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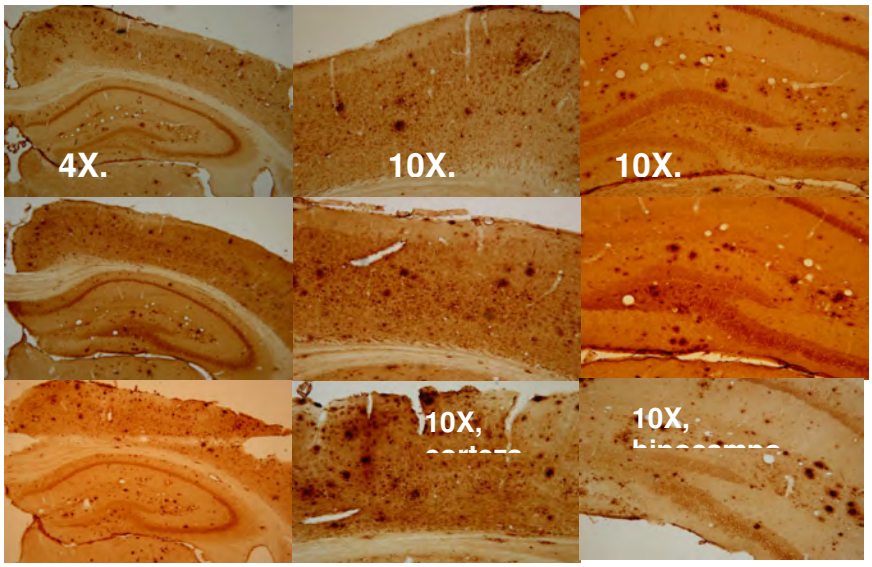
ParActin® Research Summary

(1) ParActin® is useful for preventing memory loss in Alzheimer disease by activation of PPAR-γ Receptor.

Juan L. Hancke. *Instituto de Farmacologia, Universidad Austral de Chile, Valdivia Chile.*

Study 1: PSAPP mice, which represent a transgenic mouse model of Alzheimer's disease, were used in this experiment. PSAPP mice exhibit onset of cognitive impairment and increasing amyloid-ß plaques in their brains. Mice were divided into 3 groups. Group 1 as untreated control, Group 2 were treated with ParActin®, and Group 3 were given hyperforin. Amyloid plaques in both the hippocampus and the cerebral cortex were tested at 12 weeks.

Beta Amyloid plague deposits



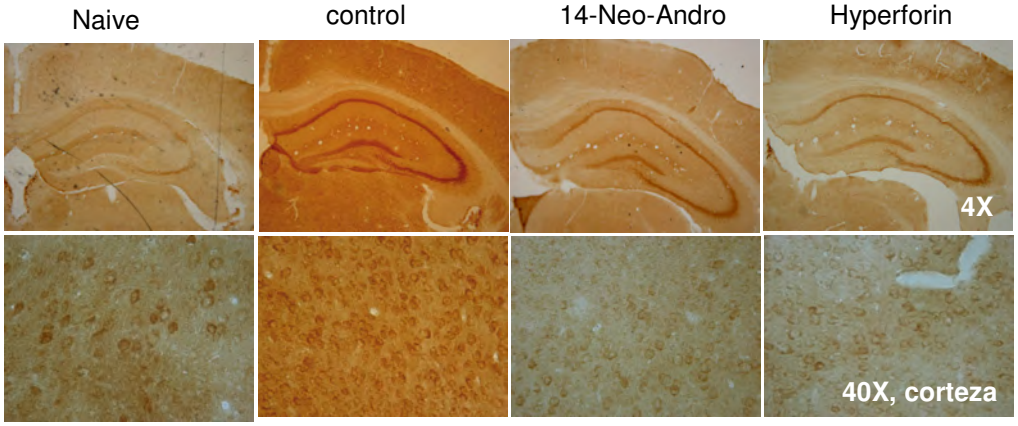
Ratón Tg (APP_{swe}+PS-1)
Untreated Control

Ratón Tg (APP_{swe}+PS-1)
(14-Neo-Andro)

Ratón Tg (APP_{swe}+PS-1)
Hyperforin

Paractin does not reduce amyloid plagues significantly. Hyperforin is effective in reducing beta amyloid plague.

Inflamation (oxidative status) of the brain



Both Paractin and Hyperforin significantly reduce the level of nitrotyrosynation (oxidative status of the brain) in transgenic mice.

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Study 2: In this experiment, the spatial memory in 25- to 30-week-old PSAPP mice was analyzed in Morris water maze (MWM) tests where the mice were trained to escape to a hidden platform.
Mice were divided into 4 groups:
Group 1 is control;
Group 2 is untreated PSAPP mice;
Group 3 is PSAPP mice treated with Hyperforin;
Group 4 is PSAPP mice treated with 14-Neo-Andro.

Group	Day 1-2	Day 3-5	Week 2	Day 12
1	20 sec	20 sec	20 sec	20 sec
2	60 sec	60 sec	60 sec	40 sec
3	40 sec	40 sec	40 sec	40 sec
4	20 sec	20 sec	20 sec	20 sec

This experiment showed that PSAPP mice treated with Paractin were able to learn the escape hidden pathway as fast as regular control mice and confirm the ability of ParActin® to improve learning ability and may reverse memory loss in AD. Although there are no apparent changes in the levels of amyloid plaques, ParActin showed the ability to stop memory and deterioration better than hyperforin, and decrease in the inflammation and oxidative status of the brain.

Study 3: ParActin® Human Clinical on Alzheimer
20 patients who manifested early stage of Alzheimer's disease (AD) were recruited. Two weeks before the clinical trial, the patients undergo appropriate psycho neurological tests such as the Mini Mental Status Exam (MMSE), the Alzheimer Disease Assessment Scale (ADAS), the Boston Naming Test (BNT), and the Token Test (TT). Neuropsychological tests are repeated on Day 0, 6 weeks and 3 months of the clinical trial. The tests are performed by neurophysiologists who are not aware of the patient's treatment regimen.

Patients are randomly assigned either 250mg of ParActin® (14-NEO-ANDRO) or placebo at the start of the study, to be taken orally twice daily. The ParActin® (14-NEO-ANDRO) and placebo results are statistically compared for all the study periods. Patients using placebo show a significant cognitive deterioration, which is the natural course of AD. Patients treated with ParActin® (14-NEO-ANDRO) ameliorate in a considerable way the test scores. This research shows that ParActin® (14-NEO-ANDRO), a PPAR-γ agonist may help in cognitive and memory deterioration and have application for maintaining healthy brain and cognitive function.

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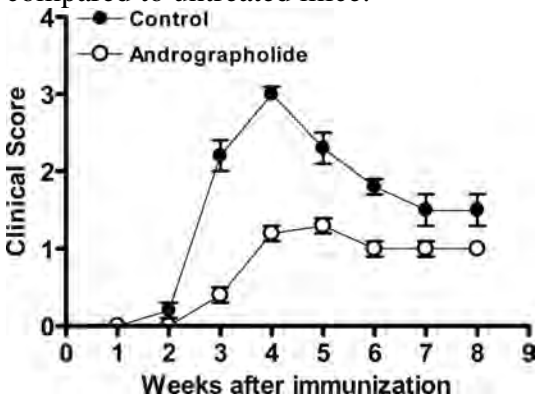
(2) ParActin® interferes with T cell activation and useful for treating Multiple Scrolesis.

Iruretagoyena MI, Tobar JA, Gonzalez PA, Sepulveda SE, Figueroa CA, Burgos RA, Hancke JL, Kalergis AM. *Universidad Austral de Chile.* ***Published: Journal of Pharmacology & Exp Therapeutics, August 26 2004.***

6 to 8 weeks old female mice were injected with 50 mg of MOG35-55 peptide. Half the mice were treated daily with 13 mg/kg of ParActin® (14-NEO-ANDRO) one week before MOG sensitization and continued through all the experiment. Clinical signs of disease were observed between day 15 and 18.. Interleukin-2 and Interferon gamma were measured with ELISA, Anti-MOG antibody response were analyzed, surface markers I-Ab, CD86 and CD40 were measured, T cell proliferation was assessed, and nonspecific IgG antibodies were measured.

In vitro T cell activation is inhibited by ParActin® (14-NEO-ANDRO)

Lymph node cells incubated with ParActin® (14-NEO-ANDRO) suppressed B and C T cell activation. ParActin® (14-NEO-ANDRO) prevented DCs from activating both CD4+ and CD8+ OVA-specific T cell by inhibiting the ability of DCs to process OVA and generate the peptide-MHC complexes required for T cell activation. ParActin® (14-NEO-ANDRO) also inhibited up-regulation of the maturation of DCs markers. Compared to untreated controls, mice treated with ParActin® significantly reduced anti-NP IgG titers compared to untreated mice.

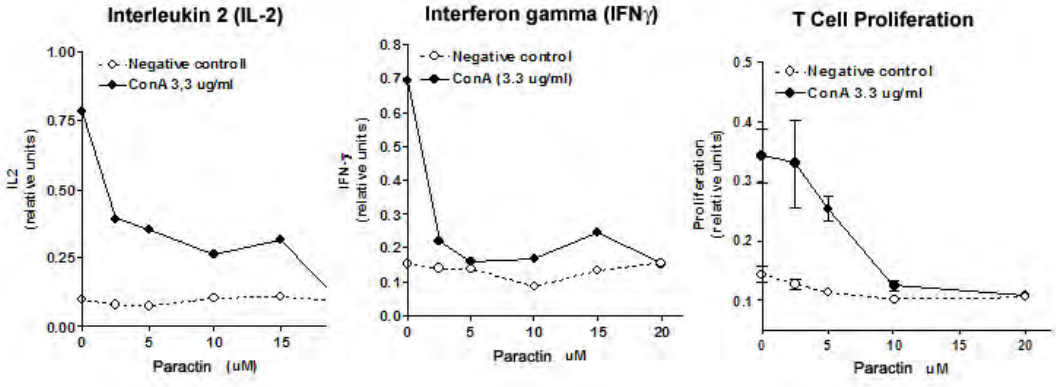


ParActin® (14-NEO-ANDRO) treatment significantly reduces severity of EAE in mice.

Symptoms of EAE were present on day 14 after sensitization. Clinical score was evaluated on a daily basis. Treatment with ParActin® (14-NEO-ANDRO) not only delayed the onset of EAE, but also significantly reduced the severity and incidence of MS.

ParActin® (14-NEO-ANDRO) reduces anti-myelin T cell and antibody response

3 weeks after EAE induction, Interferon gamma, Interleukin 2, and anti-MOG IgG were observed only in lymph node obtained from untreated mice suffering from EAE. In contrast, Interferon gamma, Interleukin 2, and anti-MOG IgG were not detected in lymph node from ParActin® treated mice.



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(3) ParActin® interferes with DNA binding of NF-κB in HL-60/Neutrophils cells.

María A. Hidalgo, Alex Romero, Jaime Figueroa, Patricia Cortés, Ilona I. Concha, Juan L. Hancke, & Rafael A. Burgos. *Uniersidad Austral de Chile.* **Published: British Journal of Pharmacology (2005) 144, 680-686**

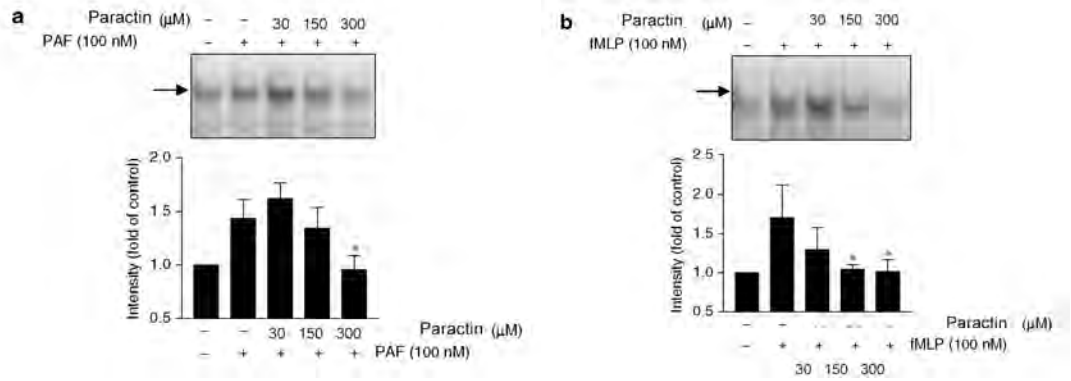
In the present study we analyzed the effect of ParActin® on the NF-kB activation induced by PAF and fMLP in human cells. We showed that ParActin® inhibited the NF-kB activity, reduced the DNA binding of NF-kB in vivo and in vitro, and reduced COX 2 expression.

ParActin® inhibits NF-κB activation by PAF

Human cells (HL-60) were pre-incubated with ParActin® (5 and 50 µM) or vehicle for 30 min, and then stimulated with PAF 100 nM for 1 hr, NF-kB activity was measured. ParActin® inhibited significantly the PAF induced luciferase activity in the NF-κB reporter, indicating that ParActin® interferes with the NF-κB activation.

ParActin® interferes with the DNA binding to NF-κB

HL-60/Neutrophils were stimulated with 100nM PAF or fMLP, and incubated with ParActin® (30,150, or 300 M) for 15 minutes. ParActin® inhibit directly the DNA binding of NF-κB stimulated by PAF and fMLP.



ParActin® reduces COX-2 expression

Research has shown that COX-2 expression is reduced by NF-κB inhibitors. In this study we demonstrated that ParActin® (14-NEO-ANDRO) reduced the COX-2 expression in HL-60/neutrophils induced by fMLP and PAF.

Conclussion: NF-κB has been described to regulate the cellular process of apoptosis. The inhibition of NF-κB by ParActin® (14-NEO-ANDRO) shown by this research could explain the anti-proliferate effect of ParActin®. We conclude that ParActin® (14-NEO-ANDRO) is a natural NF-κB inhibitor exhibiting strong potential anti-inflammatory properties.

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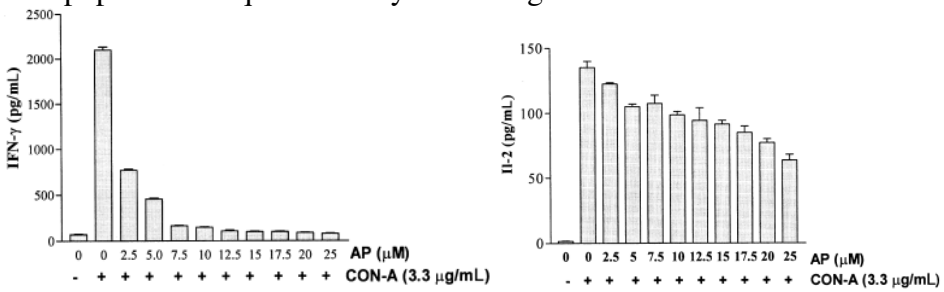
(4) ParActin® (14-NEO-ANDRO) inhibits IFNγ and IL-2 cytokines production and protects against cell apoptosis

[1]Juan L. Hancke, & [1]Rafael A. Burgos, Karina Seguel,Mirna Perez, Ada Meneses, Marcele Ortega. ***Published:*** **Planta 34/0704/416, and Biochem, Mol, Biol, 9.3.05/Summer**

In this study we present evidence that ParActin® (14-NEO-ANDRO) reduces the IFNγ and IL-2 production in murine T-cells and protects against cell apoptosis.

Method: T-cells and thymocytes were isolated from 6 to 8-week old RK mice. The. T-cells were incubated with concanavaline A (CON-A) and various concentration of ParActin®. IFNγ, IL-2 and IL-4 production was measured using ELISA. ERK1/2 phosphorylation was analyzed and apoptosis was quantified by DNA fragmentations.



All doses of ParActin® inhibited dramatically the CON-A rise of IFN-γ with maximum inhibitory response at doses of 7.5μM and IC_{50} 1.7±0.07μM. The reduction in cytokine production shown in our study could explain the anti-inflammatory effect of ParActin®

ParActin® inhibited the IL-2 production induced by CON-A. The IL-4 production was not modified either by ParActin® in T-cells.

The expression ICAM-1, iNOS, COX-2 and proinflammatory cytokine are controlled by mitogen-activated proteinkinase (MAPK). Our results show that ParActin® reduced the ERK1/2 phosphorylation induced by CON-A. ERK 1/2 is a MAPK that requires phosphorylation to become active. NF-κB regulate the cellular process of apoptosis. The DNA fragmentation of thymocytes was incubated with hydrocortisone and PMA. ParActin® alone did not induce an increase in apoptosis. 50μM of ParActin® reduced the apoptosis induced by hydrocortisone up to 20%. 100μM of ParActin® significantly reduced the apoptosis induced by PMA up to 35%. Apoptosis induced by hydrocortisone increases the production of caspase-3 activity. ParActin® was able to reduce significantly the spontaneous caspase-3 like activity. We conclude that ParActin® inhibit significantly the production of IFNγ in T-ells and ERK1/2 phosphyloration and protects against apoptosis. The antiinflammatory effect of ParActin® opens a potential use in several health imbalances associated with an increase in IFNγ and IL-2.

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(5) ParActin® EXHIBIT IMMUNOSTIMULANT EFFECT BY INCREASING CELLULAR INMUNE RESPONSE

[1]*Juan L. Hancke, &* [1]*Rafael A. Burgos*

ParActin® (14-NEO-ANDRO) exhibit immunomodulatory activities. It reduces the production of inflammatory mediators at high concentrations (micro molar) and stimulating the immune response in low concentration, both in vitro and in vivo. In previous studies, we have shown that high concentration of ParActin® reduces the IFN-γ and IL-2 in T-cells. In the present report we describe that ParActin® in low concentrations is able to increase the cellular immunity, inducing IFN-γ and IL-2 production specifically increasing the Th-1 response cell.

Method: Mice were treated intraperitoneally with a daily dose equal to 6.138 or 61.68 µg/kg of ParActin® for six days. 7 days after immunization the animals were sacrificed and T-cells were isolated from the lymph nodes and treated with concanavaline-A (CON-A), B. abortus protein, and/or with ParActin®. IFN-γ, IL-2 and IL-4 production was measured using ELISA.

Effects of ParActin® on T cell proliferation and cytokines production

ParActin® between 0.15 and 7.5 nM concentration increase T cell proliferation, and at 1.5 nM concentration showed a reduction in T cell proliferation and significantly reduce the IFN–γ and IL-2 production. There are no effects on T cell at or below 0.15 concentrations or above 15 nM concentration. RT-PCR showed that 0.15 and 1.5 nM of ParActin® induced an increase of IFN-γ mRNA.

Effect of ParActin® in mice immunized with B. abortus

T-cells from mice were treated with 5.6 and 56 µg/Kg of ParActin® and immunized with (0, 1, 5, 10 µg/ml) of B. abortus protein for 24 hours. Treatment with 5.6 and 56 µg/Kg of ParActin® significantly increase the cellular proliferation of T-cells and induces an increase of IFN-γ production. The IL-2 production was increased only in mice treated with 56 µg/Kg of ParActin®. The IL-4 production wasn't modified.

In this study we have shown the increase in the cellular proliferation and IFN-γ and IL-2 production in T-cells cultured with ParActin®, indicating the immunostimulatory effect in vitro. The present report showed ParActin® to be effective in increasing cytokine production of IFN-γ significantly in the gene expression. The IL-4 production was not modified in the concentrations used in this study. The increase of cellular proliferation and induction only of IFN-γ and IL-2, not IL-4, shown here suggest that ParActin® at low concentration could stimulate the immune system, specifically through of Th1 response. In summary, we showed clearly that ParActin® (14-NEO-ANDRO) has immunostimulatory effect at low concentration by increasing cellular proliferation and induction of Th-1 cytokine. This effect could explicate the immunostimulatory properties of ParActin® (14-NEO-ANDRO) observed *in vivo*.

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(6) ParActin® (14-Neo-Andro) Reduce Common Cold Occurrence

Location	Universidad Austral de Chile, Valdivia
Study Design	Randomized, Double Blind Placebo Controlled Study
Patients	109 healthy students during cold and flu season: 54 on ParActin®, 53 on placebo
Dosage	25mg of ParActin®/day
Duration	3 months
Evaluation	Evaluated by clinician for presence or absence of common colds
Result	No significant change between ParActin® and placebo group during the 1st month. During month 2 and 3, there is significantly decrease the incidence of common cold in ParActin® group (30%) compared to placebo group (62%)
Adverse Report	No adverse effects were reported
Conclusion	PARACTIN is effective for prevention of common cold occurrence during winter months

Previous study had shown that low concentration of ParActin® (14-NEO-ANDRO) were able to increase the production of IFN gamma and IL-2 without modifying Th2 antibody (IL-2, IL-10 cytokines) production. In addition, RNA messenger for Interferon gamma was also increased. Interferon gamma is the first line of defense against viral infection such as Influenza, and is activated by T cell.

In this study we have found that at 25mg daily dosage of ParActin® (14-NEO-ANDRO) given to healthy individuals during cold and flu season is effective in reducing the occurrence of common cold. We conclude that at low dosage, ParActin® can be used as cold weather companion and exhibit strong immunostimulating effects.

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(7) ParActin® Reduce the Intensity of Symptoms and signs of common cold.

Location	Universidad Austral de Chile, Valdivia
Study Design	Randomized, Double Blind Placebo Controlled Study
Patients	158 adult patients already contracting common cold of both sex: 79 on ParActin®, 79 on placebo
Dosage	200mg of ParActin®/day
Duration	5 days
Evaluation	Self Evaluation for: frequency & intensity of cough, expectoration, nasal Headaches, tiredness, ear ache, sleeplessness, sore throat on Day 0, 2, and 4.
Result	ParActin® significantly decrease in the intensity of all symptoms for compared with placebo group
Adverse Report	No adverse effects were reported
Conclusion	ParActin® is highly effective in reducing the occurrence and intensity of the symptoms in uncomplicated common cold beginning at day two of treatment.

ParActin® (14-Neo Andro) show pharmacological effects similar to non steroidal anti-inflammatory drugs (NSAIDs), which is widely use to treat and reduce symptoms in cold and flu. However, aspirin and paracetamol, can produce an increase in nasal symptoms. Recent study has shown that NSAIDs inhibits NFkappaB, thereby reducing cytokines production that are associated with fever, muscle aches that are commonly experienced in cold and flu symptoms. Our previous in vitro study has shown that high concentration of ParActin® is effective in inhibiting NFkappa B, thereby reducing pro-inflammatory cytokines production. This is evident in the significant reduction in all symptoms on day 2 and day 4. Interestingly, in the ParActin® treated patients, nasal secretions decreased significantly indicating an additional effect of ParActin®. This may be due to the anti-viral property of ParActin® which could help in fighting viral infection while reducing overload of cytokines. In contrast, aspirin and paracetamol suppress seroneutralizing antibody response and may increase susceptibility to colds. This current study showed the efficacy of ParActin® in reducing the intensity of common cold and its traditional use for various viral infections. High dosage of ParActin® could be an alternative to existing treatments such as nasal decongestants and antiphlogistica as it is useful in reducing the severity of various common cold symptoms.

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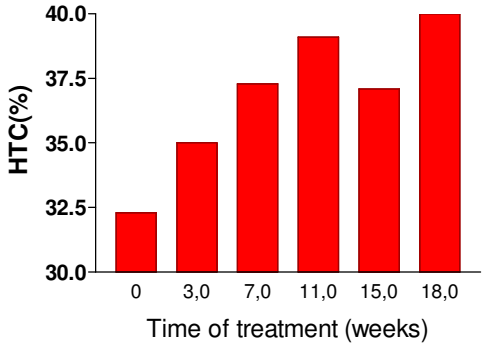
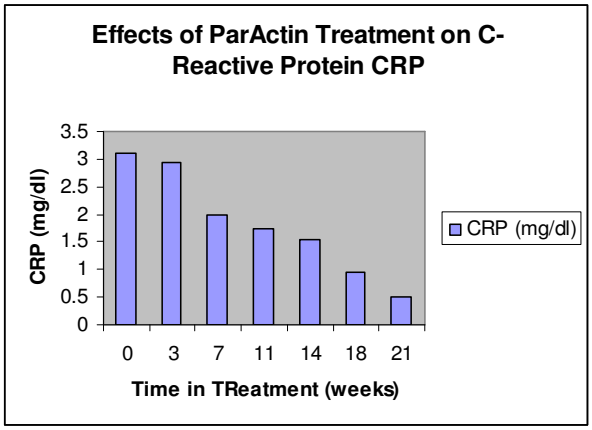
(8) ParActin® Joint Health

Study 1: 10 patients diagnosed with early stage RA were given 200mg of ParActin®, once daily for 18 weeks. A complete blood test is conducted. Hemacrit, hemoglobin, C-reactive Protein, and Rheumatoid Factor value were tested at the beginning, week 3, 7, 11, 15, and 18.

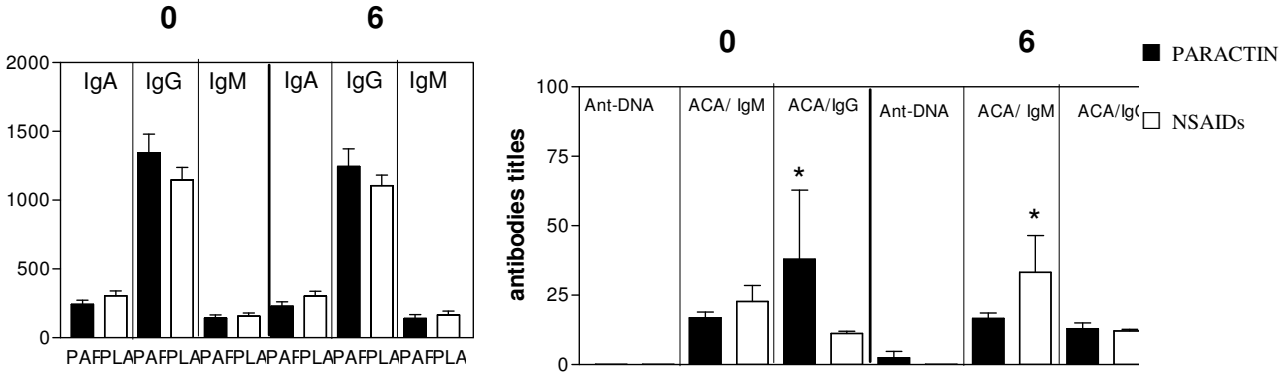


Patients taking ParActin® is showing improvement of hematocrit level from below normal level of around 32.4% (pre-treatment) to a normal average level of ~40%. Hemoglobin also showed gradual improvement from below normal level of 11% (pre-treatment) to healthy normal level of 12.6% at the end of study.

Patients treated with ParActin® is showing improvement in CRP level from above normal CRP level to a normal level of 0.5%. NSAIDs has been shown to effectively reduce CRP level. This research showed that ParActin® is effective in normalizing CRP level, and maybe useful in healthy joint support.



Study 2: 14 Patients were given either 200mg of ParActin® or NSAIDs as placebo group for 6 weeks.



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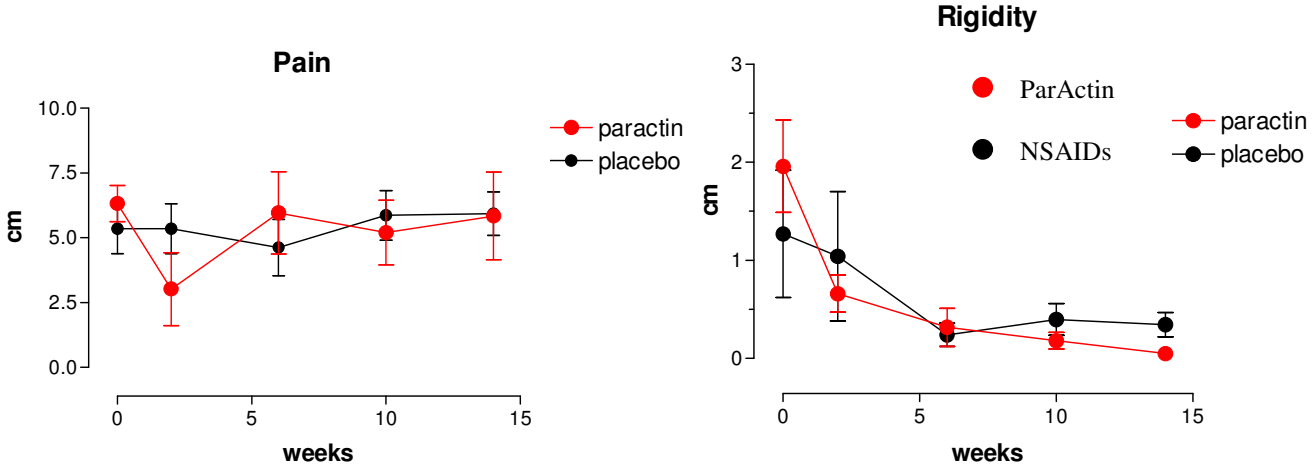
IgG and IgM anti-cardiolipin autoantibodies (ACA) are usually present in high levels in patients with rheumatoid arthritis. At the end of the 6 weeks trial, both groups are showing significant reduction in the IgG level, There are significant reductions in both classes of IgM and IgG anti-cardiolipin antibodies (ACA) in the ParActin® group, which correlate with a decrease in the RA activity. The NSAIDs placebo group is showing increase in IgM ACA titers and no change in IgG ACA titers.



ParActin® treatment significant decrease pain more than the NSAIDs group up to 2 weeks of treatment. At week 5, the analgesic effect decreases to overlap the NSAIDs group, with no change thereafter. The rigidity chart shows that both ParActin® and NSAIDs placebo groups significantly decrease the intensity of rigidity test with ParActin® group decreasing more than the placebo group throughout the 15 weeks treatment period. These findings indicate that ParActin® maybe as effective as traditional NSAIDs in reducing pain and improving rigidity in patients with arthritis.

(9) DOUBLE BLIND PLACEBO CONTROLLED STUDY ON THE EFFECT OF PARACTIN® ON RHEUMATOID ARTHRITIS

60 patients with RA were recruited in multi centric hospitals with an observation period of 14 weeks. Each patient receives either 85mg of ParActin® 3 times daily, in the morning, afternoon and night or a placebo for 14 weeks. 1. Paracetamol or other NSAIDs are given to eliminate pain if requested by patients.

On day 1, and at the end of week 2, week 6, week 10, and week 14 the following exams were performed:

1. Evaluation of the number of inflammatory and painful joints.
2. Evaluation of pain by the patient utilizing the Visual Analog Scale (VAS).
3. Improvement in the evaluation of health quality of life questionnaires
4. Duration of morning rigidity.
5. Global evaluation of symptoms by the patient and researchers.
6. Global evaluation of the tolerance by the patient and researchers.

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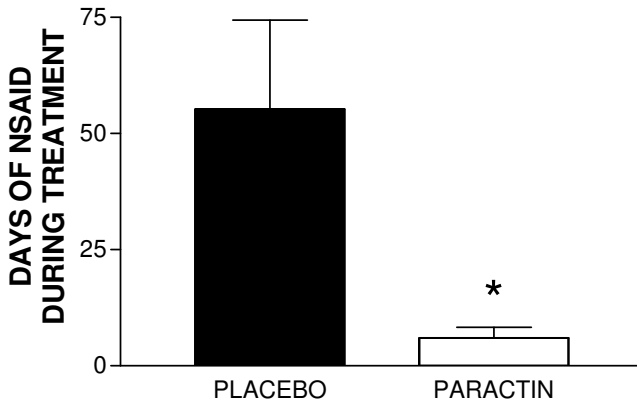
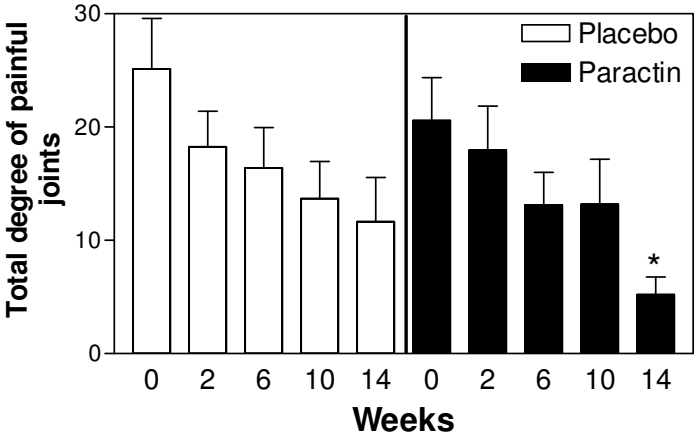
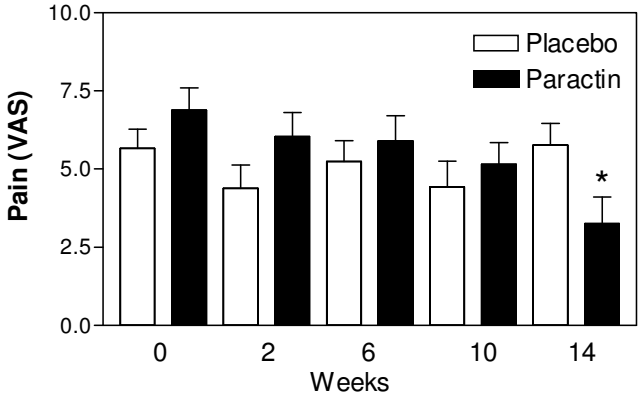


Throughout the 14 weeks of study, we allowed participants to take NSAIDs if it is necessary. The study showed that the placebo group is taking NSAIDs over 55% of the study period, while ParActin® group only take NSAIDs ~ 5% of the study period.



As observed in the figure, ParActin® group showed a significant decrease of painful joints compared to the placebo group, who is taking NSAIDs > 55% of the study period. This study indicates that ParActin® is effective in reducing painful joints.



This chart again showed that the ParActin® group experienced a gradual and significant decrease in level of pain as measured by the visual analog scale. The placebo group, who is taking NSAIDs for more than 55% of the study period do not showed any significant improvement in reducing pain.

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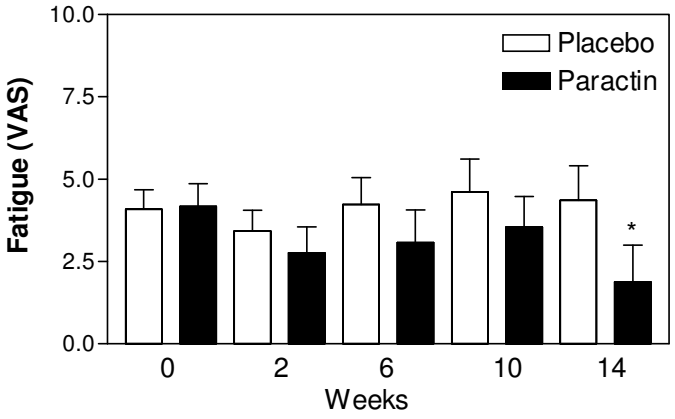
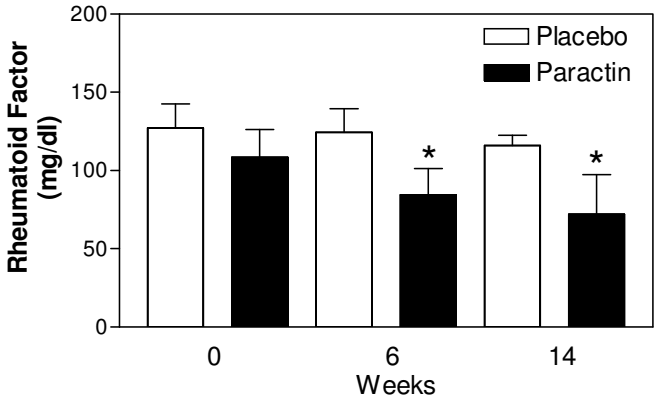
In this study, we have shown that patients taking ParActin® are experiencing significant reduction in fatigue from 4.0 to 1.25 as measured by VAS. The placebo group do not showed any improvement in fatigue even though the placebo group take NSAIDs >55% of the study period.



In this study, we showed that ParActin® significantly reduced the RF value from 110 to 70 mg/dl. The placebo group, even though taking NSAIDs most of the study period, did not experience any significant change in RF value.



Patients treated with ParActin® are showing improvement in CRP level from a above 1mg/dl to 0.5mg/dl at the end of the study. The result suggest the capacity of ParActin® to reduce C-reactive protein, which is one the most sensitive mediator reactants of acute and chronic inflammatory activity in RA. The placebo group, which is on NSAIDs most of the study period, only showed slight reduction in CRP protein level. This research showed that ParActin® is effective in normalizing CRP level.