SHORT COMMUNICATION

Growth inhibitory and cytotoxic effects of melatonin and its metabolites on human tumour cell lines *in vitro*

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It has been shown in a number of experimental studies that the pineal gland and, more specifically, the pineal indole hormone melatonin, can modulate tumour growth. Most of these reports (for example, Narita & Kudo, 1985; Regelson & Pierpaoli, 1987) refer to experimentally induced mammary carcinomas in rodents. Interestingly, while tumour growth inhibition following *in vivo* administration of melatonin has most frequently been described, there are also reports of tumour growth stimulation by the hormone (Hamilton, 1969; Stanberry *et al.*, 1983). Similarly, Meyskens & Salmon (1981), when describing the *in vitro* response of clonogenic melanoma cells from patients to melatonin, reported stimulation of colony formation in two of 11 cases, no effect in samples from three patients and a decrease in total cloning efficiency with increasing concentrations of melatonin in the remaining six cases. This activity of melatonin has been variously ascribed to a direct cell killing effect on the cancer cells, an inhibitory effect on pituitary hormones involved in mammary cancer, a regulation of polyamine synthesis or pteridine metabolism or an immunomodulatory role (Ebels, 1980; Fraschini *et al.*, 1980; Lissoni *et al.*, 1987). Although melatonin undoubtedly has an influence on tumour development, interest in its bioactivity has, until recently, centred almost entirely on its effects on reproduction. The presence of gonadal melatonin receptors suggests that melatonin can directly affect the gonads and thereby alter reproductive status (Cohen *et al.*, 1978). In view of these data, we decided to investigate whether melatonin and a series of other naturally occurring pineal indoles could directly suppress the growth of ovarian carcinoma cell lines *in vitro*.

Melatonin was first isolated from bovine pineal glands in 1969 (Lerner *et al.*, 1969) and its chemical structure was determined. It was found to have (see Figure 1) a very short biological half-life and an *N*-acetyl and a 5-methoxy group are considered structural requirements for its bioactivity. Since the C5 and C6 positions of melatonin are the sites of metabolic activity, the block of one or both of these sites should result in an analogue with an increased half-life. Melatonin can be metabolised by two main routes (Young *et al.*, 1985): (i) by 6-hydroxylation to form 6-hydroxymelatonin and (ii) by demethylation to form *N*-acetyl-serotonin (see Figure 1). Our initial laboratory investigations appeared to be consistent with these facts since they indicated that while melatonin (*N*-acetyl-5-methoxytryptamine) and *N*-acetyl-serotonin (*N*-acetyl-5-hydroxytryptamine) exerted significant *in vitro* growth inhibition against both SK-OV-3 and JA-1 ovarian carcinoma cell lines only at relatively high concentrations of approximately 0.5–1.0 mg ml⁻¹, two other pineal methoxyindoles, 5-methoxytryptamine (5-methyl-serotonin) and 6-hydroxymelatonin, proved approximately five times more effective and 5,6'-dihydroxytryptamine (a synthetic compound of similar structure to a structural isomer of 6-

hydroxymelatonin) appeared at least 10 times more potent (Leone *et al.*, 1988).

We have now confirmed these observations (see Table I) and in addition quantitated the cytotoxic effects of these compounds on the SK-OV-3 cells using soft agar clonogenic cell survival assays (Courtenay & Mills, 1978) (see Figure 2). These survival assays used conditions of continuous exposure of the cells to the test compounds, which were added into the agar. The IC₅₀ concentrations (i.e. those required to reduce survival by 50% of control, solvent-treated cells) ranged from 16 to 940 µg ml⁻¹. These values correspond well with those derived from growth inhibition assays, after 72 h *in vitro* incubation of the cells plus test compound (see Table I). Thus it would appear that these compounds have definite cytotoxic and not just cytostatic effects, as judged by the longer term clonogenic assay data. One of the major problems in using these compounds was their relative lack of solubility in aqueous solvents. This accounts for the range and, in some cases large s.e. values, of the figures quoted in Table I.

In this present study, we have also evaluated these indoles against two other human tumour cell lines, not of ovarian origin, namely the RT112 line derived from a transitional carcinoma of the bladder (Masters *et al.*, 1986) and the MCF-7 cell line derived from an adenocarcinoma of the breast (Soule *et al.*, 1973). In terms of growth inhibition, melatonin and *N*-acetyl serotonin exerted very similar effects on all the cell lines tested (see Table I). The other four compounds, however, proved considerably more growth inhibitory against the RT112 cells, as opposed to the other three cell lines. We therefore have no evidence that any of these compounds exerted any preferential growth inhibitory effects against ovarian carcinoma cells *in vitro*. We failed to observe any inhibition of cell growth in our MCF-7 cells at low concentrations of melatonin within the range of 10⁻⁸ to 10⁻¹⁰ M, as reported recently (Blask & Hill, 1986; Hill & Blask, 1988). However, since these authors attributed these effects to a complex interaction with hormones such as oestradiol and prolactin, this apparent discrepancy may be explained by differing experimental conditions using serum-containing medium and/or the source of the actual MCF-7 cells used of which there are many, as reviewed recently (Osborne *et al.*, 1987).

We showed previously (Leone *et al.*, 1988) by GCMS (gas chromatography mass spectrometry) analysis that two of the most active compounds *in vitro*, namely 6-hydroxymelatonin and 5,6'-dihydroxytryptamine, were very poorly solubilised and the former compound also proved very unstable both at 4°C and at 37°C under our *in vitro* assay conditions. Indeed, 6-hydroxymelatonin exerted significant cytotoxicity at barely detectable levels (i.e. <1 µg) obtained by GCMS analysis. In an attempt to identify melatonin derivatives that were readily water soluble and stable at 37°C, we next tested the 6-sulphate and 6-glucuronide conjugates of melatonin metabolites, normally formed in the liver and rapidly excreted in the urine. These conjugates were isolated from rat urine as described earlier by Leone *et al.* (1987). The 6-sulphatoxy-melatonin proved totally inactive in inhibiting the growth of

Figure 1 The biosynthetic pathway of the pineal indole hormone melatonin.

Table I Growth inhibitory effects of melatonin and various melatonin metabolites and related compounds, following a 72-h continuous exposure *in vitro*, on four human tumour cell lines

Test compounds ^a	SK-OV-3 cells (Mean $\pm$ s.e.) ^b	GI ₅₀ values (μ g ml ⁻¹)		
		JA cells Mean ^c (Range)	RT112 cells (Mean) ^d	MCF-7 cells (Mean)
Melatonin	505 $\pm$ 134 (450) ^e	371 (312–480)	525	400
N-Acetyl serotonin	538 $\pm$ 88 (940) ^e	455 (380–530)	325	200
5-Methoxytryptamine	158 $\pm$ 36 (125) ^e	112 (91–125)	39	130
6-Hydroxymelatonin	165 $\pm$ 78 (125) ^e	120 (100–140)	20	305
Tryptamine hydrochloride	79 $\pm$ 14 (65) ^e	100 (80–120)	24	100
5,6-Dihydroxytryptamine	18 $\pm$ 2 (16) ^e	18 (17–19)	4	23

^aThe molecular weights of the test compounds are as follows: melatonin 232; N-acetyl serotonin 218; 5-methoxytryptamine 190; 6-hydroxymelatonin 248; tryptamine hydrochloride 197 and 5,6-dihydroxytryptamine 177; ^bMean of at least 3 individual experiments; ^cMean of 2–3 individual experiments; ^dMean of 2 individual experiments; ^eIC₅₀ values derived from colony-forming assay data shown in Figure 2.

Figure 2 Survival of SK-OV-3 cells assessed by colony formation in soft agar with continuous exposure to melatonin (○—○), N-acetyl serotonin (●—●), 6-hydroxymelatonin (△—△), 5-methoxytryptamine (▲—▲), tryptamine hydrochloride (◆—◆) and 5,6-dihydroxytryptamine (□—□). Each point represents the mean of at least two estimations $\pm$ s.e.

either JA-1 or SK-OV-3 cells at concentrations up to 1 mg ml⁻¹, and although on one occasion growth inhibition was noted with the extracted 6-glucuronide conjugate (Leone *et al.*, 1988), this effect was not reproducible when further samples of this metabolite isolated from urine were tested (Leone *et al.*, 1987) or when the synthetic material (unpublished method, A.M. Leone) was evaluated at concentrations up to 100 μ g ml⁻¹.

These data therefore indicate that one of the primary metabolites of melatonin, namely 6-hydroxymelatonin appears to exert significantly greater cytotoxicity than melatonin itself, but any potential usefulness as an anti-tumour agent is severely limited by its poor solubility and instability in aqueous solution. However, in view of the anecdotal reports of benefit of melatonin in early clinical trials of some human tumours, reviewed recently (Regelson & Pierpaoli, 1987), and the fact that it can be administered *in vivo* with extremely low toxicity towards normal tissues (i.e. 3–6 g of melatonin have been given orally daily for one month with minimal side-effects (Papavasiliou *et al.*, 1972)), it may prove worthwhile to attempt to identify or synthesise more stable analogues of 6-hydroxymelatonin.

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