42566: Direct Microscopic Comparison of 5-ALA, Fluorescein Sodium, and ICG for Detection of Malignant Glioma Border

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Disclosures

DISCLOSURES: PN receives royalties from Carl Zeiss

FINANCIAL SUPPORT: The Barrow Neurological Foundation; the Newsome Chair in Neurosurgery Research to MCP; Part of this study involving endomicroscope was supported by a materials grant from Carl Zeiss AG, Oberkochen, Germany. The grantor did not have any contribution or effect on study design, data collection, analysis or paper preparation. EB acknowledges scholarship support SP-2240.2018.4.
Introduction

• Surgical resection remains the first line treatment for malignant gliomas.

• Several fluorescent dyes are currently in use to augment glioma resection, but information on their comparative effectiveness is lacking.

• We aimed to compare tumor fluorescence with fluorescein sodium (FNa) and 5-aminolevulinic acid (5-ALA) induced protoporphyrin IX (PpIX) in a malignant glioma model using advanced fluorescence imaging techniques.
Methods

• GL261 and RFP-U251 gliomas models in mice and rats were used. 5-ALA(5mg) FNa(5mg/kg) and ICG(20mg/kg) were administered perioperatively.

• 3 fluorescent signals were detected simultaneously with operative microscope, laser scanning confocal microscope and confocal laser endomicroscope.

• Fluorescence was assessed quantitatively as surface area of fluorescent positive tissue and qualitative (false/true, positive/negative) compared to H&E-stained slides.
Results

- FNa highlighted larger surface area (18.0mm$^2$) than white light (16.9mm$^2$, $p=0.016$) or PpIX (16.0mm$^2$, $p=0.035$).
- Both 5-ALA and FNa had inhomogeneous staining patterns: multiple areas of equal staining, when PpIX was present and FNa was not, and opposite.
- FNa signal was stronger (tumor to background ratio (TBR) 1.93 ± 0.56) compared to 5-ALA (1.52 ± 0.31; $p=0.002$).
- Cell-level imaging revealed PpIX positive, FNa negative migrating tumor pouches. However, some tumor areas were PpIX negative and FNa positive, or PpIX/FNa negative.
- Differences in FNa negative (8/30, 27%) and PpIX negative (12/30, 40%) tumor areas were insignificant, $p=0.27$.
- False positive FNa fluorescence (13/30, 43%) was more frequent than 5-ALA (4/30, 13%; $p=0.01$).
A. 5-ALA showed tumor areas not highlighted by FNa

B. FNa showed tumor areas not highlighted by 5-ALA

C. FNa and 5-ALA highlighted similar tumor areas

D. Surface Area of Tumor

E. Tumor to Background Ratio

Top Panel (A-C): Fluorescence of mouse glioma as seen sequentially on the operative microscope with filters. First column – white light; second column – BLUE400 filter for 5-Aminolevulinic Acid; third column – Yellow560 filter for Fluorescein Sodium; forth column – overlay of images Fluorescein sodium and 5-Aminolevulinic Acid images. A. 5-Aminolevulinic Acid showed areas not highlighted by Fluorescein Sodium (red arrows) B. Fluorescein Sodium showed areas not highlighted by 5-Aminolevulinic Acid (yellow arrows) C. Fluorescein Sodium and 5-Aminolevulinic Acid highlighted similar areas (green arrow). D. Average surface area of tumor area, as perceived under white light, Fluorescein fluorescence, and 5-Aminolevulinic Acid fluorescence. N= 39 images analyzed in each group. Images taken at the same area with different filters of operative microscope. E. Tumor-to-background ratio of 5-Aminolevulinic Acid, Fluorescein Sodium and Indocyanine Green as a measure of fluorescence strength. Ratio is a measure of fluorescent intensity of the tumor compared to that of normal brain tissue, analyzed on ImageJ. N=48 images analyzed from Kinevo operative microscope.
Laser Scanning Confocal Microscopy image. 5-ALA highlighted brain tumor cells distant to the core, while FNa did not. Top row has highlighted blue and yellow squares, corresponding to similarly outlined blue (second row) and yellow (third row) images, which are enlarged. In the middle row, FNa successfully highlighted the sample taken from the tumor core (blue square), but did not do so in the bottom row, corresponding to the sample distant to the tumor core.
Conclusions

- Confocal and surgical imaging showed inhomogeneous staining of tumor border with PpIX/FNa (-/-; +/-; -/+; ++).
- These results suggest simultaneous administration of 5-ALA and FNA may provide additional benefit to identify tumor border.
- Neither ICG, 5-ALA or FNa worked perfectly to provide consistent tumor cell delineation. Not all tumors cells were labeled with each of the fluorophores.
- 5-ALA labeled the most distant invading tumor cells, compared to FNa.