Journal of Neural Transmission (2019) 126:789–793
https://doi.org/10.1007/s00702-019-02015-x

NEUROLOGY AND PRECLINICAL NEUROLOGICAL STUDIES - SHORT COMMUNICATION

Assessment of plasma creatine kinase as biomarker for levodopa-induced dyskinesia in Parkinson's disease

Anna Delamarre[1,2,3] · François Tison[1,2,3] · Qin Li[4,5] · Monique Galitzky[6] · Olivier Rascol[6,7,8] · Erwan Bezard[1,2,3,4,5] · Wassilios G. Meissner[1,2,3,9,10]

Received: 2 January 2019 / Accepted: 11 May 2019 / Published online: 16 May 2019

Abstract
We tested in a translational approach the usefulness of plasma creatine kinase (CK) as an objective biomarker for levodopa-induced dyskinesia (LID). Plasma CK levels were measured in five dyskinetic parkinsonian non-human primates (NHP) and in ten PD patients with LID who participated in a treatment trial with simvastatin. Plasma CK levels were increased in dyskinetic NHP and correlated with LID severity while they were not affected by LID severity in PD patients.

Keywords Parkinson's disease · Levodopa-induced dyskinesia · Biomarker · Creatine kinase

Introduction

The progression of Parkinson's disease (PD) and the use of dopamine replacement therapy, in particular levodopa, are associated with the emergence of levodopa-induced dyskinesia (LID). LID is unpredictable involuntary movements that occur in most patients as peak-dose dyskinesia, i.e. when levodopa plasma levels are highest (Bastide et al. 2015). Most patients will experience LID during the disease course with negative impact on health-related quality of life (Chapuis et al. 2005; Hechtner et al. 2014; Pechevis et al. 2005).

Several LID rating scales were validated (Colosimo et al. 2010). These are challenged by the high variability of LID over time and the limited awareness of their presence, especially in patients with cognitive impairment and mood disorders (Amanzio et al. 2014). There currently exists no validated biomarker to assess LID, while such objective outcomes are needed in light of the limitations of clinical rating scales (Meissner et al. 2011). Several monitoring devices were tested in clinical studies, but none has been specifically validated for the assessment of LID (Chung et al. 2010; Lopane et al. 2015; Manson et al. 2000; Mera et al. 2012a, b; Perez-Lopez et al. 2016; Tsipouras et al. 2012; Tzallas et al. 2014). Plasma creatine kinase (CK) levels are sensitive to muscle injury and activation (Khan 2009). Rhabdomyolysis with high plasma CK levels was reported in PD patients with acute severe LID (Bektas et al. 2014; Lyoo and Lee 2011). Moreover, rhabdomyolysis induced by excessive muscle activity was also found in other medical conditions such as dystonic storm and status epilepticus (Singhal et al. 1978; Termsarasab and Frucht 2017).

✉ Wassilios G. Meissner
wassilios.meissner@chu-bordeaux.fr

1 Service de Neurologie, Hôpital Pellegrin, CHU de Bordeaux, 33000 Bordeaux, France

2 Institut des Maladies Neurodégénératives, Université de Bordeaux, UMR 5293, 146 rue Léo Saignat, 33000 Bordeaux Cedex, France

3 CNRS, Institut des Maladies Neurodégénératives, UMR 5293, 33000 Bordeaux, France

4 Institute of Laboratory Animal Sciences, China Academy of Medical Sciences, Beijing, China

5 Motac Neuroscience, Manchester, UK

6 CIC Toulouse, Toulouse, France

7 Départements de Pharmacologie Clinique et Neurosciences, INSERM CIC9302, CHU de Toulouse, Toulouse, France

8 Service de Pharmacologie, Faculté de Médecine, CHU de Toulouse, Université de Toulouse, Toulouse, France

9 Department Medicine, University of Otago, Christchurch, New Zealand

10 New Zealand Brain Research Institute, Christchurch, New Zealand

We here explored the hypothesis of increased plasma CK levels as an objective biomarker for the evaluation of LID based on a post hoc analysis of data obtained in five parkinsonian non-human primates (NHP) and ten PD patients from a small randomized, placebo-controlled, multiple cross-over *n*-of-1 trial with simvastatin (Tison et al. 2013).

Materials and methods

Animals

Five macaques were rendered parkinsonian and dyskinetic as previously described (Bezard et al. 2001; Tison et al. 2013). Briefly, animals were intoxicated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) hydrochloride (0.2 mg/kg i.v. for 15 days). After stability of induced parkinsonism (8 weeks), a daily oral administration of levodopa was administered for 12 weeks at a tailored dose (15–20 mg/kg/day) producing full reversal of parkinsonian symptoms and moderate to severe LID (Ahmed et al. 2010; Berton et al. 2009; Bezard et al. 2003; Fasano et al. 2010; Porras et al. 2012; Shen et al. 2016; Urs et al. 2015).

Motor behaviour (severity of parkinsonism and LID) was assessed in an observation cage for 240 min after oral administration of levodopa (individually tailored dose ranging between 15 and 20 mg/kg, days D2 and D14) and in OFF condition (days D1 and D21), as previously described (Tison et al. 2013). Day 1 assessment in OFF condition was performed after 1 week of levodopa wash-out. Parkinsonian symptom severity was quantified with an established scale (0 meaning no parkinsonism and a score above six corresponding to severe motor disability). LID was rated according to the NHP Dyskinesia Disability Scale (NHPDDS) from 0 (no dyskinesia) to 4 (severe and disabling dyskinesia) for both choreic and dystonic movements (Fox et al. 2012). The area under the curve (AUC) was calculated for motor disability and NHPDDS scores for each day of assessment. Blood was sampled 300 min after the oral application of levodopa for plasma CK level measure. Six macaques never exposed to MPTP or levodopa served for the measurement of CK levels in a healthy control group.

All animal experiments were performed in accordance with the European Union directive of September 22, 2010 (2010/63/EU) on the protection of animals used for scientific purposes in an AAALAC-accredited facility following acceptance of study design by the Institute of Lab Animal Science (Chinese Academy of Science, Beijing, China). The monkeys were housed in individual cages allowing visual contacts and interactions with other monkeys in adjacent cages. Food and water were available ad libitum. Animal care was supervised daily by veterinarians skilled in the healthcare and maintenance of NHPs.

Patients

PD patients participated in a randomized, placebo-controlled, multiple cross-over *n*-of-1 trial that assessed the efficacy of simvastatin against LID (Tison et al. 2013). All encountered moderately to severely disabling LID (Unified PD Rating Scale IV item 33 > 1) for more than 25% of the waking day (item 32 > 1). As part of the trial, LID were assessed by the modified Abnormal Involuntary Movement Scale (AIMS) and plasma CK concentrations were determined at each study visit (2 weeks before the beginning of the study (pre-screening), on day 1 before starting treatment, and on days 14, 28, 42, 56, 70, 84 after randomisation). Written consent of all study subjects was obtained prior to enrolment and following ethics approval (CPP Sud-Ouest et Outremer 3). The clinical trial was registered in the EudraCT database under the number 2009-011736-35.

Statistics

Clinical data and plasma CK levels in NHP were compared using a non-parametric one-way ANOVA for repeated measures, followed, if appropriate, by Dunn's test for multiple comparisons. A Spearman correlation was performed between plasma CK levels in NHP and daily NHPDDS scores. In PD patients, AIMS scores were classified into three groups, 0–2 (no or very minor LID), 3–8 (moderate LID) and > 8 (severe LID). Plasma CK levels were compared between the three defined AIMS classes using a non-parametric Kruskal–Wallis test, followed, if appropriate, by Dunn's test for multiple comparisons. A Spearman correlation was performed between plasma CK levels and AIMS score. Plasma CK levels were compared between periods on simvastatin versus placebo using a Mann–Whitney *U* test. Data are presented as median and range. A $p < 0.05$ was considered significant. Analyses were performed using GraphPad Prism 7.0 Software.

Results

Non-human primate model of LID

Five MPTP-lesioned macaques were monitored for plasma CK levels, parkinsonian motor and LID scores in OFF condition and after an acute levodopa challenge. An overall comparison revealed a significant difference in parkinsonian motor scores between the 4 days of assessment ($p = 0.0009$, Fig. 1). Post-hoc testing further showed a trend for lower parkinsonian motor scores after levodopa treatment ($p = 0.09$ for D2 and D14 compared to D1 and D21). Simultaneously,

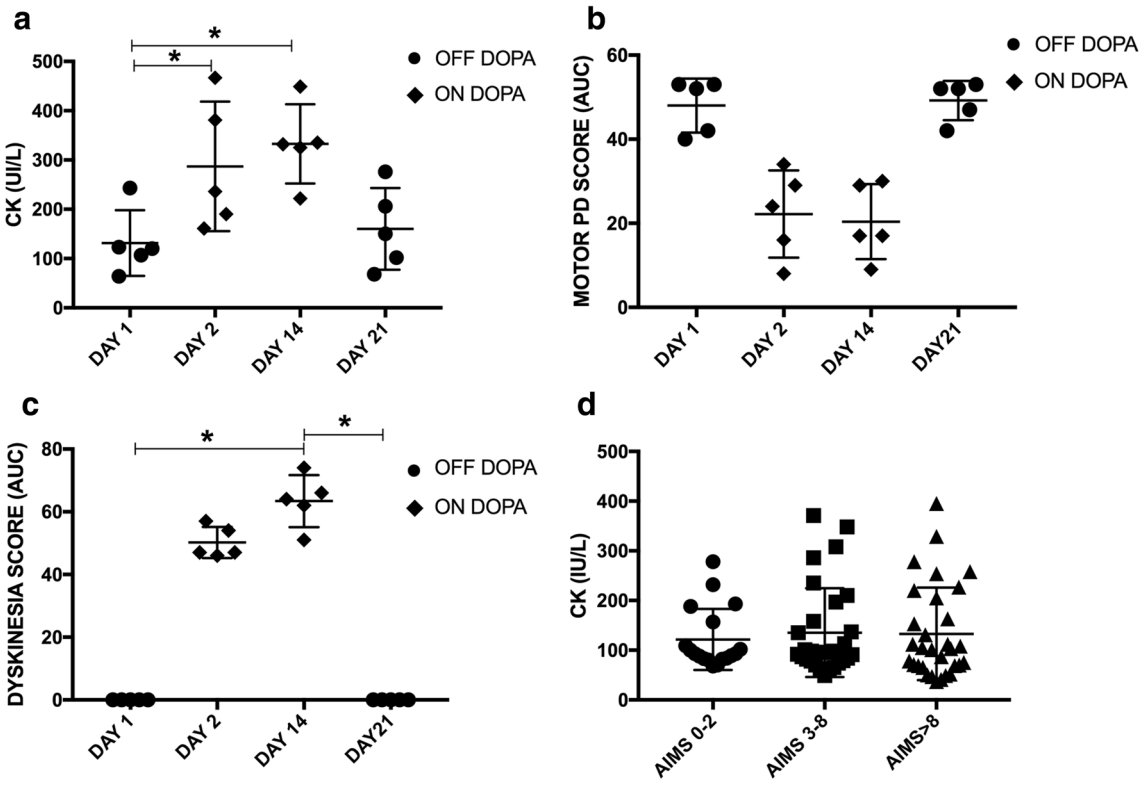

Fig. 1 Plasma CK levels and clinical scores in parkinsonian dyskinetic non-human primates and PD patients. **a** Plasma CK levels, **b** AUC of motor PD scores and **c** NHPDDS scores in OFF condition (day 1 and 21) and after an acute levodopa challenge (day 2 and 14) in parkinsonian non-human primates. **d** Plasma CK levels in patients stratified by AIMS score were not different (*$p < 0.05$ post hoc Dunn's test)

an overall comparison revealed significant differences in NHPDDS scores between the 4 days of assessment ($p < 0.0001$). Post-hoc testing further showed significantly higher LID scores after levodopa treatment (D14) compared to both days OFF (D1 and D21, $p = 0.0132$, Fig. 1).

Plasma CK levels were higher during ON condition [OFF condition: 120 (64–243) UI/L on D1 and 150 (68–276) UI/L on D21; ON condition: 236 (161–467) UI/L on D2 and 332 (222–449) UI/L on D14]. An overall comparison showed significant differences between the 4 days of assessment ($p = 0.0055$). Post-hoc testing further confirmed higher plasma CK levels in ON condition compared to OFF condition (D1 vs D2 and D14, $p = 0.042$, Fig. 1). We also found a correlation between plasma CK and corresponding NHPDDS scores (Spearman $r = 0.62$, $p = 0.0036$). Plasma CK levels in healthy NHPs were 140 (73–219) UI/L and were not different from measurements in MPTP-treated NHPs in the OFF condition on day 1.

PD patients

Data of all ten PD patients of the simvastatin treatment trial were available for the analysis with 8 days of AIMS ratings and plasma CK level measurements (Tison et al. 2013). There was no correlation between AIMS scores and plasma CK levels (Spearman $r = -0.012$, $p = 0.91$), and no significant differences when comparing the plasma CK levels of the three defined AIMS classes [no or very minor LID: 93 (68–278) UI/L; moderate LID: 97 (49–371) UI/L; severe LID: 103 (37–395) UI/L, $p = 0.9$, Fig. 1]. To exclude any effect of simvastatin on plasma CK levels, we further compared CK levels between periods on active treatment with those on placebo and found no difference [placebo: 98 (41–371) UI/L, simvastatin 98 (47–395) UI/L, $p = 0.8$].

Discussion

The aim of this pilot study was to explore in a translational approach the usefulness of plasma CK levels as biomarker for LID in parkinsonian NHP and PD patients. Indeed, plasma CK levels were higher in dyskinetic parkinsonian NHP compared to the OFF condition. However, no difference was observed in PD patients, irrespective of the LID status as assessed by the AIMS. Patients were initially enrolled in a trial assessing the efficacy of simvastatin on

LID (Tison et al. 2013). In this trial, simvastatin had no clinical effect on LID.

CK is an intracellular enzyme, mainly found in high-energy demanding cells, particularly skeletal muscular cells. Increasing serum CK levels are generally due to muscle damage or metabolic injury, leading to proteolysis and increasing membrane permeability (Bektas et al. 2014; Khan 2009; Lyoo and Lee 2011; Singhal et al. 1978; Termsarasab and Frucht 2017). Serum CK levels are supposed to peak at 48–72 h post-exercise and return to baseline within 7 days (Koch et al. 2014; Noakes 1987; Sherwood et al. 1996). Exercise intensity and duration influence CK levels in some studies, while the relationship between volume load (amount of work) during resistance exercise and serum CK levels seems weak (Koch et al. 2014; Machado et al. 2011; 2012).

Our study has several limitations. First, LID was more severe in parkinsonian NHP (all but one animal reached the maximal NHPDDS score on D2 and all on D14), thereby mimicking a more intense muscle activity than LID in the assessed PD patients (median AIMS score = 11/28). Additionally, the muscles of PD patients might have been adapted to chronic LID, limiting muscle injury and metabolic failure to such an activity. Second, the experimental conditions differed between NHP and PD patients. In NHP, we analysed CK levels after an acute levodopa challenge in NHP with a defined delay between levodopa administration and blood sampling, while in PD patients with more stable LID over time, CK was measured at each study visit with no precise timing regarding the intake of dopamine replacement therapy. Third, the number of PD patients was small with a limited power to detect significant differences.

In conclusion, plasma CK levels were increased in dyskinetic parkinsonian NHP, while we were not able to reproduce these findings in a small number of PD patients. Future studies with higher number of patients are required to further explore the usefulness of plasma CK levels for monitoring LID.

Acknowledgements The Simvastatin trial was funded by a Michael J. Fox Foundation Clinical Intervention Award 2008.

Funding The Simvastatin trial was funded by a M. J. Fox Foundation Clinical Intervention Award 2008.

Compliance with ethical standards

Conflict of interest None of the authors has a conflict of interest in relation to the research covered in the article. Outside the present study (past 12 months), QL and MG have nothing to declare. AD declares a grant from the Fondation pour la recherche médicale (FDM201806005951). FT has received a travel grant from ORKYN and research grants from the French Health Ministry (PHRC). EB has an equity stake in Motac holding Ltd and receives consultancy payments from Motac Neuroscience Ltd. Current grant support includes Agence Nationale de la Recherche, China Science Fund, Michael J Fox Foundation for Parkinson Research, France Parkinson, Fondation de France, Medical Research Council, Innovative Medicines Initiative (H2020, IMPRIND), IDEX Bordeaux, COEN, MSA Coalition, Rotary Foundation, Nouvelle-Aquitaine Regional Council, and LABEX Brain. OR has received grants from Agence Nationale de la Recherche (ANR), CHU de Toulouse, France-Parkinson, INSERM-DHOS Recherche Clinique Translationnelle, MJFox Foundation, Programme Hospitalier de Recherche Clinique, European Commission (FP7, H2020) and has served as scientific consultant for AbbVie, Adamas, Acorda, Addex, AlzProtect, Apopharma, Astra Zeneca, Bial, Biogen, Britannia, Clevexel, Cynapsus, INC Research, Lundbeck, Merck, MundiPharma, Neuroderm, Novartis, Oxford Biomedica, Parexel, Pfizer, Prexton Therapeutics, Quintiles, Sanofi, Servier, Teva, UCB, XenoPort, Zambon. EB has an equity stake in Motac holding Ltd and receives consultancy payments from Motac Neuroscience Ltd. Current grant support includes Agence Nationale de la Recherche, China Science Fund, Michael J Fox Foundation for Parkinson Research, France Parkinson, Fondation de France, Medical Research Council, Innovative Medicines Initiative (H2020, IMPRIND), IDEX Bordeaux, COEN, MSA Coalition, Rotary Foundation, Nouvelle-Aquitaine Regional Council, and LABEX Brain. WGM has received fees for editorial activities with Springer Nature, honoraria for consultancy activities from Sanofi, Lundbeck and Affiris, teaching honoraria from UCB, honoraria from MDS, as well as research support from the Michael J Fox Foundation, the University Hospital Bordeaux, the French Health Ministry, the European Community, ANR, ARAMISE, PSP-France, MSA Coalition, LABEX Excellence Initiative.

Ethical approval All animal experiments were performed in accordance with the European Union directive of September 22, 2010 (2010/63/EU) on the protection of animals used for scientific purposes in an AAALAC-accredited facility following acceptance of study design by the Institute of Lab Animal Science (Chinese Academy of Science, Beijing, China).

Written consent Written consent of all subjects of the simvastatin trial was obtained prior to enrolment and following ethics approval (CPP Sud-Ouest et Outremer 3).

References

Ahmed MR et al (2010) Lentiviral overexpression of GRK6 alleviates L-dopa-induced dyskinesia in experimental Parkinson's disease. Sci Transl Med 2:28ra28. https://doi.org/10.1126/scitranslmed.3000664

Amanzio M, Palermo S, Zibetti M, Leotta D, Rosato R, Geminiani G, Lopiano L (2014) Self-unawareness of levodopa induced dyskinesias in patients with Parkinson's disease. Brain Cogn 90:135–141. https://doi.org/10.1016/j.bandc.2014.06.014

Bastide MF et al (2015) Pathophysiology of L-dopa-induced motor and non-motor complications in Parkinson's disease. Prog Neurobiol 132:96–168. https://doi.org/10.1016/j.pneurobio.2015.07.002

Bektas H, Deniz O, Temel S, Keklikoglu HD, Akyol S (2014) Rhabdomyolysis related to dyskinesia in Parkinson's disease. J Mov Disord 7:25–27. https://doi.org/10.14802/jmd.14006

Berton O et al (2009) Striatal overexpression of DeltaJunD resets L-DOPA-induced dyskinesia in a primate model of Parkinson disease. Biol Psychiatry 66:554–561. https://doi.org/10.1016/j.biopsych.2009.04.005

Bezard E et al (2001) Relationship between the appearance of symptoms and the level of nigrostriatal degeneration in a progressive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaque model of Parkinson's disease. J Neurosci 21:6853–6861

Bezard E et al (2003) Attenuation of levodopa-induced dyskinesia by normalizing dopamine D3 receptor function. Nat Med 9:762–767. https://doi.org/10.1038/nm875

Chapuis S, Ouchchane L, Metz O, Gerbaud L, Durif F (2005) Impact of the motor complications of Parkinson's disease on the quality of life. Mov Disord 20:224–230. https://doi.org/10.1002/mds.20279

Chung KA, Lobb BM, Nutt JG, McNames J, Horak F (2010) Objective measurement of dyskinesia in Parkinson's disease using a force plate. Mov Disord 25:602–608. https://doi.org/10.1002/mds.22856

Colosimo C et al (2010) Task force report on scales to assess dyskinesia in Parkinson's disease: critique and recommendations. Mov Disord 25:1131–1142. https://doi.org/10.1002/mds.23072

Fasano S et al (2010) Inhibition of Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) signaling in the striatum reverts motor symptoms associated with L-dopa-induced dyskinesia. Proc Natl Acad Sci USA 107:21824–21829. https://doi.org/10.1073/pnas.1012071107

Fox SH, Johnston TH, Li Q, Brotchie J, Bezard E (2012) A critique of available scales and presentation of the non-human primate dyskinesia rating scale. Mov Disord 27:1373–1378. https://doi.org/10.1002/mds.25133

Hechtner MC et al (2014) Quality of life in Parkinson's disease patients with motor fluctuations and dyskinesias in five European countries. Parkinsonism Relat Disord 20:969–974. https://doi.org/10.1016/j.parkreldis.2014.06.001

Khan FY (2009) Rhabdomyolysis: a review of the literature. Neth J Med 67:272–283

Koch AJ, Pereira R, Machado M (2014) The creatine kinase response to resistance exercise. J Musculoskelet Neuronal Interact 14:68–77

Lopane G, Mellone S, Chiari L, Cortelli P, Calandra-Buonaura G, Contin M (2015) Dyskinesia detection and monitoring by a single sensor in patients with Parkinson's disease. Mov Disord 30:1267–1271. https://doi.org/10.1002/mds.26313

Lyoo CH, Lee MS (2011) Rhabdomyolysis induced by severe levodopa induced dyskinesia in a patient with Parkinson's disease. J Neurol 258:1893–1894. https://doi.org/10.1007/s00415-011-6041-x

Machado M et al (2011) Effect of varying rest intervals between sets of assistance exercises on creatine kinase and lactate dehydrogenase responses. J Strength Cond Res 25:1339–1345. https://doi.org/10.1519/JSC.0b013e3181d680d6

Machado M, Willardson JM, Silva DR, Frigulha IC, Koch AJ, Souza SC (2012) Creatine kinase activity weakly correlates to volume completed following upper body resistance exercise. Res Q Exerc Sport 83:276–281. https://doi.org/10.1080/02701367.2012.10599858

Manson AJ, Brown P, O'Sullivan JD, Asselman P, Buckwell D, Lees AJ (2000) An ambulatory dyskinesia monitor. J Neurol Neurosurg Psychiatry 68:196–201. https://doi.org/10.1136/jnnp.68.2.196

Meissner WG et al (2011) Priorities in Parkinson's disease research. Nat Rev Drug Discov 10:377–393. https://doi.org/10.1038/nrd3430

Mera TO, Burack MA, Giuffrida JP (2012a) Quantitative assessment of levodopa-induced dyskinesia using automated motion sensing technology. Conf Proc IEEE Eng Med Biol Soc 2012:154–157. https://doi.org/10.1109/EMBC.2012.6345894

Mera TO, Heldman DA, Espay AJ, Payne M, Giuffrida JP (2012b) Feasibility of home-based automated Parkinson's disease motor assessment. J Neurosci Methods 203:152–156. https://doi.org/10.1016/j.jneumeth.2011.09.019

Noakes TD (1987) Effect of exercise on serum enzyme activities in humans. Sports Med 4:245–267

Pechevis M et al (2005) Effects of dyskinesias in Parkinson's disease on quality of life and health-related costs: a prospective European study. Eur J Neurol 12:956–963. https://doi.org/10.1111/j.1468-1331.2005.01096.x

Perez-Lopez C et al (2016) Dopaminergic-induced dyskinesia assessment based on a single belt-worn accelerometer. Artif Intell Med 67:47–56. https://doi.org/10.1016/j.artmed.2016.01.001

Porras G et al (2012) PSD-95 expression controls L-DOPA dyskinesia through dopamine D1 receptor trafficking. J Clin Invest 122:3977–3989. https://doi.org/10.1172/jci59426

Shen W et al (2016) M4 Muscarinic Receptor Signaling Ameliorates Striatal Plasticity Deficits in Models of L-DOPA-Induced Dyskinesia. Neuron 90:1139. https://doi.org/10.1016/j.neuron.2016.05.017

Sherwood RA, Lambert A, Newham DJ, Wassif WS, Peters TJ (1996) The effect of eccentric exercise on serum creatine kinase activity in different ethnic groups. Ann Clin Biochem 33(Pt 4):324–329. https://doi.org/10.1177/000456329603300407

Singhal PC, Chugh KS, Gulati DR (1978) Myoglobinuria and renal failure after status epilepticus. Neurology 28:200–201

Termsarasab P, Frucht SJ (2017) Dystonic storm: a practical clinical and video review. J Clin Mov Disord 4:10. https://doi.org/10.1186/s40734-017-0057-z

Tison F et al (2013) Simvastatin decreases levodopa-induced dyskinesia in monkeys, but not in a randomized, placebo-controlled, multiple cross-over ("*n*-of-1") exploratory trial of simvastatin against levodopa-induced dyskinesia in Parkinson's disease patients. Parkinsonism Relat Disord 19:416–421. https://doi.org/10.1016/j.parkreldis.2012.12.003

Tsipouras MG, Tzallas AT, Rigas G, Tsouli S, Fotiadis DI, Konitsiotis S (2012) An automated methodology for levodopa-induced dyskinesia: assessment based on gyroscope and accelerometer signals. Artif Intell Med 55:127–135. https://doi.org/10.1016/j.artmed.2012.03.003

Tzallas AT et al (2014) PERFORM: a system for monitoring, assessment and management of patients with Parkinson's disease. Sens (Basel) 14:21329–21357. https://doi.org/10.3390/s141121329

Urs NM et al (2015) Targeting beta-arrestin2 in the treatment of L-DOPA-induced dyskinesia in Parkinson's disease. Proc Natl Acad Sci USA 112:E2517–2526. https://doi.org/10.1073/pnas.1502740112