JULY 2020

SPECIAL DELIVERY™ NEWSLETTER

Over 40 Years of Precision Dosing

SOCIAL DISTANCING WITH ALZET® OSMOTIC PUMPS

As we begin to navigate a post-lockdown world and try to return to "normal", companies have been tasked with implementing strict protocols limiting the amount of people allowed within a certain amount of space. This is especially challenging in laboratories, where space was already severely limited. Instead of prioritizing some research over others, try using an infusion pump instead.

One of the known advantages of ALZET Osmotic Pumps is that researchers are able to handle animals less often than if they were administering injections. Consequently, researchers are able to spend less time in the lab. This provides labs with the ability to limit the amount of people in the lab at one time, without limiting the amount of research being done.

IN THIS ISSUE

HEPATIC STEATOSIS AND LEPTIN

Hepatic steatosis occurs due to triglyceride overproduction in the liver relative to the liver's ability to utilize or export them as very low density lipids (VLDL). Since VLDL secretion plateaus when the body progresses to non-alcoholic fatty liver disease (NAFLD), hepatic steatosis is believed to be a critical part of the development of NAFLD (Hackl *et al.*, 2019).

Hepatic steatosis has been successfully reversed through recombinant leptin replacement therapy, although the mechanisms behind the reversal are still unknown (Oral *et al.*, 2002). Knowing that VLDL secretion is at least partially regulated by the brain and the central nervous system (Stafford *et al.*, 2008), Hackl *et al.* hypothesized that infusing leptin directly into the brain might stimulate VLDL secretion and consequently reverse the progress of hepatic steatosis. To test this, the team used ALZET Osmotic Pumps to infuse leptin both directly into the third ventricle, as well as intraperitoneally.

Central leptin infusion in rats was accomplished by connecting Model 2004 ALZET Osmotic Pumps to cannulae implanted in the third ventricle (ICV). Pumps delivered leptin (0.3 µg/day for chow diet, 0.9 µg/day for high fat diet), leptin receptor antagonist LpR (6 µg/day), or artificial cerebrospinal fluid (aCSF). The rats receiving LpR all developed hepatic steatosis, and the rats receiving leptin demonstrated a 20-30% increase in VLDL secretion compared to the aCSF control group. The increase in VLDL secretion was seen in all rats infused with leptin regardless of their diet. Notably, systemic leptin levels did not increase with ICV infusion of leptin, providing evidence for central control of VLDL secretion.

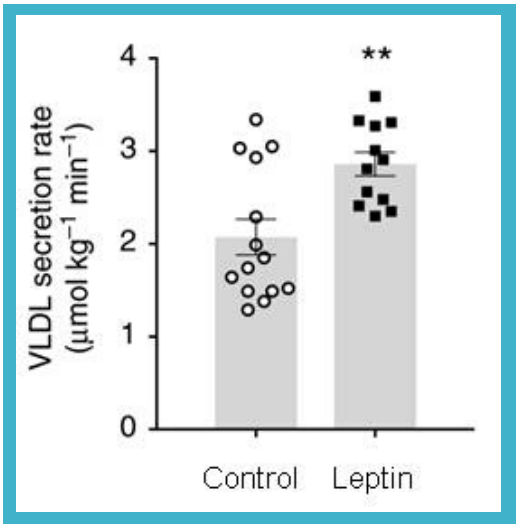

Figure 1. VLDL secretion rates seen during ICV infusion of leptin by ALZET Pumps. Notice how the leptin group is considerably higher than the control during ICV infusion (**P< 0.01); open circles are control, black squares are leptin (Hackl *et al.*, 2019).

To confirm the central effect of leptin, the team repeated the experiment, this time dosing the leptin, or saline, using intraperitonally implanted ALZET Osmotic Pumps. The team found that none of the rats showed any of the changes that were observed during the ICV infusion, proving that the increased VLDL secretion was caused by leptin in the brain and not by circulating leptin.

These discoveries led the team to conclude that leptin is a circulating peptide that is potentially counteracting hepatic steatosis by stimulating VLDL secretion via the brain rather than simply through its effects on appetite and body weight. This suggests that increasing brain leptin signaling and leptin transport across the blood-brain barrier could be strategies to reduce hepatic steatosis in obesity and other related diseases including NAFLD.

Hackl, M.T., Fürnsinn, C., Schuh, C. M., Krssak, M., Carli, F., Guerra, S., Freudenthaler, A., Baumgartner-Parzer, S., Helbich, T. H., Luger, A., Zeyda, M., Gastaldelli, A., Buettner, C., & Scherer, T. (2019). Brain leptin reduces liver lipids by increasing hepatic triglyceride secretion and lowering lipogenesis. *Nature Communications*, 10(1), 2717.

Oral, E. A., Simha, V., Ruiz, E., Andewelt, A., Premkumar, A., Snell, P., Wagner, A. J., DePaoli, A. M., Reitman, M. L., Taylor, S. I., Gorden, P., & Garg, A. (2002). Leptin-replacement therapy for lipodystrophy. *The New England Journal of Medicine*, 346(8), 570–578.

Stafford, J. M., Yu, F., Printz, R., Hasty, A. H., Swift, L. L., & Niswender, K. D. (2008). Central nervous system neuropeptide Y signaling modulates VLDL triglyceride secretion. *Diabetes*, 57(6), 1482–1490.

ANTI-INFLAMMATORY AGENTS REVERSE COGNITIVE DEFICITS OF NICOTINE WITHDRAWL

Decreased cognitive performance is prevalent during the early days of nicotine withdrawal, and is one of the most common reasons that people relapse (Saravia *et al.*, 2019). Saravia *et al.* speculated that neuroinflammation may be responsible. In fact, a seminal study using ALZET pumps in rats showed a relationship between microglia activation and altered hippocampal neurogenesis after central infusion of lipopolysaccharide (Ekdahl *et al.*, 2003). Since then, studies have gone even further to correlate impaired cognitive performance with neuroinflammation and reduced hippocampal neurogenesis (Kohman & Rhodes, 2013). Using an established mouse model of nicotine dependence based on chronic nicotine from ALZET Osmotic Pumps, Saravia *et al.* sought to determine if microglia activation and neurogenesis play a role in the cognitive deficits associated with withdrawal. They also investigated the effects of the anti-inflammatories cannabidiol (CBD) and indomethacin on the neuroinflammation.

Male C57BL/6J mice received subcutaneously-implanted Model 2002 ALZET Pumps delivering nicotine (25 mg/kg/day) for 14 days. On day 13, they were habituated to a maze for an object-recognition test of cognitive function. On day 14, they were presented with objects for the training phase followed immediately by cannabidiol injection. After a 20 minute interval, timed to allow for potential influence on memory consolidation, withdrawal was induced by mecamylamine injection. Memory for the presented objects was evaluated 24 hours after withdrawal. Memory testing was also done at 4 days post-withdrawal. For this interval, additional maze training was performed on day 17 after pump implantation, with testing on day 18 and CBD or indomethacin daily injections on days 14-17.

Saravia *et al.* found that mice treated with higher doses of CBD (10 or 30 mg/kg) did not suffer from the memory impairment found in mice receiving vehicle or low dose CBD (3 mg/kg), indicating sufficient CBD may relieve the cognitive deficits experienced in males when they first quit smoking. In addition, the team determined that CBD reduced memory impairment by modifying microglia reactivity in the hippocampus and other brain regions associated with cognitive processes.

Continued on page 7...

HSP90 INHIBITORS: THE FUTURE OF CARDIOVASCULAR RESEARCH?

Heat shock protein 90 (HSP90) inhibitor, 17-dimethylamminoethylamino-17-demethoxygeldanamycin (17-DMAG), has piqued interest in cardiovascular research due to its ability to weaken inflammatory responses and lessen oxidative stress by regulating vascular smooth muscle cells (Huang *et al.*, 2020). Since vascular smooth muscle cells are critical to the functionality of blood vessels, the teams of Zhao and Huang suspected that HSP90 is involved in blood vessel degradation. By utilizing a well established mouse model of hypertension in which ALZET Osmotic Pumps subcutaneously infused angiotensin II (Ang II), the teams were able to confidently study the therapeutic effects of 17-DMAG as well as the role of HSP90 in the mechanisms of thoracic aortic dissection (TAD) and hypertension respectively.

Over 20% of TAD patients die pre-hospital due to rapid dilation and rupture of the aorta (Zhao *et al.*, 2019). Key features of TAD are degradation of the extracellular matrix and depletion of the smooth muscle cells in the medial layer of blood vessels (Wu *et al.*, 2013). However, since the mechanisms of TAD pathogenesis are not fully understood, there is currently no reliable way to prevent them.

Zhao and his team caused aortic dissection (AD) in 80 male FVB mice by administering β-aminopropionitrile fumarate (BAPN) and Ang II in two phases. During phase one, BAPN was dosed at 1 g/kg/day in drinking water for 4 weeks, and half of the mice were injected with 10 mg/kg of 17-DMAG subcutaneously every 2 days. After phase one, Ang II was infused into all the mice at a rate of 1 µg/kg/min for 48 hours using subcutaneously implanted ALZET Osmotic Pumps.

Zhao *et al.* discovered that in comparison to untreated controls, the aortic walls of AD mice had significantly less smooth muscle cells, and more fragmentation of the elastic fibers. In addition, HSP90 in the smooth muscle cells was significantly upregulated, and in direct proportion to the amount of damage to the walls.

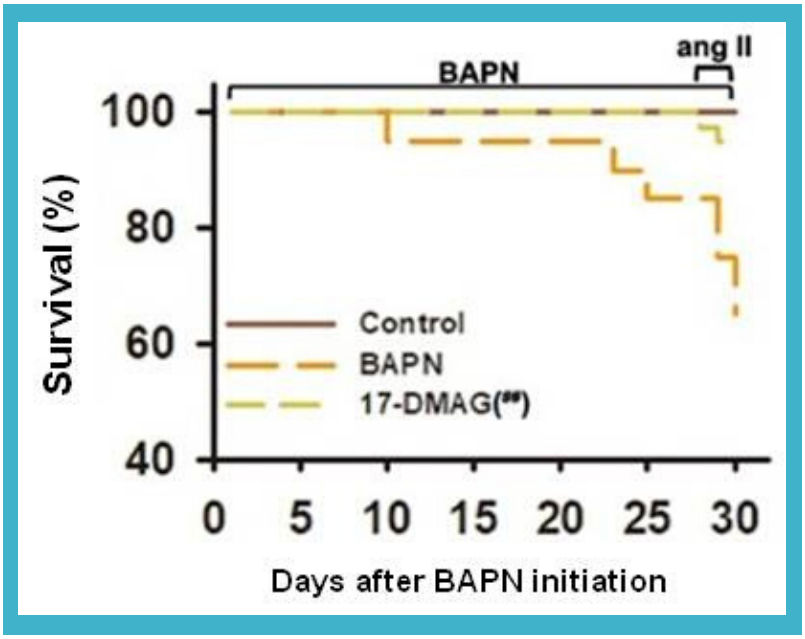

Figure 2. Survival curves of mice in each of the groups including the control. The survival rate of mice in the 17-DMAG/AD group was significantly higher than that for the mice in the AD group (## P< 0.01). Reprinted with permission from e-Century Publishing.

Aortic walls of the 17-DMAG/AD mice had considerably less damage, confirming that 17-DMAG could be used to reduce the occurrence of aortic dissection, and consequently delay or potentially avoid aortic rupture.

The team also compared the mortality rates of the 17-DMAG/AD and AD groups. By the end of the Ang II phase, 20% of the AD group had died but only 5% of the 17-DMAG/AD group. While there is more research to be done, the results of this study indicate that HSP90 plays an important role in TAD, and inhibitors like 17-DMAG have the potential to reduce the damage to aortic walls and therefore TAD mortality rates.

Huang and his team focused their research on adventitial remodeling, one of the major adaptive mechanisms of hypertension. A critical part of adventitial remodeling is a phenotype change of adventitial fibroblasts to myofibroblasts. Huang *et al.* sought to confirm that the phenotype switching was related to calcineurin, a client protein of HSP90 and known mediator of dynamin related protein (Drp1)-dependent mitochondrial fission. If calcineurin is connected, 17-DMAG could potentially regulate the transformation of adventitial fibroblasts and therefore adventitial remodeling in Ang II-induced hypertensive mice.

Huang *et al.* subcutaneously implanted Model 2004 ALZET Osmotic Pumps to infuse a continuous dose of 1400 ng/kg/min of Ang II for 28 days. Of these mice, half also received 17-DMAG (2 mg/kg injected every other day). In the Ang II-only group, the team observed a significant uptick of HSP90 in the adventitia as well as adventitial fibroblasts switching into myofibroblasts. Both of these were drastically diminished in the 17-DMAG group, indicating that 17-DMAG could potentially be used to reduce or at least slow adventitial remodeling.

After confirming this, Huang *et al.* turned their attention to calcineurin. They tested whether inhibiting HSP90 could keep it from binding to calcineurin. They discovered that 17-DMAG not only stopped HSP90 from binding to calcineurin, it reversed any subsequent progression towards adventitial remodeling. Knowing that calcineurin mediates Drp1-dependent mitochondrial fission, the team investigated if Drp1-dependent mitochondrial fission has a role in the switching process. Not only had Drp1-dependent mitochondrial fission increased within the adventitial fibroblasts, further examination revealed that the conversion of adventitial fibroblasts into myofibroblasts was successfully attenuated by Drp1 inhibitors. Together, this proved that Drp1-dependent mitochondrial fission is necessary for Ang II-induced adventitial fibroblast activation, and that activation can be controlled by regulating the binding of HSP90 to calcineurin.

This research provided first evidence of a crucial HSP90-regulated pathway including calcineurin and Drp1 that mediates Ang II-induced switching of adventitial fibroblasts and thereby adventitial remodeling. It was also the first to show that HSP90 inhibition effectively shuts down this pathway and mitigates hypertensive adventitial remodeling.

Although focused on different cardiovascular diseases, both studies show a distinct correlation between HSP90 upregulation and the deterioration of blood vessel walls. They also show the ability of the HSP90 inhibitor, 17-DMAG, to reduce and even reverse this deterioration in both the vascular adventitia and media.

Huang, G., Cong, Z., Wang, X., Yuan, Y., Xu, R., Lu, Z., Wang, X., & Qi, J. (2020). Targeting HSP90 attenuates angiotensin II-induced adventitial remodelling via suppression of mitochondrial fission. *Cardiovascular Research*, 116(5), 1071–1084.

Wu, D., Shen, Y. H., Russell, L., Coselli, J. S., & LeMaire, S. A. (2013). Molecular mechanisms of thoracic aortic dissection. *The Journal of Surgical Research,* 184(2), 907–924.

Zhao, Z., Wang, Y., Li, S., Liu, S., Liu, Y., Yu, Y., Yang, F., Xu, Z., & Wang, G. (2019). HSP90 inhibitor 17-DMAG effectively alleviated the progress of thoracic aortic dissection by suppressing smooth muscle cell phenotypic switch. *American Journal of Translational Research*, 11(1), 509–518.

HOW TO ORDER ALZET® OSMOTIC PUMPS

There are many different ways to order ALZET Osmotic Pumps. The fastest and easiest way is to use our online order form. You can also email our Customer Service Team at alzetcs@durect.com or call them at 877.922.5938. All orders are processed by 12 pm each business day, so make sure that you order before then for same day shipping!

LEPTIN AUGMENTS MINUTE VENTILATION

Since its discovery in 1994, leptin has been associated with obesity and food consumption. It has long been suspected that its influence on the body extends past this, and in the last decade research focused on its role as a respiratory stimulant has accelerated. Recent research suggests that carotid bodies have an abundance of leptin receptors, specifically the $LepR^b$ isoform. Carotid bodies sense hypoxia and relay this information to the brain via the carotid sinus nerve (CSN). The brain's hypoxic ventilatory response (HVR), is at least partially controlled by the carotid bodies. In their 2019 study, Caballero-Eraso and her team hypothesized that leptin acts in carotid bodies to stimulate HVR and increase baseline minute ventilation both while mice are asleep and awake. They tested their hypothesis using three parallel methods, including leptin infused continuously into lean mice using ALZET Osmotic Pumps.

To determine the effect of leptin on HVR, the team first measured baseline HVR in lean mice subcutaneously implanted with saline-filled ALZET pumps. After this, they explanted the pumps and implanted new pumps filled with either saline or leptin (120 µg/day) for 48 hours, a dose that matched leptin levels observed in obese mice (Caballero-Eraso *et al.*, 2019). After 48 hours the team again measured the HVR of the mice. Next the control and test groups were split and the mice underwent either CSN dissection or sham surgery. After recovery, HVR was measured again. This allowed the team to see what effect, if any, leptin has on HVR and the role of the CSN. Caballero-Eraso and her team found that leptin infusion had no effect on the minute ventilation or the HVR in denervated animals as compared with sham-operated controls.

Simultaneously, the team examined the effects of intravenous leptin on CSN activity during 100% hyperoxia, when carotid bodies are inactivated, and at 10% O_2 hypoxia. They determined that leptin, but not saline, increased CSN activity during these conditions. This indicates that leptin does in fact influence the carotid bodies to increase minute ventilation and HVR in mice. The team further concluded that any changes to minute ventilation and HVR caused by leptin, is directly related to its influence on the carotid bodies.

Continued on page 7...

ANTI-INFLAMMATORY AGENTS REVERSE COGNITIVE DEFICITS OF NICOTINE WITHDRAWAL

Continued from page 3

The mice treated with indomethacin also experienced a reversal of the cognitive deficits for the same reason. As a whole, this indicates that treating inflammation could be a way to alleviate cognitive deficits typically experienced in nicotine withdrawal. Notably, the team also concluded that inflammatory cytokines could be biomarkers for cognitive deficits since fluctuations in inflammatory cytokines in plasma seemed to rise and fall alongside the presence and then resolution of cognitive deficits.

This is the first time that an inflammatory process has been associated with cognitive deficits observed in early nicotine withdrawal. Furthermore, Saravia *et al.* build on the foundational research of Ekdahl *et al.*, and suggest that anti-inflammatory agents could be used to improve and reverse these cognitive deficits. Since reduced cognitive performance is the most common reason people relapse, this breakthrough could potentially lead to more people successfully quitting smoking.

Ekdahl, C. T., Claasen, J. H., Bonde, S., Kokaia, Z., & Lindvall, O. (2003). Inflammation is detrimental for neurogenesis in adult brain. *Proceedings of the National Academy of Sciences of the United States of America*, 100(23), 13632–13637.

Kohman, R. A., & Rhodes, J. S. (2013). Neurogenesis, inflammation and behavior. *Brain, Behavior, and Immunity*, 27(1), 22–32.

Saravia, R., Ten-Blanco, M., Grande, M. T., Maldonado, R., & Berrendero, F. (2019). Anti-inflammatory agents for smoking cessation? Focus on cognitive deficits associated with nicotine withdrawal in male mice. *Brain, Behavior, and Immunity*, 75, 228–239.

LEPTIN AUGMENTS MINUTE VENTILATION

Continued from page 6

Lastly, they examined what happened to minute ventilation and HVR during wakefulness and sleep in $LepR^b$ deficient db/db obese mice transfected with $LepR^b$ or a control sequence using an adenovirus carrier. Compared to controls, the mice transfected with $LepR^b$ showed increased minute ventilation as well as HVR both while awake and asleep. Notably, the leptin levels in these mice were similar to those in the lean mice during infusion.

By using ALZET Pumps, Caballero-Eraso *et al.* were able to provide the first evidence that leptin acting on the carotid bodies in mice augments minute ventilation in normal conditions, and that carotid sinus nerve activity responds to physiologically relevant levels of hypoxia in vivo. Taken together, the results of all three methodologies prove that leptin influences the carotid body activity in both awake and asleep mice.

Caballero-Eraso, C., Shin, M. K., Pho, H., Kim, L. J., Pichard, L. E., Wu, Z. J., Gu, C., Berger, S., Pham, L., Yeung, H. B., Shirahata, M., Schwartz, A. R., Tang, W. W., Sham, J., & Polotsky, V. Y. (2019). Leptin acts in the carotid bodies to increase minute ventilation during wakefulness and sleep and augment the hypoxic ventilatory response. *The Journal of Physiology*, 597(1), 151–172.

USED IN OVER 19,500 PUBLISHED STUDIES

Our website, www.alzet.com, has a lot of useful information about our pumps, the research applications they have been used in, as well as the agents and vehicles people are using. If you have questions about using your agent with our pumps, don't hesitate to contact our Technical Support Department at 408.367.4036, or alzet@durect.com.

It is with great regret that we announce the departure of Jose Gadea from DURECT Corporation. Jose's tenure with the ALZET family of products spanned 21 years, beginning as a Technical Information Associate providing technical support to customers using ALZET® Osmotic Pumps in their research.

To his work with ALZET, Jose brought insights from his preceding stints in both academic and biotech research. He combined these perspectives while writing for and editing this publication, the ALZET Special Delivery™, for many years.

As a firm advocate for our customers, Jose was responsible for augmenting the ALZET product portfolio with numerous ancillary products to support customers in their use of ALZET Osmotic Pumps. In addition, he was responsible for bringing the distribution of the iPRECIO® Micro Infusion Pumps to DURECT.

The ALZET team is working diligently to carry on the legacy left by Jose, and we wish him the very best in his new position in management at an established life science company.