The Role of Activated Serum in Preventing Long-Lasting Pain in a Chronic Post Surgical Pain Model in Mice

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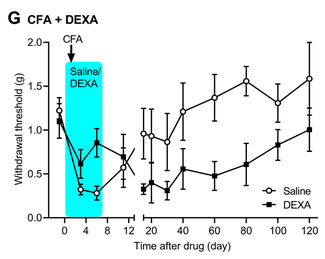
INTRODUCTION

Post-operative pain is a primary concern of patients undergoing surgical procedures and presents major challenges for patients and clinicians. Over-the-counter analgesics like non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for acute pain management. However, recent findings suggest that the inhibition of the inflammatory response in an acute pain state increases the odds of developing chronic pain[1].



Parisien et al., 2022

Activated Serum (AS) has been shown to be effective in treating different pain disorders such as osteoarthritis (OA)[2], trigeminal neuralgia[3], and neuropathic spine disease[4]. The analgesic effect and the acceleration of tissue regeneration via AS have been attributed to the enriched concentration of anti-inflammatory components such as IL-1Ra, IL-4 and IL-10 and growth factors such as TGF-beta[5]. However, an administration of an anti-inflammatory molecule (IL-1Ra) fails to provide significant analgesia in treating OA[6], suggesting that the actions of these mediators alone are insufficient to explain the long-lasting benefits of AS.

METHODS

Subject: 6–8-week-old CD-1 mice of both sexes were used. All mice were housed under standard laboratory conditions, having 12h of light/dark cycle and had access to water and food ad libitum.

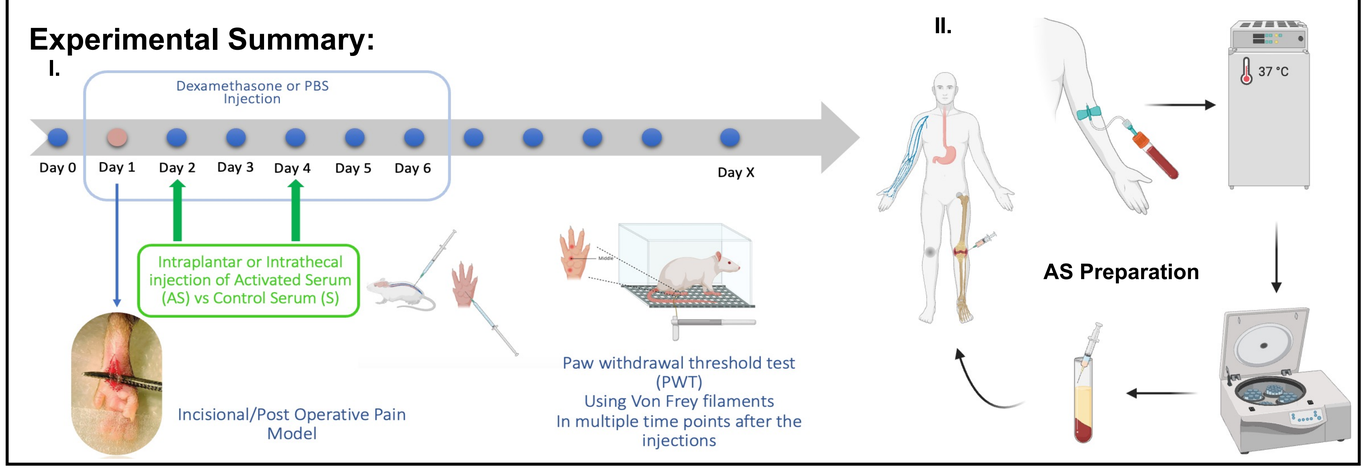
Post-Operative Pain Model and Pain Assessment: Post operative incision model is performed based on Cowie et al. protocol[7]. Tactile allodynia was measured using Von Frey filaments. 50% paw withdrawal threshold was then calculated using the up-and-down method.

Activated Serum Preparation: 50 mL of whole blood was taken from each patient using a special syringe (Orthogen, Dusseldorf, Germany). After incubation for 7 hours at 37 °C, the blood-filled syringes were centrifuged for 15 mins at 5000 rpm, the serum supernatant was then removed and aliquoted into 6 mL portions. The aliquots were stored at -20°C until use.

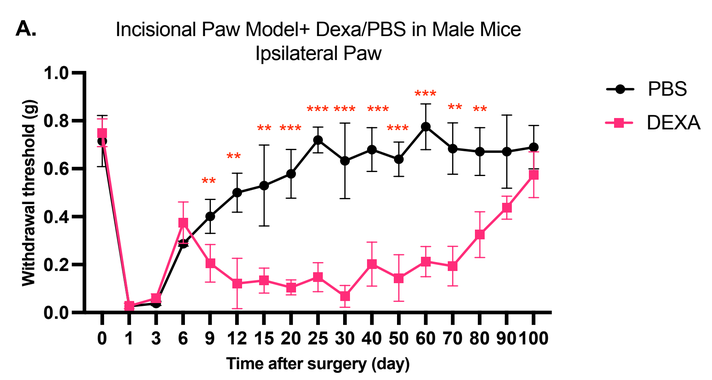
Pharmacological Treatments: Mice received 0.5 mg/kg/day of the corticosteroid dexamethasone (DEXA), or 1% DMSO subcutaneously for six consecutive days. On days 2 and 4 post-surgery, 10 microliters of Activated Serum (AS) or Control Serum (S) were injected intraplantar (i.pl.) or intrathecally (i.t.).

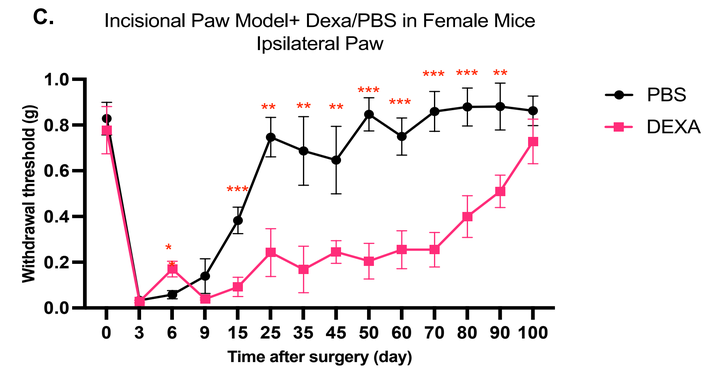
Statistical Analysis: Data collected individually from each subject has been calculated into group means and analyzed using Student's t-test or analysis variance (ANOVA) followed by Tukey's or Dunnett's post hoc testing as appropriate.

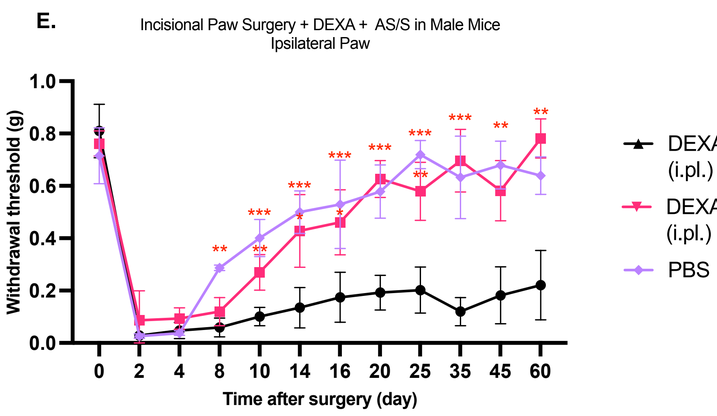
Experimental Summary:

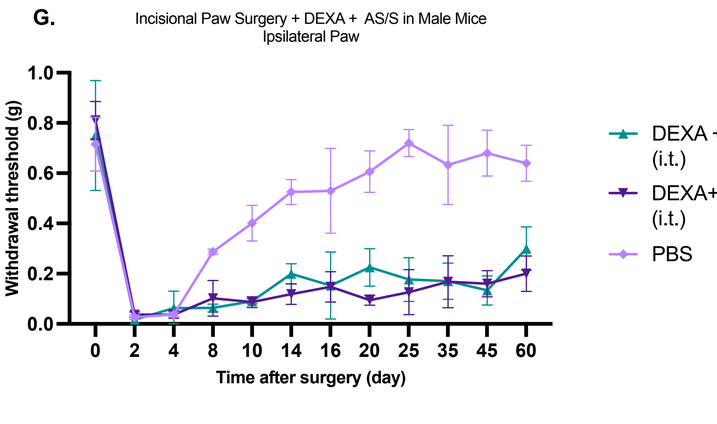


RESULT: The Dexamethasone Treatment Led to the Prolongation of Pain Behaviour in the Post-Operative Model Which Can be Prevented by AS Intraplantar Injections









The first two rows show mechanical pain threshold and days to return to baseline in male mice **(A, B)** and in female mice **(C,D) t**reated with PBS (Control) or DEXA. The last two rows show mechanical pain threshold and days to return to baseline after AS or S injection intraplantar **(E, F)** or intrathecally **(G,H)** in mice treated with DEXA. * Indicates Statistical Differences with DEXA and PBS group *p<0.05, **p<0.01, *** p<0.001

CONCLUSIONS

- Our results validated the previously reported observation that inhibiting active biological processes at the acute state of injury greatly prolongs pain resolution in a new and commonly used clinically relevant mouse model of postoperative pain.
- Dexamethasone injections acutely inhibit pain behaviour but greatly prolong pain resolution in the post-operative model.
- AS intraplantar injections prevent long-lasting pain induced by dexamethasone in post-surgical models.
- AS intrathecal injections did not prevent long-lasting pain induced by dexamethasone in post-surgical models.
- All the detected effects were observed in both male and female mice.
- **Pain behaviour in our model resolves spontaneously unless the immune/inflammatory response is inhibited by DEXA. AS, but not control serum, restores this immune response and protects from transitioning to chronic pain.**

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REFERENCES

1.Parisien, M., Lima, L. V., Dagostino, C., El-Hachem, N., Drury, G. L., Grant, A. V., Huising, J., Verma, V., Meloto, C. B., Silva, J. R., Dutra, G. G., Markova, T., Dang, H., Tessier, P. A., Slade, G. D., Nackley, A. G., Ghasemlou, N., Mogil, J. S., Allegri, M., & Diatchenko, L. (2022). Acute inflammatory response via neutrophil activation protects against the development of chronic pain. Science Translational Medicine, 14(644). https://doi.org/10.1126/scitranslmed.abj9954
2.. Motaal, F. K. A.-E., Elganzoury, A. M., Fathalla, M. M. & Abdulkareem, O. Low-dose intra-articular autologous conditioned serum in treatment of primary knee osteoarthritis. Egypt Rheumatol Rehabil 41, 98–102 (2014).
3. Aghamohammadi, D., Saharifi, S., Shakouri, S. K., Eslampour, Y. & Dolatkhah, N. Autologous conditioned serum (Orthokine) injection for treatment of classical trigeminal neuralgia: results of a single-center case series. J Med Case Reports 16, 183 (2022).
4. H. S., R. K., Goni, V. G. & Y. K., B. Autologous Conditioned Serum as a Novel Alternative Option in the Treatment of Unilateral Lumbar Radiculopathy: A Prospective Study. Asian Spine J 9, 916 (2015).
5. Kerscher, M., Hertz-Kleptow, D., Drabik, A., Kaptan, T. & Reinecke, J. Cell-Free Blood Cell Secretome (BCS) Counteracts Skin Aging and Restores Firmness and Elasticity. 20, (2021)..
6. Chevalier, X. et al. Intraarticular injection of anakinra in osteoarthritis of the knee: a multicenter, randomized, double-blind, placebo-controlled study. Arthritis Rheum 61, 344–352 (2009)
7. Cowie AM, Stucky CL. A Mouse Model of Postoperative Pain. Bio Protoc. 2019 Jan 20;9(2):e3140. doi: 10.21769/BioProtoc.3140. PMID: 30820443; PMCID: PMC6390978.

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