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THE PINEAL AND REGULATION OF FIBROSIS: PINEALECTOMY AS A MODEL OF PRIMARY BILIARY CIRRHOSIS: ROLES OF MELATONIN AND PROSTAGLANDINS IN FIBROSIS AND REGULATION OF T LYMPHOCYTES.

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ABSTRACT

Pinealectomy leads to increased formation of fibrous tissue in the abdominal cavity, increased skin pigmentation and elevated cholesterol and alkaline phosphatase levels. It also leads to reduced formation and/or action of prostaglandin (PG) E1 and thromboxane (TX) A2. PGE1 plays an important role in enhancing function of T suppressor lymphocytes which control overactive antibody-producing B lymphocytes. In primary biliary cirrhosis there are increased skin pigmentation, hepatic fibrosis, elevated cholesterol and alkaline phosphatase levels, defective T lymphocytes and hyperactive B lymphocytes. Primary biliary cirrhosis may be a pineal deficiency disease. Serotonin is important in the pineal and the serotonin antagonist methysergide may cause retroperitoneal fibrosis by interfering with pineal function. There is a good deal of other evidence which suggests that melatonin, PGE1 and TXA2 are important in the regulation of fibrosis in other situations such as "collagen" diseases, lithium-induced fibrosis and cardiomyopathies. This suggests that enhancement of formation of PGE1 and of TXA2 may be of value in diseases associated with excess fibrosis and defective T suppressor cell function. PGE1 levels may be raised by zinc, penicillin, penicillamine and essential fatty acids. TXA2 levels may be raised by low dose colchicine. These new approaches to treatment may prove safer and more effective than existing ones. They may be of value in disorders such as cardiomyopathy, Hodgkin's disease and other lymphomas, multiple sclerosis, Crohn's disease, atopy and other diseases in which defective T cell function is suspected.

INTRODUCTION

Prolactin, zinc, penicillin and penicillamine have actions in the perfused superior mesenteric vascular bed of the rat which are consistent with stimulation of the formation of prostaglandin (PG) E1 from its precursor, dihomo-gamma-linolenic acid (DGLA). The agents may enhance conversion of esterified DGLA to free DGLA which can then rapidly be converted to PGE1 (1-4). The effects of the agents vary markedly with the season and in an attempt to identify

the source of this variation we investigated the effect of pinealectomy (5,6). Pinealectomy in summer had little effect but in winter converted the responses to a summer pattern. Melatonin in winter had relatively little effect but in summer and in pinealectomized animlas at any time it converted responses to the winter pattern. Melatonin had actions which were consistent with increased formation of thromboxane (TX) A2 and with enhanced effects of prolactin, zinc and penicillin on PGE1 (Fig. 1).

Because we were cannulating the superior mesenteric artery we could not avoid noticing that many of the pinealectomized animals had excessive fibrous tissue formation in the abdominal cavity. Instead of being freely mobile the organs were frequently firmly stuck together and the liver and spleen were hard in consistency and sometimes fixed to the under surface of the diaphragm (7). The picture was remarkably similar to that of the retroperitoneal and other forms of fibrosis which can occur in humans during prolonged treatment with methysergide (8,9). Methysergide fibrosis has never been satisfactorily explained. It may be relevant that methysergide is a serotonin antagonist and that serotonin plays a major role in the pineal.

These observations led us to consider the possibility that disorders of melatonin, PGE1 and TXA2 regulation could be important in human disease in which fibrous tissue production is disordered. The most dramatic example is primary biliary cirrhosis.

PRIMARY BILIARY CIRRHOSIS

Primary biliary cirrhosis is a disease of unknown origin mainly affecting women. The intrahepatic bile ducts are destroyed and much of the liver is replaced by fibrous tissue leading to death within 2-10 years in most cases. Itching and darkening of exposed areas of the skin are the common early features being followed by progressively severe indications of liver damage. Apart from the expected changes in bilirubin, the outstanding laboratory findings are elevated alkaline phosphatase and cholesterol levels, defective T lymphocyte function and excess formation of a variety of antibodies, notably an unusual antimitochondrial antibody (10-13).

The increased pigmentation has a variety of possible causes but a lack of the skin-lightening substance, melatonin, is one of them. Our rat studies of pinealectomy suggest that fibrosis could also be related to a melatonin deficiency. We have recently shown that even relatively early after pinealectomy blood alkaline phosphatase and cholesterol levels are markedly elevated (7).

There is a great deal of evidence that PGE1 is a major factor in the regulation of T lymphocyte function. This has been summarised elsewhere (2,14) but includes the following observations. 1. PGE1 itself may cause T cell maturation in vitro (15). 2. Prolactin and zinc, which are both able to enhance PGE1 synthesis, cause T cell maturation and thymus growth (16,17). 3. Cortisol, which inhibits formation of all PGs, inhibits T cells and causes thymus atrophy. 4. Lithium,which selectively inhibits formation of 1 series PGs (2,18), causes thymus atrophy (19). 5. PGE1 treatment controls adjuvant arthritis, an experimental syndrome associated with defective T cell and excessive B lymphocyte activity (19). 6. PGE1 treatment controls auto-immune disease in NZB/W mice which again is thought to be due to defective T and

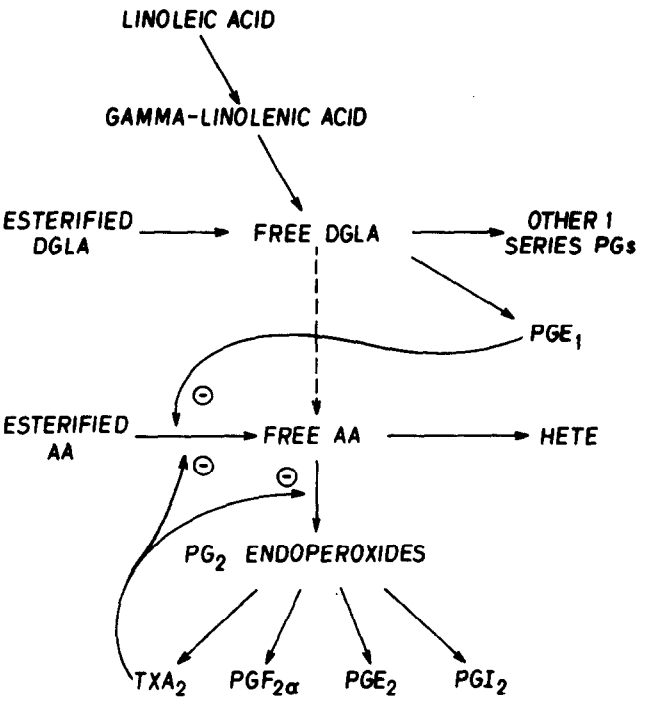

Fig. 1. An outline of prostaglandin biosynthesis and its regulation. The work of Brenner indicates that the conversion of linoleic acid to gamma-linolenic acid is inefficient. Feinste et al (27) have shown that PGE1 may be able to inhibit formation of 2 series PGs and we have proposed that thromboxane (TX) A2 may be a negative feedback regulator of the 2 series PG pathway (2, 26). Zinc, penicillamine and colchicine may, by different mechanisms, all enhance conversion of esterified DGLA to free DGLA.

and excess B cell function (20,21). 7. Penicillamine which appears to enhance PGE1 formation (4), can reduce excess antibody formation in primary biliary cirrhosis (13).

When considered together these observations strongly suggest the possibility that primary biliary cirrhosis may be related to inadequate melatonin production with consequent reductions in PGE1 and TXA2 synthesis. The female preponderance may also be explained on this basis since it has recently been demonstrated that progesterone is able to antagonise the actions of PGE1 (22). This may account for the curious finding that the thymus has a potent system for metabolising progesterone (23). This system might protect the thymus against progesterone-induced damage due to PGE1 antagonism (2). Another curious observation which also points to a role for the pineal in regulation of fibrosis is that collagen synthesis in the skin of patients with scleroderma is significantly higher in the summer (24) when pineal activity is reduced. This indicates that primary biliary cirrhosis may not be the only disorder in which defective regulation of fibrous tissue formation by melatonin, PGE1 and TXA2 play a part.

OTHER FIBROUS TISSUE DISORDERS

In these other situations the evidence is much less striking than in the case of primary biliary cirrhosis. However in most cases there have not been any specific investigations directed towards finding roles for melatonin, PGE1 and TXA2 in fibrosis. In view of this the amount of evidence is rather surprising.

1. The evidence that human and mouse systemic lupus erythematosus (SLE) may be related to defective formation of TXA2 and PGE1 has been reviewed in detail elsewhere (2,25). Since both TXA2 and PGE1 seem to be involved in inhibition of the synthesis of other 2 series PGs (Fig. 1) (2, 26,27, 28) lack of TXA2 and PGE1 will be associated with excess formation of other PGs such as PGE2 and PGF2α. Of all the pieces of evidence in favour of this concept of SLE, the most striking are the successful treatment of mouse SLE by PGE1 (20) and the finding that drugs which cause the SLE syndrome in humans behave as TXA2 synthesis inhibitors or antagonists (25).

2. In animals which have reduced essential fatty acid intake, collagen biosynthesis in inflammatory lesions is strikingly enhanced (28).

3. Lithium treatment, which selectively inhibits synthesis of 1 series PGs, is associated with renal fibrosis (29).

4. In patients with cardiomyopathy there is a failure of T suppressor cell function, a factor which is probably involved in the high incidence of lymphomas following cardiac transplantation in this group (30).

5. Penicillamine, which seems able to enhance PGE1 formation, can reduce collagen levels in human skin (31,32).

THE PROBLEMS OF ZINC, GLUCOCORTICOIDS AND SCHIZOPHRENIA

There are three groups of observations which initially seem to argue strongly against the idea that PCE1 deficiency can lead to excessive fibrosis.

1. Zinc deficiency is associated with defective collagen formation. Zinc seems to be important in enhancing PGE1 biosynthesis and many of the features of zinc deficiency can be prevented by stimulating PGE1 formation in other ways (33).

2. Glucocorticoids inhibit formation of all PGs including PGE1 and are associated with defective collagen formation.

3. Schizophrenics seem unable to form PGE1 normally (34,35,36) and they often have fragile skin with formation of striae on stretching.

These three observations all point to the idea that far from *inhibiting* excess collagen formation, PGE1 may be *necessary* for normal collagen formation. Can the two series of observations be reconciled?

Fortunately they can and in a surprisingly logical way. A number of the biological actions of PGE1 follow what is sometimes called a "bell-shaped" dose response curve. This has been best documented in the case of the PGE1 effect on vascular smooth muscle (37,38). In this model, as PGE1 concentrations are increased, so the amount of intracellular calcium released on activation rises, reaches a peak and then is reduced again. This means that the effects of very low and very high concentrations of PGE1 are identical. If PGE1 effects on collagen are similar then it is to be expected that collagen formation will be reduced both by very high and very low levels of PGE1. In Fig. 2 is shown a hypothetical model which could account for the observations. Peak muscle effects of PGE1 seem to be in the 10^{-11} - 10^{-12}M range whereas PGE1 levels in body fluids may be in the 10^{-10}M range. We therefore suggest that in normal individuals PGE1 levels are to the right of the peak of the curve. If a similar curve is involved in regulation of collagen, then as PGE1 levels fall, collagen synthesis will first of all be increased. If the fall progresses and very low PGE1 levels occur, then collagen synthesis will fall again and may become defective. The model therefore predicts that excess fibrosis will be associated with moderately reduced PGE1 levels and that both very low and very high PGE1 concentrations will lead to defective connective tissue formation.

THERAPEUTIC STRATEGIES FOR REGULATING FIBROSIS AND T LYMPHOCYTE FUNCTION

The preceding arguments suggest that fibrosis may be inhibited and T lymphocytes activated by enhancing PGE1 formation. It is possible that penicillamine, whose mechanism of action in these two areas is not understood, could be acting by enhancing PGE1 synthesis. If so, some of the difficulties of using penicillamine may be explained because penicillamine also chelates divalent cations, including zinc, an action which would be expected to interfere with the effect on PGE1 synthesis. The work discussed in this paper suggests safer strategies for achieving an enhanced synthesis of PGE1.

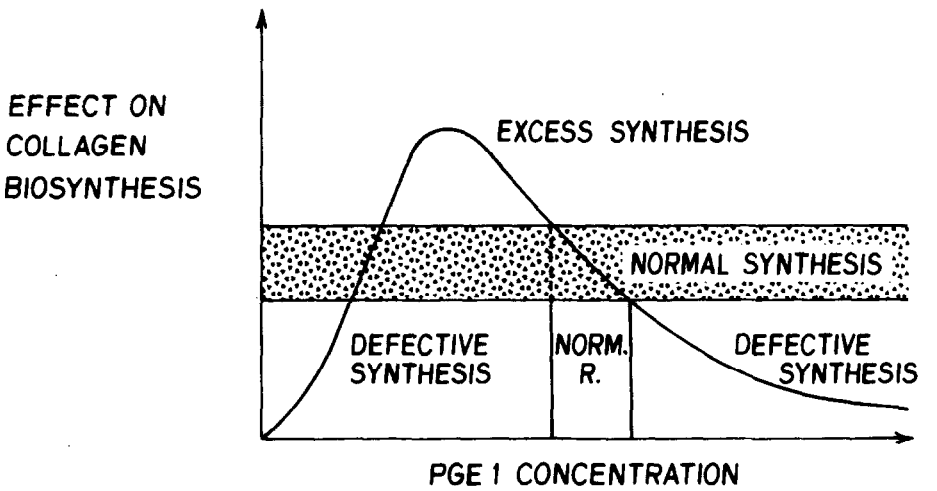

Fig. 2. The proposed relationship between PGE1 levels and collagen biosynthesis. As with many other effects of PGE1 we suggest that the dose/response curve is bell-shaped with the effects of PGE1 excess and PGE1 deficiency being similar. There is at present inadequate information relating to the precise PGE1 concentrations which constitute the normal range but we believe that they are in the 10^{-10} to 10^{-11} M region. The model predicts that collagen synthesis will also be normal at a second, lower range of PGE1 concentrations. Norm. R. indicates normal range.

1. Increased intake of essential fatty acids (EFAs). EFAs are the precursors of PGs with DGLA giving rise to 1 series PGs and arachidonic acid to 2 series PGs (Fig. 1). There is virtually no DGLA or gamma-linolenic acid (GLA) in most foods and so most of the DGLA in the body must be formed from linoleic acid. Until recently it was believed that linoleic acid intake was adequate but Brenner in particular has shown that this may not be the case (39,40,41). Only cis-linoleic acid can serve as a PG precursor and as much as 30-40% of the linoleic acid in Western diets may be in the trans form. Moreover conversion of cis-linoleic acid to cis-GLA can be blocked by high fat and carbohydrate intake, by cholesterol, by lack of insulin, by ageing and by the trans acids. Because of this inefficient conversion it is possible that many people on a Western diet are deficient in GLA and therefore in DGLA. It therefore seems appropriate to by-pass this block by giving GLA or DGLA directly. One way in which this can be done is to give evening primrose oil which is unique among vegetable oils in containing 8-9% of GLA (2,55). The oil can be administered even to those with inadequate digestion since it can be absorbed through the skin surface. An appropriate dose might be 2-4 ml of the oil per day.

2. Zinc. DGLA is stored in the body in various lipid esters. In order to be utilised it must be converted to free DGLA and zinc ions seem to be important in this process (3). Increased zinc intake should therefore accompany an increased EFA intake. It has already been shown that in malnourished humans zinc can enhance thymus growth and restore normal lymphocyte function (42). In the disease acrodermatitis enteropathica in which there is a defect in zinc absorption there is also lymphocyte failure which can be overcome by high zinc doses which overcome the absorption block (43). In otherwise normal individuals 30-40 mg of zinc per day in the form of zinc sulphate or zinc gluconate should be sufficient

3. Penicillamine and penicillin. Both penicillin and penicillamine seem able to increase the formation of a substance with the characteristics of PGE1. Penicillamine has already been shwon to activate T lymphocytes and to reduce collagen formation in humans. It is possible that the much less toxic penicillin, which does not chelate divalent cations, may have similar effects but to our knowledge this has not been tested. Penicillin, particularly when combined with evening primrose oil, has recently been found to have a therapeutic effect in schizophrenia in which there seems to be a PGE1 deficiency (44,45).

4. PGE1 or an analogue. PGE1 is unlikely to be effective therapeutically because it is almost all removed from the circulation during one passage through the lung but more stable analogues might be active. However the surprising success of PGE1 in treating NZB/W mice raises the possibility that it could be used itself.

5. Melatonin or cholchicine. Melatonin is readily available and could be administered to humans , although a short half life may be a problem. An alternative approach may be to use colchicine. We screened a large number of drugs in the mesenteric preparation and to our surprise found that low concentrations of colchicine had actions like melatonin which were consistent with enhancement of TXA2 synthesis (46) and enhancement of the effects of zinc and prolactin on PGE1 formation. Only later did we find that colchicine and melatonin can bind to the same sites on

microtubules (47). Relatively high doses of colchicine far above the therapeutic range in human body fluids have the opposite effect to melatonin on melanocytes (48,49). It is possible that this may be an example of an agonist/antagonist effect of colchicine with much lower colchicine concentrations having a melatonin-like effect on TXA2 and PGE1. Colchicine may also enhance the biological actions of PGE1 since it has been shown to enhance substantially the effect of PGE1 on cyclic AMP in fresh human granulocytes (50). Whatever the mechanism there is already excellent empirical evidence that in vivo colchicine in low doses has highly desirable effects in inhibiting inflammatory disorders, enhancing defective T cell function and suppressing overactive B lymphocytes. Colchicine is effective in Behçet's syndrome (51) and in familial Mediterranean fever: in the latter disease it is remarkably successful in preventing renal failure due to immune complex deposition (52). In animals it can suppress amyloid formation following casein injections (53,54) and there is preliminary evidence that it may be of value in multiple sclerosis (55,56). In preliminary experiments we have shown that colchicine given with evening primrose oil can suppress formation of excess fibrous tissue in pinealectomised rats.

In summary therefore it seems that an appropriate safe strategy to enhance T lymphocyte function, suppress overactive B lymphocytes and reduce fibrosis would be to combine evening primrose oil, zinc and colchicine. Penicillin or penicillamine, the latter possibly in much lower doses than are now used, could further enhance the efficacy of this regime. It is possible that this approach could be of value in a wide variety of diseases associated with defective T lymphocyte function, including primary biliary cirrhosis, various "collagen" disorders, cardiomyopathies, atopic disorders, Crohn's disease and Hodgkin's disease and other lymphomas.

Finally it should be noted that although in this paper we have referred to collagen biosynthesis we are aware of the possibility that some of the effects discussed may relate to collagen degradation rather than synthesis. However at present there is no adequate information about prostaglandin or melatonin effects on collagen breakdown.

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