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THE REGULATION OF PROSTAGLANDIN E1 FORMATION: A CANDIDATE FOR ONE OF THE FUNDAMENTAL MECHANISMS INVOLVED IN THE ACTIONS OF VITAMIN C.

D.F. Horrobin, M. Oka, M.S. Manku, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal H2W 1R7, Canada.

ABSTRACT

Vitamin C stimulates the formation of PGE1 in human platelets. The effect occurs over the physiologically relevant range of concentrations. PGE1 is required for T lymphocyte function and plays a major part in the regulation of immune responses. PGE1 is also important in the regulation of collagen and ground substance metabolism, in cholesterol metabolism and in regulation of responsiveness to insulin. It is proposed that defective formation of PGE1 could account for many of the features of scurvy and for many of the reported therapeutic effects of vitamin C. If correct, vitamin C will be of value only in conjunction with an adequate supply of dihomogammalinolenic acid, the precursor of PGE1. Essential fatty acids, pyridoxine and zinc are all required to achieve this.

INTRODUCTION

Although the clinical features of vitamin C deficiency are well known, there is still no fully convincing explanation of its actions at the cellular level. This lack of understanding of a fundamental biochemical mechanism may have contributed to resistance to the use of vitamin C as a therapeutic agent even though there is relatively good evidence of its value in a number of situations. We have recently discovered that over a physiological range of concentrations vitamin C enhances the conversion of dihomogammalinolenic acid (DGLA) to prostaglandins (PGs), particularly PGE1, in human platelets (1). We suggest that this mechanism can account for many of the known actions of vitamin C and reveals that therapeutic results are to be expected only if adequate supplies of DGLA are available.

By far the best documented effects of vitamin C are those on collagen and ground substance and on resistance to infections. Severe vitamin C deficiency in humans and other species which cannot manufacture it themselves leads to a widespread disruption of collagen, susceptibility to a variety of infections, weakness, depression, elevated cholesterol levels, resistance to insulin with diabetes, and anemia. Most of the other described changes seem to be secondary to these fundamental effects (2,3,4,5). A high proportion

of the changes can be accounted for on the basis of defective formation of PGE1.

SYNTHESIS AND ACTIONS OF PGE1

PGE1 is formed from DGLA and like all the 1 series PGs has 1 double bond in its side chains. Relatively little attention has been paid to PGE1 because only relatively small amounts of DGLA are found in the body in contrast to the very large amounts of arachidonic acid (AA) the precursor of 2 series PGs. The ready availability of radioactive AA, in contrast to radioactive DGLA which must be obtained by expensive special order has contributed to the neglect. However some DGLA seems to be present in almost all tissues. The kidneys (6), human liver (7), brain (7), thymus (8) and seminal fluid (9) contain relatively large amounts of DGLA and/or PGE1.

Both DGLA and AA are essential fatty acids (EFAs) and both are stored in membranes in esterified forms. Both are believed to be liberated to the free form by the action of a phospholipase and to be converted to prostaglandins by a series of reactions, the first of which is transformation to unstable endoperoxides by an aspirin-sensitive cyclo-oxygenase enzyme system (10). It is widely assumed that the mechanisms converting the esterified to the free acids and the free acids to endoperoxides are fundamentally similar and are regulated by similar factors. However this is a theoretical assumption which until recently has not been experimentally tested. It has now been shown using rat vascular tissue and human platelets that while some agents have similar effects on both EFAs, others are highly specific. For example the liberation of free AA and DGLA seem to be blocked by cortisol to an equal degree (11,12,13,14) whereas lithium seems to block DGLA liberation while having a much weaker effect on AA. Angiotensin and vasopressin appear to liberate AA while prolactin and zinc appear to liberate DGLA. Ascorbic acid causes a dose dependent rise in conversion of free DGLA to PGE1 in human platelets while having no effect on the conversion of AA to PGE1 (1). Alcohol also enhances PGE1 formation and simultaneously inhibits formation of thromboxane B2 from AA (15). It is therefore clear that regulation of the 1 and 2 series PGs can in some circumstances be independent, while in others is similar.

In vascular tissue, the actions of PGE1 are highly specific and are not shared by any other known PG (16). Low concentrations of PGE1 seem to enhance intracellular calcium release while high concentrations inhibit it. This characteristic bell-shaped effect is very important as will be seen later. Its implications have been explored in detail (17,18).

In relation to ascorbic acid the actions of PGE1 which are of particular interest are those on the immune system and those on connective tissue. PGE1 is found in relatively high concentrations in the thymus (8) and there is good evidence that it plays an important role in T lymphocyte maturation and function. This has been reviewed in detail elsewhere but includes the following: 1. PGE1 can imitate thymic hormone in maturing T lymphocytes in vitro (19). 2. Prolactin (20) and zinc (21,22), both of which enhance PGE1 formation, can enhance thymic growth and development. 3. Cortisol and lithium (23) both of which inhibit PGE1 formation cause thymus atrophy. 4. In animals with acquired or inherited defects in T lymphocyte function, PGE1 injections can largely restore normal responses (24,25,26,27).

With regard to collagen, PGE1 seems to have a biphasic effect. Moderate reduction in PGE1 levels seems to be associated with increased fibrosis due to either enhanced collagen formation or decreased collagen destruction or a combination of both. On the other hand severe reduction in PGE1 formation, such as may perhaps occur in zinc deficiency, is associated with defective collagen and all the associated problems such as inadequate wound healing (28) PGE1 seems to be an effective regulator of formation of glycosaminoglycans (GAG) which are important in maintaining the stability of collagen and intracellular ground substance (59,60,61). Here again there is evidence of a biphasic effect with moderate concentrations enhancing formation and high concentrations inhibiting it. PGE1 also enhances cAMP formation but whether this is a cause of or only associated with the GAG effects is uncertain.

Among other relevant actions of PGE1 are its inhibition of platelet aggregation (10,29) and its enhancement of a peripheral action of insulin (30). PGE1 is, of course, formed from the essential fatty acids which have been used in the reduction of cholesterol levels (31,32) and in the treatment of diabetes (33,34).

It is important to note that many cancer cells and virally transformed cells seem to be severely deficient in PGE1. This is because a marker of many such cell lines seems to be loss of the ability to convert linoleic acid to gamma-linolenic acid (GLA), a necessary precursor of PGE1 (35,36). This key reaction is also defective in those on a high fat diet, in old age and in diabetes (37,38) suggesting that many in Western societies may have defective PGE1 formation.

There is recent evidence that PGE1 may inhibit the mobilization of arachidonic acid and so reduce formation of 2 series PGs. The effect was first shown in human platelets (39). A consequence is that a partial deficiency of EFAs is likely to lead to increased formation of 2 series PGs. This is because stores of DGLA are so small in relation to those of AA. During partial deficiency DGLA stores will be depleted first leading to loss of PGE1 formation at a time when AA stores are still very large. Loss of the control of AA will lead to increased 2 series PG formation. That this is not a purely theoretical assumption is shown by children with cystic fibrosis who do have a partial EFA deficiency with high circulating levels of 2 series PGs (40). Repletion with EFAs brings the high PG levels down to normal.

RELEVANCE OF PGE1 TO VITAMIN C ACTIONS: COLLAGEN

In scurvy there seem to be severe defects in both collagen and the intercellular ground substance. Failure of hydroxylation of proline and lysine has been thought to be involved but this single defect is unlikely to be the explanation for all the problems (41,43). For example, in women with metastatic breast cancer a single dose of vitamin C can sharply reduce urinary excretion of hydroxyproline, i.e. it stops the loss of already hydroxylated collagen (42). Attention has therefore been paid to the glycosaminoglycans (GAG) which seem to play a major role in stabilising collagen fibrils and possibly protecting them from degradation. There could be either reduced GAG synthesis or enhanced GAG degradation due to hyaluronidase and a variety of lysosomal enzymes. The evidence that GAG breakdown may be enhanced has recently been reviewed (43).

An aspect of vitamin C deficiency which has been largely ignored is the major contrast between marginal vitamin C deficiency and severe deficiency. In a first class study of voluntary vitamin C deficiency in humans all the subjects developed some or all of the features of Sjögren's syndrome (44). Sjögren's syndrome consists of the triad of dry eyes, dry mouth and chronic arthritis. The salivary and lacrimal glands become heavily infiltrated with lymphocytes and fibrosed. It is often very difficult to distinguish between the pathological lesions and malignant lymphomas. Salivary secretions are sharply reduced in contrast to the excess salivation which is characteristic of scurvy. The syndrome is often associated with other "connective tissue" diseases in which there is excessive fibrosis. That this is not an isolated report is shown by the observation that collagen synthesis is also exaggerated in marginal vitamin C deficiency in guinea pigs (45). There is some evidence that the lymphocytic infiltrations and fibrous tissue formation in Crohn's disease are associated with partial vitamin C deficiency (46).

There is thus a remarkable parallel between the apparent effects of PGE1 deficiency on fibrous tissue formation and those of vitamin C deficiency. Moderate PGE1 deficiency and moderate vitamin C deficiency are both associated with excess fibrous tissue formation. On the other hand severe deficiencies of both are associated with failure of normal connective tissue function and wound healing. Since vitamin C is able to regulate PGE1 synthesis the idea that the vitamin effects are mediated via changes in PGE1 levels is an attractive one. This is particularly so when it is remembered that moderate levels of PGE1 enhance GAG synthesis and so are likely to stabilize collagen and connective tissue whereas high PGE1 levels may inhibit it (29,30,31). The effects of vitamin C on connective tissue may therefore be explained by its effects on PGE1 and possibly ultimately by the effects of PGE1 on cyclic nucleotides and calcium.

PGE1, VITAMIN C AND THE IMMUNE SYSTEM

There is no doubt that severe vitamin C deficiency leads to susceptibility to infections, possibly particularly those caused by viruses. It also leads to a defect in the ability to reject transplanted organs (47). There is evidence that vitamin C is required for formation of a thymic factor which is necessary for immune function (48,49). Partial vitamin C deficiency leads to Sjögren's syndrome (44). Sjögren's syndrome is particularly associated with failure of T lymphocyte function often with excess activity of the B lymphocytes (54). This is believed to be because of a failure of cells known as T suppressor cells whose role is to regulate B cell function and to prevent excess antibody formation. A failure of T cell function may be responsible for many allergies and T cell defects have been described in a wide variety of diseases including the so-called auto-immune diseases rheumatoid arthritis, various cancers, Crohn's disease, multiple sclerosis, diabetes and so on.

The evidence that PGE1 may be able to activate T cells and may be required for normal cellular immune responses and for control of B cells was briefly reviewed earlier. If ascorbic acid is required for PGE1 synthesis, then a deficiency in PGE1 formation could account for a high proportion of the immunological defects which have been associated with vitamin C deficiency.

PGE1 AND OTHER FEATURES OF SCURVY

The features of scurvy described in the literature are legion but they include ready bruising and haemorrhage possibly due to platelet deficiencies because of excess platelet consumption, hair loss and skin abnormalities, dental caries and loss of teeth, diabetes and elevated cholesterol. All of these might be explained by a failure of adequate PGE1 formation.

PGE1 is produced by platelets (50,10). It inhibits platelet aggregation and may be important in preventing abnormal aggregation with a consequent fall in platelet count and failure of normal haemostatic mechanisms. 1 series PGs seem particularly important in the maintenance of normal skin. This has been conclusively demonstrated in cats where the hair loss and skin problems which occur in essential fatty acid deficiency can be controlled by provision of precursors of 1 series PGs alone (51,52). In the cat this is possible because DGLA cannot be converted to arachidonic acid and the 2 series PGs in this species. The hair loss and skin problems which occur in zinc deficiency may also be related to a failure of PGE1 formation.

The effects of vitamin C deficiency on saliva production and on dental caries are of extraordinary interest. While vitamin C deficiency seems to cause progressively increasing periodontal disease and dental damage, the action on saliva is biphasic. One of the first features of vitamin C deficiency in otherwise healthy individuals is dryness of the mouth with reduction of saliva production (44). In contrast Lind in his major treatise and all other descriptions of scurvy mention that excessive salivation is a feature of the disease. How does the PGE1 concept relate to the dental problems? There is rather striking evidence from a completely unexpected direction which indicates that PGE1 may be important for dental health. Lithium at concentrations used clinically has a selective and potent action in reducing PGE1 formation (14). It has recently been observed that the use of lithium leads to a substantial increase in dental caries coupled with reduction in salivation, a situation similar to that in moderate vitamin C deficiency (53).

The control of elevated cholesterol levels by vitamin C has been described (57,58). Part of the effect may relate to mobilisation of cholesterol deposits and it is therefore important to note that in atherosclerotic individuals there may be an initial rise in cholesterol levels (58). There is good evidence that an adequate intake of essential fatty acids can reduce cholesterol levels. The recent finding that loss of the ability to form GLA (and therefore PGE1), and of the ability to control by a negative feedback mechanism the activity of HMGCoA reductase occur simultaneously in cancer cells is of particular interest (35,36). HMGCoA reductase is a key rate limiting enzyme in cholesterol biosynthesis. One of a number of possible explanations is that PGE1 is necessary to allow the feedback to operate.

Similarly the reduced glucose tolerance and resistance to insulin characteristic of scurvy may be related to PGE1 deficiency. PGE1 and insulin have a number of common actions on metabolism and PGE1 seems necessary for a recently described effect of insulin on smooth muscle (30).

CANCER

The relationship between vitamin C and cancer is of major scientific and public interest and has recently been the subject of a major review (43). Early descriptions of scurvy suggest that, contrary to what might have been expected, there is enhanced cell proliferation with lack of the normal lines of demarcation between organs. Not mentioned in the review was the relationship between vitamin C deficiency and Sjögren's syndrome (44). This is a notorious example of a situation where the lymphocyte proliferation in the salivary or other glands is often extremely difficult to distinguish from malignant change. For example the latest edition of Harrison's Principles of Internal Medicine states of those with Sjögren's syndrome: "These patients are considered to have a disorder falling between neoplasia and hyperplasia which is diagnosed as pseudolymphoma" (54).

The roles of vitamin C deficiency in the development of cancer and of high vitamin C intake in the treatment of cancer (43,55,56) are subjects of major controversy. However the case for an adequate trial of such a non-toxic substance is very strong. The concept that vitamin C enhances PGE1 formation strengthens this case in two major ways. First there is strong evidence that the mechanisms whereby the immune system may naturally eliminate cancer are dependent on effective T lymphocyte function. PGE1 activates T lymphocytes. Second it has recently been demonstrated that a number of transformed cell lines lose the ability to convert linoleic acid to gammalinolenic acid (35,36). Loss of this enzyme may be a marker of malignant change. One consequence of it is that cancer cells are unable to make their own GLA, DGLA or PGE1 because they cannot utilise precursor linoleic acid. The significance of this observation is only beginning to be explored but the possibility that it is related to defective GAG formation and excess proliferation is a real one. High vitamin C levels would be expected to counteract the defect, partly by enabling cancer cells to make the best of any residual DGLA available to them and possibly also by enabling non-cancer tissues to increase their formation of PGE1 which might possibly regulate cancer growth. At present it must be stressed that these are no more than plausible possibilities.

CONCLUSIONS

The effect of vitamin C on PGE1 formation provides a plausible explanation for a number of the consequences of vitamin C deficiency and for some of the reported desirable effects of high vitamin C intake. If the reaction is important in vitamin C action, the potential desirable effects of vitamin C will take place only if adequate amounts of DGLA are available. In the absence of DGLA, the administration of megadoses of vitamin C will be the equivalent of attempting to squeeze blood from a stone. The nutritional factors which determine DGLA availability are discussed in detail in a companion paper (38). Zinc, pyridoxine and the essential fatty acids themselves are obviously important. In cancer it may be necessary to provide GLA or DGLA directly and natural sources of these acids such as plants of the borage family and the seeds of the evening primrose deserve full exploration as both nutrients and therapies.

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