STRESS HORMONES IN CHRONIC MIGRAINE ARE MODULATED BY BOTULINUM TOXIN -TYPE A

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Summary

Failures in chronic migraine (CM) treatments are often due to superimposed medication overuse headaches (MOH), a new form of secondary headache which is defined as excessive medication use in a susceptible patient.
In order to increase personal well-being investigate potential natural preventive measures, the aim of the present research was to study hypothalamus-pituitary-adrenal (HPA) axis activity in patients severely affected by chronic migraines and MOH after subcutaneous treatment with botulinum toxin type A (BoNT-A). The HPA axis activity was specifically monitored by measuring cortisol, testosterone and DHEA-S in saliva, thus allowing the stressful event of venipuncture to be avoided.
One month after the MOH rehabilitation procedure, the cortisol level was significantly higher than controls in CM patients. With regard to the cortisol to DHEA-S ratio, an inverse marker of psycho-physical well-being, the CM group showed significantly higher values than controls. Moreover, testosterone levels, as well as testosterone to cortisol ratios (anabolic/catabolic index of physical performance) were significantly lower than controls in CM patients. Further studies are required to elucidate the clinical relevance of HPA derangement in CM: the increase in cortisol level can be considered a trigger factor of migraine attacks but may also appear as a reaction to chronic migraine attacks. Treatment with BoNT-A counteracted the CM-induced increase in cortisol levels. Moreover the treatment produced a significant reduction, in comparison to placebo, of DHEA-S and testosterone and not significant variations of their ratio to cortisol.
Therefore, despite the encouraging results reported in the present research, the inclusion of BoNT-A treatment to counteract CM-induced derangement of the HPA axis and/or as preventive treatment in patients suffering from MOH needs to be more clearly established in future studies.

Key words: chronic migraine and medication overuse headache; stress hormones in saliva; botulinum toxin type A (BoNT-A).

Introduction

Chronic migraines (CM) represent a disease of high social impact in terms of both direct and indirect costs. It is a complex syndrome with several associated conditions such as major depression and insomnia (1, 2, 3). Moreover a superimposed medication-overuse-headache (MOH) has been described as a new form of secondary headache. This is associated with clinical manifestations of variable nature and intensity, which appear after the overuse of ergot-derived analgesics, barbiturates, and triptans, taken singularly or in combination (4, 5, 6). A primary-assistance study revealed that MOH is third among the most common causes of migraines. In European headache centres, 10% of patients suffer from MOH, and in American clinics 80% of chronic daily headache patients suffer from symptomatic drugs overuse (7, 8).
Botulin toxin type A (BoNT-A) is a focally acting neurotoxin that inhibits the release of acetylcholine and other neurotransmitters from presynaptic nerve endings (9, 10). There is emerging scientific and clinical evidence to support the advantages and limitations of BoNT-A use in the pharmacological prevention of CM (11, 12).
Migraines are often associated with distress, which is highlighted as a trigger factor of headaches but may also appear as a consequence of migraine attacks (13-15).
Therefore, the aim of the present research was to study HPA axis activity in CM patients treated with BoNT-A to identify potential risks of HPA axis derangement. Such aims may be useful for the personal well-being of patients (16) and for the future prevention of CM prevention by "natural" medication options.
To avoid the "stress" of blood taking, cortisol, testosterone and DHEA-S have been measured in saliva (17-21). Since DHEA-S has antiglucocorticoid properties that might offer protection against a stress-induced increase of cortisol, the cortisol to DHEA-S ratio has been calculated as a measure of the degree to which an individual is buffered against the negative effects of stress (22-24). Finally, to evaluate asthenia, a symptom very frequently attributed to CM patients, the testosterone to cortisol ratio has been calculated as an indicator of muscular defatigation and anabolic/catabolic index of physical performance (25-27).

Methods

Patient population and Study design.
Among the outpatients admitted over a period of time from July 2005 to march 2006 at the Regional Referral Headache Centre at S. Andrea University Hospital, we selected a group of 50 patients with chronic migraines and also affected by MOH.
CM diagnosis fulfilled the 2004 IHS criteria (28). The enrolled patients attended an in-hospital rehabilitation procedure ranging from 5 to 10 days, to recover from MOH (29). Twenty four of the 44 eligible patients declined to give salivary samples. The remaining 20, all non-smoker women, were not consumers of any vasoactive drugs which could influence cortisol secretion (i.e. anti-hypertensives, antidepressants, thyroid agents, etc.). Among the hospital staff, 20 age-matched healthy women were enrolled as control

(Control Group). Patients and controls were matched for age (healthy controls 48.7 ± 3.3; CM: Placebo treated n=10: 47.9 ± 4.7; BoNT-A treated n=10: 52.3 ± 4.7) and Body Mass Index (healthy controls 23.2 ± 1.0; CM: Placebo treated n=10: 22.7 ± 1.8; BoNT-A treated n=10: 24.4 ± 1.4), which was considered because it may affect the concentration of hormone-binding globulines and testosterone (30, 31). None of these patients had received any immunosuppressive/corticosteroidal drugs in the previous six months. The main hematological and hematochemical parameters of all subjects were within the normal range for our laboratory. All partecipants were fully informed concerning the procedure and gave their written consent. The study protocol was approved by our Institutional Ethics Board and informed written consent was obtained also from controls. The recommended principles of the Declaration of Helsinki, September 1989, were closely observed during this clinical research.

Subjects selected in the present study, all triptan abusers, attended MOH rehabilitation in the outpatient regime for 5 days: the treatment consisted of infusion therapy with tiapride, ketorolac, ademetionine sulphate, reduced glutation, granisteron, ranitidine (29).

Participants were instructed on how to collect saliva samples at home, which was performed at about 08:00 a.m. whithin 1 hour of awakening. Salivary cortisol, DHEA-S and testosterone were measured in controls (Control Group) and in patients one week after the end of the MOH rehabilitation procedure (CM). Prior to enrolment and subsequent to the MOH rehabilitation procedure, 10 out 20 patients of the CM Group were randomly assigned to subcutaneous injection with 100 IU BoNT-A (BoNT-A) or placebo (Placebo). Subcutaneous injections were performed following the protocol described by Blumenfeld (32) and salivary hormones were also measured one month later. The samples were stored in the home refrigerator until the participants' return to the laboratory.

At each study visit, several pieces of information were collected including: a) the subject's rating of worst, least and usual headache-associated pain as measured on a 11-point scale (0=no pain to 10= pain as bad as can be; b) the subject's response to treatment (rated on a 9-point Global Assesment Scale from -4 (very marked worsening) to +4 (clearance of signs and symptoms). Other efficacy measures were documented in the patient diary regarding days of usual headache medication usage and presence/absence of associated headache symptoms, and no-headache days.

Salivary sampling procedure and Measurement of Cortisol, DHEA-S and Testosterone.

Samples of saliva were collected by the Salivette (Sarstedt, Italy), a sampling device which allows quick and hygienic saliva recovery from an inert polyester wool swab by centrifugation at 3,000 rpm for 5 min. For each sample, duplicate measurements were performed on 50-100 µl of saliva by means of commercial immunoenzymatic kits (Diametra, Italy) for direct salivary assay of cortisol, testosterone and DHEA-S. The inter-assay coefficient of variation was <10%, and the intra-assay coefficient of variation was <7%, with a minimum detectable concentration for cortisol of 0.5 ng/ml, for testosterone of 5 pg/ml, for DHEA-S of 25 pg/ml.

Statistical procedure
Analysis of variance (ANOVA) was applied, followed by Fisher's LSD multiple comparison test: statistical significance was set at $p< 0.05$ (33).

Results

One month after the MOH rehabilitation procedure, all CM patients reported their headaches to be much better, with a drastic decrease of functionally incapaciting headache days per month, from 15 or more to 4. However, no significant differences were found between BoNT-A and Placebo treated patients or in referred treatment-related adverse events (data not reported).
In Figure 1, panel A, salivary cortisol levels are depicted which were significantly increased in CM patients at the end of the MOH rehabilitation procedure; an injection of 100 IU of BoNT-A counteracted the CM-induced cortisol increase.
Panel B reports salivary DHEA-S levels that are not affected by CM itself but resulted in a significant reduction one month after treatment with 100 IU of BoNT-A. The same course of DHEA-S is followed by Testosterone (panel C of the figure 1).
Panel D shows that the cortisol to DHEA-S ratio calculated in CM patient is significantly higher than in controls. BoNT-A treatment did not modify the increase in the CM induced ratio.
Finally, in the panel E of Figure 1, the testosterone to cortisol ratio is reported. CM is characterized by a statistically significant reduction of the ratio that was not modified by BoNT-A treatment.

Discussion

This study shows that CM patients, one week after relapse from MOH, presented an increase of salivary cortisol secretion. Cortisol is a "flight or fight" stress hormone - it helps us fight inflammation, puts us on alert, opens our pupils, and rushes blood into the head and heart. On the other hand, it suppresses our immune system and it is catabolic. Experimental data have suggested that chronic exposure to high levels of corticosteroids produces neurotoxic effects by several mechanisms, including the metabolic vulnerability of neurons due to exacerbation of glutamate toxicity (34-36), a pathogenetic mechanism also involved in the CM (29, 37).

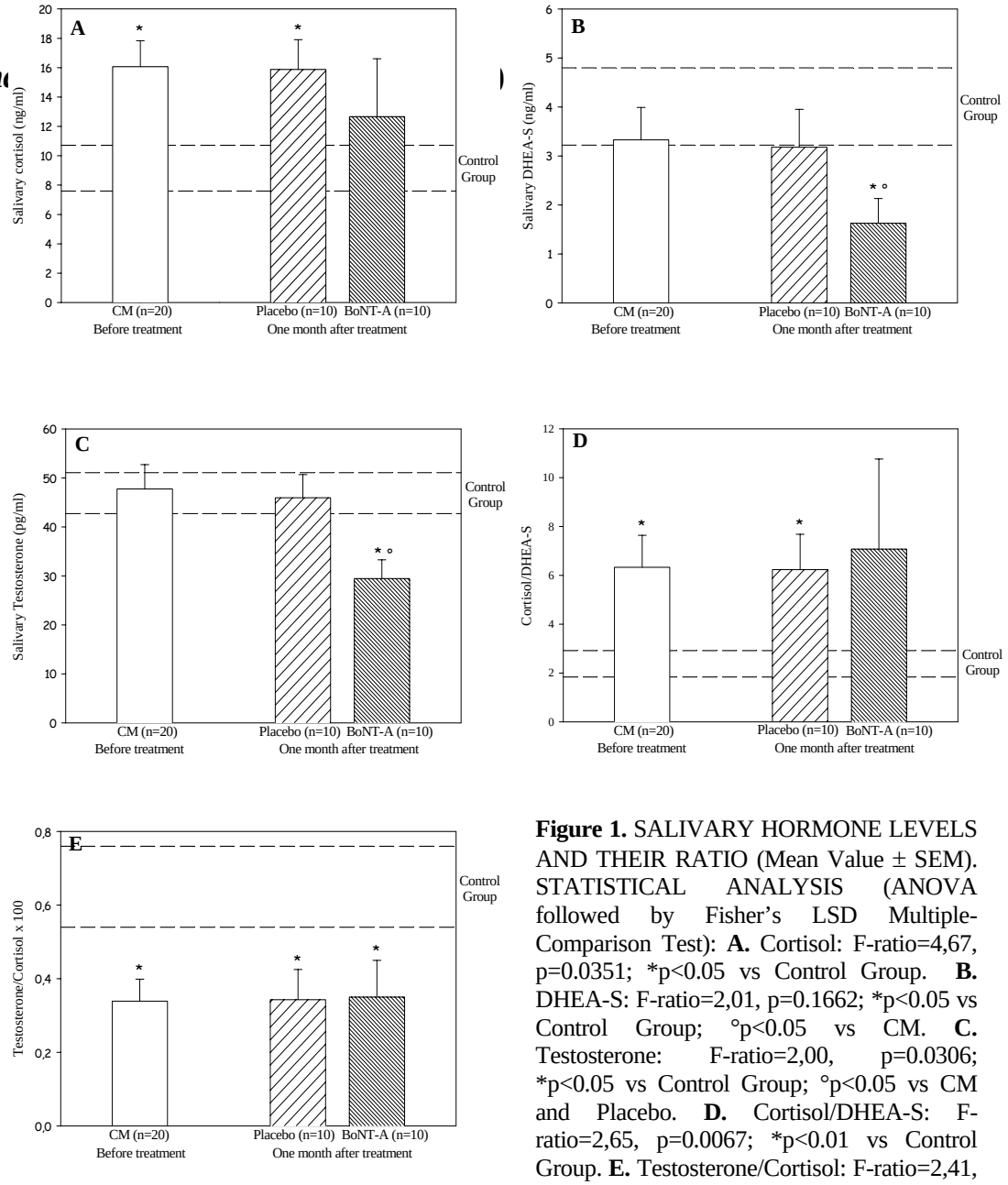

Figure 1. SALIVARY HORMONE LEVELS AND THEIR RATIO (Mean Value ± SEM). STATISTICAL ANALYSIS (ANOVA followed by Fisher's LSD Multiple-Comparison Test): **A.** Cortisol: F-ratio=4,67, p=0.0351; *p<0.05 vs Control Group. **B.** DHEA-S: F-ratio=2,01, p=0.1662; *p<0.05 vs Control Group; °p<0.05 vs CM. **C.** Testosterone: F-ratio=2,00, p=0.0306; *p<0.05 vs Control Group; °p<0.05 vs CM and Placebo. **D.** Cortisol/DHEA-S: F-ratio=2,65, p=0.0067; *p<0.01 vs Control Group. **E.** Testosterone/Cortisol: F-ratio=2,41, p=0.0117; *p<0.05 vs Control Group.

CM is a disorder affecting a larger proportion of women than men, therefore research has mainly focused on female sexual hormones and their effects on CM, whereas little attention has been paid to androgens (30, 38, 39). Moreover, there is a lack of data concerning modifications of adrenal hormones other than cortisol during the disease. A number of potential actions has been recently postulated for DHEA-S, in that it might moderate the effect of glucocorticoids and protect neurons from their neurotoxicity (22, 23).

As far as we know this is the first report in which the relationship between CM and BoNT-A prophylactic therapy has been explored, considering cortisol levels both as

an absolute marker of HPA axis activity, and its relation with the concomitant release of testosterone and DHEA-S.
Neither testosterone nor DHEA-S were modified during CM, while both hormone levels were significantly lower than placebo one month after BoNT-A.
The higher cortisol to DHEA-S ratio in CM patients, which was not modified by BoNT-a treatment suggested that the antiglucocorticoid action usually exerted by DHEA-S (22-24) is not working in CM disease. Moreover, the lower testosterone to cortisol ratio measured in CM patients in comparison with controls confirms the presence of tiredness, a symptom frequently reported by CM patients.
Botulinum toxin injections, already hugely popular for removing wrinkles, could perhaps one day become a novel therapy for the reduction in both the severity and frequency of headaches (40). In the present report we have shown that BoNT-A treatment was able to counteract CM-induced increase of cortisol. Consequently the treatment could reduce the overexposure to potentially damaging stress hormones. It is difficult to discuss the mechanism of this action, and we can only argue that it can be exerted by altering the workings of pain-carrying neurotransmitters as well as relaxing muscles.
Further studies are clearly needed to establish whether the HPA derangement in CM has not only a pathogenetic relevance but can also be considered as a target of novel treatment strategies. Such studies may better orient treatment strategies to improve the well-being of migraine sufferers by damage prevention than by symptomatic therapies.

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References

1. Osterhaus JT, Gutterman DL, Plachetka JR. Healthcare resource and lost labour costs of migraine headache in the US. Pharmacoeconomics 1992; 2:67-76
2. Verri AP, Proietti CA, Galli C, et al. Psychiatric comorbidity in chronic daily headache. Cephalalgia 1998;18 (suppl 21):45-49.
3. Nagtegaal JE, Smits MG, Swart AC, et al. Melatonin-responsive headache in delayed sleep phase syndrome: preliminary observations. Headache 1998; 38:303-307
4. Headache Classification Subcommittee of the International Headache Society. The International Classification of Headache Disorders: 2nd Edition. Cephalalgia 2004; 24 [Suppl 1]:9-160.
5. Silberstein SD, Olesen J, Bousser MG et al. On behalf of International Headache Society. The International Classification of Headache Disorders, 2nd Edition (ICHD-

II) – revision of criteria for Medication-overuse headache. Cephalalgia 2005; 25:460-465.

6. EURO-MED-STAT Group. Monitoring expenditure and utilization of medicinal products in the European Union countries: a public health approach. Eur J Public Health 2003; 13 [Suppl 3]:95-100.
7. Capri S, Ceci A, Terranova L et al. Guidelines for economic evalutations in Italy: recommendations from the Italian group of pharmacoeconomic studies. Drug Inf J 2001; 35:189-201.
8. Martelletti P., Leonardi M (2003) The global impact of migraine. J Headache Pain 4: S1-S2 (editorial)
9. Silberstein S, Mathew N, Saper J, Jenkins S. Botulinum toxin type A as a migraine preventive treatment. Headache 2000; 40:445-50.
10. Silberstein SD, Göbel H, Jensen R et al. Botulinum toxin type A in the prophylactic treatment of chronic tension-type headache: a multicentre, double-blind, randomized, placebo-controlled, parallel-group study. Cephalalgia 2006; 26:790-800.
11. Blumenfeld A. Botulin toxin Type A for the treatment of Headache: pro. Headache 2004; 44: 825-830.
12. Welch KMA. Botulin toxin Type A for the treatment of Headache: con. Headache 2004; 44: 831-833.
13. Martelletti P., Patacchioli FR. Neuroendocrine derangement in chronic migraine: fact or fiction? Headache 2006 (in press)
14. Rainero I, Ferrero M, Rubino E, Valfrè W, Pellegrino M, Arvat E, Giordano R, Ghigo E, Limone P, Pinessi L. Endocrine function is altered in chronic migraine patients with medication overuse. Headache 2006; 46: 597-603
15. Patacchioli FR, Monnazzi P, Simeoni S, De Filippis S, Salvatori E, Coloprisco G, Martelletti P. Salivary cortisol, dehydroepiandrosterone sulphate (DHEA-S) and testosterone in women with chronic migraine. J Headache Pain 2006; 7: 90-94
16. McEwen BS. Protective and damaging effects of stress mediators. N Eng J Med 1998, 3: 171-179.
17. Giubilei F, Patacchioli FR, Antonini G, et al. Altered circadian cortisol secretion in Alzheimer's disease. Clinical and neuroradiological aspects. J Neurosci Res 2001, 66: 262-265.
18. Hubert W and De Long-Meyer R. Emotional stress and salivary cortisol response. J Clin Chem Clin Biochem 1989, 27:235-237.
19. Kirschbaum C and Hellhammer D. Response variability of salivary cortisol under psychological conditions. J Clin Chem Clin Biochem 1989, 27:237.
20. Kirschbaum C and Hellhammer D. Salivary cortisol in psychoneuroendocrine research: recent developments and applications, Psychoneuroendocrinology 1994, 19:313-333.
21. Patacchioli FR, Angelucci L, Dell'Erba G, Monnazzi P and Leri O. Actual stress, psychopathology and salivary cortisol levels in the irritable bowel syndrome (IBS). J Endocrinol. Invest. 2001; 24:173-177.

22. Wolf OT, Kirschbaum C. Actions of dehydroepiandrosterone and its sulphate in the central nervous system: effects on cognition and emotion in animals and humans. Brain Res Rev 1999, 30:264-288.
23. Morgan III CA, Southwick S, Hazlet G, Rasmusson A, Hoyt G, ZimoloZ, Charney D. Relationship among plasma dehydroepiandrosterone sulfate and cortisol levels, symptoms of dissociation, and objective performance in humans exposed to acute stress. Arch. Gen. Psychiatry 2004, 61: 819-825.
24. Van Niekerk JK, Huppert FA, Herbert J. Salivary cortisol and DHEA: association with measures of cognition and well-being in normal older men, and effects of three months of DHEA supplementation. Psychoneuroendocrinology 2001, 26:591-612.
25. Bosco C, Tihanyi J, Viru A. Relationship between field fitness test and basal serum testosterone and cortisol levels in soccer players. Clinical Physiology 1996;16: 317-322.
26. Hoogeveen AR e Zonderland ML. (1996). Relationship between testosterone, Cortisol and performance in professional cyclist. Int. J. Sport Med. 17: 423-428.
27. Urhausen A, Gabriel H, Kindermann W. Blood hormones as markers of training stress and overtraining. Sports Med 1995; 20: 251-276.
28. The International Headache Society. "The International Classification of Headache Disorders, 2nd Edition." (ICHD-II) September, 2004).
29. Martelletti P. Health status after detoxification in medication overuse headache. J Headache Pain 2004; 5:215-216.
30. Silberstein SD Headache and female hormones: what you need to know. Curr Opin Neurol 2001; 14: 232-333.
31. Martelletti P, Andreoli A, Bernoni RM, Di Sabato F, Del Bolgia F, Baldi A, Sasso GF, Barra P, De Lorenzo A, Giacovazzo M Biolectrical Impedance Assay (BIA) of total body composition in alcohol-induced migraine patients. Preliminary report. Headache1991; 31:41-45.
32. Blumenfeld AM, Binder W, Silberstein SD, Blitzer A. Procedure for administering botulin toxin type A for migraine and tension-type headache. Headache 2003; 43: 884-891.
33. Winer BJ (1990) Statistical Principles in experimental design, Fourth edition. McGraw-Hill, Tokio
34. Sapolsky RM. Stress, glucocorticoids, and damage to the nervous system: the current state of confusion. Stress 1996, 1: 1-19
35. Simonian, N.A. and Coyle, J.T. Oxidative stress in neurodegenerative diseases. Annu. Rev. Pharmacol. Toxicol. 1996; 36: 83-106.
36. Mc Intosh LJ, Sapolsky RM. Glucocorticoids may enhance oxygen radical-mediated neurotoxicity. Neurotoxicology. 1996, 17: 873-882
37. Dolly O. Synaptic transmission: inhibition of neurotransmitter release by botulinum toxins. Headache 2003; 43: S16-S24.
38. Steward WF., Simon D., Shechter A. and Lipton RB (1995) Population variation in migraine prevalence: a meta-analysis. J. Clin. Epidemiol. 48: 269-280.

39. Lundberg PO Endocrine headache. In: Rose FC ed. Headache, vol 44 Elsevier Science Publisher New York 1996:431-440.
40. Mathew NT, Frishberg BM, Gawel M, Dimitrova R, Gibson J, Turkel V, and the BOTOX CDH Study Group. Botulinum toxin type a (Botox®) for the prophilactic treatment of chronic daily headache: a randomized, double blind, placebo controlled trial. Headache 2005; 45:293-307.