Autophagy impairment is involved in midazolam-induced lipid droplet accumulation and consequent phagocytosis decrease in BV2 cells

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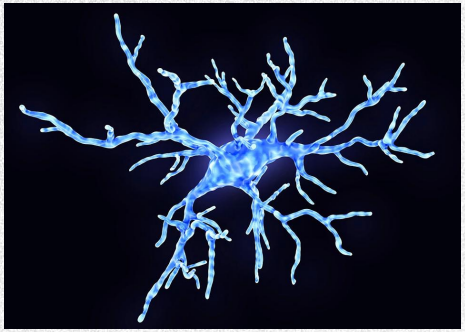
In a nutshell...

What is the link between anesthetics and post-operative central nervous system complications and diseases?

Background Information

What are microglial cells?



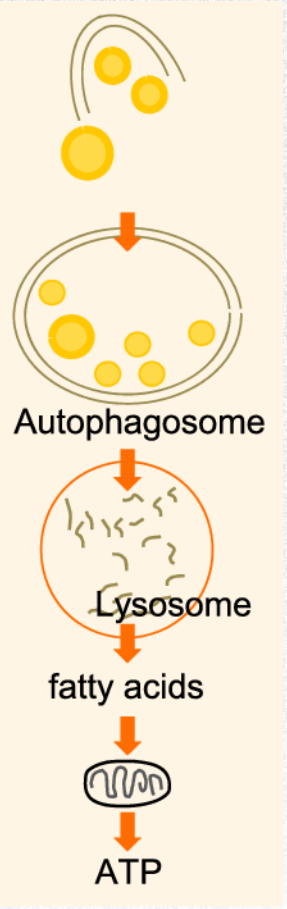
- Brain cells that help in central nervous system (CNS) homeostasis by conducting phagocytosis
    - Amyloids
    - Damaged myelin sheaths
    - Damaged neurons
- Lipid metabolism plays a significant role in their function of phagocytosis because lipids are needed in the formation/maturation of phagosomes
- Disordered lipid utilization in microglia down-regulates phagocytosis and, in turn, causes a build-up of lipids in lipid droplets

Lipids → Phagocytosis → Microglial cell function

Background Information

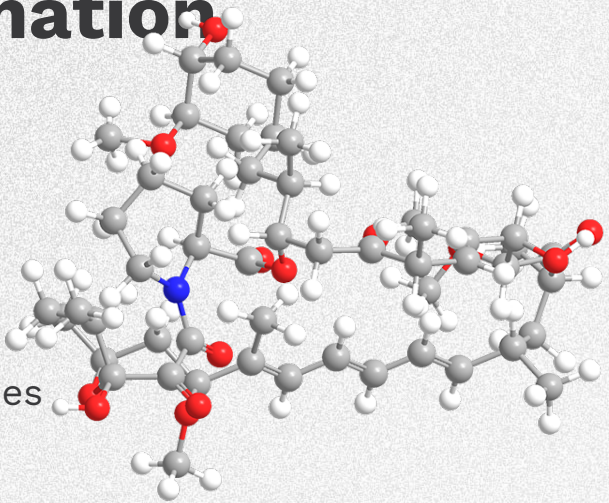
Lipids and microglial cells

- Lipids also provide microglial cells with energy via lipid droplets (LDs)
- How do we access energy from LDs? Autophagy
- What happens if autophagy becomes impaired?
    - LD accumulation
    - [Energy] ↓
    - Impaired phagocytosis
- Understanding the relationship between the lipid droplets and phagocytosis in microglia is important for the treatment of post-operative CNS complications



Background Information

Rapamycin



- is a natural product isolated from Streptomyces hygroscopicus
- A drug that is used to coat coronary stents, prevent organ transplant rejection, and treat rare lung diseases and perivascular epithelioid cell tumor
- Rapamycin is a commonly used as an inducer of autophagy, the process by which cells degrade dysfunctional organelles and obtain molecules for energy. This reduces the accumulation of the waste that normally clogs up our tissues as we get older, and hence slows or even reverses the ageing process.
- Aka the anti-aging drug

Overview of Previous Studies

Re: anesthetics are closely related to post-operative CNS complications

01 Mitophagy impairment, altered synapse structure, and dysregulation of Ca^{2+} homeostasis cause post-operative cognitive dysfunction development following exposure to anaesthetics

02 Autophagy regulates LD degradation and defects in autophagy may result in LD accumulation

03 Midazolam, a popular intravenous general anaesthetic, can impair autophagy and disrupt lysosomal homeostasis

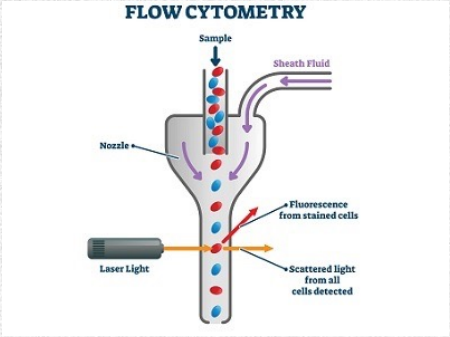
Research Objectives

Therefore, their objectives were to investigate the following:

- Does midazolam impair autophagy and in turn induce LD accumulation in BV2 cells?
- If so, does the LD accumulation reduce phagocytosis from occurring in microglial BV2 cells?
- Can rapamycin treatment be used to reverse midazolam-induced impaired autophagy in microglial BV2 cells?

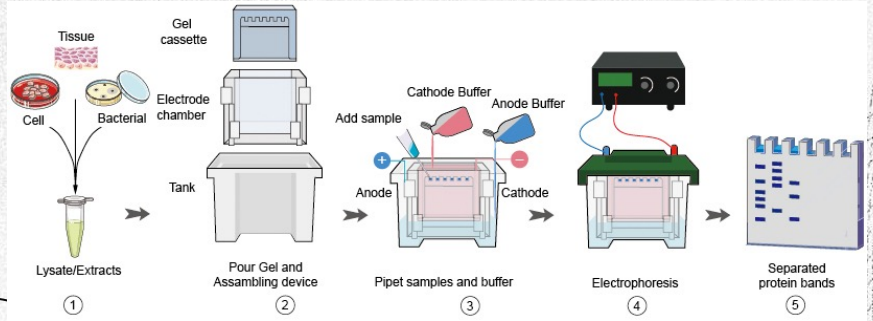
Research Procedure

They wanted to see the effect of midazolam (anesthetic) on lipid droplets and phagocytosis on microglial BV2 cells



- **Lipid droplets:** used flow cytometry and triglyceride quantification

- **Microglial BV2 cells:** used by detecting phagocytosis by latex beads, western blot analysis, and microscopy



Procedure in Detail for Lipid Droplets

1

Staining LDs

- Treated with midazolam
- Cells were washed
- BODIPY* staining
- Flow cytometry
- Histogram was produced

2

Triglyceride measurement

- Cells were seeded in wells dishes
- Treatment with midazolam
- Cells were trypsinised
- TG measurement using TG assay kit

3

Autophagy flux assay

- Cells were seeded in petri dishes
- Infected with adenovirus
- Treatment with midazolam/rapamycin
- Fluorescence observed with microscope

4

Statistical analysis

- T-tests were performed to compare differences between the two groups

*BODIPY can be used as a stain for lipids and as a tracer for oil and other nonpolar lipids

Procedure in Detail for BV2 cells

1

Microglial BV2 cells

2

Western blot analysis

- Cells were harvested
- Protein was separated
- Membrane was incubated with antibodies
- Immunoreactive bands were visualized
- Density of band was quantified

3

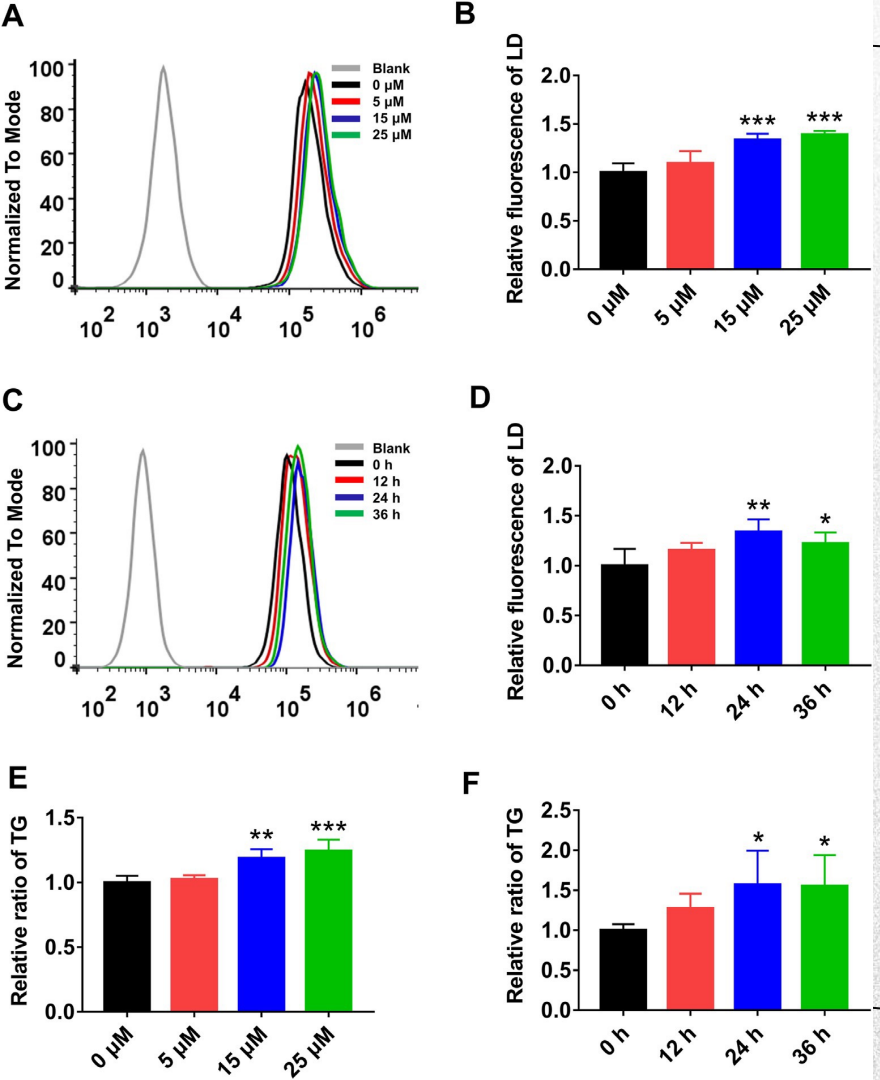
Immunofluorescence

- Cells were plated
- Culture medium was removed
- DAPI staining
- Images captured by microscope

4

Phagocytosis Assay

- Fluorescence-labelled latex beads were added after treatment with midazolam
- Cells were washed to remove non-phagocytised beads
- Images captured by microscope



Results & Significance

Fig. 1. Does midazolam induce LD accumulation by impairing autophagy?

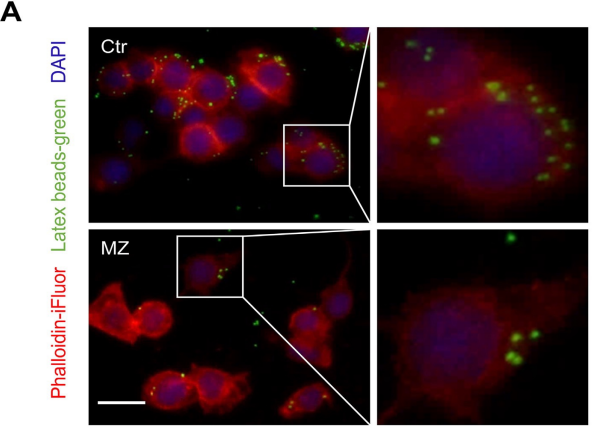
(A, B) → BV2 cells were treated with varying concentrations midazolam (24 hrs)

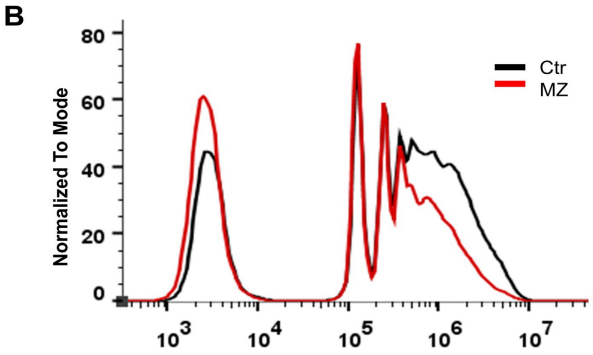
(C, D) → BV2 cells were treated with midazolam for varying amounts of time (15 mM)

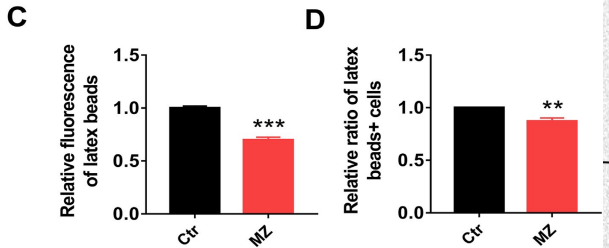
(A, C) → Flow cytometry images

(E, F) → Triglyceride quantification

Midazolam induces lipid droplet accumulation in BV2 cells and, in turn, impairs autophagy







Results & Significance

Fig. 2. Does LD accumulation caused by midazolam inhibits BV2 cells' function of phagocytosis?

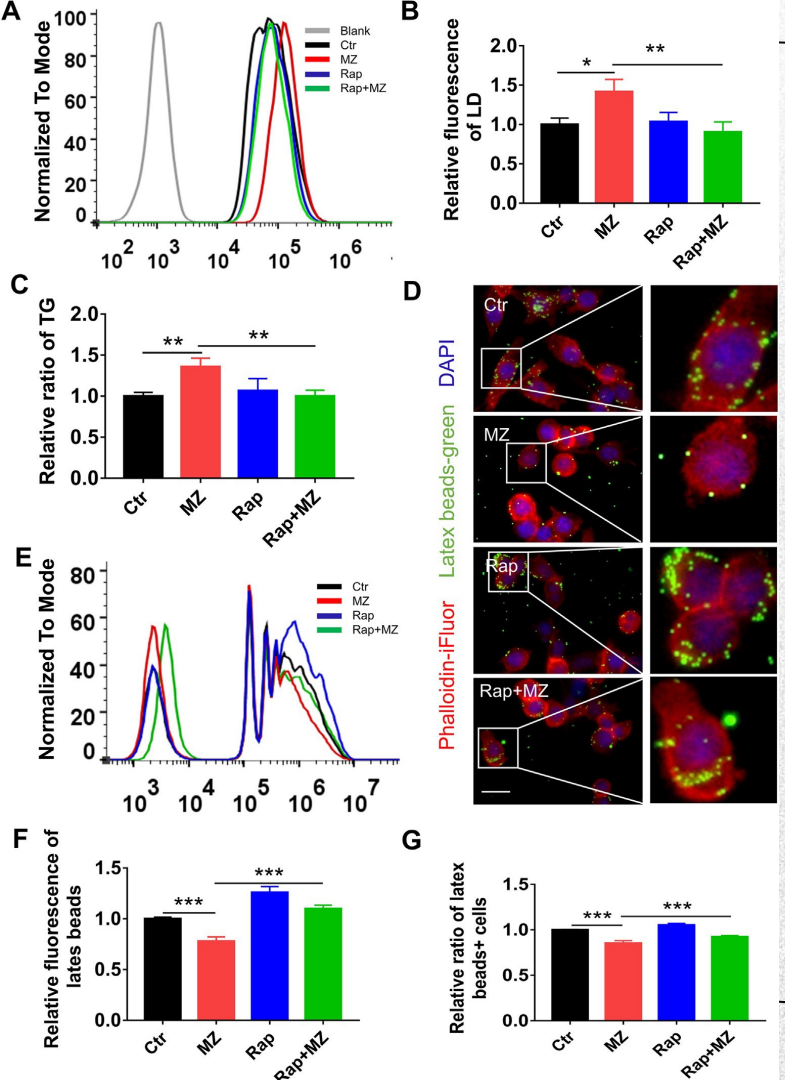
These cells were pre-treated with midazolam and then incubated with latex beads-green. Phagocytosis of was measured using immunofluorescence and flow cytometry assays.

(A) → Immunofluorescence; more latex beads were observed in the control BV2 cells than in the midazolam-treated cells

(B-D) → Flow cytometry; Fluorescence intensity and the amount of latex bead-positive cells were significantly decreased in midazolam-treated cells

(C, D) → Statistical chart of (B)

<u>Midazolam inhibits the phagocytosis of BV2 cells.</u>



Results & Significance

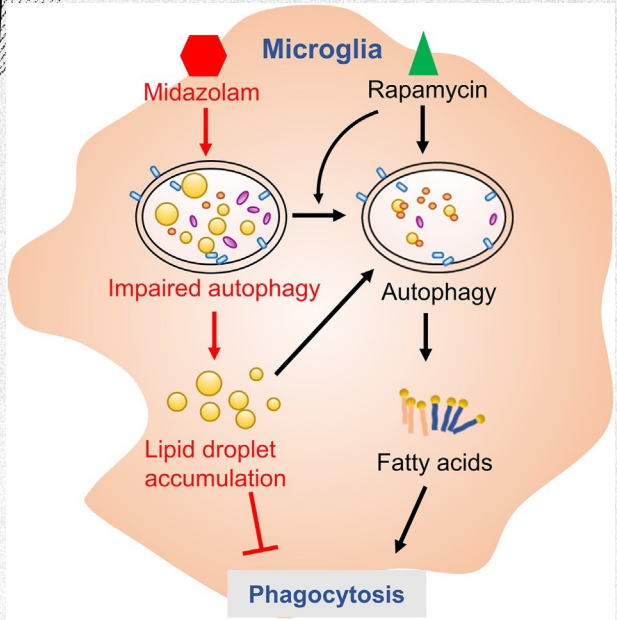
Fig. 5. Can rapamycin treatment be used to reverse midazolam-induced impaired autophagy in microglial BV2 cells?

(A - C) → Rapamycin + midazolam treatment significantly decreased the fluorescence intensity of LDs and reduced the TG content compared with midazolam treatment alone

(D) → Rapamycin treatment increased the engulfment of latex beads in midazolam- treated cells

<u>Rapamycin alleviates midazolam-induced lipid droplet accumulation</u>

Discussion



Previous studies → midazolam inhibits autophagic degradation and disturbs lysosomal homeostasis

This study → the LD accumulation is why midazolam inhibits autophagy in BV2 cells but rapamycin can be used to stop that

Researchers concluded that the LD accumulation caused by midazolam has occurred because the anesthetic inhibited the lipid degradation pathway.

Objectives? → After promoting autophagy with rapamycin, we found that LD accumulation returned to normal levels thus **validating** our hypothesis.

Conclusions

If we a take a look at the objectives...

- Does midazolam impair autophagy and in turn induce LD accumulation? YES
- If so, does the LD accumulation reduce phagocytosis from occurring in microglial BV2 cells? YES
- Can rapamycin treatment be used to reverse midazolam-induced impaired autophagy in microglial BV2 cells? YES

Therefore, anesthetics cause autophagy impairment, LD accumulation, and a decline in the phagocytosis of unwanted molecules which can lead to post-operative CNS complications and diseases.

Thank you

Any Questions?