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# Oral human administration of red grape polyphenol in nickel-mediated allergic contact dermatitis: an in vitro study

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## Abstract

Nickel (Ni)-mediated allergic contact dermatitis (ACD) is a very common disease worldwide. Our previous findings demonstrated that in vitro supplementation of polyphenols, extracted from seeds of red grape (Nero di Troia cultivar), to peripheral lymphomonocytes from Ni-mediated ACD patients could reduce release of T helper (h)1 [interferon (IFN)-γ] and Th2 [interleukin (IL)-4] cytokines, on the one hand. On the other hand, IL-10 (an anti-inflammatory cytokine) levels increased with a reduction of IL-17 (an inflammatory cytokine). Also levels of nitric oxide (NO) decreased in response to polyphenol pretreatment (1). On these grounds, 25 Ni-mediated ACD patients were orally administered with 300 mg polyphenols prodie extracted from seeds of red grape (Nero di Troia cultivar) (NATUR-OX®) for 3 months. Other 25 matched Ni-mediated ACD patients received placebo only for the same period of time. Serum biomarkers analyzed before (T0) and after (T1) treatment were represented by IFN-γ, IL-4, IL-10, IL-17, pentraxin (PTX)3 and NO. Patch test scores to evaluate sensitization to Ni were recorded at T0 and T1, respectively. In both groups seven drop outs were recorded. At T1 in comparison to T0, in treated patients, values of IFN-γ, IL-4 and IL-17 decreased, while IL-10 levels increased. Also PTX3 and NO concentrations decreased at T1 when compared with T0 values. Conversely, in placebo-treated patients elevated amounts of IFN-γ, IL-4, IL-17, PTX3 and NO at T0 did not change at T1. Also decreased levels of IL-10 at T0 remained unmodified when compared to those determined at T1. Clinically, in 16 out of 18 treated patients patch test positivity decreased at T1, while in placebo patients positivity score remained unchanged. Conclusively, present laboratory and clinical data are justified by the anti-oxidant, anti-inflammatory and anti-allergic properties of polyphenols, as also demonstrated in previous experimental and clinical settings.

## Patients and Methods

A total of 50 females patients with Ni-mediated ACD were enrolled in the Section of Dermatology

Department of Biomedical Sciences and Human Oncology, University of Bari, School of Medicine, Bari (Italy). At the time of enrollment (T0), data about, age, onset of the related eczematous events, date of diagnosis, magnitude of the positivity of the reaction to the nickel patch test were collected.

Patients were orally administered for three months (T1) with one capsule daily of NATUR-OX® [Farmalabor, s.r.l., Canosa (Italy)] which is a dietary supplement containing grape seed extracts from Nero di Troia (*Vitis vinifera*). Each capsule contains 280 mg of proanthocyanidins where Ni contamination of capsule is below 0.24 ppm. The other group of patients was administered with placebo one capsule/daily for three months. The placebo capsules had the same appearance and composition of the supplement except for the active ingredient (polyphenols). At T1, all patients were examined by dermatologists for patch test evaluation.
In both groups peripheral venous blood sampling (5 ml test tubes without anticoagulant) was carried out before T0 and after T1 treatment.
Serum samples were collected and stored at -30°C until use.

**Patch testing**

Patch tests were performed with the S.I.D.A.P.A. (Italian Society of Allergological, Occupational and Environmental Dermatology) baseline series (Euromedical, Calolziocorte, Italy) by Al Test® (Euromedical) on Scanpor® Tape (Norgesplaster, Vennesla, Norway). They were applied on the back and left in occlusion for 2 days. Readings were performed at days 2, 4 and 7 following European Society of Contact Dermatitis guidelines (2). All patients were positive and degree of sensitization to Ni was indicated with (+).

**Clinical evaluation**

In particular, positive patients were graded as following: i. asymptomatic patients at the moment of the enrollment but with a past history of Ni-mediated ACD; ii. patients with weak erythema; iii. patients with even erythema, edema and possibly papules or hints of vesicles; iv. patients with erythema, edema, papules and vesicles which can flow to form bubbles (3).
In both groups of patients, 7 dropouts were recorded not due to side effects but spontaneous discontinuation of the treatment.

**Determination of serum cytokines**

Serum samples collected by both groups and at different times, T0 and T1 were used to detect by means of an ELISA kit (TEMA Ricerca, Bologna, Italy) according to manufacturer's instructions the following cytokines: IFN-γ, IL-4, IL-10, IL-17 and PTX3. Cytokine concentrations were read at 550 nm by means of an ELISA reader (iMarkTM Microplate Absorbance Reader, BioRad, Hercules, California, U.S.A.).

**Determination of serum nitric oxide**

Determination of serum nitric oxide (NO) was performed by a colorimetric assay.

**Evaluation of serum cytokines**

Polyphenols significantly diminish levels of IFN-γ and IL-4, thus, suggesting that Ni-mediated ACD is a disease characterized by the involvement of both Th1 and Th2 cell subsets. Moreover, in the same patients, investigation on the IL-10/IL-17 ratio demonstrates in a clear cut way that the polyphenol-mediated enhancement of IL-10 production with the parallel dampening of IL-17 amounts may represent one of the key mechanisms through which these substances switch off the inflammatory/allergic reactions in Ni-mediated ACD.

**Evaluation of serum PTX3**

In our experiments, PTX3 is significantly reduced by polyphenol administration. Conversely, in the placebo group, no modifications of the above cited immune parameters are detectable at the end of the trial.

**Evaluation of serum NO**

Serum NO concentration decreased at T1 in the group of patients who assumed polyphenols. Conversely, NO serum levels in the placebo group were unchanged at both times.

**Conclusion**

The present study clearly demonstrates that polyphenol administration to Ni-induced ACD patients is able to reduce the inflammatory allergic pathway, while enhancing the tolerogenic response mediated by IL-10. Of note, NO is also able to inhibit cutaneous DCs, thus, down modulating the effect related to their interaction with Ni. Laboratory findings are corroborated by clinical improvement of symptomatic patients.

**References**

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