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Investigation of Farnesyltransferase Inhibitors as Antiprotozoal and Antifungal Agents

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ABSTRACT

BACKGROUND

Currently, plants are components of many drugs. Of particular interest is the wormwood genus Artemisia glabella. Or rather a compound isolated from it. Which was converted into a water-soluble dosage form and named "Arglabin"- farnesyl transferase inhibitor, depressing process of cell proliferation. It is now regarded as an antitumor agent. FPT participates in the synthesis of sterols that are an important part of the cell membrane. FPT inhibitors may therefore be used to eliminate pathogenic microorganisms.

METHODS

For the isolation and differentiation of Candida Albicans and C.tropicalis we used Nickerson`s nutrient medium, which allows you to differentiate Candida Albicans from other species of Candida by morphological features of the colonies. Incubate at 25 ± 2 ° C for 18-72 hours and to 5 days, if necessary. Then, the method of "stroke" passaged four Petri dishes with nutrient Saburo for different concentrations of drugs. Each petri dish is divided into two halves(experiment and control). Incubated for 24 hours at 37 ° C in a thermostat, and within five days at room temperature.

RESULTS

After a day in the control area there was negative growth. We conducted a comparative analysis of the impact arglabin in concentrations of 20mg, 40mg, 60mg and 80mg and fluconazole (control) in concentrations of 25 mg, 50mg, 75mg and 100mg on the growth of fungi C.albicans C.tropicalis. On Arglabin at concentrations of 20mg, 40mg, 60mg and 80mg, notes the continuous growth of C.albicans and C.tropicalis around wells. Lack areas stunting.

CONCLUSION

Farnesyltransferase inhibitors has a unique activity. Studies show that with further study and refinement, IFT could become major drugs against fungi and protozoa.

KEYWORDS

Arglabin, Artemisia glabella, Antifungal Agents, Microbial Secondary Metabolites

INTRODUCTION

Plants are a natural source of most biologically active substances. They are part of many medications. Of great interest is the wormwood genus Artemisia glabella. In particular it`s compound which name is Arglabin. Arglabin [1 (R), 10 (S) -epoxy-5 (S), 5 (S), 7 (S) -guaia-3 (4), 11 (13) -dien-6, 12-olide] - herbal remedies, which are based on sekviterpen`s gamma-lactone isolated from Artemisia smooth Artemisia glabella, germinating in the Karaganda region of Kazakhstan. The compound has been transformed to convert it into a water-soluble drug by attaching dimethylamine hydrochloride to a group of C (13) karbogid fragment to form eventually arglabin-DMA. Kazakhstan has developed the drug "Arglabin" - farnesyl transferase inhibitor, a depressing process of cell proliferation, and therefore recommended as an antitumor chemotherapeutic agent [1, 2, 4-8].

As is well known, protein farnesylation is a form of post-translational modification that is present in most eukaryotic concerning; researchers are now exploring the use of farnesyl transferase inhibitors (hereinafter IFT- farnesyl transferase inhibitors) in the treatment of diseases caused by pathogenic eukaryotes [3, 6]. IFT has already proven effective in the treatment of eukaryotic pathogens eksperimental models, including Trypanosoma brucei, the causative agent of African sleeping sickness, and Plasmodium falciparum, the causative agent of malaria. Due to the fact that protein prenyltransferase are included in the most fungi and protozoa, as well as considering the fact that the eukaryotic parasites are more vulnerable for inhibiting farnesyl than the host tissue, they can be used as therapeutic targets, as me think. Sterols are essential elements in the structure of cell membranes, providing their functioning, communication with other cells and active substances, as well as performing a protective function of the membrane. These components are present in virtually all eukaryotic cells in some form, for example in the human body

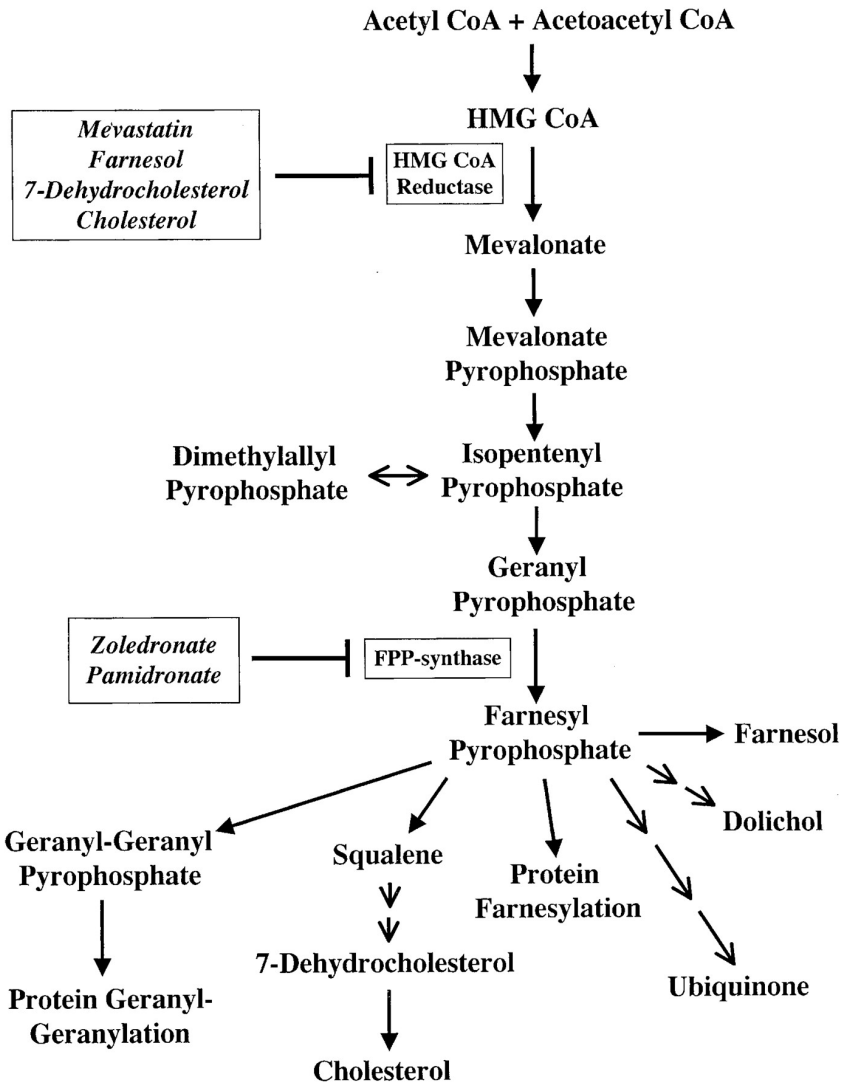

Fig 1. Relationship of posttranslational protein isoprenylation pathways to the mevalonate pathway of cholesterol biosynthesis

in the form of cholesterol in fungi and protozoa in the form of a special class of sterols, including ergosterol and other 24-metilsteroly (Fig. 1) [5, 6].

Knowing the mechanism of the synthesis of sterols, we can influence the growth and activity of pathogenic protozoa and fungi. For the synthesis of sterols requires a minimum of 20 steps in the synthesis of cholesterol 48 [7-10]. The main ones are:

1.Condensation two units of acetyl CoA to form acetyl-CoA acetyl.

2.Formation 3-hydroxy-3-metilglyuta- reel-CoA (HMG-CoA)

3. Recovery with the formation of mevalonic acid. This is the end of mevalonic way.

4. Conversion mevalonate in isoprentenyldiphosphate (IPP) (+ one two phosphorylation decarboxylation).

5. Conversion using isoprentenyl-diphosphate isomerase in dimetylallildiphosphate (DMAPP).

6. Condensation IPP with DMAPP and geranyldiphosphate to form farnezyldiphosphate (FPP), catalyzed farnezydiphosphatsynthetase (FPPS). This ends the isoprenoid pathway. Before this step the sterol synthesis is identical in all organisms studied [2].

Farnezyldiphosphate is a substrate for further synthesis of various enzymes of sterol, and a donor for prenylation. Prenylation - posttranslational lipid modification of proteins with cysteine residues

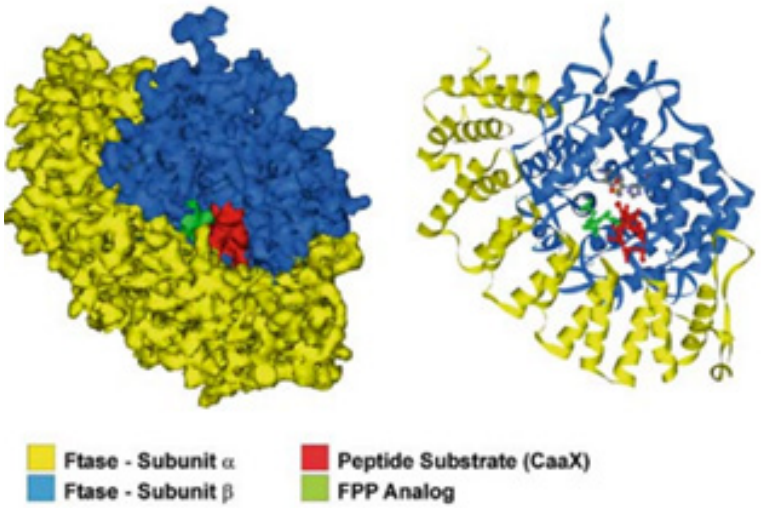

Fig 2. Farnesyltransferase enzyme (FTase) ternary complex (FTase-Peptide-Substrate-FPP anlog)

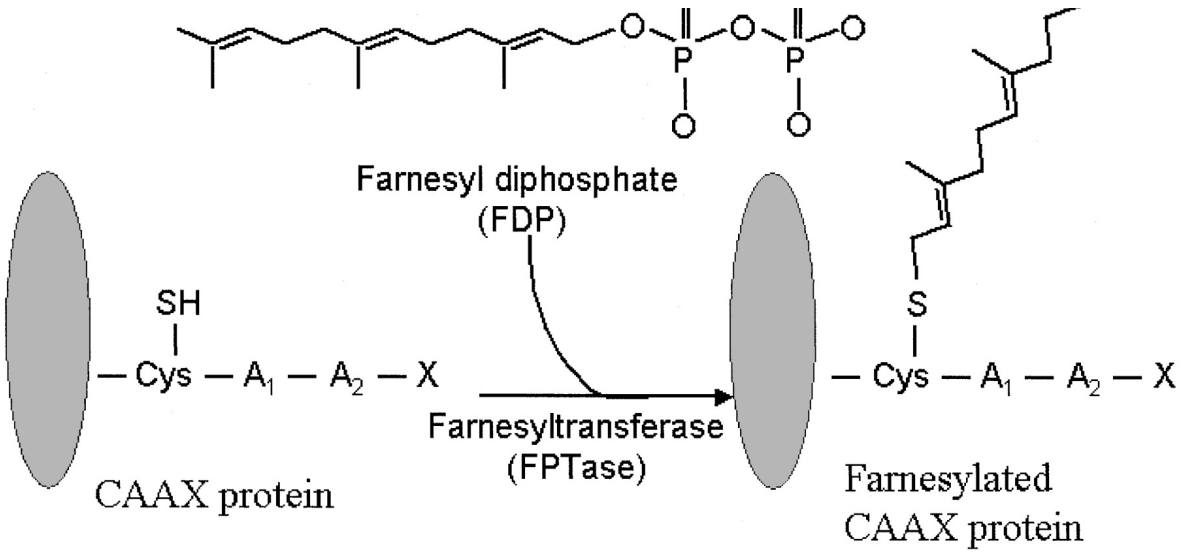

Fig 3. Schematic representation of farnesyl protein transferase (FTase) reaction

required for the association of the protein with lipophilic membrane as well as protein-protein interactions. For prenylation substrate proteins are H-, N-, K-Ras, Rac, Rho, Rab, involved in vesicle transport, signal transduction and cell cycle life activity [3-8].

To date, three known enzyme providing prenylation: farnesyltransferase (FPT), geranyltrasferase type I (GGPT-I) and geranyltransferase type II (GGPT-II). FPT is a substrate proteins containing Ca- ah motif at the C-terminus, where C is cysteine, aa - no aromatic amino acids, x - terminal amino acid which may be methionine, serine, alanine or glycine. FPT carries farnesyl group from FPP to the cysteine thiol group [9].

FPT - heterodimer composed of two subunits:

• alpha subunit of 48 kDa, consists of 379 residues.

• Beta-subunit of 46 kDa, consists of 437 residue

FPT - is the zinc`s enzyme. A zinc ion in the protein dimer is necessary for its catalytic activity. Zinc ion

is not required for isoprenoid-binding but is required for coordination peptides. It was also proved that for the maximum activity FPT needed millimoles of magnesium, and some scientists believe that magnesium helps to stabilize a pyrophosphate group [4, 13, 15, 16].

GGPT-I is also a substrate proteins containing sugar motif, but here's - leucine or phenylone. Another difference is that a beta- subunit (just 30% similar to the amino acid content), in contrast to alpha, both of which enzymes are identical. Also for their optimal activity GGPT-I does not require magnesium ions. Because of the strong similarity of these enzymes has been proved their cross-action, that is, the absence of one, the other completely takes over the job.

GGPT-II adds two geranyl-geranyl group to the terminal residues of proteins, ending SS SSKHH, LDL-C motifs. Now found that GGPT-II catalyzes the only Rab-proteins [5, 9, 11-14]. Given that Ras-oncogenes are farnesylations proteins were created and investigated the effects of a significant number of inhibitors farnesyltransferase (IFT) as a cancer treatment, Arglabin was no exception. However, as indicated above farnesyltransferase involved in the synthesis of sterols and thus exerting influence on it is possible to prevent the growth of pathogenic eukaryotic protozoa and fungi [6, 17, 18].

Although the reason for more effective inhibition of cancer and parasitic farnesyltransferase, as compared to normal mammalian cells is not clear, it can be assumed that this selectivity is based on biological differences between these cells. Action IFT on protozoa and fungi can also be based on the presence and absence of farnesyl transferase only a workaround in the form of geranylgeranyltransferase [11, 12, 15-18].

Anyway, IFT drugs are effective against fungi and protozoa, the only difficulty is to ensure the delivery of the inhibitor into the cell pathogens. If you provide such a modification of IFT, which will help deliver the inhibitor to a substrate, having a barrier membrane, we can get a complete elimination of the pathogen [7-10]. One of the problems encountered when creating drugs with antimicrobial activity, is the presence of toxic side effects and high cost of these drugs. In this regard, the proposed research is interesting in terms of a new approach to the ability of the drug to the known mechanism of action.

The aim of the study

The possibility of using domestic farnesyl transferase inhibitor - "Arglabin" as an antifungal drug.

METHODS

For isolation and differentiation of Candida albicans and C.tropicalis we used Nickerson`s nutrient medium as this medium makes it possible to distinguish Candida albicans from other Candida species based on morphological features of the colonies. Incubate at 25 ± 2 ° C for 18-72 hours and to 5 days, if necessary. On Nickerson`s nutrient medium we have identified museum strains of Candida tropicalis, and Candida albicans. On the second day we received a colony. Differentiation was founded on colony morphology. C.albicans - with smooth edges, protruding, of medium size, black colonies; C.tropicalis - with smooth edges, protruding, of medium size, brown colony. From grown crops we made smear, fixed and stained by Gram. Microscopic - large, slightly oval in shape, arranged in groups, Gram-positive. Thus, we confirmed the purity of the grown cultures. Then, the method of "stroke" passaged four Petri dishes with nutrient Saburo for different concentrations of drugs. Just before planting in each cup were prepared by two crescent for making drugs (experiment and control). Each petri dish is divided into two halves. As well, the left half of the solution was introduced arglabin (experience) in concentrations of 20 mg - Petri dish - №1, 40mg - №2, 60mg - №3 and 80mg - №4. As well, the right half of a solution made of fluconazole (control) at a concentration of 25mg - Petri dish - №1, 50mg - №2, 75mg - 100mg №3i - №4. Incubated for 24 hours at 37 ° C in a thermostat, and within five days at room temperature.

RESULTS AND DISCUSSION

Within five days, we observed daily for growth of microorganisms area. After a day in the control area there was negative growth. We conducted a comparative analysis of the impact arglabin in concentrations of 20mg, 40mg, 60mg and 80mg and fluconazole (control) in concentrations of 25 mg, 50mg, 75mg and 100mg on the growth of fungi C.albicans C.tropicalis. The antifungal activity of fluconazole observed in our work too. As can be seen from the table, in a therapeutic concentration of fluconazole reduced the growth of C.albicans, negative zone was 4cm: 3cm; reducing growth C.tropikalis not.

Fungi\drugs	Arglabin				Fluconazole			
	20mg	40mg	60mg	80mg	25mg	50mg	75mg	100mg
C.albicans	Lack areas stunting				2cm : 1-1.5cm	4 cm : 3 cm	3 cm : 3.5 cm	3cm : 2cm
C.tropicalis	Lack areas stunting				3.3cm:3cm	-	3.5 cm : 1.5 cm	uniform reduction in growth

At a concentration of 75 mg of fluconazole marked reduction in growth of C.albicans C.tropicalis-3cm: 3.5 cm and 3.5 cm: 1.5 cm, respectively. It is interesting to note that a minimum concentration of fluconazole identified zone stunting C.albicans 2cm: 1-1,5sm and 3,3sm: 3cm area was stunting C.tropicalis. On Arglabin at concentrations of 20mg, 40mg, 60mg and 80mg, notes the continuous growth of C.albicans and C.tropicalis around wells. The table shows that, in the concentration zone arglabin delay fungal growth there.

Possible reasons for the negative result:

1. The use of museum strains, but not freshly isolated cultures of C.albicans and C.tropicalis;

2. Instill drugs instead of using diffuse disc or broth microdilution. Scientists JianjunQiao, PeipingGao, XiaolingJiang in their experiences using methods diffuse disc (on a determination of antibiotic resistance), and the broth microdilution. Farnesyltransferase inhibitors were manumycin A and tipifarnib. But unfortunately, it was also proven that the minimum inhibitory drug's concentrations to fungi were significantly higher concentrations inhibit vitality and proliferation of mammalian cells themselves.

3. Studying only fungi of C.albicans and C.tropicalis.

Thus, in experiment of JianjunQiao, PeipingGao, XiaolingJiang, and HongFang [8] action against farnesyl transferase inhibitors of growth Aspergillus species and Candida has been proven. Also, we received information that farnesyltransferase inhibitors can lead to growth reduction Cryptococcusneoformans. And deletion of genes encoding subunits of farnesyltransferase lead to death C. neoformans and Candida Albicans and growth defect of C. Glabrata.

CONCLUSION

Farnesyltransferase inhibitors - unique products having an activity on quite different substrates. A detailed study of them with the aim of expanding the range of application will enable us in the future, perhaps, the first line drug against fungi, protozoa. You do not need to create anything, it is already there, you just figure out how many useful things we can learn from it.

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