The effect of a Protein Kinase C inhibitor (PKCi) on steroid hormones: a 2-week rat oral (gavage) investigative study focusing on pathology and hormone analysis in plasma and tissues

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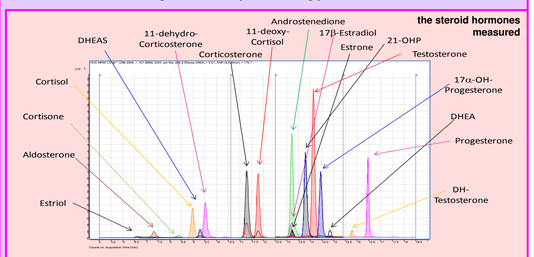
INTRODUCTION

- The Protein Kinase C (PKC) family is a family of serine/threonine kinases with various classical and novel isoforms.
- Some of these isoforms are expressed in T and B cells and have a key role in T-lymphocyte activation, downstream of the T-cell receptor and CD28 co-receptor signaling, therefore being used as immunosuppressive agents (Matz et al, 2011).
- PKC could also have a potential role as a receptor and/or transducer of the non-genomic effects of steroid hormones which regulate a wide variety of cellular responses (regulation of ion transport and cell proliferation/migration/differentiation, and death) (Alzamora and Harvey, 2008).
- The inhibition of PKC could alter steroid hormone actions and regulations leading to disturbances in multiple organ systems, particularly the reproductive system.
- To investigate the potential effects of PKC inhibitors on steroid hormones and its consequences, a 2-week oral mechanistic study in rats was conducted with a PKC inhibitor, focusing on pathology and hormone analysis in plasma and tissues.

STUDY DESIGN & PARAMETERS

STUDY DESIGN	
Groups & Doses	Group 1: Vehicle Control Group 2: 100 mg/kg/day Group 3: 200 mg/kg/day
No of Animals	10 rat / sex / group
Duration	2 weeks
Vaginal smear collection	On Pre-test Days: -2 and -1 On Dosing Days: 1, 2, 3, 9, and 10
Hormone measurements	LH, FSH, Prolactin and steroid hormones: On Pre-test Day: -1 On Dosing Days: 3, 9 and 14 (~ 3 hours post-dose) Oxytocin: after the last dose (~ 3 hours post-dose)

Reproductive organ histopathology
Endocrine organ histopathology



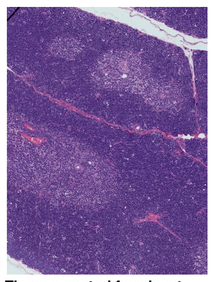
Hormone analysis

Gene expression (thymus)

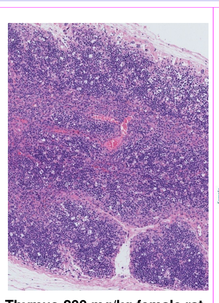
Lymphoid organ histopathology

RESULTS

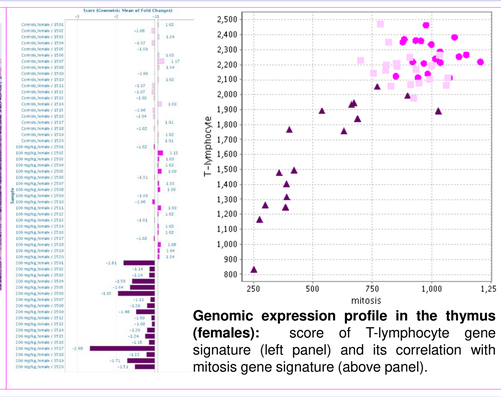
PKCi Effects on Lymphatic Organs



Thymus-control female rat (H/E x10)
Densely-cellular, dark cortex



Thymus-200 mg/kg female rat (H/E x10)
Lymphoid depletion (reduced cortex thickness and paler due to reduced cellularity)

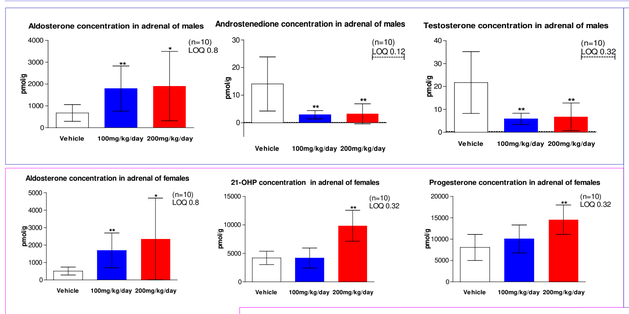


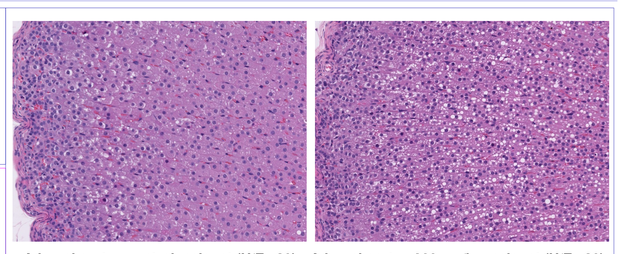
Genomic expression profile in the thymus (females): score of T-lymphocyte gene signature (left panel) and its correlation with mitosis gene signature (above panel).

- Lymphoid depletion in the thymus, lymph nodes, and spleen of both sexes at 100 and 200 mg/kg/day.
- Strong down-regulation of T-lymphocyte specific genes (CD3, CD6, CD8) and mitosis genes at 200 mg/kg/day.

Findings are consistent with the immunosuppressive effect of PKCi. Stress has possibly contributed to the extent of the lymphoid depletion at 200 mg/kg/day [poor clinical condition of the animals (reduced body weight gain, piloerection and chromodacryorrhea)].

PKCi Effects on Endocrine Organs





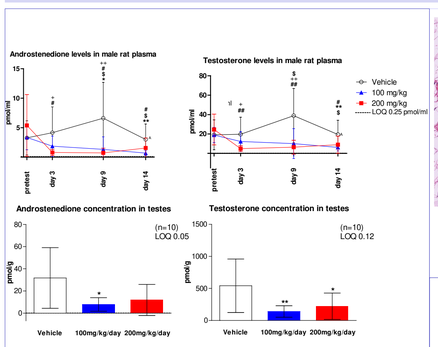
Adrenal cortex-control male rat (H/E x20)
Scattered cytoplasmic vacuoles, predominantly in the zona fasciculata

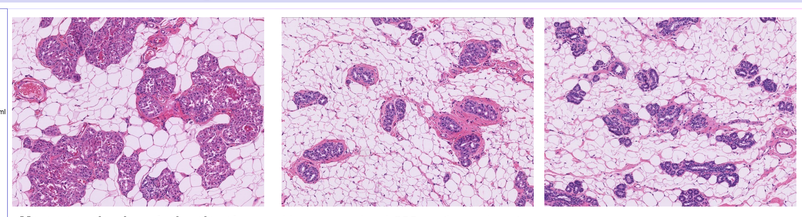
Adrenal cortex-200 mg/kg male rat (H/E x20)
Increased (in number and size) cytoplasmic vacuoles, predominantly in the zona fasciculata

- Increased cortical vacuolation in the adrenals of both sexes at 100 and 200 mg/kg/day associated with:
  - an increase in adrenal aldosterone in both sexes
  - elevated progesterone, 11-deoxycorticosterone (21-OHP) and corticosterone in female adrenals
  - decreased testosterone and androstenedione in male adrenals

Vacuolation can be consistent with stress (Greaves, 2012) and/or with the observed statistically significant increase in aldosterone and cortisol content in adrenal tissue and plasma concentrations in both sexes.

Male Accessory Reproductive Organs





Mammary gland-control male rat (H/E x10)
Lobuloalveolar pattern (florid growth)

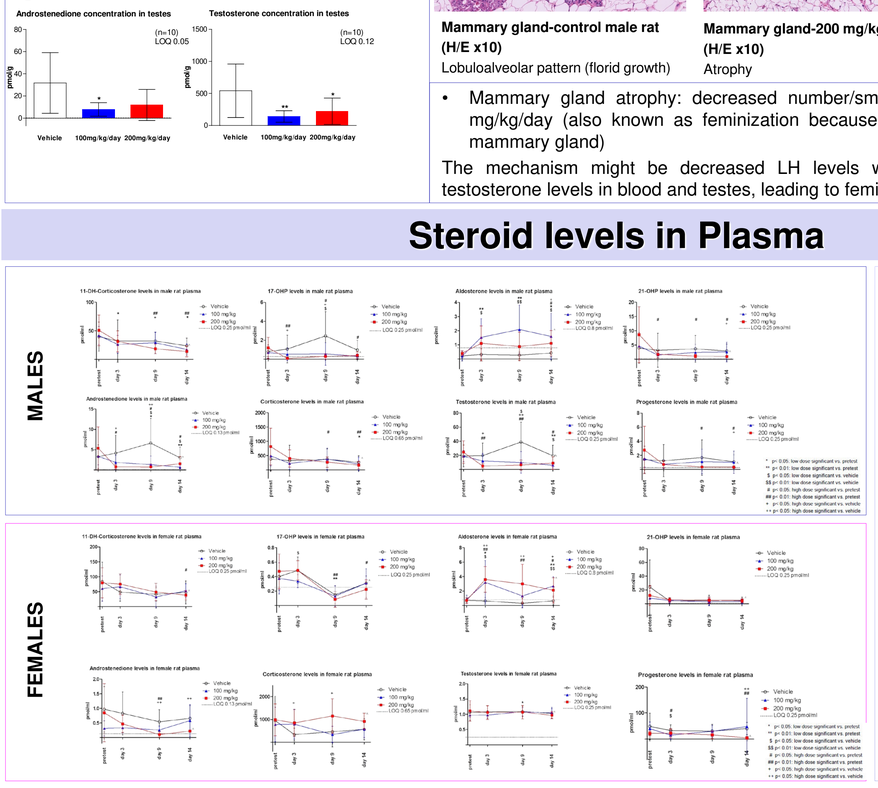
Mammary gland-200 mg/kg male rat (H/E x10)
Atrophy

Mammary gland-control female rat (H/E x10)
Tubuloalveolar pattern

- Mammary gland atrophy: decreased number/smaller alveolar profiles in males at 200 mg/kg/day (also known as feminization because mimicking the histology of the female mammary gland)

The mechanism might be decreased LH levels with secondary reduction of measured testosterone levels in blood and testes, leading to feminization of the male mammary gland.

Steroid levels in Plasma

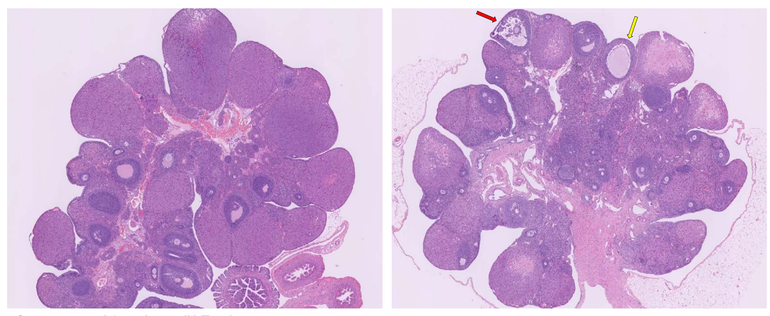


Changes in steroid hormones:

- slight to marked increases in aldosterone (1.6- to 10.5-fold pretest) at ≥ 100 mg/kg/day from Day 3 in males and females
- slight to marked decreases in progesterone (-27 to -89% vs. pretest) at ≥ 100 mg/kg/day from Day 3 in males and at 200 mg/kg/day on Day 14 in females
- moderate to marked decreases in androstenedione (-44 to -88% vs. pretest) at ≥ 100 mg/kg/day from Day 3 in males and at 200 mg/kg/day from Day 9 in females
- slight to marked decreases in 21-hydroxyprogesterone (-45 to -88% vs. pretest), 17-hydroxyprogesterone (-29 to -92% vs. pretest) and in testosterone (-35 to -80% vs. pretest) in males at ≥ 100 mg/kg/day from Day 3

PKCi Effects on Female Reproductive Organs

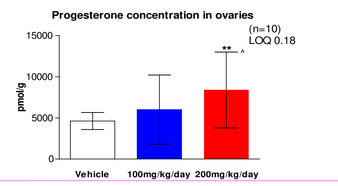
Vaginal histology and smears: Panel (a) to (d): Different stages of estrus cycle in controls
Panel (e): Atrophy/degeneration of the mucosal epithelium at 200 mg/kg/day

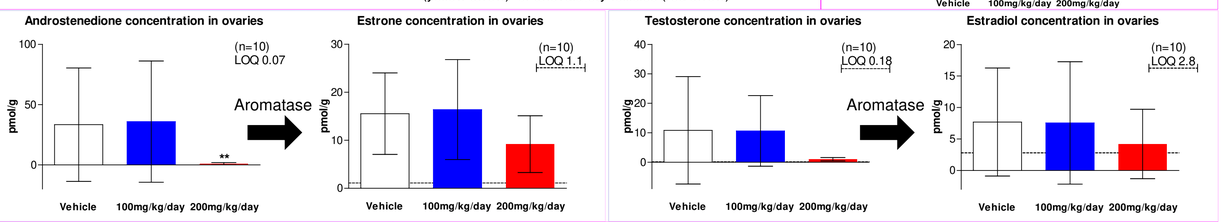


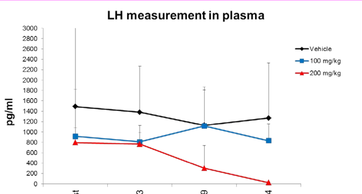
Ovary-control female rat (H/E x4)
Corpora lutea at different stages (basophilic: developing; eosinophilic: matured or maturing) with some developing ovarian follicles

Ovary-200 mg/kg female rat (H/E x4)
Degenerating corpora lutea [all appearing eosinophilic (matured/maturing)] with atretic follicles, and cystic (yellow arrow) or anovulatory follicles (red arrow)

At 200 mg/kg/day atretic follicles/corpora luteal degeneration of ovaries were noted, in association with disturbances in estrous cycle, as observed by female genital system staging and vaginal cytology. A reduction in androgen and estrogen levels, and an increase in progesterone in the ovary were also seen at 200 mg/kg/day







A comparison between female estrus cycle and sex hormone concentrations in gonads revealed in general a good correlation of progesterone, estrone, and estradiol levels in ovaries with the phase of the estrus cycle in controls and 100 mg/kg animals, showing higher estrogen levels in proestrus phase females compared to other phases (data not shown).

LH was markedly reduced in females at 200 mg/kg/day.

In consequence, reduced progesterone levels in gonads would have been expected. However, although progesterone was decreased in blood, it was elevated in ovaries of these animals.

SUMMARY & CONCLUSIONS

- Treatment of Wistar rats with a PKC inhibitor orally at 100 and 200 mg/kg/day for 2 weeks resulted in changes in female reproductive organs (atretic follicles and corpora luteal degeneration consistent with disturbed estrus cycle), male mammary gland (atrophy/feminization), and adrenal glands (increased cortical vacuolation) associated with various hormonal changes in tissues and circulation.
  - These results indicate that PKC inhibitors can induce hormonal imbalances leading to disturbances in multiple organ systems, particularly the reproductive system in the rat. In this study a direct effect on the hypo-pituitary-gonad axis was demonstrated with very likely additional direct effect on ovaries and adrenals.
- Lymphoid depletion observed in various lymphoid organs confirmed the known immunosuppressive effects of PKC inhibitors (Matz et al, 2011).
  - It should also be noted that stress (poor clinical condition at 200 mg/kg) could have contributed to the extent of lymphoid depletion.

References

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- Greaves, 2012. Endocrine glands. In: Histopathology of preclinical toxicity studies. 4th edition, published by Elsevier, Amsterdam, Netherlands, p. 750-751
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