"ABSORPTION OF CALCIUM IN THE ABSENCE OR PRESENCE OF VITAMIN D, PRESENTING VANILLIN (VANNILOIDS) TO ENHANCE THE FUNCTION OF MEMBRANE CALCIUM CHANNEL TRPV6 FROM THE FORTIFIED MILK- A NOVEL STUDY".

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Abstract: Calcium metabolism can occur in the presence of Vitamin D but it takes long path to absorb the calcium in the intestine and kidney, herewith old age people are facing vitamin D deficiency likewise hyper-vitaminosis- D, to change this approach an alternative pathway is studied in the presence of vanniloids (vanillin) receptors in the fortified milk (absence and presence of vitamin D) that could bye-pass calbindin protein and react with TRPV6 membrane calcium channel for easy absorption calcium.

Key words: Calcium, Vitamin D, Vanniloids, TRPV6, Fortified milk, Membrane calcium channel, Calbindin Protein.

I. Introduction

Detail of the study:

This study is an alternative approach to enhance the calcium level retention in the blood plasma of old age people. Due to aging apoptosis (cell death) would be taken place on the skin, merely the skin cells are not take more Vitamin D from the sun light as younger one could do, so that the aged people should be depend on diet and supplements to meet the vitamin D requirements. As we know, that the deficiency of vitamin D is depreciate in elderly people cause bone deformities because the incumbent calcium in bones used by the blood serum to standardise the nutrient level in the body. Absolute calcium metabolism deferred in functional level, so that the study seeks bye pass pathway in the absence of vitamin D i.e., there is no necessity of Calbindin (Vitamin D dependent calcium binding proteins in the intestine and kidney to start the calcium metabolism) therefore, the direct presence of the TRPV6 protein is required to start the calcium metabolism without the involvement of Calbindin. To initiate these reaction Vanniloid receptors is essential to start the calcium absorption mechanism and directly contact with TRPV6 protein to start the membrane calcium channel to absorb the calcium channel.

Aims and problems are to be focused;

1. Quick calcium absorption.
2. Free from hypervitaminosis D (Dehydration, vomiting, decreased appetite, irritability, constipation, fatigue, muscular cramping and metastatic calcification.
3. To find normal values of calcium range from 8.5 to 10.2 mg/dL in both fortified milk (from the blood sample of mice)

a) Protein+ Vitamin A + Vitamin D

b) Protein + Vitamin A + Vanillin.

c) Protein + Vitamin A + Vanillin + Vitamin D

4. To bring Vitamin D in normal range using vanillin in fortified milk.
5. Vanillin's other biological uses.

II. Components of the study

Fortified milk

Fortified milk made more nutritious by addition of milk protein, vitamin A, or vitamin D.

Calcium metabolism

It refers to all the movements (and how they are regulated) of calcium atoms and ions into and out of various body compartments, such as the gut, the blood plasma, the interstitial fluids which bathe the cells in the body, the intracellular fluids, and bone.

Vitamin D

Vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone.

As vitamin D is mainly obtained from the action of sunlight on the skin, people who are housebound or live in institutions may be at risk of deficiency.

Vanilloids

The vanilloids are compounds which possess a vanillyl group. They include vanillyl alcohol, vanillin, vanillic acid, capsaicin, vanillylmandelic acid etc.

Vanillin

Natural vanillin, produced from vanilla beans and other naturals is one of the most common flavor chemicals and is used in a broad range of flavors. It occurs in the vanilla bean at level of 20 g per kg dry weight. A minimum vanillin content of 1.18-2 % and a moisture content ranging between 20 to 22 % are preferred by importers.

Functions of vanillin

(i)Antimicrobial

Antibacterial and antifungal actions have been demonstrated in vitro for vanilla and vanillin, suggesting a role in food preservation.

(ii) Antioxidant High pressure liquid chromatography studies and in vitro experiments show antioxidant activity primarily for vanillin, but also for naturally derived vanilla.

(iii) Blood

An antisickling effect has been demonstrated for vanillin in vitro in animal experiments and limited clinical studies. In mice, increased survival in hypoxic conditions and a decrease in the percentage of sickle cells was shown, while in a clinical study, vanillin 1 g daily produced measurable antisickling effects.

(iv) Cancer

Vanillin may exert antimutagenic and anticarcinogenic activity by inhibiting a DNA repair process leading to the production of mutagenic cells. Antioxidant action may also contribute to this effect. The ability to suppress phototoxic DNA damage, as well as potentiate cisplatin cytotoxicity, has also been demonstrated in vitro. Clinical studies are lacking.

Membrane Calcium Channel (TRPV6)

Two members of the transient receptor potential vanilloid family, TRPV5 and TRPV6, are calcium selective ion channels and are known to be expressed in calcium-transporting epithelial tissues.

TRPV6 is a membrane calcium channel which is particularly involved in the first step in calcium absorption in the intestine. The protein is located in the apical brush-border membrane of the intestinal enterocyte where it regulates calcium entry into the cell. It is most abundant in the proximal small intestine (duodenum and jejunum).

Transient receptor potential vanilloid subfamily member 6 (TRPV6) is a highly selective Ca^{2+} channel that exercises its normal physiological function via Ca^{2+} absorption in the intestine and kidney.

Transient receptor potential vanilloid subfamily member 6 (TRPV6) is a highly selective calcium channel that has been considered as a part of store-operated calcium entry (SOCE).

The transient receptor potential cation channel subfamily V member 6 (TrpV1), also known as the vanillin receptor and the vanilloid receptor 6, is a protein that, in humans, is encoded by the *TRPV6* gene. This protein is a member of the TRPV group of transient receptor potential family of ion channels.

This gene encodes a member of a family of multipass membrane proteins that functions as calcium channels. The encoded protein contains N-terminal ankyrin repeats, which are required for channel assembly and regulation. Translation initiation for this protein occurs at a non-AUG start codon that is decoded as methionine. This gene is situated next to a closely related gene for transient receptor potential cation channel subfamily V member 5 (TRPV5)

III. Materials and methods

Mice

Mice as model organisms for this study procured from Pandit Ravi Shankar Shukla University, Raipur. There are 4 pairs of mice required for this study.

Composition of milk

Fresh cow milk procured from the farm and vitamin D3 powder procured (Emergen – Vitamin D3) through Nutrition express herewith Vitamin A obtained from Nutrilite and Vanillin powder 15 grams procured from Food chem.

Homemade fortified milk preferred with the addition of Vitamin D and Vitamin A and Vanillin.

For the purpose of the study three groups were divided.

1. Control group (only fresh farm milk)
2. Milk + Vitamin D (add vitamin A)
3. Milk + Vitamin D + Vanillin (add vitamin A)
4. Milk + Vanillin

- Vitamin D 1 IU = 0.3 mcg retinol or 0.6 mcg Beta-Carotene.
- Vitamin D 1 IU = 0.025 mcg (Cholecalciferol or Ergocalciferol)
- Vanillin powder = 0.06 mcg per 8 ounces.

TABLE SHOWS DIFFERENT CATEGORY TO FEED MILK.

S.NO	MICE	MILK (ML)	VITAMIN D	VITAMIN A	VANILLIN
1	CONTROL GROUP	226.796	-	-	-
2	GROUP 1	226.796	100 IU	100 IU	-
3	GROUP 2	226.796	100 IU	100 IU	0.06 mcg
4	GROUP 3	226.796	-	-	0.06 mcg

Consumption of Milk by mice:

Mouse can consume 5 gm of food per day and 3 to 5 ml of water per day considered that preparing fortified milk for 226 ml with different compositions but only 1 ml of milk from the composition given to each mouse of different group.

Blood Sample Collection

A month later with a continuous feed of milk to mice blood sample collected through intra venous and sample had been used for different molecular studies.

Molecular studies

Reverse transcriptase PCR was used to examine the presence of TRPV6 mRNA in blood sample. Protein expression was assessed by western blotting using TRPV 6 – specific antibodies. Immunocytochemistry was employed to examine sub cellular localization of TRPV6 in frozen, formaldehyde-fixed sections of intestine and kidney. Finally TRPV6 activity was assessed in intestine and kidney, using Ca^{2+} indicator dyes to follow $(Ca^{2+})_i$as a function changes in $[Ca^{2+}]_o$ with and without addition of the TRPV6 inhibitor ruthenium red.

IV. Result

Reverse transcriptase PCR showed the presence of TRPV6 mRNA in the blood sample of group 2 and group 3 that implies the vanilloid indicators presence and the membrane calcium channels responsible for quick absorption of calcium from fortified milk. Protein expression from the western blot showed the specific antibodies that were not appeared in control group. Western blot analysis of TRPV6 in blood sample, kidney and intestine detected a distinct band at approximately 85 kDa. In live-cell imaging experiments, [Ca(2+)](i) was lower in the presence of the TRPV6 inhibitor ruthenium red.

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