Activated Serum Prevents Chronic Post-Surgical Pain in Rodent Model

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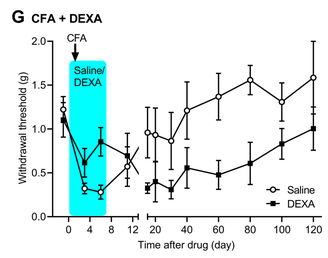
INTRODUCTION

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 increase the odds of developing chronic pain[1]. In ref. 1 prevention of pain chronification was pinpointed mechanistically on Neutrophils and their release of S100A8/A9 proteins.



Ref. 1: Parisien et al., 2022

An Activated Serum preparation (AS, also called *autologous conditioned serum, ACS*) has been shown to be effective in treating different chronic 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, administration of anti-inflammatory cytokine 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. We tested whether AS can revers or prevent pain in a post surgery model. Recent ELISA measurements confirm that AS contains S100A8/A9 (±4.8µg/mL)

METHODS

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

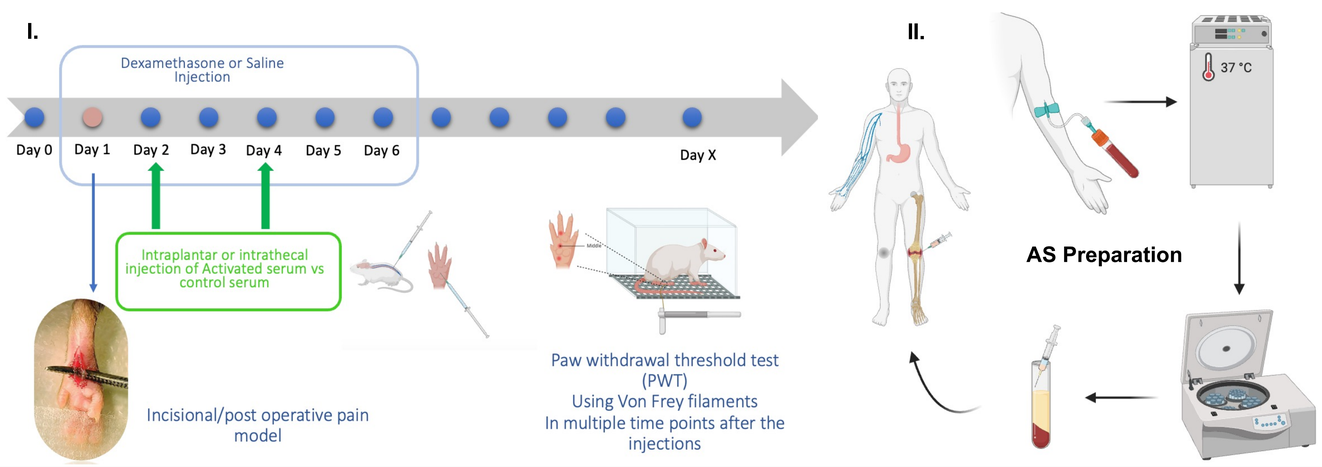
Post-Operative Pain Model and Pain Assessment: Post-operative incision model is performed based on Cowie et al.[7]. Tactile allodynia was measured using Von Frey filaments. 50% paw withdrawal threshold was then calculated using the up-and-down method.

Activated Serum Preparation: 50 mL of whole blood was taken from each patient using a special syringe type (Orthogen, Dusseldorf, Germany). After incubation 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.

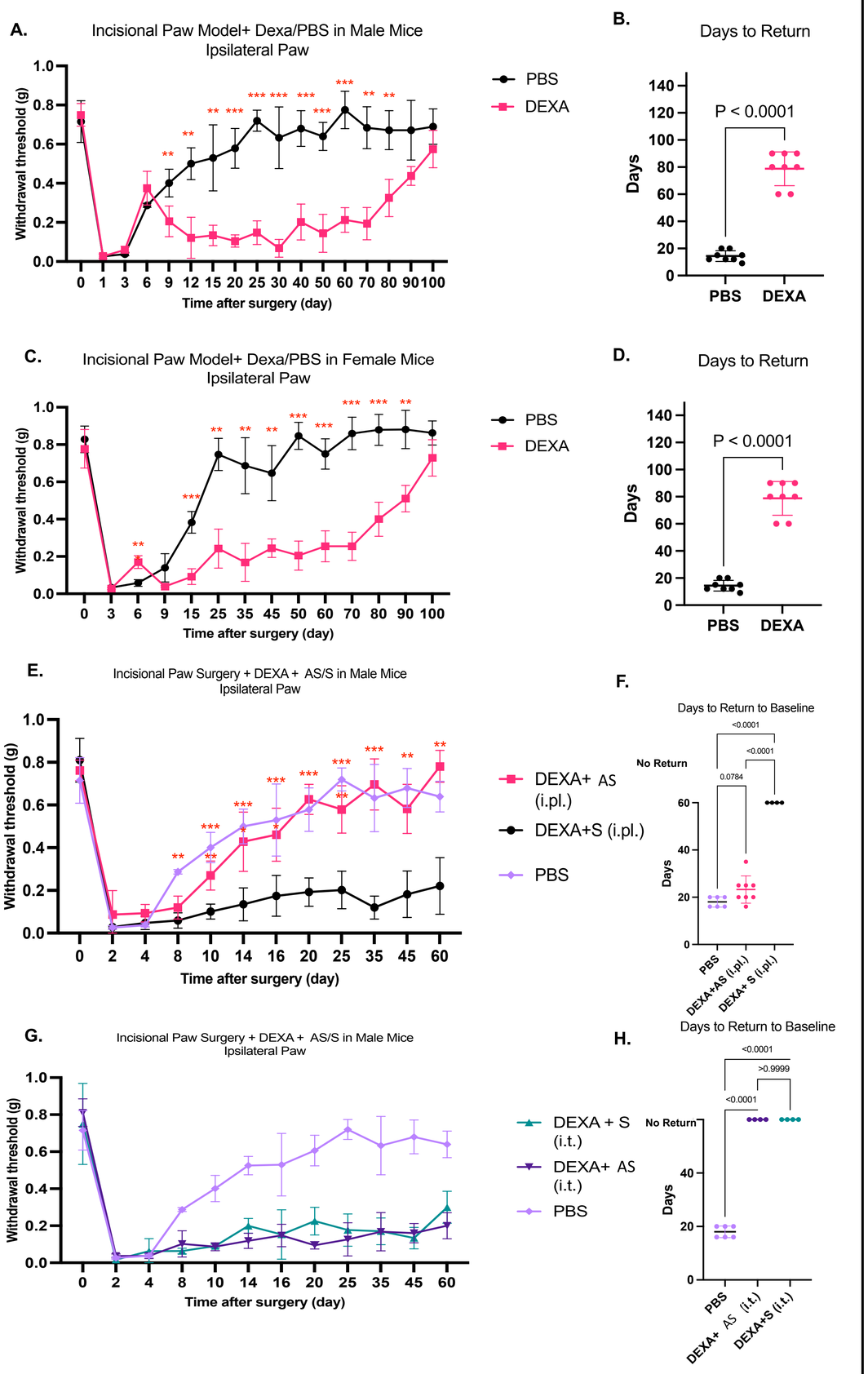
Pharmacological Treatments: Mice received 0.5 mg/kg/day of the corticosteroid dexamethasone (DEXA), or 1% DMSO subcutaneously for six consecutive days. On days 2 and 4 post-surgery, 10 microliters of activated serum (AS) or control serum (S) were injected intraplantar (i.pl.) or intrathecally (i.t.).

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: Dexamethasone Treatment Led to 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

- These results validate previously reported observations, namely that inhibition of active biological processes at the acute state of injury creates a state of chronic pain in a commonly used and clinically relevant mouse model of post-surgical pain. All detected effects were observed in both male and female mice.
- Dexamethasone injections acutely inhibit pain behavior but greatly prolong pain resolution in the post-surgical model.
- AS intra-plantar injections did prevent long-lasting pain induced by dexamethasone in this post-surgical model.
- AS intra-thecal injections did not prevent long-lasting pain induced by dexamethasone in this post-surgical model.

- **Pain behaviour in this 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.**
- **Recent ELISA measurements confirm that AS contains S100A8/A9 (4.8µg/mL)**

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