Original Article

It is Time to Carpe Diem with Porcine ACTH – A Comparison of Porcine Sequence Corticotropin to Tetracosactide Hexaacetate in Testing the Hypothalamic Pituitary Adrenal Axis in Healthy Individuals

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Abstract

Context: Our literature search revealed that the use of porcine sequence corticotropin has not been validated against tetracosactide hexaacetate in a healthy population. **Aims:** To study the efficacy of using porcine sequence corticotropin in comparison with tetracosactide hexaacetate in the evaluation of hypothalamo pituitary adrenal (HPA) axis. **Materials and Methods:** Fifteen healthy volunteers were enrolled. Serum cortisol was measured at 0 minute in all subjects and at 30 and 60 minutes after tetracosactide hexaacetate 250 μg intravenously. Four weeks later, serum cortisol level was measured at 0 minute and at 30 and 60 minutes following 24 units of porcine sequence corticotropin given intramuscularly. **Results:** Mean serum cortisol values with tetracosactide were 30.3 (±7.83) μg/dl and 31.27 (±7.36) μg/dl at 30 and 60 minutes, respectively. The mean cortisol values with porcine sequence corticotropin were 26.33 (±5.47) μg/dl and 31.59 (±6.40) μg/dl at 30 and 60 minutes, respectively. All subjects had a response qualified as normal or adequate at 30 minutes itself. Mean peak serum cortisol response was 32.65 (±7.76) μg/dl in tetracosactide group and 31.59 (±6.4) μg/dl in porcine sequence corticotropin group, and the responses in two groups were comparable ($P = 0.686$). There were no immediate side effects in both groups, with a lower cost of procedure in the porcine corticotropin group. **Conclusion:** Our study established the efficacy of porcine sequence corticotropin in testing the adequacy of HPA axis in healthy individuals. Our study also revealed that, the intactness of the HPA axis could be confirmed as early as 30 minutes in healthy individuals.

Keywords: ACTH stimulation test, HPA axis suppression, Synacthen

Introduction

Cortisol insufficiency is a frequent and life-threatening conundrum that doctors encounter in endocrinology clinics and hospitals.[1,2] Primary adrenal insufficiency (PAI) is defined as decreased or absent glucocorticoid and mineralocorticoid secretion by adrenal glands. Most common cause for PAI in India is tuberculosis.[3] Insulin tolerance test (ITT) is used in the diagnosis of adrenal insufficiency but unfortunately the test is associated with risks and complications which may be unacceptable.[4,5] ITT is contraindicated in patients with seizure disorder and ischemic heart disease.[6,7] Short synacthen test (SST) is standardized in the diagnosis of adrenal insufficiency and is relatively safer, quicker and cheaper.[1,2]

SST involves administration of 250 μg of cosyntropin (tetracosactide hexaacetate), a synthetic adrenocorticotropic hormone (ACTH) composed of first 24 amino acids of ACTH 1-39 peptide, intravenously or intramuscularly. Serum cortisol is measured at 0, 30 and 60 minutes after ACTH administration. A previous study done in our institution showed that dilution and refrigeration of tetracosactide hexaacetate does not affect the validity of the testing.[8]

ACTH acts on MC2R receptors on adrenal glands and stimulates production of glucocorticoids, mineralocorticoid

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and adrenal androgens but cortisol responds most by a 64 fold increase.[9]

The use of synacthen (tetracosactide hexaacetate) in India is constrained by lack of availability and expense. Acton prolongatum (porcine sequence corticotropin) is freely available throughout India for subcutaneous and intramuscular use. It is marketed as a multi-dose vial at a cost that is one-twelth to one-fifteenth of synacthen when individual patient is concerned.

We undertook this study to validate the efficacy of synthetic corticotropin carboxymethylcellulose in saline of porcine sequence in comparison to tetracosactide hexaacetate for evaluation of hypothalamic pituitary adrenal (HPA) axis in healthy normal controls. A previous study has documented the efficacy of porcine sequence corticotropin in healthy volunteers and those with adrenal insufficiency without documenting a comparison to gold standards (ITT and SST).[10] However, we felt that the response of HPA axis to porcine sequence corticotropin in healthy controls has to be documented and compared with the existing standards of synacthen stimulation testing before we apply the test to evaluate the adequacy of HPA axis function.

Subjects and Methods

Our study was conducted in the Department of Endocrinology, St. John's Medical College Hospital, Bengaluru after the approval of the ethics committee. Written informed consent was taken from all the subjects after explaining the procedure to them. The study costs were met by the investigator team themselves. Considering a mean difference of 0.43, standard deviation of 6.6 and equivalence margin of 5 units in the cortisol level with tetracosactin and porcine sequence corticotropin with 80% power and 5% error, the minimum required sample size was 13.[11]

Serum cortisol was measured at 0 minute in all subjects following which they received tetracosactide hexaacetate 250 μg intravenously. Serum cortisol level was measured at 30 and 60 minutes after the tetracosactide hexaacetate. After 4 weeks, serum cortisol level was measured at 0 minute and 24 units of porcine sequence corticotropin was given intramuscularly. Serum cortisol was measured at 30 and 60 minutes. Volunteers were questioned about any immediate discomfort and adverse events.

Acton prolongatum is a synthetic corticotropin in porcine sequence. Reversible binding of ACTH to caboxymethylcellulose protects the peptide against enzymatic breakdown and prolongs its duration of action. It is available for intramuscular or subcutaneous use in a 5 ml multi-dose vial. Each ml contains 60 IU of ACTH. For diagnostic testing we have used 0.4 ml of acton prolongatum containing 24 units intramuscularly to deltoid in accordance with a previous study.[10]

Synacthen is available as tetracosactide hexaacetate in 1 ml ampoules. Cortisol response at 30 and 60 minutes in porcine sequence corticotropin and tetracosactide hexaacetate groups were compared. A cut-off value of serum cortisol >18 μg/dl at 30 or 60 minutes were considered as normal or adequate response.

Serum cortisol was measured using CLIA with ADVIA Centaur XP by Siemens and the test had an analytical sensitivity of 0.5 μg/dl. Reference range for assay was 0.5–75 μg/dl. Normal reference range for AM cortisol was 4.3–22.4 μg/dl. Blood sample was collected by venipuncture in a golden-yellow capped vacutainer and was allowed to clot completely at room temperature for 15–30 minutes and then centrifuged at 3000 rpm for 10 minutes. 20 μl of serum was pipetted automatically by the machine and reagent was added.

Data analysis and statistical methods

Data were analyzed using SPSS v. 24. Cortisol values were described using mean and standard deviation. The change and peak response of cortisol between the drugs were compared using independent sample *t*-test and the change in cortisol values between the drugs over the period of 0, 30 and 60 minutes were compared using repeated measures of analysis of variance. *P* value was considered to be significant at 5% level of significance for all comparisons.

Results

Our study included 15 healthy volunteers. Among the volunteers nine were female and six were male. None of the volunteers were on any form of chronic or traditional medications. Mean basal serum cortisol was 14.21 (±6.18) μg/dl in tetracosactide hexaacetate group and mean basal cortisol value in porcine sequence corticotropin group was 11.07 (±4.22) μg/dl which was not significantly different ($P = 0.117$).

All subjects had serum cortisol levels >18 μg/dl at 30 minutes itself with both porcine sequence corticotropin and tetracosactide hexaacetate [Figure 1].

Mean serum cortisol levels with tetracosactide hexaacetate were 30.3 (±7.83) μg/dl and 31.27 (±7.36) μg/dl at 30 and 60 minutes, respectively. The mean serum cortisol values with porcine sequence corticotropin were 26.33 (±5.47) and 31.59 (±6.40) μg/dl at 30 and 60 minutes, respectively. The trend of rise in cortisol between the two groups was not significantly different [Table 1 and Figure 2].

Mean peak response of cortisol was 32.65 (±7.76) μg/dl in tetracosactide hexaacetate group and 31.59 (±6.4) μg/dl in porcine sequence corticotropin group, respectively. The peak response in two groups were similar ($P = 0.686$) [Table 2].

Delta increase in serum cortisol value after stimulation was calculated as rise from basal-to-peak response (30 or 60 minutes) [Table 2].

Mean delta increase in serum cortisol from 0 to peak response was 18.44 (±7.39) μg/dl in tetracosactide hexaacetate group and 20.92 (±4.55) μg/dl in porcine sequence corticotropin, respectively [Table 2]. Mean delta increase in serum cortisol in both groups were comparable ($P = 0.28$).

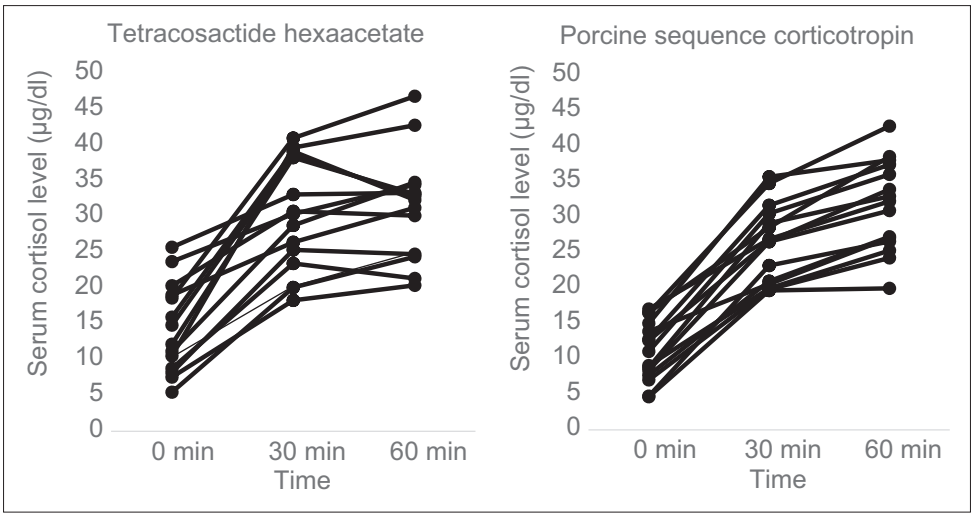

Figure 1: Serum cortisol values at 0, 30 and 60 minutes in both groups

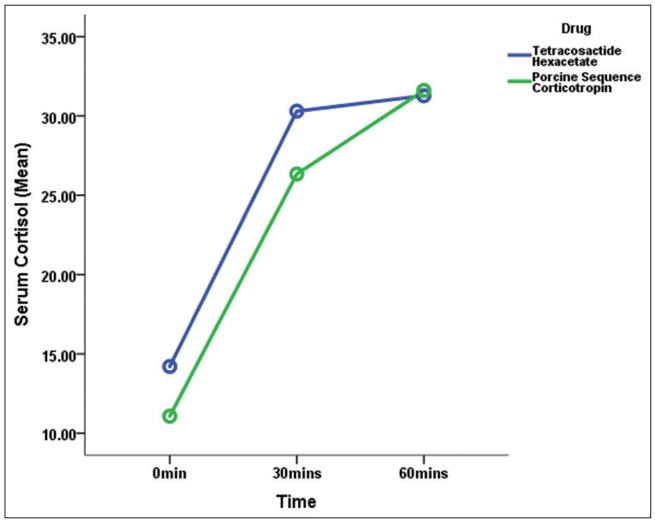

Figure 2: Comparison of trend of rise in serum cortisol (µg/dl) with time in the two groups

We also compared the cortisol response in overweight volunteers with normal BMI individuals and the response was not significantly different [Tables 3 and 4].

We did not come across any immediate adverse events like anaphylaxis, rash, nausea, vomiting, giddiness and syncope with porcine sequence corticotropin or tetracosactide hexaacetate in the volunteers. The cost of porcine sequence corticotropin administration was Rs. 170.33 per individual as compared to Rs. 2012 for tetracosactide hexaacetate administration.

Discussion

The cornerstone in assessment of an intact adrenal response is the stimulation of HPA axis using ITT or SST with tetracosactide hexaacetate. In view of the difficulties in the procurement of tetracosactide in India we decided to test the efficiency of porcine sequence corticotropin with carboxymethylcellulose. Animal studies comparing porcine ACTH and synthetic ACTH did not find any difference in the two preparations with regard to adrenal response.[12] We hypothesized that porcine sequence corticotropin would be non-inferior to tetracosactide hexaacetate in testing the adrenal reserve of healthy volunteers.

In our study all healthy volunteers initially received tetracosactide hexaacetate intravenously followed by porcine sequence corticotropin 4 weeks later in order to prevent any residual action of intramuscular porcine sequence corticotropin on further

Table 1: Comparison of trend of rise in serum cortisol with time in the two groups

Drug	Mean serum cortisol level (µg/dl) ± SD	*n*	*P**
0 min			
Tetracosactide hexaacetate	14.21±6.19	15	0.108
Porcine sequence corticotropin	11.08±4.22	15	
30 min			
Tetracosactide hexaacetate	30.30±7.83	15	
Porcine sequence corticotropin	26.34±5.47	15	
60 min			
Tetracosactide hexaacetate	31.27±7.36	15	
Porcine sequence corticotropin	31.59±6.40	15	

**P* calculated by repeated measures of ANOVA

Table 2: Comparison of mean peak increase in cortisol and delta increase in both groups

Drug	*n*	Mean cortisol level (µg/dl) ± SD	*P**
Peak response			
Tetracosactide hexaacetate	15	32.66±7.76	0.686
Porcine sequence corticotropin	15	31.60±6.40	
Delta increase 0 to peak			
Tetracosactide hexaacetate	15	18.45±7.40	0.28
Porcine sequence corticotropin	15	20.92±4.56	

*Independent sample *t*-test

Table 3: Comparison of peak and delta increase in cortisol response with tetracosactide hexaacetate in normal and overweight/obese group

BMI	*n*	Mean cortisol level (μg/dl) ± SD	*P**
Peak response			
Normal	8	32.42±9.05	0.905
Overweight/obese	7	32.93±6.69	
Delta increase 0 to peak			
Normal	8	17.85±6.88	0.752
Overweight/obese	7	19.13±8.45	

*Independent sample *t*-test

Table 4: Comparison of peak and delta increase in cortisol response with porcine sequence corticotropin in normal and overweight/obese group

BMI	*n*	Mean cortisol level (μg/dl) ± SD	*P**
Peak response			
Normal	8	31.08±8.10	0.753
Overweight/obese	7	32.18±4.28	
Delta increase 0 to peak			
Normal	8	20.65±5.74	0.818
Overweight/obese	7	21.23±3.13	

*Independent sample *t*-test

testing with tetracosactide. All subjects had serum cortisol values >18 μg/dl at 30 minutes itself in both groups, suggesting that a normal cortisol response to intramuscular corticotropin could be expected even at 30 minutes in healthy individuals. Previously Virginia *et al*. had shown that porcine sequence of ACTH produced a more rapid response, but human ACTH had a more lasting effect. However, the porcine ACTH was given as a continuous infusion in the above study.[11] We also noted that the peak response and the delta increase in serum cortisol between the two groups did not differ ($P = 0.686$ and $P = 0.28$, respectively). Hence we validated our hypothesis that HPA axis stimulation in healthy volunteers using porcine sequence corticotropin was non-inferior to tetracosactide stimulation.

In healthy volunteers the mean cortisol level at 30 and 60 minutes with porcine sequence corticotropin was not significantly different from tetracosactide group. The increment in cortisol after 30 minutes was minimal. Also, there was no significant difference in cortisol increment between obese and lean people in the porcine sequence corticotropin and the tetracosactide group. Since all volunteers had an adequate response at 30 minutes, it can be assumed that porcine sequence corticotropin stimulation testing can adequately test HPA axis at 30 minutes in healthy volunteers. It was also noted that porcine ACTH did not produce adverse events in the immediate period after its administration.

Porcine ACTH is used in some countries instead of tetracosactide hexaacetate. However, the studies that compare the two molecules are limited.[1] Our literature search revealed only one study done in India using porcine ACTH to assess the HPA axis in healthy individuals. The said study assessed the HPA axis response in healthy volunteers and in patients with adrenal insufficiency using porcine sequence corticotropin. The cortisol incremental response in the healthy volunteers in this study was similar to the increment seen in our population (20.92 ± 4.55 μg/dl vs 19.27 ± 6.46 μg/dl, respectively). The strength of our study was the comparison with the existing gold standard of tetracosactide stimulation, which was lacking in the above mentioned study by Abhay *et al*.[10] Porcine sequence corticotropin was also used to identify adrenal insufficiency in critically ill patients from a center in India.[13]

Our study has a few limitations. First of all, we analyzed and validated the adequacy of HPA axis response in healthy individuals only. We need to validate the same in HPA axis suppression, Addisonian and CAH patients. Secondly, a dose titration of porcine ACTH was not performed. We used a dose of 24 units of porcine sequence corticotropin based on previous studies. With a dose of 24 units all subjects in our study had a normal cortisol response at 30 minutes. Lowest adequate dose of porcine sequence corticotropin for adequate stimulation testing needs to be assessed in future studies. We have only assessed the immediate side effects with porcine corticotropin and we have not collected any data on long-term adverse events. ACTH antibodies have been reported with repeated use of depot preparation of porcine sequence corticotropin and antibodies were not measured in our study.[14] Finally, there is a lack of data on efficacy with the storage and reuse of multi-dose vial of porcine corticotropin. However the stored vial produced a similar trend of cortisol response in all our volunteers when compared to tetracosactide acetate at 30 and 60 minutes. This trend was similar in both groups with no statistical difference demonstrating that there is no loss of efficacy with at least short duration of storage.

As we know, in patients with PAI, we would not expect normal cortisol response at 30 or 60 minutes. A variety of herbal remedies and exogenous glucocorticoids used for long duration in treatment of rheumatoid arthritis and other chronic conditions can lead to HPA axis suppression.[15] However, HPA axis suppression with exogenous steroids is dependent on dose, route of administration and duration of treatment which produces a variable picture in clinical presentation. Generally, HPA axis recovers in 6–12 months; however, cases with delayed recovery up to 18 months have been reported.[16] HPA axis suppression with exogenous steroids is the one of most common cause of cortisol insufficiency and the dynamics of cortisol increment with porcine sequence corticotropin at 30 and 60 minutes needs to be clarified in this population. It would also be illustrative to see whether cortisol increment could occur later at 120 minutes or during infusion of porcine sequence corticotropin in patients recovering from HPA axis suppression [Figure 3].

Porcine sequence corticotropin is easily available compared to tetracosactide hexaacetate. The cost of investigations at

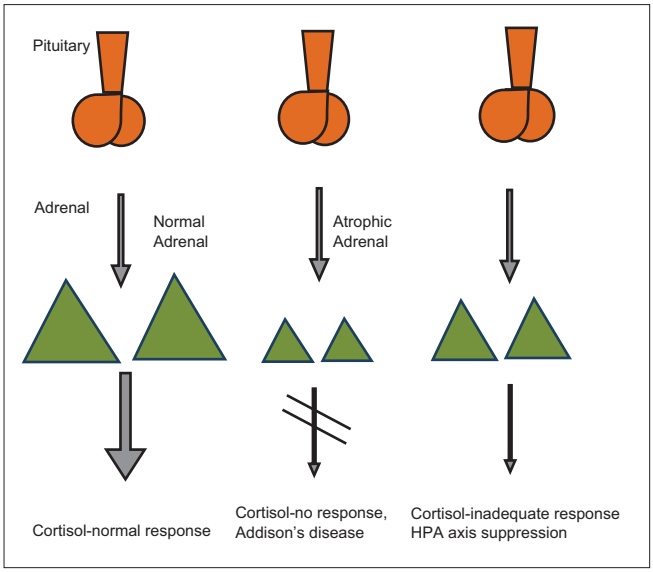

Figure 3: Cortisol responses to ACTH stimulation in normal individuals, Addison's disease and HPA suppression

the individual level with porcine sequence corticotropin is considerably less compared to tetracosactide hexaacetate, making it an attractive option in our setting. Further studies with porcine sequence corticotropin would contribute considerably to fill the existing knowledge deficits.

Conclusion

Our study established the short-term safety and efficacy of porcine sequence corticotropin with lower procedure costs in testing the adequacy of HPA axis response in healthy individuals. Porcine sequence corticotropin stimulation testing was non-inferior to the gold standard tetracosactide hexaacetate stimulation testing and the intactness of the HPA axis could be confirmed as early as 30 minutes in healthy individuals.

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Conflicts of interest

There are no conflicts of interest.

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