; on structural assessment, visible contusion of their cord was observed. In the treatment arm, the animals receiving SQAd demonstrated coordination between their hind limbs and forelimbs during ambulation without a visible contusion on ultrastructural studies. Finally, with the use of the the Basso, Beattie, and Bresnahan grading scale, SQAd animals scored a 14.4, a sign of axonal transduction through a lesion site.

The exact neuroprotective mechanism of these SQAd nanoassemblies remains unclear. In the stroke model, ischemic microvessels were found to be patent 6 hours after middle cerebral artery reperfusion, whereas in the control arm, ischemic capillaries were occluded by erythrocytes. Furthermore, SQAd-treated mice lacked swelling of astrocytic end feet around microvessels and endothelial nuclei compared with control mice, an indication that adenosine released from the nanoassemblies penetrates endothelium to interact with the microcirculation and pericyte receptors. Most important, the effective dose of adenosine in the SQAd compound did not cause systemic vasodilation and cardiovascular collapse, a side effect that would have been unavoidable with an unbound nucleoside administered at a neuroprotective dose.

Nanotechnology and squalenoylation techniques have been avidly applied to anticancer and antiretroviral compounds but have been used less in stroke and SCI. Managing this technology without restricting pharmaceutical design will prove to be a difficult but essential task in advancing the therapeutic use of nanotechnology in neurological diseases.

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## Focused Ultrasound With Microbubbles Increases Temozolomide Delivery in U87 Transfected Mice

**M**alignant astrocytomas, particularly glioblastoma multiforme, are among the deadliest of all human cancers, with a median survival of approximately 15 months. Aggressive treatment with maximal resection followed by chemotherapy and radiation has prolonged survival a matter of months.<sup>1</sup> The mechanism of resistance of these tumors to traditional treatment strategies is multifactorial, but the inability to effectively deliver therapeutic agents across the blood-brain barrier (BBB) is clearly one of the major causes of treatment failure. As a result, penetration of endothelial tight junctions of the BBB to provide increased drug delivery is an area of intense research.<sup>2-4</sup> In this report, we review a recent study demonstrating increased delivery of temozolomide (TMZ) to implanted glioma tumor lines in mice using focused ultrasound with microbubbles (FUS).<sup>5</sup>

Initially, the investigators examined the effects of 2- and 5-W delivery of FUS on tumor-free mice. The 2-W delivery of FUS was shown to open the BBB and did not induce damage to normal tissue via hematoxylin and eosin and Evans Blue staining. The 5-W treatments opened the BBB in a wider distribution but caused extravasation of erythrocytes on gross examination and hematoxylin and eosin and Evans Blue staining. For this reason, all FUS treatments in the subsequent experiments were carried out with 2-W strength.

U87 glioma cells were grown in culture and stereotactically implanted into the striatum of 5- to 7-week-old immunocompromised male mice. Thirty-nine mice received TMZ treatment alone, and 43 received TMZ treatment after FUS. Treatment began when the tumor mass was detectable on magnetic resonance imaging, and all mice were sacrificed after treatment. Implanted tumor, contralateral normal brain, and peripheral blood were harvested and analyzed via liquid chromatography/mass spectroscopy to

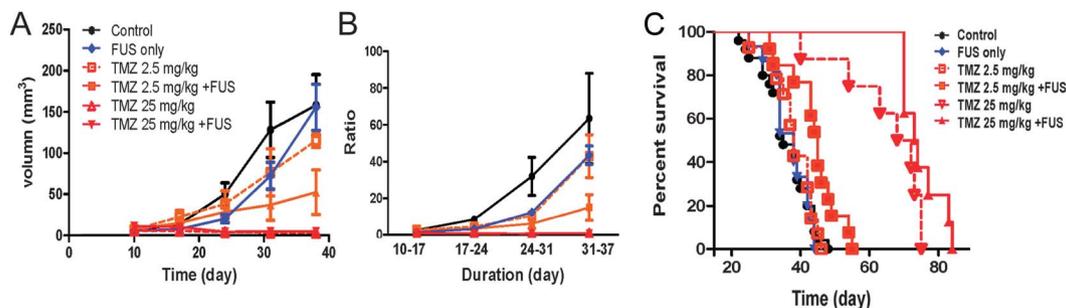


Figure. The effects of temozolomide (TMZ) and focused ultrasound (FUS) on tumor volume (A), growth ratio (B), and overall survival (C) of nude mice with stereotactically implanted U87 glioma xenografts. Note the marked decrease in tumor burden and increased overall survival in both groups treated with TMZ at 25 mg/kg. Modified from Liu et al.<sup>5</sup>

determine TMZ levels. Within plasma and contralateral brain, the concentration and rate of degradation of TMZ were identical between FUS-treated and untreated groups. Within tumor samples, TMZ levels were higher in the FUS-treated group, but this difference did not reach statistical significance. The rate of degradation was significantly slower in the FUS-treated group.

In a second arm of the study, implanted mice were divided into 7 groups: no TMZ or FUS and 2.5, 5, and 25 mg/kg TMZ with or without FUS. Treatment was started 10 days after implantation and carried out for 3 consecutive days. Mice were sacrificed when tumors reached a volume of  $\geq 200$  mm<sup>3</sup> or when 20% body weight was lost. In mice treated with FUS and no TMZ, the authors observed a statistically significant decrease in tumor growth between days 14 and 28, but no differences were noted in long-term tumor volume or growth rates. These mice showed a similar survival (35 days) compared with those that were not treated with TMZ (38 days). The addition of FUS to TMZ was most notable on tumor growth at a dose of 2.5 mg/kg. Tumor volume at 38 days in the TMZ + FUS group was 52.6 mm<sup>3</sup> compared with 115.2 mm<sup>3</sup> in the TMZ without FUS group, with a tumor growth ratio reduction of

42.87 to 14.95 ( $P < .05$ ). Mice in the 2.5 mg/kg TMZ + FUS group showed a significantly longer median survival of 45 days compared with 38 days in the control group. At 25 mg/kg, both groups showed near-complete resolution of radiographic tumor burden. The 25 mg/kg TMZ + FUS group demonstrated a median survival of 74 days, whereas the TMZ without FUS group demonstrated a median survival of 70 days. The authors also point out that all mice survived  $>70$  days in the TMZ + FUS group, whereas only 50% survived  $>70$  days in the TMZ without FUS group (Figure).

The ability of chemotherapeutics to evade the BBB is critical to their efficacy. In the above study, the authors demonstrated the ability of FUS to increase BBB permeability in a localized fashion, increasing tumoral concentration of TMZ and slowing the degradation of TMZ. There also appears to be a survival benefit from this therapy compared with no treatment, but the benefit of TMZ + FUS over TMZ alone is unclear from this work. FUS may be useful in systemic dose reduction in patients with intolerable side effects at higher doses, but more work is needed to support its widespread use. It is also possible that longer treatment regimens and the addition of radiation therapy in line with current treatment paradigms may

show a more significant survival benefit of TMZ + FUS over TMZ alone.

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