Review

Hepatic proliferation inhibitor

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Summary

Although a long held tenet of biology has been that endogenous inhibitors can modulate cell proliferation, little progress was made in purifying any such inhibitor. This was largely due to the rarity of non-malignant cell cultures in which regulation of cell division was still operative, and to problems in separating cytotoxic and cytostatic effects in the complex biological extracts which were being studied. During the last decade, hepatic proliferation inhibitors of varying degrees of purity have been isolated using regenerating rat liver or hepatoma cell cultures as test systems. In these early studies, a number of inhibitors with differing molecular weights, physicochemical properties and biological responses were purified from liver cytosol and/or serum. Some of them could inhibit DNA synthesis or mitosis and thus were considered to be G1 or G2 inhibitors. However, experiments which could give precise answers about mechanisms of action could not be done until an inhibitor purified to homogeneity was available.

Using well-characterized rat liver diploid epithelial cell cultures, which maintain a number of liver properties and which do not possess any transformation markers or malignant properties, we recently purified an hepatic proliferation inhibitor to a homogenous protein. It has a molecular weight of 26 000 daltons and an isoelectric point of 4.65. It specifically inhibits cell division and DNA synthesis in a number of non-malignant rat liver epithelial cell types, and has no effect on transformed liver cells, or hepatoma cells, in culture. Its effect is not mediated through destruction or sequestration of essential nutrients or calcium ions. Nor have preliminary experiments shown the hepatic proliferation inhibitor to interfere with the binding of epidermal growth factor to its receptors. The majority of the cells treated with the inhibitor are blocked in the G1 phase. Further experiments to study its mechanism of action and the inter-relationship, if any, between the cell cycle block induced by serum or nutrient deprivation, and the inhibitor-induced cycle block are in progress.

1. General

It is becoming increasingly clear that cell proliferation and differentiation are, to a major extent, regulated by the microenvironment in which the cells are localized (22, 33, 38, 94, 95). Many of the recent studies in the area of proliferation control have been done in model systems in vitro using a variety of cultured cells. Such studies have shown that cell division can be inhibited and cells made quiescent by the removal of either serum (growth factors), or nutrients, from the culture medium. However, relatively little is known about the effects of adding proliferation inhibitors to the medium. The sparsity of well-executed studies of the latter type using inhibitors of cell proliferation is due to the difficulty of separating the cytotoxic and cytostatic effects of such factors, as well as to the stringent requirement that tissue-specific cell types in which the regulatory mechanism(s) are still opera-
tive be used as target cells, i.e., availability of non-malignant cells. In contrast, growth stimulatory factors, nutrients and ions which have been shown to be important in the control of cell proliferation (4, 11, 28, 29, 51) can be tested for their positive effect on cells rendered quiescent by the removal of serum or the appropriate nutrients. As a result, a number of factors that stimulate cell proliferation have been isolated from various tissues and well characterized. Indeed, many of these purified growth factors are commercially available and are being used extensively to investigate various aspects of cell biology.

Despite experimental difficulties in investigating proliferation inhibitors, such studies cannot be neglected since it is believed that negative control of cell proliferation (51, 92, 94) plays an important role in the carcinogenic process (8, 37, 81–83). Although much has been published about inhibitors of cell proliferation in different cells and tissues, almost all of these inhibitors were far from pure, and studies involving inhibition of cell proliferation have been the subject of both controversy and criticism (1). Recently, a number of research groups have succeeded in overcoming many of the problems and are making significant progress in this area.

2. Hepatic proliferation inhibitors

This category includes (a) inhibitors isolated from and acting on hepatic cells, (b) inhibitors from plasma and serum which act on hepatic cells and (c) inhibitors isolated from liver but which act only on extrahepatic cells. In this review, we dealt in detail only with the first two types. Examples of type c include factors derived from human and rat liver which were found to inhibit DNA synthesis in HeLa cells (68), and L-929 fibroblasts (21, 30, 46).

2.1. Hepatic inhibitors of hepatic cell proliferation

In a number of reviews on chalone (15, 31, 53, 68, 78, 103, 104), hepatic proliferation inhibitors (of type a and b) were also described. Work done before 1973 was described in detail by Lozzio et al. (53). Recently, Nadal (68) and Verly (104), who were involved in active research on serum and liver-derived hepatic chalone respectively, have summarized their work and that of others who worked specifically with hepatic proliferation inhibitors. We shall not attempt to describe all of them here, but have tabulated the salient features, together with some more recent publications, in Tables I and 2. Information on factors of type a, i.e., derived from, and acting upon, hepatic cells is given in Table 1. In this Table both 'inhibitor' and 'assay' are separately described, since a clear understanding of both of these is essential to an understanding of the validity of the research done in this area. The parameters used have been taken either directly from the publication, or have been extrapolated from the data presented therein. The effective dose for in vitro assays is given as units/ml of the culture medium, and for in vivo assays as units/g of body weight of the animal. In cases where the route of injection, weight of the animal and the dose injected were not reported, the entry 'not clear' is made. From Table 1, it can be seen that there are a number of variables with respect to the inhibitor, as well as the assay, used by different authors. In some cases, crude homogenates of rat liver (5, 17, 45, 59, 70) were tested in chicken embryo liver (17, 59), livers of baby rats (70), or in rat hepatoma cells in culture (5, 45). In all other studies, at least some degree of purification was attempted. The initial isolation or homogenization of the liver tissue was generally done in water, with very few exceptions (5, 23, 45, 80), where either phosphate buffered saline or sucrose was used. In two cases, effluent fluid from isolated perfused liver was used as the starting material (63, 79). After high-speed centrifugation, the supernatant fluid was further purified using a number of separation techniques based mainly on molecular size. The resultant materials, after one or more of the separation techniques, possessed different degrees of biological activity as reflected by the large variation seen in the effective doses (Table 1), which in most cases were inversely proportional to the degree of purity. The biological activity is also dependent on the type of target cells and the assay systems used (see Section 4). Large increases in inhibitor activity could also be due to the removal of proliferation stimulators known to co-exist in some of these preparations (18, 23, 56, 60).

The physicochemical properties of the material purified by different methods and by different research groups did show considerable variation. Most of the hepatic proliferation inhibitors isolated were polypeptides or proteins, but some were found