THE CRANBERRY (VACCINIUM MACROCARPON): ITS POTENTIAL USE IN THE TREATMENT OF RECURRING URINARY TRACT INFECTIONS.

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Introduction

The English term "Cranberry" is used to indicate the group of small evergreen shrubs that form the Oxycoccos sub-genus of the Vaccinium genus, of the Ericaceae family, and which grow on acid soils in the cold regions of the northern hemisphere of the planet. Four species are associated with this sub-genus: V. erythrocarpon, V. macrocarpon, V. microcarpon and V. oxycoccos. These are prostrate plants with slim branches which are not completely woody, bearing small coriaceous leaves, and can grow up to two metres in length. The flowers are white with shades of dark pink and have well separated petals which are somewhat inverted; the stigma and the stamens are exposed and protruding. The fruit is a berry which is larger than the flowers and initially white before turning bright red when ripe. The berries are edible and have a sourish flavour which can conceal its sweetness. Cranberries were part of the diet of the Native American Indians and were also used as dyes and, in medicine, to heal wounds. They also became popular with the European settlers and today the plants are grown in a variety of countries, but mainly in some states of the USA where the berries are used by the food industry to produce drinks, sauces and jams for wide consumption.

Cranberries contain several flavonoids, vitamins and mineral salts, but in particular they are a rich source of condensed flavonoids known as "proanthocyanidins" (or procyanidins, oligomeric proanthocyanidins (OPCs), pycnogenol, leucocyanidin).

Seven flavonoids have been identified as forming the monometric units of the proanthocyanidins, the commonest of which include catechin, epicatechin, gallocatechni and epigallocatechin. Independently of the specific structure - dimeric, trimeric or oligomeric – these substances which are also produced by many other plants, such as the common vine (Vitis vinifera L., Vitaceae), are well known for their antioxidant properties and they are also attributed with the capacity of blocking some risk factors of contracting diseases including cardiovascular and tumoral diseases. For these reasons, products containing concentrated sources of proanthocyanidins have been marketed since the 1980s as beneficial for the health. For a few decades now, interest has been converging on the proanthocyanidins of one of the species of cranberry, Vaccinium macrocarpon Aiton (called "Large cranberry", "American cranberry" or "Bearberry"; it differs from the other species belonging to the sub-genus Oxycoccos by its larger leaves), as it has been observed, first through empirical experiments and then through studies carried out according to the criteria of modern experimental science, that eating the berries of this plant and then taking its extracts limited the relapse of subjects with recurring urinary tract infections, especially if supported by Escherichia coli. As these infections are the cause of high rates of morbidity in the population and as the possibility that the proanthocyanidins of V. macrocarpon can be useful in controlling recurring urinary tract infections of different aetiologies has emerged, knowledge on their biological and therapeutic properties is increasing.

Chemical and biological characteristics of the proanthocyanidins produced by Vaccinium macrocarpon

Thee are two types of proanthocyanidins, A and B, which differ by the bond that joins the monomeric units. Type B is the more widespread in nature, but V. macrocarpon produces type A (Fig. 1).

Proanthocyanidins of Vaccinium The proanthocyanidins in the fruit of the plant do not have an antibacterial activity, but interfere in the mechanisms used by E. coli and by other bacteria to adhere to the cells of the epithelium and colonize the tissues of the urinary tract. This gram-negative bacteria adheres to the membrane of the epithelial cells using proteins called adhesines located at the distal extremity of fine filaments, called pili or fimbriae, which emerge from its wall; there are type 1 mannose-sensitive fimbriae, which bond with glycoproteins containing mannose, and type P mannose-resistant fimbriae which bond with a disaccharide of galactose, -D-Gal(1,4)-,-D-Gal, present on the surface of the epithelial cells.

In 1998, S. Ahuja and coll. (1) of Tulane University in the USA showed with an in vitro experiment that the juice of the fruit of V. macrocarpon prevents agglutination between the cells of P-fimbriated E. coli strains, irreversibly preventing the expression of the fimbriae. More recently, a similar experiment was carried out to ascertain directly on the urogenital system the anti-adherent effect shown by the proanthocyanidins of V. macrocarpon, using a new experimental model based on bladder cells and vaginal cells cultivated in vitro (2). For this experiment, a pulverized dry extract of the fruit available on the market in capsules and standardized to 9 mg of proanthocyanidins per gram, an extract enriched in proanthocyanidins obtained by column fractionating, isolates of E. coli of the uropathogenic strain IA2 which expresses P type fimbriae and primary cultures of bladder and vaginal epithelial cells (respectively BECs and VECs) taken from consenting patients, were used. The V. macrocarpon products were added to the culture medium in increasing concentrations and the adhesion of E.coli to the cells was tested before and after the addition.

The powder extract decreased the adhesion of E. coli to the vaginal cells from 18.6 to 1.8 bacteria per cell ($p < 0.001$), whilst at a concentration of proanthocyanidins of 50 Ìg/ml, the adhesion to the bladder cells decreased from 6.9 to 1.6 bacteria per cell ($p < 0.001$). The ratio between the concentrations of proanthocyanidins and the number of bacteria adhering to the cells tested was linear between 5 and 75 Ìg/ml. The results of the study provide mechanistic evidence and biological plausibility on the role of the products of V. macrocarpon in the prevention of urinary tract infections.

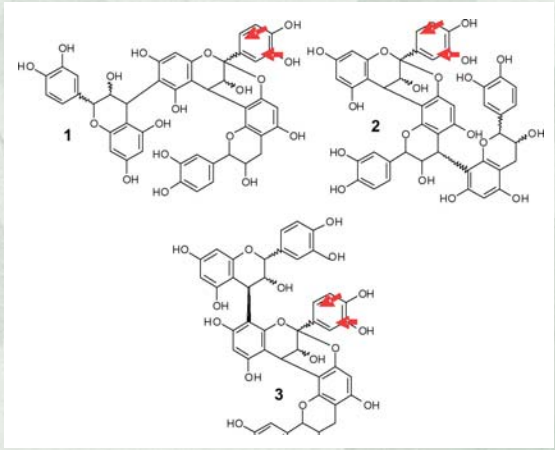

Fig. 1: Proanthocyanidins of Vaccinium macrocarpon: 1) epicatechin-(4,?6)-epicatechin-(4,?8,2,?0-7)-epicatechin; 2) epicatechin-(4,?8,2,?0-7)-epicatechin-(4,?8)-epicatechin; 3) epicatechin-(4,?8)-epicatechin-(4,?8,2,?0-7)-epicatechin (the arrows indicate the type A bods).

A different type of study (3) showed for the first time that the proanthocyanidins of V. macrocarpon in particular, but also those produced by other plant species (for example Camellia sinensis and Vitis vinifera), are capable of bonding, neutralizing them to the lipopolysaccharides (LPS) of many bacterial species in a way which is apparently similar to that shown for the antibacterial polymyxin B. The data obtained has shown that the recognition of the LPS by the proanthocyanidins is greatly mediated by interactions with the lipid fraction of the former and has demonstrated the capacity of the latter to inhibit the interactions between the proanthocyanidins with the cells of mammals that express the receptors of LPS (TLR4/MD2 and CD14). This interaction also mediates the inhibition of the activation of the NF-ÎB factor induced by the same LPS. Using a thermo-dynamic approach, it has also been possible to demonstrate that the inhibition of the capacity of E. coli to adhere to the cells of the urinary tract provoked by the proanthocyanidins of V. macrocapon is accompanied by the modification of the chemical and physical characteristics of the bacterial wall (4). This demonstration has been produced by calculating the changes of free energy of adhesion according to Gibbs (DeltaG(adh)) through measuring the corners of contact between one strain of the P-fimbriated bacteria and a non-P-fimbriated strain and the aforementioned cells exposed to juice of the plant's fruit. The data obtained has allowed ascertaining that in the case of the

non-P-fimbriated strain, Delta G(adh) is negative and that specific strong bonds fostering adhesion are formed between the Gal-Gal disaccharide fractions of the cell surface receptors of the urogenital tract and the fimbriae expressed by the bacteria; the P-fimbriated strain, on the other hand, was sensitive to V. macrocarpon, with positive DeltaG(adh) and non-specific weak bonds.

Clinical studies to evaluate the efficacy and safety of Vaccinium macrocarpon preparations for the prevention of urinary tract infections

Some preparations of V. macrocarpon have been the object of studies with the aim of ascertaining their efficacy and safety on patients sensitive to recurring urinary tract infections. Clinical literature published up to 2008 was systematically reviewed, through a bibliographical study in the main biomedical data banks and contacting the companies that market products based on the plant and carry out research (5). For the analysis 10 controlled or almost controlled studies (a total of 1049 patients) were selected, 5 of which crossover trials and 5 for parallel groups. In seven studies, the fruit juice of V. macrocarpon was tested in comparison with a placebo consisting of water or other general fruit juices, whilst in three, extracts in tablets were tested against inert materials as the placebo (in one study both the juice and tablets were evaluated).
The data collected from the studies analysed has shown overall that the V. macrocarpon preparations tested significantly reduced, compared to the controls, the rate of urinary tract infections over 12 months of treatment. The preparations in question are more effective in non-elderly women than in men and women of an advanced age or with catheters. All the patients suffered side effects and several had to drop out of the studies. This systematic review of the clinical studies carried out with preparations of the plant in question has nevertheless highlighted that the type of preparation and the doses correlated with the highest level of efficacy cannot yet be defined.

A subsequent study published in 2009 compared the efficacy and safety of an extract in capsules of the fruits of V. macrocarpon with those of the antibiotic trimethoprim in elderly women with recurring urinary tract infections (6). For this purpose, 137 women were enrolled, aged 45 or above with a history of at least 2 infectious episodes (cystitis) treated with antibiotics in the previous year. The patients were randomized to receive 500 mg/die of the extract (n = 69) or 100 mg/die of trimethoprim (n = 68) for 6 months. The primary end point was the number of patients of each group who suffered a relapse after the start of the treatment and the time that had passed (after the infection reappeared, the patients had to drop out of the study).

The data collected showed the absence of a statistically significant difference between the two treatments. Thirty-nine patients suffered a relapse out of a total of 137 (28%), 25 in the V. macrocarpon group and 14 in the trimethoprim group. The average time between the start of the treatment and the first infection was 84.5 days in the V. macrocarpon group and 91 days in the trimethoprim group. There were 17 drop-outs from the study due to adverse reactions (12%), 6 in the V. macrocarpon group (9%) and 11 in the trimethoprim group (16%). In both groups the adverse reactions were mainly of a gastrointestinal nature (4 cases each), but trimethoprim was also responsible for a certain rate of skin rashes and prurient irritation (3 cases).

This study was the first that compared the efficacy of an extract of V. macrocarpon with an antibiotic in the prophylaxis of recurring urinary tract infections in double blind and for parallel groups. The results lead us to consider that the prophylaxis with the antibiotic trimethoprim enjoys a slight advantage over that based on the natural product, counter-balanced however by a greater rate of adverse reactions; in addition, in favour of V. macrocarpon there is the absence of risks of resistance and of bacterial superinfections.
A population with a high risk of recurring polymicrobial urinary tract infections, with an average rate estimated at 1.8 episodes per year, is that made up of patients with a neurogenic bladder due to a lesion in the spinal marrow. Considerable progress has been made in controlling this condition, but the problem of recurring infections can still not be effectively solved with the antibiotic prophylaxis.

As a consequence, a randomized clinical study has been carried out, in a double blind crossover trial, to check the possible benefit deriving from the prophylaxis with V. macrocarpon (7). Fifty-seven patients with lesions to the spine and a neurogenic bladder (on average 1.2 infections in the previous year) were randomized to receive 500 mg/die of a fluid extract in tablets of the fruit of V. macrocarpon or a placebo for 6 months; at the end of this period, the treatments were alternated without washout and continued for a further 6 months. The study was completed by 47 patients, who reported a reduction in the rate of infections and symptomatology (especially spasms in the bladder) during the 6 months of treatment with the extract (6 patients had 7 episodes of infection) compared to the 6 months of treatment with the placebo (6 patients with 21 episodes). The frequency of infections was reduced from 1 a year in correspondence with the placebo treatment to 0.3 a year in correspondence with the treatment with the extract. The patients with a degree of glomerular filtration of more than 75 ml/min benefited most from the treatment with the extract.

The results of this study suggest that the extract of V. macrocarpon should be taken into consideration for the prophylaxis of urinary tract infections in patients with lesions to the spinal marrow affected by a neurogenic bladder.
The most frequent complications following the radiation therapy treatments to which patients with prostate cancer are subjected are bacterial infections of the urinary tract, which cause a complex symptomatology which seriously compromises the quality of life. Also in a case such as this, the efficacy of an extract of the fruit of V. macrocarpon has been assessed, in order to ascertain its possibility of use in the prevention of side effects of an infective type (8).
For this purpose, 203 patients with prostate adenocarcinoma were selected, prepared by catheterization and opacification of the bladder. A part of these patients (n = 88) were treated with one capsule/die of a commercial product containing 200 mg of an extract of V. macrocarpon standardized to 30% of proanthocyanidins, whilst the remaining patients (n = 115) did not receive any treatment and acted as the control group. The patients were then given 2.3 Gy of radiation treatment a day and for 6-7 days, directed on the prostatic cavity (and on the pelvis in the case of lymphonodal risk); the pharmacological treatment was given throughout the cycle of radio therapy.

Amongst the patients treated with the extract, there were 8 cases of urinary infection (9.1%), against 19 amongst the non-treated patients (16.5%); 4 of the non-treated patients had relapses of the infection (about 45% reduction of the rate of infections caused by the active treatment). The difference between the two groups was statistically significant. No side effects attributable to the extract were reported (gastralgia occurred in only one patient suffering from chronic gastritis). The patients treated with the extract benefited from a reduction of urinary symptomatology, especially dysuria, nycturia and urgent stimulus.

The results of this preliminary study show that the preparations of V. macrocarpon can also be useful in controlling urinary tract infections contracted in non-physiological situations, such as acute bladder mucositis caused by radiation therapy in prostate cancer patients, limiting the damage caused by radiation on the bladder.
Bacterial contamination of the invasive prostheses, such as urinary catheters and cardiac pacemakers, are the cause of a broad spectrum of nosocomial pathologies. According to estimates made in the USA by the National Nosocomial Infection Surveillance system, bacterial infections of the urinary tracts associated with the use of bladder catheters and for urostomy represent a third all nosocomial infections. Two-thirds of these infections progress from extraluminal contaminations, which often occur during insertion operations or by perineal bacteria that are spread through the mucus film contiguous with the outer surface of the catheters. This kind of urinary tract infection is mainly caused by Escherichia coli and Enterococcus faecalis (together, about 40% of urinary infections). The colonization of urinary catheters by uropathogenic bacteria causes the formation of a biofilm on the surface of the catheters, from where the infection then proceeds towards the tissues of the urinary tract. The antibiotic prophylaxis currently used is often ineffective in preventing the formation of the biofilm and also boosts the development of multi-resistant bacterial strains.

The infections associated with catheters start from the adhesion of the bacteria to the biomaterials used to make these devices. It has been shown that the predominant physical-chemical forces that control the interactions between the bacteria and solid surfaces are the Lifshitz-Van der Waals bonds and the electric double layers. The Derjaguin-Landau-Verwey-Overbeek (DLVO) theory provides the basis to interpret these interactions. Understanding the phenomena of interaction and adhesion of bacteria to the cells of mammals is equally important.
Although the proanthocyanidins of V. macrocarpon mainly interfere in the adhesion mechanisms of bacteria with the eukaryotic cells, the evidence exists that these substances also prevent bacterial

adhesion to inert materials such as glass and silicone, which is a resin widely used to make urinary catheters; however, the mechanism with which this effect is exercised is still unknown. A recent study has investigated, by using a model based on a parallel-plate flow chamber, whether these proanthocyanidins can also prevent the adhesion of various gram-positive and gram-negative bacteria to other biomaterials commonly used in urinary catheters such as polyvinyl chloride and polytetrafluoroethylene (9). The results obtained have shown the efficacy of these high molecular weight tannins in preventing bacterial adhesion to the tested materials, independently of biological mechanisms. The experimental data has suggested that the effect is mediated by physical mechanisms of steric interference.

Conclusions

The adhesion of bacteria to the receptors of the surface of eukaryotic cells involves a considerable number of biomolecules and macromolecules. Escherichia coli, for example, adheres to the uropithelial cells to start urinary tract infections through type 1 and P fimbriae which bond to receptors with molecules of mannose in their structure or the polysaccharide -D-Gal(1,4)-,-D-Gal respectively. Numerous pharmacological studies have shown that the adhesion of uropathogenic bacteria to the epithelial cells of the urinary tract is prevented in the presence of products obtained from the fruit of the Vaccinium macrocarpon plant, through the loss of the fimbrial expression and conformational changes caused in the macromolecules of the cell surfaces. The anti-adhesive activity of these products is attributed to the presence of proanthocyanidins in which, the only case in nature, the monomer flavonoids are joined by type A bonds. Some clinical studies have shown that the V.macrocarpon products can effectively reduce the rate of urinary tract infections contracted in physiological and non-physiological conditions with considerable advantages compared to the current antibiotic treatments.

The data showing the property of the V. macrocarpon products comes from uncoordinated studies which at times can be criticised for their quality; for example, the type of preparation tested and the dosages given are not always clearly specified, whilst the titre of the preparations in proanthocyanidins is seldom indicated. Nevertheless, the potential of these products for the prophylaxis of urinary tract infections in subjects at risk seems to have been sufficiently shown.

Health and regulating authorities ought to give up their mistrust of phytotherapy by offering incentives for and contributing to the coordinated and rational development of plant products which, like those of V. macrocarpon, promise to be able to play a positive role in pathologies of important social significance.

SOCIETÀ ITALIANA DI FITOTERAPIA

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