Endocrine (2017) 57:46–50
DOI 10.1007/s12020-017-1330-5

ENDOCRINE METHODS AND TECHNIQUES

Performance of low-dose cosyntropin stimulation test handled via plastic tube

Leonard Saiegh[1] · Asala Abu-Ahmad[2] · Mohammad Sheikh-Ahmad[1] · Maria Reut[1] · Limor Chen-Konak[1] · Nizar Jiries[2] · Carmela Shechner[1]

Received: 13 March 2017 / Accepted: 15 May 2017 / Published online: 22 May 2017

Abstract
Purpose Studies on 1 µg low-dose test showed that among 1 µg cosyntropin samples pushed through long IV plastic tubing, some adrenocorticotropic hormone dosage was not recovered, and in healthy volunteers it provided subnormal cortisol responses. The aim of the current study is to assess whether there is any loss in adrenocorticotropic hormone 1–24 concentration when pushed through a short plastic tube, and to assess serum and salivary cortisol responses in low-dose test among healthy volunteers, using a similar short plastic tube vs. direct intravenous consyntropin injection.
Methods We evaluated in vitro if adrenocorticotropic hormone was absorbed in a 2.5 cm plastic tube by measuring adrenocorticotropic hormone 1–24 concentration in a 1 µg/ml adrenocorticotropic hormone aliquot solution before and after being flushed through the plastic tube. For the in vivo study, we recruited 20 healthy adult volunteers. Each subject underwent low-dose test via 2.5 cm plastic tube via plastic tube and via direct intravenous injection by a metal syringe via direct intravenous injection, and cortisol responses were determined.
Results Mean adrenocorticotropic hormone 1–24 concentration did not differ significantly when flushed via plastic tube or measured in the aliquot solution ($P = 0.25$). In vivo, mean 30-min serum cortisol concentrations were 20.47 ± 2.87 and 21.62 ± 3.89 µg/dl in via plastic tube and in via direct intravenous injection tests, respectively, and did not show a significant difference ($P = 0.16$).
Conclusions In low-dose test, using a 2.5 cm plastic tube ensures completeness of the intravenous adrenocorticotropic hormone injection dosage and provides equivalent cortisol responses.

Keywords Cosyntropin · Hypoadrenalism · Cortisol · ACTH

Introduction

The cosyntropin (adrenocorticotropic hormone (ACTH) 1–24) stimulation test is a widely used test for diagnosing adrenal insufficiency. Initially, the test was performed using the 250 µg cosyntropin dose [1]. However, in light of strong evidence demonstrating that the 250 µg testing dose is a supra-physiologic stimulus, investigators developed a lower-dose cosyntropin test, the 1 µg low-dose test (LDT) [2–5]. While there is no consensus as to which diagnostic test may be optimal, several meta-analyses have agreed that LDT, especially in central and mild adrenal insufficiency, might show higher sensitivity. As a result, use of the latter has been encouraged [6–8].

Few studies have examined the potential effect of the way in which low-dose cosyntropin is intravenously administered, on cortisol responses. One review has emphasized the importance of minimizing or eliminating catheter length [9], yet this issue was not addressed in other reviews [7, 10]. Few papers explicitly cite catheter length; and in those that do, investigators used both short [11] and

✉ Leonard Saiegh
leonard.saiegh@gmail.com

[1] Department of Endocrinology, Bnai-Zion Medical Center, Haifa, Israel

[2] Department of Internal Medicine B, Bnai-Zion Medical Center, Haifa, Israel

long catheters [12]. Murphy et al. showed that significant loss of ACTH occurs when cosyntropin injections are given via plastic devices, and extent of loss increases in proportion to the length of the device. They concluded that their findings indicate the need to standardize the injection procedure for the LDT via plastic tubes, as loss of the dose injected may significantly reduce the increment in plasma ACTH in blood and, hence, the quality and duration of the cortisol response [13]. In the same way, Wade et al. examined the technical details that may influence the accuracy of LDT, and showed that among 1 µg cosyntropin samples pushed in vitro through 20.3 cm IV tubing, some ACTH dosage was not recovered. Moreover, they showed that in vivo, using the same tube length, 25 out of 60 healthy volunteers had subnormal cortisol responses. As a result, they recommended use of the shortest possible length of tubing, or alternatively, direct venous injection [14].

Given the above, it is important to clarify whether a short plastic tube during LDT can guarantee completeness of the intravenous injection and provide equivalent cortisol response. For this purpose, the current study assessed, in vitro, whether would be loss in ACTH 1-24 concentration when pushed through a 2.5 cm short plastic tube. In addition, the study assessed serum and salivary cortisol responses during LDT in healthy volunteers using the same short plastic tube, and compared the results to the direct intravenous consyntropin administration.

Methods

Participants

Twenty healthy adult volunteers were recruited for the study. Exclusion criteria included pregnancy, lactation, recent use of glucocorticoid medications, oral contraception use, or the presence of signs or symptoms of adrenal insufficiency (unintentional weight loss, nausea, fatigue or joint pain). Participants with well-controlled chronic non inflammatory illnesses (e.g., hypertension) were eligible to be included. Authorization by The National Institute of Child Health and Development Institutional Review Board was obtained for the study (ClinicalTrials.gov identifier NCT02413944) and all subjects provided written informed consent.

Preparation of 1 µg cosyntropin for in vivo an in vitro study

A stock ACTH solution was prepared by adding 1 ml ampoule of 250 µg ACTH 1–24 solution (Synacthen, Sigma-Tau Industrie Farmaceutiche Riunite S.p.A, Italy) to 49 ml of sterile physiologic saline (0.9%), yielding a 5 µg/ml cosyntropin solution. One µg cosyntropin was prepared just before administration as follows: using 1 ml syringe, 0.2 ml was drawn from the stock solution and then 0.8 ml of physiologic saline (0.9%) was added, yielding 1 µg/ml ACTH aliquot stock solution for the in vivo LDT and for the in vitro study.

In vivo study

Each subject recruited in the study underwent LDT via plastic tube (VPT) and via direct intravenous injection (VIV). VPT test: At 0800 to 0900 h, a 25 mm plastic intravenous line (Polyurethane cannula, BD Venflon™ Pro Safety Shielded IV Catheter 22GA 0.9×25 mm) was inserted in an antecubital vein. Then, 1 µg/ml ACTH aliquot stock solution was pushed through, followed by 5 ml physiologic saline (0.9%). VIV test: At 0800–0900 h, 1 µg/ml ACTH aliquot stock solution connected to metal needle was administered through direct antecubital venous acupuncture. For each subject, VPT and VIV tests were performed at least 3 days apart. In both tests, serum cortisol (SC) was measured just before ACTH administration and 30 min later, and salivary free cortisol (SFC) was measured just before ACTH administration, 30 and 60 min later.

In vitro study

We evaluated if ACTH was absorbed by the 2.5 cm plastic IV tubing. This was done by measuring ACTH 1–24 concentration in the following samples: (a) 1 µg/ml ACTH aliquot stock solution followed by 2 ml saline flushed through the plastic tubing, (b) 1 µg/ml ACTH aliquot stock solution added to 2 ml saline using no plastic tubing. Samples (a) and (b) were collected and assayed for ACTH 1-24. Samples (a) and (b) were prepared separately 10 times, and ACTH 1-24 concentrations in (a) samples and in (b) samples were averaged out.

Assays

SC was measured using solid phase competitive chemiluminescent enzyme immunoassay by an automated analyzer Immulite 2000 (Siemens). The method's sensitivity was 0.20 µg/dl and intra-assay and inter-assay coefficients of variations were 9.4 and 7.4%, respectively. SFC was measured by enzyme linked immunosorbent using salivary RE52611 kit (IBL International, Hamburg, Germany), measurement range 0.015–3.000 µg/dl. Inter-assay and intra-assay coefficients of variation were below 9.3 and 7.3%, respectively.

ACTH 1-24 concentration was measured by radioimmunoassay kit (MP Biomedicals, Orangeburg, NY-Catalog No. 07-106101). Samples were assayed in duplicate at dilutions of 1:1000 using zero ACTH standards as diluents, to give an assayed ACTH result of 25–500 pg/ml

Table 1 Summary of mean baseline and stimulated cosyntropin test results

Administration mode	Results	Basal SC[c] (μg/dl)	30-min SC[c] (μg/dl)	Basal SFC[d] (μg/dl)	30-min SFC[d] (μg/dl)	60-min SFC[d] (μg/dl)
VPT[a]	Mean ± SD***	12.90 ± 3.60	20.47 ± 2.87	0.365 ± 0.152	0.956 ± 0.254	0.620 ± 0.232
	Range	7.86–20.30	15.40–26.70	0.078–1.074	0.585–1.481	0.325–1.194
	95% CI**	11.22–14.59	19.12–21.81	0.300–0.502	0.837–1.075	0.511–0.729
VIV[b]	Mean ± SD***	12.37 ± 3.99	21.62 ± 3.89	0.420 ± 0.250	0.979 ± 0.345	0.790 ± 0.384
	Range	6.16–19.80	12.90–30.20	0.109–1.066	0.181–1.720	0.178–1.895
	95% CI**	10.50–14.23	19.80–23.43	0.300–0.540	0.818–1.141	0.610–0.970
Sig.*	*P*-value	0.52	0.16	0.39	0.72	0.06

* statistical significance between VPT and VIV mean values

** confidence interval

*** standard deviation

[a] cosyntropin test via plastic tube

[b] cosyntropin test via direct intravenous injection

[c] serum cortisol

[d] salivary free cortisol

(standard curve range: 10 to 1000 pg/ml). Intra-assay and inter-assay coefficients of variations were 4.1–6.8 and 3.9–10.7%, respectively.

Data analysis

All statistical analyses were performed by IBM-SPSS statistics version 12 software. The results are presented as mean ± standard deviation. Normal distribution of the results was validated by Kolmogorov–Smirnov test. The Student *t* test was used for data comparison between groups and McNemar's test was used to analyze differences in nonparametric data between groups. Statistical significance was defined as a P-value < 0.05. Cortisol response to cosyntropin below 18 μg/dl at 30-min time point, was regarded a subnormal response.

Results

In vitro study

Mean ACTH 1–24 concentration in "a" samples (flushed VPT) and in "b" samples (aliquot stock solution) did not differ significantly (217 ± 86.7 vs. 174 ± 63.4 ng/ml, respectively; $P = 0.25$).

In vivo study

Subjects

20 subjects (10 females and 10 males) were studied, yielding 40 tests (each subject underwent 1 VPT test and 1 VIV test). Mean age was 32.2 (range 23–54) and 32.3 (range 26–40) years for males and females, respectively. No subject received medications known to affect cortisol or corticosteroid-binding globulin (CBG) levels. One subject had well-controlled hypertension and all others were healthy volunteers taking no chronic medications.

Cortisol responses

SC and SFC in all time points showed statistically normal distribution ($P > 0.3$). Mean baseline and stimulated SC and SFC concentrations are presented in Table 1. Mean 30-min SC concentrations were 20.47 ± 2.87 and 21.62 ± 3.89 μg/dl in VPT and in VIV tests, respectively; and did not show a significant difference ($P = 0.16$). No significant difference was observed in the 30-min mean SFC concentrations between VPT and in the VIV tests (0.956 ± 0.254 and 0.979 ± 0.345 μg/dl, respectively; $P = 0.72$). Moreover, between VPT and VIV tests, no significant difference was observed in the mean absolute increment between baseline and 30-min time point, in SC concentration (7.56 ± 3.42 and 9.25 ± 4.76 μg/dl, respectively; $P = 0.11$), and in SFC concentration (0.610 ± 0.287 and 0.580 ± 0.383 μg/dl, respectively; $P = 0.72$).

Mean SFC concentrations were lower in the 60-min time point than in the 30-min time point in both VPT ($P < 0.001$) and in VIV ($P = 0.017$) tests, and only in 10% of the VPT and in 35% of the VIV tests, 60-min SFC concentration was higher than the 30-min concentration (data not presented).

One subject slightly failed the VPT test (30 min SC = 17.00 μg/dl) but passed the VIV test (30 min SC = 18.50 μg/dl), and another subject failed the VIV test (30 min SC = 17.60 μg/dl) but passed the VPT test (30 min SC = 19.20

μg/dl). Surprisingly, one male subject significantly failed to pass both VPT and VIV tests (30-min SC was 15.40 and 12.90 μg/dl in VPT and in VIV tests, respectively). He did not suffer from any symptoms of hypo-adrenalism or used any steroids, and had normal ACTH, testosterone and electrolyte levels. His baseline SC levels were 12.60 and 10.20 μg/dl in VPT and in the VIV tests, respectively. His 30-min SFC levels were 0.641 μg/dl in VPT test and 0.580 μg/dl in VIV test, both below the 95% confidence interval received from this cohort (Table 1).

Discussion

Earlier studies have claimed that the subnormal cortisol response using plastic tubes might result from cosyntropin adherence to the tube and loss of the delivered dosage [13, 14]. Wade et al. showed that 21.6–58.6% of ACTH dosage had not been recovered when pushed through 20.3 cm plastic tube [14], and Murphy et al. showed loss of up to 70% of ACTH when cosyntropin was delivered through a plastic 30 cm scalp vein set. However, unlike earlier research, the current study employed plastic tubes that were by far shorter than those used by previous studies [13]. The current study confirmed, in vitro, that no ACTH loss took place when cosyntropin was pushed through a 2.5 cm plastic tube. Furthermore, in healthy volunteers, we observed no difference in 30-min SC concentration, in 30-min and 60-min SFC concentration, when 1 μg cosyntropin was delivered directly intravenously or through the same short plastic tube. As our study did not use long plastic tubes to assess recovery of ACTH dosage and cortisol responses, it is not possible to rule out the chance that differences of our results from previously published studies using long tubes might have been derived from different types of plastic tubes.

The current study demonstrated that, subnormal SC response was observed only in 2/20 subjects during VPT and in 2/20 subjects during VIV tests, giving a specificity of 90% of both modalities, higher than reported in most other studies (mean 79%, CI 74–84%) [15]. We argue that several reasons might underlie subnormal responses. As no commercial 1 μg cosyntropin preparations are available, we could not rule out technical dilution errors or different ACTH formulations used in the different studies. Moreover, despite the fact that the current study has adopted a commonly used cortisol threshold in defining normal cortisol response, differences between cortisol assays might give diverse results, so it is important to validate diagnostic threshold criteria at each center [16]. In addition, even though the study did not recruit volunteers with medical situation that could have altered CBG levels, these levels were not directly measured. As a result, we cannot rule out low CBG levels as a cause for some of the subnormal results observed, a possible reason reported in some studies [17]. It was previously proposed that SFC assessment during LDT can be used in particular in situations where abnormal CBG levels are suspected [18–21]. In our study, the subject who failed both tests did not reach the 95% CI of 30-min SFC concentration, a fact standing against the assumption that low CBG levels might be a cause of these subnormal SC levels.

Many studies in LDT, have shown cortisol value at 60-min time point be consistently lower than 30-min point [7]. On the other hand, in their study, Cartaya et al. showed that in some cases 60-min cortisol can be higher than 30-min cortisol [22], and another study showed that in LDT, peak SC response can occur at the 20-min time point [6]. However, as was stated in the "letter to the editor" regarding Cartaya's paper, "authors specified neither catheter length nor cosyntropin administration mode; and both issues are considered to be highly important when using LDT" [23]. In the current study, we did not assess SC concentration at the 20-min and 60-min time points; however, we assessed 60-min SFC and showed that in 10–35% of cases 60-min SFC was higher than at the 30-min point.

Park et al. showed in their study that during LDT, afternoon 30-min SC levels were normal in all eight healthy volunteers, still levels were lower than morning 30-min SC [11]. Another study that compared morning and afternoon LDT 30-min cortisol responses in healthy subjects after dexamethasone pretreatment, did not show these differences [3]. In their study, Wade et al. showed that afternoon testing was associated with a sevenfold increased likelihood of failing the 1 μg test. However, in their study they used a 20.3 cm plastic tube, which might have led to uncompleted cosyntropin delivery [14]. As we showed, using a 2.5 cm plastic tube did not alter cosyntropin dosage delivered or cortisol stimulation. It may be worthy to study afternoon cortisol stimulation in LDT using that tube, in order to verify whether results in the morning and in the afternoon are comparable.

In conclusion, we have shown that use of a 2.5 cm plastic tube does not alter delivered cosyntropin dosage or cortisol stimulation. Regardless of injection technique, and in accordance with previously published data, LDT might yield false positive results, and this has the potential of subjecting healthy individuals to life-long glucocorticoid replacement therapy.

Compliance with ethical standards

Conflict of interest All authors declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of

the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. J.B. Wood, A.W. Frankland, V.H. James, J. Landon, A rapid test of adrenocortical function. Lancet. **30**, 243–245 (1965)
2. B. Ambrosi, L. Barbetta, T. Re, E. Passini, G. Faglia, The one microgram adrenocorticotropin test in the assessment of hypothalamic-pituitary-adrenal function. Eur. J. Endocrinol. **139**, 575–579 (1998)
3. G. Dickstein, C. Shechner, W.E. Nicholson, I. Rosner, Z. Shen-Orr, F. Adawi, M. Lahav, Adrenocorticotropin stimulation test: effects of basal cortisol level, time of day, and suggested new sensitive low dose test. J. Clin. Endocrinol. Metab. **72**, 773–778 (1991)
4. K. Tordjman, A. Jaffe, N. Grazas, C. Apter, N.J. Stern, The role of the low dose (1 microgram) adrenocorticotropin test in the evaluation of patients with pituitary diseases. J. Clin. Endocrinol. Metab. **80**, 1301–1305 (1995)
5. S. Rasmuson, T. Olsson, E. Hagg, A low dose ACTH test to assess the function of the hypothalamic-pituitary-adrenal axis. Clin. Endocrinol. **44**, 151–156 (1996)
6. T.A. Abdu, T.A. Elhadd, R. Neary, R.N. Clayton, Comparison of the low dose short synacthen test (1 microg), the conventional dose short synacthen test (250 microg), and the insulin tolerance test for assessment of the hypothalamo-pituitary-adrenal axis in patients with pituitary disease. J. Clin. Endocrinol. Metab. **84**, 838–843 (1999)
7. R. Kazlauskaite, A.T. Evans, C.V. Villabona, T.A. Abdu, B. Ambrosi, A.B. Atkinson, C.H. Choi, R.N. Clayton, C.H. Courtney, E.N. Gonc, M. Maghnie, S.R. Rose, S.G. Soule, K. Tordjman, Corticotropin tests for hypothalamic-pituitary- adrenal insufficiency: a metaanalysis. J. Clin. Endocrinol. Metab. **93**, 4245–4253 (2008)
8. A. Beishuizen, J.H. van Lijf, J.F. Lekkerkerker, I. Vermes, The low dose (1 microg) ACTH stimulation test for assessment of the hypothalamo-pituitary-adrenal axis. Neth. J. Med. **56**, 91–99 (2000)
9. G. Dickstein, L. Saiegh, Low-dose and high-dose adrenocorticotropin testing: indications and shortcomings. Curr. Opin. Endocrinol. Diabetes. Obes. **15**, 244–249 (2008)
10. G. Reimondo, S. Bovio, B. Allasino, M. Terzolo, A. Angeli, Secondary hypoadrenalism. Pituitary. **11**, 147–154 (2008)
11. Y.J. Park, K.S. Park, J.H. Kim, C.S. Shin, S.Y. Kim, H.K. Lee, Reproducibility of the cortisol response to stimulation with the low dose (1 microg) of ACTH. Clin. Endocrinol. **51**, 153–158 (1999)
12. J.G. Gonzalez-Gonzalez, N.E. De la Garza-Hernandez, L.G. Mancillas-Adame, J. Montes-Villarreal, J.Z. Villarreal-Perez, A high-sensitivity test in the assessment of adrenocortical insufficiency: 10 microg vs 250 microg cosyntropin dose assessment of adrenocortical insufficiency. J. Endocrinol. **159**, 275–280 (1998)
13. H. Murphy, J. Livesey, E.A. Espiner, R.A. Donald, The low dose ACTH test--a further word of caution. J. Clin. Endocrinol. Metab. **83**, 712–713 (1998)
14. M. Wade, S. Baid, K. Calis, H. Raff, N. Sinaii, L. Nieman, Technical details influence the diagnostic accuracy of the 1 microg ACTH stimulation test. Eur. J. Endocrinol. **162**, 109–113 (2010)
15. L.K. Nieman, Dynamic evaluation of adrenal hypofunction. J. Endocrinol. Invest. **26**, 74–82 (2003)
16. M.G. Burt, B.L. Mangelsdorf, A. Rogers, J.T. Ho, J.G. Lewis, W. J. Inder, M.P. Doogue, Free and total plasma cortisol measured by immunoassay and mass spectrometry following $ACTH_{1–24}$ stimulation in the assessment of pituitary patients. J. Clin. Endocrinol. Metab. **98**, 1883–1890 (2013)
17. L.C. Smans, P.M. Zelissen, Is diagnosis and subclassification of adrenal insufficiency as easy as it looks? Front. Horm. Res. **46**, 146–158 (2016)
18. Y. Marcus-Perlman, K. Tordjman, Y. Greenman, R. Limor, G. Shenkerman, E. Osher, N. Stern, Low-dose ACTH (1 microg) salivary test: a potential alternative to the classical blood test. Clin. Endocrinol. **64**, 215–258 (2006)
19. I. Perogamvros, L.J. Owen, B.G. Keevil, G. Brabant, P.J. Trainer, Measurement of salivary cortisol with liquid chromatography-tandem mass spectrometry in patients undergoing dynamic endocrine testing. Clin. Endocrinol. **72**, 17–21 (2010)
20. W.J. Inder, G. Dimeski, A. Russell, Measurement of salivary cortisol in 2012 - laboratory techniques and clinical indications. Clin. Endocrinol. **77**, 645–651 (2012)
21. M.P. Cornes, H.L. Ashby, Y. Khalid, H.N. Buch, C. Ford, R. Gama, Salivary cortisol and cortisone responses to tetracosactrin (synacthen). Ann. Clin. Biochem. **52**, 606–610 (2015)
22. J. Cartaya, M. Misra, The low-dose ACTH stimulation test: is 30 minutes long enough? Endocr. Pract. **21**, 508–513 (2015)
23. A. Salcido-Montenegro, K.E. Carlos-Reyna, H.E. Tamez-Pérez, J. G. González-González, Low-dose ACTH stimulation test: dose, sampling time, and technical issues. Endocr. Pract. **21**, 1079 (2015)