The Effect of LPS on Phosphorylation of AKT Signaling in Schwann Cells

Nicholas Wilde, Mackenzie Wilcox, and Angela Asirvatham, Ph.D.
Department of Biology, Misericordia University, Dallas, PA

INTRODUCTION

Schwann cells (SC) are important glial cells in the peripheral nervous system that produce a myelin sheath to wrap around axons. Myelination of axon increases the speed of electrical impulses throughout the body. When SCs are injured, the myelin sheath becomes damaged, and the cell releases inflammatory molecules to allow macrophages to clear cellular debris. Once the debris is cleared, neuronal growth factors activate the cAMP pathway to promote neuronal growth. In the lab, forskolin (F) activates the cAMP pathway and a bacterial endotoxin known as lipopolysaccharide (LPS) is used to simulate nerve injury. Activating the cAMP pathway inhibits inflammation[2] in some systems and promotes pro-/anti-apoptotic[4] behavior in dose dependent experiments. Similarly, LPS has been shown to influence cell viability based on dose and time. A potential solution to the lack of neuronal growth after an injury could be in the protein kinase B (AKT) cell survival signal. When phosphorylated, the AKT pathway becomes activated and plays a critical function in the regulation of myelin[1] and activating the transcription factor NFκB[3], which is a proinflammatory signal. It was hypothesized that RT4-D6P2T rat Schwannoma cells treated with increasing doses of LPS will have an increase in the upregulation of phospho-AKT and cells treated with both F and LPS would have a synergistic effect on expression of phospho-AKT.

ACKNOWLEDGEMENTS

The researcher would like to thank the Misericordia University Summer Undergraduate Research Fellowship and Student Research Grant for financial assistance. The researcher would also like to thank their research mentor Dr. Angela Asirvatham and research partner Mackenzie Wilcox as well as Leo Carr, Jill Dillon, Helen Bogdon, and the Misericordia University Biology Department for their assistance in the lab.

RESULTS

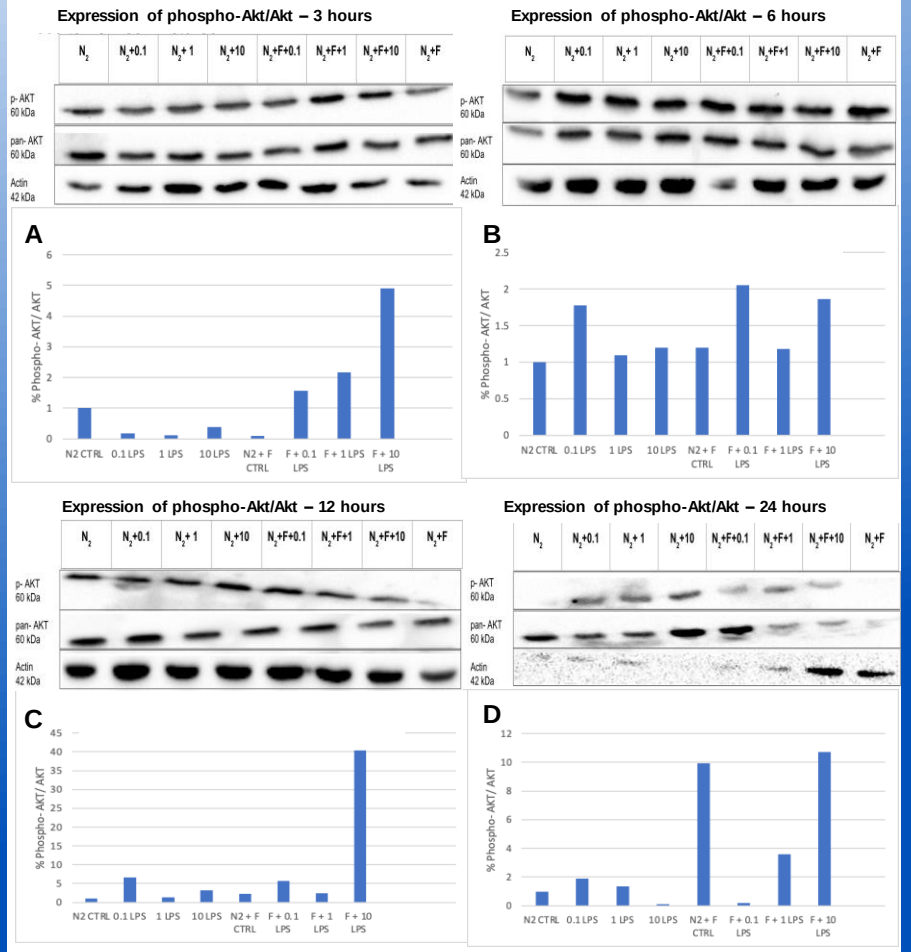
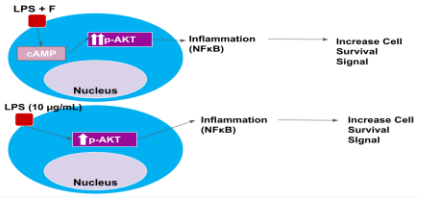


Figure 1. Temporal expression of phospho-AKT/ AKT for 3 (A), 6 (B), 12 (C) and 24 hours (D). The RT4- D6P2T rat Schwannoma cells were treated with either varying concentrations of LPS (01, 1, and 10 µg/mL), LPS (01, 1, and 10 µg/mL) + F (2 µM), or without LPS or F. Protein content was detected using Western blot and expressed as a percent of phospho-AKT by AKT after accounting for the background protein actin. Experiments were replicated twice for 3, 6 and 12 hours and once for 24-hour time-point.

CONCLUSION

It was discovered that, without activating the cAMP pathway, the upregulation of phospho-AKT was dose and time dependent, while activating the cAMP pathway (LPS + F) had a synergistic effect as LPS dosage increased at all time periods. In LPS alone, an increase in the upregulation of phospho-AKT was seen from 1 µg/mL to 10 µg/mL across all time periods besides 24 hours. In LPS + F, a synergistic effect was seen from F + 1 µg/mL to F + 10 µg/mL across all time periods when compared to control.



REFERENCES

1. Ishii A, Furusho M, Bansal R. 2021. Mek/ERK1/2-MAPK and PI3K/akt/mTOR signaling plays both independent and cooperative roles in schwann cell differentiation, myelination and dysmyelination. Glia. 69(10):2429- 2446.
2. Makranz, C., Cohen, G., Reichert, F., Kodama, T., Rotshenker, S. 2006. cAMP cascade (PKA, EPAC, adenylyl cyclase, Gi, and phosphodiesterases) regulates myelin phagocytosis mediated by complement receptor-3 and scavenger receptor-AI/II in microglia and macrophages. *Glia* 53(4): 441-8.
3. Saponaro C, Cianciulli A, Calvello R, Dragone T, Iacobazzi F, Panaro MA. 2012. The PI3K/akt pathway is required for LPS activation of microglial cells. Immunopharmacol Immunotoxicol. 34(5):858- 65
4. Insel PA, Zhang L, Murray F, Yokouchi H, Zambon AC. 2012. Cyclic AMP is both a pro-apoptotic and anti-apoptotic second messenger. Acta Physiol (Oxf). (2):277-87.
5. Schmitt JM, Stork PJ. 2001. Cyclic AMP-mediated inhibition of cell growth requires the small G protein Rap1. Mol Cell Biol. 21(11):3671-83.

Misericordia University Printing Services