Food and Chemical Toxicology 92 (2016) 1–7

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Unfermented grape juice reduce genomic damage on patients undergoing hemodialysis

Zuray Corredor [a], Lara Rodríguez-Ribera [a], Elisabeth Coll [b], Rosario Montañés [b], Juan Manuel Diaz [b], José Ballarin [b], Ricard Marcos [a, c, *], Susana Pastor [a, c, **]

[a] Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Edifici C, Universitat Autònoma de Barcelona, 08193 Bellaterra, Cerdanyola del Vallès, Spain
[b] Fundació Puigvert, Barcelona, Spain
[c] CIBER Epidemiología y Salud Pública, ISCIII, Madrid, Spain

ARTICLE INFO

Article history:
Received 27 December 2015
Received in revised form
14 March 2016
Accepted 21 March 2016
Available online 23 March 2016

Keywords:
Hemodialysis
Unfermented grape juice (UGJ)
Comet assay
Micronucleus assay
Trolox equivalent antioxidant capacity (TEAC) assay
Oxidative damage

ABSTRACT

Chronic kidney disease (CKD) patients in dialysis (HD) are considered to be submitted to a continuous oxidative stress. This stress can cause damage on DNA and, consequently, contribute to the high levels of DNA damage observed in these patients. Due to the well-known role of polyphenols as antioxidant agents we proposed its use to reduce the levels of genotoxicity present in HD-CKD patients. The objective of this study was to evaluate the antigenotoxic effects of unfermented grape juice (UGJ) on HD-CKD patients. The levels of DNA damage were analyzed using different biomarkers, such as breaks and oxidized DNA bases by the comet assay, chromosome damage by the micronucleus test. In addition, TEAC (Trolox equivalent antioxidant capacity) was also evaluated. Thirty-nine patients were followed for six months, of whom 25 were supplemented by UGJ and 14 were not supplemented. The obtained results showed a significant decrease in the underlying levels of oxidative DNA damage, in the supplemented group. Regarding the clinical parameters, LDL and cholesterol, were significantly reduced in the patients studied after the supplementation period, although cholesterol was also decreased in the non-supplemented patients. In conclusion, in our studied group the supplementation with UGJ reduced the levels of oxidative DNA damage of HD-CKD patients.

1. Introduction

Oxidative stress is the result of an accumulation of reactive oxygen species (ROS), together with a low antioxidant capacity, that leads to biochemical alterations causing structural and functional modifications of these biomolecules (Massy et al., 2009). Many studies have focused on the detection of oxidative stress in patients with renal alterations to determine whether this is an underlying cause/effect mechanism in chronic kidney disease (CKD). At this point, it must be indicated that CKD patients under dialysis (HD) are submitted to a continuous oxidative stress (McDonald et al., 2014). The HD process contribute to the elimination of plasma antioxidants and together with the dietary restrictions of these patients, the reduced antioxidant enzyme activity, and iron and erythropoietin supplements, these patients became a group with potentially high levels of ROS (Spormann et al., 2008). These reasons support the view that HD process induces oxidative stress in CKD patients and it has been reported that HD-CKD patients show significantly higher levels of oxidative DNA damage than CKD predialysis (PD) patients (Corredor et al., 2015). One of the hypotheses explaining this fact is that, during the dialysis session, the contact blood-membrane induced the activation of macrophages, losing antioxidant capacity and contributing to enhanced oxidative stress in CKD patients. In this scenario the use of antioxidants supplements can be helpful to this type of patients.

It is well known that phenolic compounds act as antioxidant, specially due to their ability to donate hydrogen or electrons and prevent the oxidation of various compounds, particularly fatty

* Corresponding author. Grup de Mutagenesi, Departament de Genetica i de Microbiologia, Edifici C, Universitat Autonoma de Barcelona, 08193 Bellaterra, Cerdanyola del Valles, Spain.
** Corresponding author. Grup de Mutagenesi, Departament de Genetica i de Microbiologia, Edifici C, Universitat Autonoma de Barcelona, 08193 Bellaterra, Cerdanyola del Valles, Spain.
E-mail addresses: ricard.marcos@uab.es (R. Marcos), susana.pastor@uab.es (S. Pastor).

http://dx.doi.org/10.1016/j.fct.2016.03.016
0278-6915/

acids and oils (Gülçin, 2012). Fruits, especially those with red or blue color (such as grapes, plums and cherries), are the most important sources of polyphenols (Stratil et al., 2007). In grapes, flavonoids are primarily located in the epidermal layer of berry skin and in the seeds (Waterhouse, 2002). Flavonoids are the main group of soluble phenolic compounds in grapes, as well as the main contributors of the biological activities in products derived from grapes (Conde et al., 2007). It has been reported that ingestion of unfermented grape juice (UGJ) as a polyphenol-rich dietary supplement exerts hypolipidemic, antioxidant, and anti-inflammatory effects in hemodialysis patients (Castilla et al., 2006). In fact, increased uptake of food-based antioxidants can be a promising alternative measure to reduce oxidative cell damage and stress response (Weisel et al., 2006). In HD patients, few studies (Castilla et al., 2006, 2008; Spormann et al., 2008; Alipour et al., 2012; Janiques et al., 2014) have evaluated the effects of polyphenolic-rich fruit juices on antioxidant capacity, and oxidative stress. No previous studies have been carried out to determine its effect on the levels of genetic damage in such patients. Therefore, the aim of this study was to evaluate the effect of UGJ on the levels of genomic damage in CKD patients under HD by analyzing markers such as genomic/oxidative DNA damage (comet assay) and chromosome damage (micronucleus test). Additionally, variations on TEAC (Trolox equivalent antioxidant capacity) values were also determined.

2. Materials and methods

2.1. Study population

The study involved a total of 39 CKD patients undergoing hemodialysis three times per week, with 3:30—4 h per session. Patients were recruited at the hospital Fundació Puigvert (Barcelona, Spain). Two blood samples were obtained for each patient before the HD session, before and after 6 months. Patients were randomly distributed in 2 groups: UGJ and reference. During that period of time, 25 CKD patients were supplemented, during the last half hour of each dialysis session, with 100 mL of unfermented grape juice (UGJ), assuming that they did not changed their food intake habits during the studied period. A descriptive of the general characteristics of the studied population is indicated on Table 1. Medications and supplements administered to these patients during the follow up are indicated in Table 2. Clinical data was recovered directly from medical history, and clinical parameters (Table 3).

Standard blood analysis included the determination of calcium, phosphorus, glucose, cholesterol, triglycerides, albumin and hemoglobin, among other parameters. Moreover ferritin, iron, transferrin saturation index, parathyroid hormone and C-reactive protein were also analyzed. The erythropoiesis stimulating agents (ESA) resistance index (ERI) was determined as the weekly weight-adjusted ESA dose (IU/week/kg) divided by the product of the patient's weight (Kg) and the hemoglobin level (g/dL). A conversion ratio of 1:200 was used to convert the darbepoetin dose (mcg) to international units (IU) of erythropoietin. All individuals participating in the study provided written informed consent, and blood samples were collected under protocols approved by the Ethics Committee of the Puigvert Foundation. Blood samples were sent to the Universitat Autònoma of Barcelona and immediately processed to analyze the levels of genomic damage and the antioxidant capacity.

2.2. Unfermented grape juice

The UGJ concentrate administered to the patients, was purchased from Concentrados Pallejà S.L. (Riudoms-Tarragona, Spain). The UGJ was unpasteurized, fresh concentrate to avoid losing the antioxidant properties. The polyphenol composition was assessed at the Instituto de Investigación en Ciencias de la Alimentación (CSIC, Madrid). To determine the composition of the UGJ, two different methods were applied (Monagas et al., 2006): (1) the method of total polyphenols, based on oxidation in basic medium of phenol hydroxyl groups fear the Folin-Ciocalteu reagent; and (2) the method of total anthocyanins, based on a colorimetric pH change. The results are expressed in mg of gallic acid/L. From these studies it was concluded that the UGJ contained: total polyphenols 5888 ± 262 mg/L and total anthocyanins 1515 ± 98 mg/L. The levels of potassium were 7.5 mEq/100 mL and correspond to the 6.6% of the estimated daily intake of potassium in hemodialysis patients. The UGJ concentrate was bottled in 1 L-cans, stored in a refrigerator at 4 °C, and administered by the nurses before the end of each hemodialysis session.

2.3. Comet assay

DNA breaks present in peripheral blood lymphocytes were measured using the comet assay performed following the standard protocol, as previously described (Singh et al., 1988; Stoyanova et al., 2010) with minor modifications. Briefly, isolated lymphocytes from 2 mL of blood from each patient were cryopreserved until use, in 500 μL of medium containing 90% serum and 10% DMSO. Comet assay was carried using Gelbond® films (GF) instead of microscopic slides as a support for the agarose gel. The use of hydrophilic films facilitates the rapid processing of numerous samples, increasing the efficiency of the alkaline comet technique, without sacrificing the reliability or sensitivity of the assay (McNamee et al., 2000; Azqueta et al., 2013). Lymphocytes were isolated using Ficoll—Paque density gradient from 500 μL of whole blood; cells were adjusted to a concentration of 17,800 cells in 25 μL

Table 1
General description of the studied groups.

	Supplemented patients (N = 25)	Reference patients (N = 14)
Gender (men/women) (%)	15 (60)/10 (40)	9 (64.3)/5 (35.7)
Age (years) (mean ± SE)	66.16 ± 2.55	59.71 ± 4.61
BMI (mean ± SE)	25.47 ± 1.22	23.93 ± 1.06
Time in HD (months) (mean ± SE)[a]	36.24 ± 5.50	16.57 ± 7.36*
RT previous (% yes/no)	8/92	21.4/78.6
Hypertension (%yes/no)	92/8	92.9/7.1
CV pathology (%yes/no)	56/44	71.4/28.6
Previous cancer (%yes/no)	44/56	35.7/64.3
Diabetes mellitus (%yes/no)	32/68	35.7/64.3
Dyslipidemia (% yes/no)	76/24	64.3/35.7

SE, standard error;; *t*-test, $^*P < 0.05$.
[a] HD time before treatment.

Table 2
Medication and supplementation received during the study in the studied groups.

	Supplemented CKD patients (N = 25)[a]		Reference CKD patients (N = 14)[a]	
	Before UGJ supplement	6 months after UGJ supplement[b]	First sample	Second sample
Folic acid	8/92	8/92	28.6/71.4	41.7/58.3*
Vitamin B and C	16/84	16/84	21.4/78.6	8.3/91.7
L-carnitine	52/48	56/44	14.3/85.7	41.7/58.3
ACE inhibitor	36/64	36/64	42.9/57.1	50/50
Statins	68/32	68/32	78.6/21.4	83.3/16.7
Sevelamer	80/20	76/24	42.9/57.1	58.3/41.7
Calcium	12/88	12/88	57.1/42.9	42.9/57.1
Vitamin D	88/12	92/8	50/50	66.7/33.3
Venofer dose (mg/month)	264 ± 28.21	304 ± 45.63	142.85 ± 25.05	191.66 ± 39.32
ESA (μg darbepoetin/mes)	201.20 ± 28.78	187.60 ± 29.86	297.14 ± 63.31	261.66 ± 55.23

*$P < 0.05$.
[a] (% yes/no); ACE inhibitor: angiotensin-converting-enzyme inhibitor; Venofer: iron sucrose injection; ESA: Erythropoiesis stimulating agents; ERI: Erythropoietin resistance index.
[b] No differences were observed between sampling periods (two-tailed Fisher test) or paired-samples t-test; Two-tailed Fisher test.

Table 3
Comparison of blood biochemical data between both periods of sampling.

	Supplement patients (N = 25)		Non-supplement patients (N = 14)	
	Before UGJ supplement	6 months after UGJ supplement	First sample	Second sample
Hemoglobin (120–160 g/L)	119.76 ± 2.56	121.68 ± 2.97	117.58 ± 4.34	118.66 ± 3.38
Glucose (4–5.8 μmol/L)	4,81 ± 0.15	4.91 ± 0.21	5.18 ± 0.35	5.11 ± 0.36
Albumin (37–47 g/L)	41.02 ± 0.55	41.25 ± 0.66	36.69 ± 1.08	39.08 ± 1.33
Calcium (2.1–2.55 mmol/L)	2.24 ± 0.03	2.17 ± 0.02*	2.21 ± 0.04	2.25 ± 0.05
Phosphorus (0.8–1.3 mmol/L)	1.35 ± 0.07	1.52 ± 0.08*	1.81 ± 0.16	1.63 ± 0.13
Ca × P ($mmol^2/L^2$)	3.05 ± 0.19	3.29 ± 0.18	3.98 ± 0.34	3.66 ± 0.30
Parathyroid hormone (7–53 ng/L)	214.95 ± 30.04	307.59 ± 32.36***	149.50 ± 29.43	201.46 ± 46.47
Ferritin (25–250 μg/L)	265.53 ± 94.14	306.26 ± 89.34	175.14 ± 57.71	276.42 ± 58.00
Transferrin saturation (12–44%)	24.25 ± 0.02	21.54 ± 0.02	21.90 ± 0.02	28.10 ± 0.03
Iron (9–27 μmol/L)	10.88 ± 0.97	10.10 ± 0.72	9.80 ± 1.21	11.51 ± 1.31
ERI (IU/week/kg/g/dL > 10)	13.80 ± 2.16	12.60 ± 2.21	20.31 ± 4.13	9.64 ± 4.48[a]
C-reactive protein (<10 mg/L)	9.14 ± 2.21	15.57 ± 5.78	7.98 ± 3.71	10.28 ± 4.65
HDL (>1.40 mmol/L)	1.35 ± 0.09	1.24 ± 0.07	1.14 ± 0.08	1.08 ± 0.08
LDL (<4.13 mmol/L)	2.08 ± 0.15	1.75 ± 0.13*	2.47 ± 0.32	1.85 ± 0.22
Cholesterol (3.20–5.20 mmol/L)	4.01 ± 0.19	3.59 ± 0.17**	4.51 ± 0.36	3.58 ± 0.26*
Triglycerides (0.30–1.40 mmol/L)	1.26 ± 0.13	1.42 ± 0.20	1.68 ± 0.24	1.59 ± 0.20
Kt/V (>1.3)	1.58 ± 0.05	1.61 ± 0.05	1.50 ± 0.08	1.63 ± 0.07
Systolic blood pressure (<140 mmHg)	138.80 ± 3.91	138.00 ± 4.01	124.62 ± 8.52	137.37 ± 5.87
Diastolic blood pressure (<90 mmHg)	72.40 ± 2.00	73.0 ± 2.08	68.37 ± 5.99	81.62 ± 4.97*

Paired-samples t-test: *$P < 0.05$, **$P < 0.05$, ***$P < 0.001$; Ca x P, calcium phosphorus product; ERI, ESA resistance index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PRU, Urea reduction percentage.
[a] Values determined only for 3 patients.

PBS and carefully re-suspended in 225 μL of 0.75% low melting agarose (LMA) at 37 °C and dropped onto a GF (10.5 × 7.5 cm). Forty-eight drops (7 μL each) were placed on each GF and samples of eight donors were run simultaneously, each donor being represented by six drops. Lymphocytes were lysed for a minimum of 1 h at 4 °C in a dark chamber containing a cold fresh lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris–HCl and 1% Triton X-100, adjusted to pH 10). To allow DNA denaturation, unwinding and exposure of alkali-labile sites, GF were placed in a horizontal gel electrophoresis tank filled with freshly cold (4 °C) electrophoresis solution (1 mM Na_2EDTA and 300 mM NaOH, adjusted to pH 13) for 35 min. Electrophoresis was performed in the same buffer for 20 min at 20 V and 300 mA. After electrophoresis, GF were neutralized with two 5-min washes with PBS 1X, followed by 1 min wash with water and then incubated overnight in 100% ethanol for fixation. Sheets were then dried and stored in the dark at room temperature until scoring. Just before the microscopic analysis, GF were stained with 20 μL of SybrGold. The images were examined at 20× magnification with a Komet 5.5 Image Analysis System (Kinetic Imaging Ltd, Liverpool, UK) fitted with an Olympus BX50 fluorescence microscope equipped with a 480–550 nm wide band excitation filter and a 590 nm barrier filter. A total of one hundred randomly selected cells were analyzed per patient and the % tail DNA was used as a measure of DNA damage.

2.4. Detection of oxidative damage

To determine the levels of oxidized bases present in the lymphocytes, GF were washed two times (10 and 50 min, 4 °C) after cell lysis in an enzyme buffer solution (40 mM HEPES, 0.1 M KCl, 0.5 mM EDTA, 0.2 mg/mL BSA, pH 8.0) containing the enzyme FPG (formamidopyrimidine DNA glycosylase) (Azqueta et al., 2013). FPG enzyme was produced in our laboratory and a concentration of 5.76 μg/μL of enzyme extract was used in each treatment.

Each sample was analyzed using two GF. One GF remained in the cell lysis solution in order to assess basal DNA damage. The second was treated with the enzyme buffer without FPG, to control for any effects of the buffer alone. GF were incubated with enzyme buffer (with and without FPG) for 30 min at 37 °C, after that, samples were processed as in the standard alkaline comet assay procedure. Net oxidative DNA damage was calculated by subtracting the damage scored in the samples incubated with buffer from those incubated

with FPG.

2.5. Lymphocyte culture and micronucleus assay

Blood samples from heparinized vacutainers were processed as described previously using cytochalasin-B to arrest cytokinesis (Rodríguez-Ribera et al., 2014). Two of the four cultures set up were irradiated with 0.5 Gy ^{137}Cs gamma rays in an irradiator IBL 437C, type H, No. 701 (SCHERING CIS Bio International) at the Unitat Tècnica de Protecció Radiològica (UTPR-UAB). To determine the frequency of binucleated cells with micronuclei (BNMN), 1000 binucleated lymphocytes per sample (irradiated and non-irradiated) were blind scored on coded slides, according to standard criteria (Fenech, 2007). In addition, 500 cells with one, two or more nuclei were scored to determine the cytokinesis-block proliferation index (CBPI) (Surrallés et al., 1995). The net effect of irradiation was calculated by subtracting the background BNMN values in the non-irradiated samples from the values obtained in the irradiated samples (Rodríguez-Ribera et al., 2015). The resulting net BNMN value represents the frequency of BNMN induced by IR. In the same way, the net CBPI was also calculated.

2.6. Trolox equivalent antioxidant capacity (TEAC) assay

The plasma antioxidant capacity was measured using the TEAC assay as already described (Kambayashi et al., 2009) with minor modifications. Venous blood samples from EDTA tubes were centrifuged at 170 g during 5 min to obtain plasma and, after that, it was stored at −80 °C. Ten μL of plasma or Trolox standard reacted with 6.20 μM myoglobin solution (20 μL), 183 μM ABTS solution (150 μL) and 10 mM H_2O_2 (25 μL) on a microplate. Reaction was followed at 405 nm with the plate reader Sunrise (Tecan Trading AG, Switzerland). Lag time from kinetic curves and Trolox calibration curve was calculated and plasma TEAC was expressed as Trolox equivalent.

2.7. Statistical analyses

Continuous variables (MN, BNMN, CBPI, TEAC, and comet assay) were assessed for normality using the Kolmogorov–Smirnov test. For the statistical analysis of the continuous data t-test for paired samples was used. For qualitative data, Fisher's test was used (Table 2). *Data is presented as mean* ± standard error of the mean (SEM) *if not* otherwise *indicated*. Pearson and Spearman correlations were used to determine relationship between variables. For the statistical analysis of the genomic and oxidative damage obtained by the comet assay, non-parametric tests were used. Comparisons between the two groups (before and after 6 months supplementation with unfermented grape juice) were analyzed using the Wicolxon test for continuous variables, and Mann–Whitney test for discrete variables (Table 4). All the analyses were done using the Statistic Package of Social Sciences (SPSS) software for Windows version 19.0.

3. Results

All selected patients were included in the HD program of the Fundació Puigvert and underwent conventional hemodialysis for 3.5–4 h, on a thrice weekly dialysis schedule. HD was carried out using synthetic low permeability membranes (ultrafiltration coefficient of 13 mL/h/mm Hg) of polyethersulfone with a surface between 1.8 and 2 m^2, with a bicarbonate dialyzate. The average time they have been subjected to conventional HD previous to the study was 36.24 ± 5.50 months for the treated group, and 16.57 ± 7.36 months for the reference group. Patients included in the study drank 100 mL of UGJ, in the last 30 min of their hemodialysis session for 6 months.

General characteristics of CKD patients are shown in Table 1. The main causes of disease in supplemented and reference patients, respectively were: vascular nephropathy (4%, 14.3%), diabetes mellitus (24%, 14.3%), glomerulonephritis (36%, 43%), polycystic (12%, 7.1%), and others (20%, 14.3%). CKD is more common in male than in female and, consequently, about 60% of the CKD patients were men. Both groups of study are homogenous for most of the parameters tested. The most remarkable difference was in the time that patients have been in hemodialysis. The reference group showed approximately half of the time in HD than the supplemented group. It is interested to remark the high incidence of hypertension, cardiovascular pathologies and dyslipidemia among the participants, something well reported for these types of patients.

Table 2 list the medication and supplements received by the CKD patients previous and during the study. No significant changes were found regarding the treatment received before and at the end of the study for the supplemented group. Slight but significant differences were showed between for periods of time within the reference group regarding the levels of folic acid, vitamin B and C, and L-carnitine. We assume that these differences are mainly due to the sampling size more than changes in the supplements provided to HD patients.

Blood analysis is summarized in Table 3. The only changes attaining statistical significant differences correspond to calcium ($P = 0.021$), phosphorus ($P = 0.029$), parathyroid hormone ($P < 0.001$), LDL ($P = 0.012$), and cholesterol ($P = 0.004$), after 6 months under UGJ supplement. In the reference group, only differences are observed in diastolic blood pressure ($P = 0.014$) and cholesterol ($P = 0.016$), while some parameters showed borderline effect, as albumin ($P = 0.063$), ferritin ($P = 0.077$), LDL ($P = 0.057$), and Kt/V ($P = 0.061$).

The levels of genetic damage before and after 6 months supplemented with UGJ, and for the HD patients without supplementation are described in Table 4. As observed, no statistically

Table 4
Levels of DNA damage and antioxidant capacity before and after 6 months of supplementation with unfermented grape juice.

	Supplement CKD patients (N = 25)			Reference CKD patients (N = 14)		
	Before UGJ supplement	6 months after UGJ supplement	Paired *t*-test *P*	First sample	Second sample	Paired *t*-test *P*
BNMN	11.24 ± 1.28	9.92 ± 1.42	0.374	6.50 ± 1.01	5.91 ± 1.19	0.704
BNMN net After irradiation	39.44 ± 20.54	35.56 ± 14.84	0.334	24.90 ± 5.69	20.72 ± 3.92	0.591
CBPI	1.54 ± 0.15	1.65 ± 0.03	**0.005***	1.45 ± 0.05	1.44 ± 0.05	0.910
% DNA tail Basal damage	16.15 ± 0.96	15,61 ± 1.03	0.594	13.52 ± 1.83	13.13 ± 2.13	0.902
% DNA tail Oxidative damage	26.36 ± 1.30	22.41 ± 1.18	**0.025***	8.12 ± 1.68	14.61 ± 2.80	0.125[a]
TEAC (mMol/L)	0.21 ± 0.02	0.17 ± 0.01	0.072	NDA		

Mean ± SE; NDA: no data available. *$P < 0.05$
[a] Paired Wilcoxon test.

significant differences were observed neither in the basal BNMN frequency nor in the individual radiosensitivity (BNMN net) between sampling times, for both studied groups. In the same way, no differences were observed between sampling periods in the comet assay, used to determine the basal levels of DNA breaks under alkaline conditions, nor in the Trolox equivalent antioxidant capacity (TEAC) assay. Nevertheless, when the comet assay was complemented with FPG enzyme to determine the levels of oxidized DNA bases, a significant decreased was observed when patients were supplemented with UGJ. Regarding the cytokinesis block proliferation index (CBPI), an increase in cell proliferation was also observed when patients were supplemented with UGJ.

When different associations between parameters were determined, a good correlation was observed between the levels of BNMN and net-BNMN before and after the supplementation ($r = 0.426$, $P = 0.034$; $r = 0.417$ $P = 0.038$, respectively). This would confirm the lack of effect of UGJ supplementation on chromosome damage. On the other hand, the levels of CBPI ($r = 0.135$, $P = 0.530$), oxidative DNA damage measured by the comet assay ($r = 0.123$, $P = 0.556$), nor TEAC ($r = 0.303$, $P = 0.141$) did not show any correlation, what would indicate that UGJ supplementation interfere on these parameters. In addition, if we focus in factors modulating genetic damage in patients supplemented with UGJ, a negative correlation was observed between the antioxidant capacity and the oxidative net damage ($r = -0.422$, $P = 0.035$). This would indicate that those patients with initial high damage have less antioxidant capacity.

4. Discussion

In our study we found slight decreases, but not significant changes, in the basal frequencies of primary DNA damage (comet assay) or in chromosomal damage (micronucleus assay) when CKD patients submitted to HD were supplemented with UGJ for six months. Nevertheless, significant decreases in the underlying levels of oxidative DNA damage were obtained (comet assay plus FPG) in such patients after UGJ supplementation. This would suggest an antioxidant role of UGJ at least in this type of patients.

Under normal conditions ROS (which include various compounds such as superoxide anions, hydrogen peroxide, and hydroxyl radical) are generated in the mitochondria of mammalian cells in the course of energy production, by reducing oxygen during aerobic respiration. However, excessive ROS levels can produce cellular damage by interacting with biomolecules (such as proteins, lipids, and nucleic acids), having negative effects on tissue function and structure. This oxidative stress plays an important role in different pathological situations such as cardiovascular diseases (Jeremy et al., 2004), aging (Dugan and Quick, 2005) and Alzheimer (Muhammad et al., 2009), as well as in renal diseases (Dobashi et al., 2000). Another effect associated to oxidative stress is inflammation, also typical of CKD patients.

In CKD patients it has been indicated that the negative effects of oxidative stress are associated with the progression of the disease; finding a correlation with the level of renal function (Dounousi et al., 2006). Our group has found that oxidative DNA damage measured by Comet with FPG is higher in HD patients with respect to pre-dialysis patients (Corredor et al., 2015). Recent studies comparing oxidative stress under various types of dialysis modalities showed increased levels of advanced oxidation protein products in conventional HD patients compared with those submitted to peritoneal dialysis (Zhou et al., 2012; Marques de Mattos et al., 2012). Nevertheless, other studies found similar or lower levels of oxidative stress markers (advanced oxidation protein products, myeloperoxidase, and 8-OHdG levels) in HD patients when compared to patients submitted to peritoneal dialysis (Castoldi et al., 2010; Samouilidou et al., 2012). Thus, the contribution of the oxidative damage to CKD disease and/or vice versa is still controversial.

Dialysis treatment, by itself, appears to contribute to oxidative stress by creating alterations in the balance between free radicals generation and antioxidant protection systems (Sung et al., 2013). In fact, previous studies have shown increased levels of the lipid peroxidation product malondialdehyde and decreased levels of the primary lipid-soluble antioxidant α-tocopherol, suggesting that HD procedure is associated with oxidative stress (Westhuyzen et al., 1997). It should be taken into account, that dialysis procedure does not eliminates efficiently all advanced glycation end-products (AGEs) and non-important changes in total AGEs and lipoperoxide levels are observed after dialysis treatment (Gugliucci et al., 2007). Therefore, it is assumed that alterations of plasma lipoprotein levels and lipid peroxidation of low density lipoproteins (LDL), occurs in HD patients (Kaysen and Eiserich, 2004).

In front of this situation different antioxidant supplementation therapies have been proposed to be used in CKD patients. Among these supplements grape juice has been indicated as a suitable proposal to reduce the levels of oxidative stress in these type of patients (Castilla et al., 2006, 2008), and also to decrease the levels of genetic damage (Weisel et al., 2006). Grapes contain a large number of secondary metabolites such as flavonoids, which constitute a large group of polyphenolic compounds also found in fruits and other foods (Rice-Evans et al., 1996; Georgiev et al., 2014), and the consumption of foods rich in flavonoids is associated with a reduced risk of various chronic diseases (Hertog et al., 1995). The protective benefits of dietary flavonoids may be due in part to their antioxidant properties and their ability to reduce oxidative stress (Rice-Evans et al., 2000). In fact, as reviewed by Kumar and Pandey (2013) significant antioxidant activity of specific dietary flavonoids, and of some of the major metabolites and conjugated derivatives that occur in the circulation after consumption of dietary flavonoids has been reported.

In this context, the aim of this study was to evaluate the effect of supplementation, with unfermented grape juice, in a group of hemodialysis patients over a period of six months. We found that some of the clinical parameters analyzed, such as LDL and cholesterol, were significantly reduced in the patients studied after the supplementation. Nevertheless, since cholesterol levels were also reduced in controls we cannot attribute this decrease uniquely to the ingestion of UGJ.

Several studies have demonstrated that ingestion of polyphenols rich extracts reduced plasma LDL-cholesterol concentrations in humans (O'Byrne et al., 2002; Castilla et al., 2006), and a similar effect was found for red wine polyphenols in hamsters (Vinson et al., 2001). This has been explained because polyphenols may act like a HMG-CoA reductase inhibitor agent, and treatment with these inhibitors usually results in large increases in the mRNA and protein for HMG-CoA reductase, the LDL receptor, as well as in other genes (Endo, 1992). A previous study demonstrated that hyperlipidemic hamsters fed with dealcoholized red wine had a 45% reduction in plasma LDL concentrations relative to control hamsters drinking water (Vinson et al., 2001). Another *in vivo* study suggested that the decrease observed in circulating lipoproteins in hamsters fed dealcoholized red wine may be a result of increased LDL receptor binding activity and a suppression of hepatic lipoprotein production (Pal et al., 2003). These results would agree with the prospective study reporting that anthocyanidins, flavanones, and foods rich in flavonoids were associated with lower cardiovascular disease mortality (Mink et al., 2007). Although we observed a higher decrease in LDL values in UGJ supplemented patients than in controls we failed to find an increase in plasma HDL-cholesterol as described by some other authors who found

that red grape juice improves the lipoprotein profile, reduces plasma concentrations of inflammatory biomarkers and oxidized LDL in both HD patients and controls (Castilla et al., 2006). Differences in the dose of UGJ concentrate could be an explanation of this discrepancy: 1766 mg/week with respect to the 4508 mg/week administered by Castilla et al. (2006). Supplements with fruit juices in HD patients have already proved its protective effects. HD patients supplemented for one year with pomegranate juice showed significant reduction of protein oxidation, lipid oxidation, and inflammation biomarkers (Shema-Didi et al., 2012). In spite of those reported studies our results did not support the view that lipidic profile changes are uniquely attributed to the ingestion of UGJ. This view would agree with the meta-analysis results reported by Liu et al. (2013) who did not found a direct effect of fruit juices with antioxidant properties on cholesterol levels and LDL values.

Interestingly, in our studied group of HD patients the intake of UGJ significantly reduced the levels of oxidative DNA damage, while these levels remained stable in the reference group. Although the antioxidant effect of grape juice on HD patients has been previously demonstrated (Castilla et al., 2006, 2008) no previous studies have evaluated the effect of UGJ on the levels of genomic damage in HD patients. Others studies have already observed changes in the levels of genomic damage on HD patients after supplementation with vitamin E (Kan et al., 2002), vitamin C (Tarng et al., 2004) or selenium (Zachara et al., 2011). Even, it has been observed that in healthy people, grape juice continuously exerted persistent antioxidant activity up to 2 h after supplementation (Ko et al., 2005). These suggest that the consumption of fruit juice rich in polyphenols can reduce the oxidative stress, causing DNA damage, and that this effect may be the result of theirs antioxidant activity of the over the reactive oxygen species generated in human plasma. Overall, these studies show that supplementation with antioxidants reduced genomic damage levels.

5. Conclusions

In our study we have detected a slight but non-statistically significant reduction in the levels of the biomarkers used (MN, comet, and TEAC assays) after 6 month of UGJ supplementation. Nevertheless, a significant decrease in the levels of oxidative DNA damage was observed, what would support the antioxidant role of UGJ and suggest its potential benefits in reducing in some extension the levels of genomic damage and, consequently, their potential hazardous effects.

Conflict of interest

There are no conflicts of interest, and the results presented in this paper have not been published previously in whole or part.

Acknowledgments

First of all we thank all the volunteers that have participated in this study. Z. Corredor and L. Rodriguez-Ribera were supported by postgraduate fellowships from Colciencias Departamento Administrativo de Ciencia Tecnología e Innovación (Colombia), and the Universidad Autónoma de Barcelona, respectively. We appreciate the collaboration of Begoña Bartolomé from the Instituto de Investigación en Ciencias de la Alimentación (CSIC, Madrid), in the analysis of the polyphenol composition. The research group belongs to a Consolidated Research Group (AGAUR 2009/SGR-1116) and to REDINREN (Spanish Renal Network for Research 16/06 RETICS, ISCIII). This investigation has been supported in part by the Generalitat de Catalunya (CIRIT, 2014SGR-202) and the Instituto de Salud Carlos III (FIS, PI12/02559).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fct.2016.03.016

References

Alipour, M., Davoudi, P., Davoudi, P., 2012. Effects of unripe grape juice (verjuice) on plasma lipid profile, blood pressure, malondialdehyde and total antioxidant capacity in normal, hyperlipidemic and hyperlipidemic with hypertensive human volunteers. J. Med. Plants Res. 6, 5677–5683.

Azqueta, A., Langiec, S., Slyskovad, J., Collins, A., 2013. Measurement of DNA base and nucleotide excision repair activities in mammalian cells and tissues using the comet assay- A methodological overview. DNA Repair 12, 1007–1010.

Castilla, P., Dávalos, A., Teruel, J.L., Cerrato, F., Fernández-Lucas, M., Merino, J.L., Sanchez-Martín, C.C., Ortuño, J., Lasunción, M., 2008. Comparative effects of dietary supplementation with red grape juice and vitamin E on production of superoxide by circulating neutrophil NADPH oxidase in hemodialysis patients. Am. J. Clin. Nutr. 87, 1053–1061.

Castilla, P., Echarri, R., Dávalos, A., Cerrato, F., Ortega, H., Teruel, J.L., Milagros, L., Gómez-Coronado, D., Ortuño, J., Lasunción, M., 2006. Concentrated red grape juice exerts antioxidant, hypolipidemic, and antiinflammatory effects in both hemodialysis patients and healthy subjects. Am. J. Clin. Nutr. 84, 252–262.

Castoldi, G., Antolini, L., Bombardi, C., Perego, L., Mariani, P., Viganò, M.R., Torti, G., Casati, M., Corti, A., Zerbini, G., Valsecchi, M.G., Stella, A., 2010. Oxidative stress biomarkers and chromogranin a in uremic patients: effects of dialytic treatment. Clin. Biochem. 43, 1387–1392.

Conde, C., Silva, P., Fontes, N., Dias, A.C.P., Tavares, M.R., Sousa, M.J., Agasse, A., Delrot, S., Gerós, H., 2007. Biochemical changes throughout grape berry development and fruit and wine quality. Food 1, 1–22.

Corredor, Z., Stoyanova, E., Rodríguez-Ribera, L., Coll, E., Silva, I., Diaz, J.M., Ballarin, J., Marcos, R., Pastor, S., 2015. Genomic damage as a biomarker of chronic kidney disease status. Environ. Mol. Mutagen 56, 301–312.

Dobashi, K., Ghosh, B., Orak, J.K., Singh, I., Singh, A.K., 2000. Kidney ischemia-reperfusion: modulation of antioxidant defenses. Mol. Cell Biochem. 205, 1–11.

Dounousi, E., Papavasiliou, E., Makedou, A., Ioannou, K., Katopodis, K.P., Tselepis, A., Siamopoulos, K.C., Tsakiris, D., 2006. Oxidative stress is progressively enhanced with advancing stages of CKD. Am. J. Kidney Dis. 48, 752–760.

Dugan, L.L., Quick, K.L., 2005. Reactive oxygen species and aging: evolving questions. Sci. Aging Knowledge Environ. 2005 pe20.

Endo, A., 1992. The discovery and development of HMG-CoA reductase inhibitors. J. Lipid Res. 33, 1569–1582.

Fenech, M., 2007. Cytokinesis-block micronucleus cytome assay. Nat. Protoc. 2, 1084–1104.

Georgiev, V., Ananga, A., Tsolova, V., 2014. Recent advances and uses of grape flavonoids as nutraceuticals. Nutrients 6, 391–415.

Gugliucci, A., Mehlhaff, K., Kinugasa, E., Ogata, H., Hermo, R., Schulze, J., Kimura, S., 2007. Paraoxonase-1 concentrations in end-stage renal disease patients increase after hemodialysis: correlation with low molecular age adduct clearance. Clin. Chim. Acta 377, 213–220.

Gülçin, I., 2012. Antioxidant activity of food constituents: an overview. Arch. Toxicol. 86, 345–391.

Hertog, M.G., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., Giampaoli, S., Jansen, A., Menotti, A., Nedeljkovic, S., Pekkarinen, M., Bozidar, S., Simic, B.S., Toshima, H., Edith, J.M., Feskens, E.J.M., Hollman, P.C.H., Katan, M.B., 1995. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch. Intern. Med. 155, 381–386.

Janiques, A.G., Leal de, V.O., Stockler-Pinto, M.,B., Moreira, N.X., Mafra, D., 2014. Effects of grape powder supplementation on inflammatory and antioxidant markers in hemodialysis patients: a randomized double-blind study. J. Bras. Nefrol. 36, 496–501.

Jeremy, J.Y., Shukla, N., Muzaffar, S., Handley, A., Angelini, G.D., 2004. Reactive oxygen species, vascular disease and cardiovascular surgery. Curr. Vasc. Pharmacol. 2, 229–236.

Kambayashi, Y., Binh, N.T., Asakura, H., Hibino, Y., Hitomi, Y., Nakamura, H., Ogino, K., 2009. Efficient assay for total antioxidant capacity in human plasma using a 96-well microplate. J. Clin. Biochem. Nutr. 44, 46–51.

Kan, E., Undeğer, U., Bali, M., Başaran, N., 2002. Assessment of DNA strand breakage by the alkaline Comet assay in dialysis patients and the role of Vitamin E supplementation. Mutat. Res. 530, 151–159.

Kaysen, G.A., Eiserich, J.P., 2004. The role of oxidative stress-altered lipoprotein structure and function and microinflammation on cardiovascular risk in patients with minor renal dysfunction. J. Am. Soc. Nephrol. 15, 538–548.

Ko, S.H., Choi, S.W., Ye, S.K., Cho, B.L., Kim, H.S., Chung, M.H., 2005. Comparison of the antioxidant activities of nine different fruits in human plasma. J. Med. Food 8, 41–46.

Kumar, S., Pandey, A.K., 2013. Chemistry and biological activities of flavonoids: an overview. Sci. J. 2013, 1–16.

Liu, K., Xing, A., Chen, K., Wang, B., Zhou, R., Chen, S., Xu, H., Mi, M., 2013. Effect of fruit juice on cholesterol and blood pressure in adults: a meta-analysis of 19 randomized controlled trials. PLoS One 8, e61420.

Marques de Mattos, A., Marino, L.V., Ovidio, P.P., Jordão, A.A., Almeida, C.C., Chiarello, P.G., 2012. Protein oxidative stress and dyslipidemia in dialysis

patients. Ther. Apher. Dial. 16, 68–74.
Massy, Z.A., Stenvinkel, P., Drueke, T.B., 2009. The role of oxidative stress in chronic kidney disease. Semin. Dial. 22, 405–408.
McDonald, C.I., Fraser, J.F., Coombes, J.S., Fung, Y.L., 2014. Oxidative stress during extracorporeal circulation. Eur. J. Cardiothorac. Surg. 46, 937–943.
McNamee, J.P., McLean, J.R., Ferrarotto, C.L., Bellier, P.V., 2000. Comet assay: rapid processing of multiple samples. Mutat. Res. 466, 63–69.
Mink, P.J., Scrafford, C.G., Barraj, L.M., Harnack, L., Hong, C.P., Nettleton, J.A., Jacobs Jr., D.R., 2007. Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am. J. Clin. Nutr. 85, 895–909.
Monagas, M., Núñez, V., Bartolomé, B., Gómez-Cordovés, C., 2006. Phenolic content of blends of tempranillo with graciano or cabernet sauvignon wines produced in Spain. Food Technol. Biotechnol 44, 507–513.
Muhammad, S., Bierhaus, A., Schwaninger, M., 2009. Reactive oxygen species in diabetes-induced vascular damage, stroke, and Alzheimer's disease. J. Alzheimers Dis. 16, 775–785.
O'Byrne, D.J., Devaraj, S., Grundy, S.M., Jialal, I., 2002. Comparison of the antioxidant effects of concord grape juice flavonoids and alpha-tocopherol on markers of oxidative stress in healthy adults. Am. J. Clin. Nutr. 76, 1367–1374.
Pal, S., Ho, N., Santos, C., Dubois, P., Mamo, J., Croft, K., Allister, E., 2003. Red wine polyphenolics increase ldl receptor expression and activity and suppress the secretion of ApoB100 from human HepG2 cells. J. Nutr. 133, 700–706.
Rice-Evans, C.A., Miller, N.J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids catherine. Free Radic. Biol. Med. 20, 933–956.
Rice-Evans, C., Spencer, J.P., Schroeter, H., Rechner, A.R., 2000. Bioavailability of flavonoids and potential bioactive forms in vivo. Drug. Metabol. Drug Interact. 17, 291–310.
Rodríguez-Ribera, L., Stoyanova, E., Corredor, Z., Coll, E., Silva, I., Diaz, J.M., Ballarin, J., Marcos, R., Pastor, S., 2014. Time in hemodialysis modulates the levels of genetic damage in hemodialysis patients. Environ. Mol. Mutagen 55, 363–368.
Rodríguez-Ribera, L., Corredor, Z., Sandoval, S.B., Coll, E., Silva, I., Díaz, J.M.L., Ballarin, J.E., Pastor, S., Marcos, R., 2015. Radiosensibility in patients suffering from chronic kidney disease. Int. J. Radiat. Biol. 91, 172–178.
Samouilidou, E.C., Karpouza, A.P., Kostopoulos, V., Samouilidou, T., Karpouza, E.C., Kostopoulos, A.P., Bakirtzi, T., Pantelias, K., Petras, D., Tzanatou-Exarchou, H., Grapsa, E.J., 2012. Lipid abnormalities and oxidized LDL in chronic kidney disease patients on hemodialysis and peritoneal dialysis. Ren. Fail. 34, 160–164.
Shema-Didi, L., Sela, S., Ore, L., Shapiro, G., Geron, R., Moshe, G., Kristal, B., 2012. One year of pomegranate juice intake decreases oxidative stress, inflammation, and incidence of infections in hemodialysis patients: a randomized placebo-controlled trial. Free Radic. Biol. Med. 53, 297–304.
Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175, 184–191.
Spormann, T.M., Albert, F.W., Rath, T., Dietrich, H., Will, F., Stockis, J.P., Eisenbrand, G., Janzowski, C., 2008. Anthocyanin/polyphenolic-rich fruit juice reduces oxidative cell damage in an intervention study with patients on hemodialysis. Cancer Epidemiol. Biomark. Prev. 17, 3372–3380.
Stoyanova, E., Sandoval, S.B., Zúñiga, L.A., El-Yamani, N., Coll, E., Pastor, S., Reyes, J., Andrés, E., Ballarin, J., Xamena, N., Marcos, R., 2010. Oxidative DNA damage in chronic renal failure patients. Nephrol. Dial. Transpl. 25, 879–885.
Stratil, P., Klejdus, B., Kubáň, V., 2007. Determination of phenolic compounds and their antioxidant activity in fruits and cereals. Talanta 71, 1741–1751.
Sung, C.C., Hsu, Y.C., Chen, C.C., Lin, Y.F., Wu, C.C., 2013. Oxidative stress and nucleic acid oxidation in patients with chronic kidney disease. Oxid. Med. Cell Longev. 2013, 301982.
Surrallés, J., Xamena, N., Creus, A., Catalán, J., Norppa, H., Marcos, R., 1995. Induction of micronuclei by five pyrethroid insecticides in whole-blood and isolated human lymphocyte cultures. Mutat. Res. 341, 169–184.
Tarng, D.C., Liu, T.Y., Huang, T.P., 2004. Protective effect of vitamin C on 8-hydroxy-2'-deoxyguanosine level in peripheral blood lymphocytes of chronic hemodialysis patients. Kidney Int. 66, 820–331.
Vinson, J.A., Teufel, K., Wu, N., 2001. Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. Atherosclerosis 156, 67–72.
Waterhouse, A.L., 2002. Wine phenolics. Ann. N.Y. Acad. Sci. 957, 21–36.
Weisel, T., Baum, M., Eisenbrand, G., Dietrich, H., Will, F., Stockis, J.P., Kulling, S., Rüfer, C., Johannes, C., Janzowski, C., 2006. Anthocyanin/polyphenolic-rich fruit juice reduces oxidative DNA damage and increases glutathione level in healthy probands. Biotechnol. J. 1, 388–397.
Westhuyzen, J., Saltissi, D., Healy, H., 1997. Oxidation of low density lipoprotein in hemodialysis patients: effect of dialysis and comparison with matched controls. Atherosclerosis 129, 199–205.
Zachara, B.A., Gromadzinska, J., Palus, J., Zbrog, Z., Swiech, R., Twardowska, E., Wasowicz, W., 2011. The effect of selenium supplementation in the prevention of DNA damage in white blood cells of hemodialyzed patients: a pilot study. Biol. Trace Elem. Res. 142, 74–83.
Zhou, Q., Wu, S., Jiang, J., Tian, J., Chen, J., Yu, X., Chen, P., Mei, C., Xiong, F., Shi, W., Zhou, W., Liu, X., Sun, S., Xie, D.I., Liu, J., Xu, X., Liang, M., Hou, F., 2012. Accumulation of circulating advanced oxidation protein products is an independent risk factor for ischaemic heart disease in maintenance haemodialysis patients. Nephrology 17, 642–649.