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HUMAN INTESTINAL BACTERIAL INTERACTIONS - POTENTIAL RELEVANCE IN PAIN CONTROL

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Abstract
Discovering the positive effects of the animal intestinal inhabitant bacteria such as synthesis of a group of vitamins especially group B, vitamin K, vitamin C, lead from the beginning, to the desire of using these advantages into the benefit of the human being. According to existent research, riboflavin, also known as vitamin B2 and ascorbic acid known as vitamin C have antinociceptive proprieties. Treatment of pain has a great significance because of the physical discomfort that it creates, but more important the psychological one. The hypothesis of producing your own vitamins through bacteria inoculation has actively gained field towards the possibility of self pain control by using the compounds resulted after bacteria activity. In this paper, it will be presented the diversity of interactions in the human intestinal ecosystem leading to vitamin production known to possess antinociceptive effects.

Key words*:*gut bacteria, bacterial production of vitamins, probiotics, vitamin synthesis.

Introduction

The human gut can be described as a complex bacterial ecosystem. There are estimated at least 50 bacteria types that inhabit the colon including several hundreds of species[1-5]. Because of these inhabitant species, positive biotic interactions can be encountered, such as the role they play in fiber digestion and synthesis of several vitamins[6] or negative effects on the host, like peritonitis or abscesses when bacteria penetrate the intestinal wall[6]. These effects depend on which is the majority bacteria population.

The pain phenomenon has without exception been of great interest, people always engaging in the hunt of searching explanations to why pain occurs which conducted to the appearance of multiple hypotheses. A more official point of view coming from the International Association of the Study of Pain describes pain phenomenon as "an unpleasant sensitive and emotional experience associated with potential or existing tissue damage"[7].

Following a study which investigated the effect of administering ascorbic acid (vitamin C) and riboflavin (vitamin B2) to animal models of nociception and induced inflammatory pain in mice, it reached the conclusion that when thermal pain stimuli are applied, ascorbic acid and riboflavin have antalgic proprieties. The registered results indicated that the effects are dose-dependent, time variations have been recorded, and efficiency is higher on supraspinal than spinal structures. Also, inflammatory pain shown more sensitivity to vitamin B2 compared to vitamin C and orofacial pain was found to be more resistant to the antalgic effect of the two vitamins[8,9].

Having these results in mind and knowing that the human organism is not capable of synthesising these vitamins on its own, it appeared the idea of searching a new possibility of producing naturally these vitamins and bringing them into the human organism by means of a live vehicle aiming a constant synthesis of the aforementioned vitamins.

Throughout this mini-review there will be highlighted different aspects of biotic interactions between bacteria and human organism, following especially the influence of intestinal bacteria on vitamin synthesis with known antinociceptive effects.

Materials and Methods

For this mini-review the main scientific databases (e.g. Sciencedirect, Scopus, Pubmed, Oxford Journals) were researched from inception until 12 January 2016 using the following keywords “gut bacteria”, “bacterial production of vitamins”, “probiotics”, “vitamin synthesis”. Cross-references for the selected words were also taken in consideration. The selection steps included browsing through titles relevant for the mentioned key-words. After extracting the most relevant researches, their abstracts were analyzed. Afterwards, the remaining studies were used in this review. The selection process was conducted by two independent researchers (I.A.).

Results and Discussions

Intestinal interactions and their global effect on the human organism

The human organism aside from its complex and varied composition is an intricate biological community. A biological community grows based on the interactions that take place in some populations of some species.

It is well known that he colon is by far the most inhabited region of the digestive tract, being the host of over 10^{12} bacteria for every gram of intestinal content[10,11].

But this location is not the only one that is abundant in bacteria population, the oral cavity, skin, respiratory airways are a few other examples.

Defining the relationship between the human organism and these bacteria species focuses on the interactions that develop between the two of them. The relationship status varies from cooperation, found in health conditions, until antagonist relation which lead to unbalance, generating associated pathology.

The relationships that define biotic interactions are intraspecific relations, manifested between individual of the same population and interspecific relations, found between different populations. There are several main types of relationships which can be distinguished: competition, pray-predator relation and symbiosis.

In the context of the human body there can be observed all types of the aforementioned relationships. When we are talking about competition different bacteria populations are fighting over the same nutrients, elements without which their survival is not possible.

Keeping this complex competition in equilibrium plays a key spot in maintaining human health. This microbial community that stands behind the interactions proceeding at any level of our organism, but mostly in our intestinal tract, is often called our hidden metabolic `organ`[12]. The reasoning backing up this choice of words is attributed to the paramount influence it has on human wellbeing, including here metabolism, physiology, nutrition and immune function[13]. Recent findings are linking changes in this population with considerable consequences to the human body, both favourable and disastrous[12]. If an event occurs and disrupts intestinal microbiota (phenomenon also called dysbiosis) there are signs that these might be strongly connected with pathological situations, such as obsesity[14, 15], diabetes[16], inflammatory bowel disease (IBD), ulcerative colitis (UC) and Crohn`s disease (CD)[17]. Therefore, it is imperiously necessary to keep this mechanism at its optimal functioning levels.

The human gastrointestinal tract is inhabited by a great variety of microorganisms known as intestinal microbiota, containing up till 10^{11} bacteria on a gram of intestinal contain[18,19]. The majority of microorganisms populating the human colon poses a strictly anaerobiotic metabolism, the number of optional anaerobiotic bacteria being less more than the ones strictly anaerobiotic [2,4,11,20]. This information with regard to the composition of the gut microbiome was deducted from the study of the faeces. For instance, data brought by Finegold and contributors[3] display the distribution of different bacteria groups in a study including 141 individuals randomly selected with various eating habits and health states. The prevalent ones were anaerobes gram-negative rods belonging to the *Bacteroides* group. These microorganisms represent 30% from the total of faeces flora and in this way they can have an important impact on the fermentative proceses developed in the gut. Other numerically representative groups are embodied by bifidobacteria (gram-positive rods), eubacteria (gram-positive rods), clostridia (gram-positive rods), lactobacilli (gram-positive rods) and gram-positive cocci. There are also other groups present, but in smaller amounts, along with enterococci, coliforms, methanogens and dissimilatory sulphate-reducing bacteria[11].

Generally speaking there can be made a classification of gut bacteria in species that act in the benefit or deficit of the host. Among the harmful effect there can be mentioned: diarrhea, infection, hepatic damage, carcinogenesis and intestinal putrefaction. On the other hand, the positive consequences might be caused by inhibition of pathogenic bacteria, enhancement of immune functions, decrease in gas distension trouble, vitamins synthesis, and improvement of digestion and absorption of fundamental nutrients[11].

Vitamins C and B2

Vitamins are fundamental nutrients that can be normally found as precursors of different enzymes that are required for the progress of various biochemical reactions that take place in all live cells. Because the human body is incapable of synthesising most vitamins it needs its exogenous contribution[19].

Vitamins are generally separated in two groups, ones that are fat-soluble including the group of vitamins A,D, E and K and others that are water-soluble incorporating vitamin C, biotin (vitamin H or B7) and some other vitamins belonging to group B-thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folic acid (B11) and cobalamin (B12). Looking into the roles of these vitamins it can be evidenced that fat-soluble vitamins appear to work as integrated parts of cellular membranes, while hydrosoluble vitamins behave as coenzymes, often performing the part of carriers of a certain chemical group[21,22].

Vitamin B2, also called riboflavin because of its yellow colour (flavus)[23] is absolutely necessary in the diet of human individuals and ironically their organisms lacks the capacity of producing it on their own[22]. The necessity of this vitamin resides in the fact that without its presence the flavoprotein functions would be impaired, considering that vitamin B2 is the central component of the cofactors flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)[23]. FAD and FMN are operating as electron carriers in oxidation-reduction reactions[22]. The relevance of flavoproteins is determined by their essential role in amino acids metabolism, energy production and activating coenzymatic types of folate and pyridoxine[24,25].

There are two sources of riboflavin available to fill the human organisms need: from food and produced by the colon microflora[22,26,27]. To the recommended daily dose of vitamin B2 is 1,3 mg/day for men and 1.1 mg/day for women[24] are contributing in the western countries mostly milk and dairy products[28] and also grains, meat, fish fat and green vegetables[29].

Vitamin C is another compound that the human body is not capable of synthesising, but that is essential in the diet. Also called L-ascorbic acid, vitamin C is an important antioxidant, considered to poses prophylactic or delaying proprieties of some types of tumors, cardiovasculary diseases or other disorders where oxidative stress plays a crucial part[23]. Other known or presumptive functions of vitamin C are: free radicals scavenger[30,31,32], cofactor for a number of enzymes[31,33,34].

Vitamins C and B2 synthesising bacteria

The idea of using microorganisms that produce vitamins to replace the necessary amount of vitamins C and B2 is a viable natural alternative coming to help the consumer, comparing to the usage of synthesised pseudo-vitamins through chemical procedures[19]. The concept of ingesting useful bacteria for the purpose of manipulating the intestinal bacteria ensemble in the benefit of the human health is not new. For instance, the Russian biologist Ilya Ilyich Mechnikov has suggest in 1907 that by "adopting measures which would lead to altering the human body flora and replacing the pathological germs with useful ones" it would create an equilibrium translated into health state. He hypothesized that through ingestion of lactic acid producing bacteria as the ones encountered in yoghurt it would increase the life span[35,36].

Even from far back there was noticed that some bacteria species have vitamin synthesis proprieties. Advocating for this claim, it has been demonstrated that lactic acid producing bacteria can synthesize riboflavin[37]. Coming to back up the same cause, it has been proved that several dysenteric bacillary strains have been reported to be gifted with the capacity of forming thiamine, coenzyme I or II, riboflavin and possible biotin[37]. These scientists came with the idea by observing that important quantities of riboflavin, pyridoxine and antihemorrhagic vitamin develop in the sheep's and cow`s rumina considering that their diets are low on these vitamins and it has been thought that the source of the vitamins comes from inside due to the commensal microorganisms that colonize the rumina[37]. Base on the assumption that the rumina of animals host bacteria capable of producing certain vitamins, even since 1942 it has been proved that intestinal bacteria such as *Bacterium aerogenes, Escherichia coli* have this mentioned capacity in vitro conditions[37]. This discovery was perceived to have great potential and a bright future perspective. Even if the effects of these investigated bacteria such as *Bacillus acidophilus* – now known as *Lactobacillus acidophilus*[38] were not fully known, Cheplin and Retteger fed them to people who offered to be volunteers in their experiments and also to rats. Afterwards, examining the faeces it was noted the presence of changes in the composition of the faeces microbiota, described as being "the conversion of intestinal flora"[36,39,40]. In this way, trying to define this procedure through a term, probiotic came up, probably used for the first time by Werner Kollath for describing organic and anorganic nutritive supplements and also a clear distinction between probiotics and antibiotics was established[36,41].

Probiotics relevance in the context of vitamins production

Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) have consulted experts in evaluating nutritive proprieties and health state influences cause by the presence in the diet of probiotics and they reach the conclusion that probiotics can be defined as live microorganisms which administered in adequate quantities grants a benefit to the host's health[42]. Following FAO and WHO`s definition the International Institute of Life Sciences (ILSI)[43] and European Food and Feed Cultures Association (EFFCA)[44] came up with a similar definition for the term probiotic: a live bug part of an aliment which consumed in adequate quantities confers health benefits to the consumer`s health[45].

Probiotics have been conceived for the purpose of improving certain physiological states in various parts of the body. Even if the gastrointestinal tract is the most targeted area from most of the probiotics, other parts of the body, like the oral cavity, urogenital tract and skin are also of interest to the action of probiotics[45]. Considering the mentioned situations, probiotics could play an important role in oral medicine and dentistry. The purpose of using probiotics in the context of the gastrointestinal tract follows some objects such as decreasing colonization with pathogens, increasing vitamin synthesis, optimizing intestinal transit, lowering intolerance to lactose, reducing bloating sensation and promoting some modulatory effects of the immune system[45].

Of great importance is the way the probiotics reach their site of action. Most of the times probiotic strains need a special matrix which would guaranty their survival through the digestive tube. Several methods of assuring their highest rate of continuity are by introducing them in a chocolate matrix[46] or in conventional product as milk[47], kefir[48] and yoghurts or other special matrixes for example grains, cheese, sausage, cookies. Other vectors that would bring them safely in their destinations are using them as nutritive supplements frequently following specific pathologies[45].

Their benefits on the health are only obtained if the probiotic reaches the targeted spot, where it can manifest its metabolic proprieties and there are a generous number of probiotic strains. The survival of the strain is heavily put to the test until it reaches the colon. First, it must face the acid attack encountered in the stomach. Therefore, this situation highlights the importance of having a matrix determining the pH the probiotic strain will have to survive[45]. Afterwards, it encounters a new stress generated by the bile salts. Only later intervenes the situation where it would be necessary to prove the ability of colonizing the colon paying attention to two components: ecological and mucosal. The ecological component is represented by the completion with the bacterial community residing there for the nutrients, obliging the probiotic to find and occupy a functional niche in the bacterial ecosystem of the gut. Of great help in this mission would be if in the formula of the product would be added the specific nutrients[45]. These added nutrients are called prebiotics defined as being a sum of alimentary ingredients that cannot be processed by the human digestive system, but can be digested by specific intestinal bacteria, in this way stimulating the process of selective gastrointestinal proliferation[36,49]. The ensemble complied by the blend between probiotics and prebiotics represents a synbiotics[36,50]. The mucosal component of the colonization ability necessary for the probiotic is represented by its capacity of adhering to the surface of the mucosa that covers the intestinal epithelium[45].

The method of obtaining vitamin C using the synthetic process applying Reichstein method starts from glucose which is chemically transformed into sorbitol. Currently this method has been simplified and consists of seven steps, beginning with transforming L-sorbitol into D-sorbitol. Afterwards *Gluconobacteroxydans* oxidates specifically D-sorbitol into L-sorbitol. Afterwards, the resulted reaction product from the last process is crystallized and condensed with acetone and sorbose-diacetone is obtained. Later on, this is oxidazed to 2-keto-L-gluconic acid (2KLGA) and this is enolized and lactonized to form L-ascorbic acid. Because this process has several steps it was found another method to shorten the procedure using microbial methodologies that lead to the production of 2-KLGA utilizing cultures with single and mixed strains. An example of this process is made through utilizing in the first phase of *Erwinia* and *Acetobacter* and in the second phase, *Corynebacterium*[23,51,52].

Through genetic and metabolic bioengineering there were developed other procedures of obtaining 2-KLGA from different organisms. When employing *Erwinia herbicola* genetically modified with *Corynebacterium* it reaches a production of 120gl^{-1} [23,53]. Knowing that the yeast *S. cerevisiae* is capable of producing D-erithroascorbic acid it was genetically altered with a gene from *Acetobacter thaliana* resulting an yeast capable of producing 100 mg l^{-1} of L-ascorbic acid[23,54]. Also, there are researched for their ability to directly produce ascorbic acid some microalgae named *Prototheca moriformis* and *Chlorella pyrenoidosa.* These synthesize 2g l^{-1} of ascorbic acid but their great inconvenient is determined by decreased rates of metabolism and development[23].

The biosynthetic way of obtaining riboflavin has been extensively studied in the context of *Bacillus subtilis* and *Escherichia coli.* For the biosynthesis of riboflavin it is necessary a precursor of guanosin-5`-triphospate (GTP) and of ribuloz-5-phospate. First phase on the GTP dependent branch in the biosynthetic way is coded by ribA in *E. coli.* In the case of *B. Subtilis* it is also coded by ribA, but in this situation ribA is a bifunctional coenzyme which catalyzes also the development of 3,4- dihyroxi-2-butanone-4phospate from ribulose 5-phospate[22,55]. For being biologically active, riboflavin needs to be converted in its types of coenzymes FAD and FMN. This process is made possible by the flavokinase/ FAD synthetase which is encoded in *B. subtilis* by ribC or by ribF in *E. Coli*[22,56,57]. Nowadays, the microorganisms exploited for the production of riboflavin are *A. Gossypii*, *Candida famata* and *B. Sutilis* with productions of riboflavin that reach 15 g l^{-1}, 20 g l^{-1} and 40 g l^{-1} [22,58,59,60].

Today, there are several vitamins that are almost exclusively produced by microorganisms and these vitamins are B2 and B12. Vitamin C is still chemically obtained but also microbiologically[23]. Future developments in the biotechnological field should lead us to succeed in producing vitamins entirely by using microorganism systems as if they were cell factories. Obtaining this extraordinary success there will be created the favourable circumstances of surveying and attentively evaluating the antinociceptives effects of vitamins C and B2.

Future perspectives of research

The main idea of this mini-review initiated from the hypothesis of a future research on the vitamin synthesizing bacteria acquired biotechnologically which could be administered to human individuals and through their proprieties of synthesizing vitamins in the colon they would gain a therapeutically antinociceptive effect.

For avoiding competition it will be pursued the selection of specific commensally species inhabiting the colon. With the help of biotechnologically methods of genetic altering with genes from the aforementioned bacteria that possess vitamin synthesizing capacities it will be targeted to generate a synbiotic product (meaning prebiotic and probiotic as presented above). Afterwards, it will be followed by administering this product to human individuals. It is believed that this product will coexist with the flora of the gut, but in case it doesn`t it will be necessary to speculate a time frame until the next administration is possible.

After the product is attached, the next supervised parameter is the possibility of vitamin absorption through the intestinal mucosa, so that they could fulfil their antalgic purpose highlighted in the introduction part.

Conclusions

The bilateral interactions that appear to take place in the intestine manifest proven mutual benefits for the involved parties. These symbiotic benefits could bring in the future through the development of vitamin production systems, new perspectives in pain management, based on the antinociceptive effects that vitamins C and B2 manifest.

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