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Case report

Hepatotoxicity induced by paclitaxel interaction with turmeric in association with a microcystin from a contaminated dietary supplement

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ABSTRACT

A 67-year-old Caucasian male with lung cancer was presented to the Emergency Department with asthenia, anorexia, jaundice and choluria. The patient's lung cancer was being treated medically by a combination of paclitaxel/carboplatin with bi-monthly frequency. The patient was also self-medicating with several natural products, including *Chlorella* (520 mg/day), *Silybum marianum* (total of 13.5 mg silymarin/day), zinc sulphate (5.5 mg), selenium (50 μg) and 15 g/day of *Curcuma longa*. In first chemotherapy cycle no toxicity was observed even he was taking other medications as budesonide and sitagliptin. The toxic events started only after the introduction of the dietary products. *Chlorella* had contamination with cyanobacteria (Oscillatoriales) and 1.08 μg of cyanotoxin Microcystin-LR (MC-LR) per gram of biomass was found. Patient was consuming ca 0.01 μg MC-LR/kg/day. This case report describes the first known case of paclitaxel toxicity probably related to pharmacokinetic interaction with Turmeric and a contaminated *Chlorella* supplement resulting in an acute toxic hepatitis and the impact on oncologic patient health.

1. Introduction

The combination of paclitaxel (Taxol, Abraxane) and carboplatin (Paraplatin) have become major antineoplastic treatments for lung cancer over the past two decades. In the meantime, also the use of herbal remedies and/or dietary supplements during cancer treatment is growing among these patients many times contributing for fatal events.

Among many medicinal (or non-medicinal) species sold with for this purpose: aloe, algae, fungi as Coryolus or Reishi (mushrooms), Milk thistle (*Sylibum marianum*), and Turmeric (*Curcuma longa*) are the most popular. The latter is toxic above 8 g/day and inhibits cytochrome P450 (CYP) 1A2, 2C9 and 3A4 enzymes (Al-Jenoobi et al., 2015), which are required to prevent plasma concentrations of paclitaxel from reaching levels which can compromise liver function. Milk thistle it is also an important inhibitor of CYP 2C9 (Budzinski et al., 2007; Etheridge et al., 2007) compromising the metabolization of this drug. Similarly, dietary supplements such as *Spirulina* (cyanobacteria) and *Chlorella* have been marketed due to their alleged beneficial effects in “detoxification,” stimulating immune function and fighting cancer (Gilroy et al., 2000; Cavaliere et al., 2010). However, products with strains of cyanobacteria non-toxin producers have been seen to contain the cyanotoxin microcystin-LR (MC-LR) (Iwasa et al., 2002; Valério et al., 2016). MC-LR is the most studied cyanotoxin (Solter et al., 1998) being a potent inhibitor of Phosphatase 1 (P1) and 2A (P2A), leading changes in the microfilament structure of hepatocytes (Solter et al., 1998; Fujiki and Suganuma, 2009). MC-LR has the potential to promote tumor growth during chronic low-level exposure (Al-Jenoobi et al., 2015; Solter et al., 1998; Fujiki and Suganuma, 2009). MC-LR contamination in drinking water has been associated with high incidence of liver cancer (Lawton et al., 1994; Pinheiro et al., 2013).

2. Materials and methods

2.1. Methods for Chlorella supplement analysis

The sample of *Chlorella* was observed by optic microscope to detect

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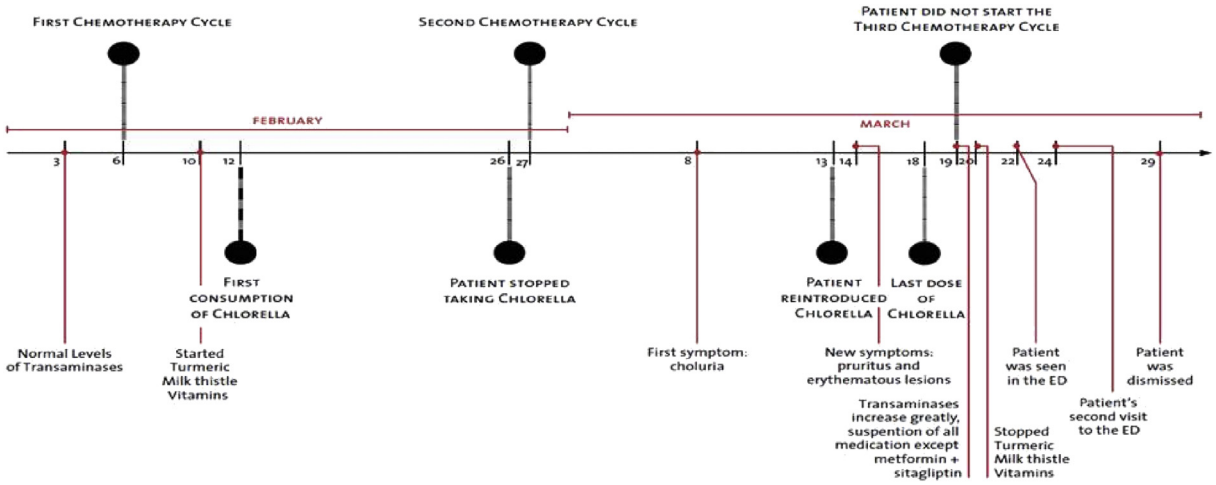

Fig. 1. Timeline of treatment and dietary supplement consumption.

the presence of other species of microalgae.

Extraction for ELISA analysis and LC-MS/MS: Ten capsules of *Chlorella* were opened and their contents homogenized and weighted. An average mass of 623.4 mg ± 55 mg (CV = 8.7%) per capsule was obtained. The sample extract was prepared according to Ramanan et al. (2000), Lawton et al. (1994) and Pinheiro et al. (2013) with some modifications. Three extracts with different masses (141 mg, 258.3 mg and 597.1 mg) were made with a solution of 50% methanol. The extracts were sonicated in an ice bath for 5 min at 60 Hz for 5 × 1 min (VibraCell 50-sonics & Material Inc. Danbury, CT, USA) in order to achieve cell lyses and centrifuged (4 °C, 4495 g/10 minutes) to remove cell debris. This process was repeated and the supernatant was totally evaporated by Speed Vac. The pellet was re-suspended in water for ELISA analysis and 50% methanol for LC-MS/MS. Immunoassay ELISA was performed using a microcystins/nodularins (ADDA) ELISA Abraxis kit with a detection limit of 0.1 µg/l.

A system of HPLC Surveyor (Thermo Scientific), an automated pump and sampler Surveyor LC, a photodiode detector (PDA) Surveyor combined with a mass spectrometer of Thermo Scientific containing an electrospray was used to identify MC-LR. The chromatograph column was Hypersil Gold (4.6 nm ID x 100 mm, 5 µm Thermo Scientific) at 25 °C, the flux 0.8 ml/min and the volume of injection was 25 µl. The mobile phase was water and methanol, both acidified with 0.1% formic acid. The gradient used was 55–90% of methanol in 12 minutes and then it was increased to 100% in 0.5 minutes and in 2.5 minutes it was reduced to 55%. The mass spectrometer was run with positive ion electrospray. The capillary and lens tube voltage were maintained at 22 and 120V, respectively. Nitrogen was used as the auxiliary gas. Helium gas was used as the collision for the ion trap. The gas flow rate was fixed at 80 units (ARB) and capillary temperature was 350 °C. MC-LR was analyzed using the mass-to-charge ratio (m/z) of transition 995 > 599 (Zhang et al., 2004; Spoof et al., 2003).

3. Case report

A 67-year-old Caucasian male patient presented at Emergency Department (E. D.) with symptoms of asthenia, anorexia, jaundice and choluria, in late March. Approximately one week earlier, the patient came to the hospital with complaints of pruritus and erythematous lesions, successfully treated with anti-histamine therapy. Initial testing revealed significantly elevated liver enzymes (AST: 2036 U/l, normal: < 34 U/l; ALT: 3127 U/l, normal < 55 U/l) and serum bilirubin levels (6.1 mg/dl, normal < 1.2mg/dl; conjugated bilirubin: 4.8 mg/dl, normal < 0.5 mg/dl). Results of viral hepatitis markers (HBs Antigen, anti-HBs, total anti-HBc, anti-HCV) were negative and renal function and coagulation parameters were normal. The patient had history of pulmonary emphysema (without respiratory insufficiency), type 2 diabetes (controlled), dyslipidemia, benign prostatic hyperplasia and lung cancer (pulmonary adenocarcinoma, T3N0Mx). The patient had no history of previous liver disease or alcohol abuse. He was taking chronic medication: metformin + sitagliptin, alfuzosin, atorvastatin, budesonide, formoterol, tiotropium bromide and was prescribed with six weeks of paclitaxel (165 mg/m2) and carboplatin (275 mg/m2) (BSA: 1.82 m2) with bi-monthly frequency (q2w). Four days after the beginning of the first cycle of chemotherapy, the patient started taking orally Turmeric (15 g/day) and various pills of dietary supplements such as Milk thistle (300mg with 1.5% of silymarin/3 × day – eq to 13.5 mg/day) (30 minutes before meals), vitamins and minerals (vitamin-C 60 mg, vitamin-E 20 mg, vitamin-A 1.5 mg, zinc sulphate 5.5 mg and selenium 50 µg). Two days after he add *Chlorella* (520 mg/day) and colostrum (650 mg/day) intake (what makes one week after the first treatment with the antineoplastic). The last product, colostrum, was taken only for about one week. All the others, were taken 14 days, until one day before the second dose of chemotherapy (end February). Two weeks later (middle March) he started to take *Chlorella* pills again and, the following day, pruritus and erythematous lesions were observed. However they were associated to secondary effects of paclitaxel. At that time in E. D. no information was given about the dietary supplements. Five days later, before the third dose of chemotherapy the evaluation carried out to the patient reveals elevated transaminases and the treatment was not done. The patient stopped to take *Chlorella* pills the day before but continued the intake of Turmeric until late March (Fig. 1), without the knowledge of the doctors.

At the time in E.D. visit (March 22nd), the patient had been endorsed to stop all medications (alfuzosin, atorvastatin, budesonide, formoterol, tiotropium bromide), except for metformin + sitagliptin, because of elevated transaminases, as mentioned above at the routine oncology appointment three days earlier (March 19th). Those drugs were, at this moment, considered the cause of his elevated liver enzymes (Fig. 1).

Subsequent evaluation of the clinical and laboratory tests indicated the diagnosis of toxic hepatitis, which was initially attributed to the chemotherapy regimen and its chronological relationship with the event. The patient was seen and dismissed the same day (late March), but re-admitted two days later due to choluria and jaundice worsening. Repeat liver tests showed evidence of worsening living function (AST: 2784 U/l; ALT: 4188 U/l; total serum bilirubin: 9.0 mg/dl; conjugated bilirubin: 6.8 mg/dl). All drugs and the remain dietary supplements were suspended at that time and the patient's rapid recovery led to his discharge five days later without permanent liver injuries (Fig. 2).

Despite the exposure to potentially hepatotoxic cancer therapy, the patient's supplemental use of Turmeric could be responsible for the