Biochemical Pharmacology, Vol.32, No.15, pp. 2363-2365, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

PRELIMINARY COMMUNICATIONS

1-NAPHTHOL: A POTENTIAL SELECTIVE ANTITUMOUR AGENT

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(Received 8 April 1983; accepted 14 April 1983)

A major problem in cancer chemotherapy is the lack of selective toxicity of many commonly used anti-cancer agents towards tumour tissue compared to normal tissue.[1] This is due primarily to the fact that many of these drugs, such as the alkylating agents, attack rapidly dividing tissues of the host as well as the tumour. Another area of concern is the refractory nature of many solid tumours to established drugs. In particular, colorectal cancer patients have a poor prognosis and only 5-fluorouracil is the standard single agent with a significant, although limited, benefit in advanced disease.[2] In this study, 1-naphthol is shown to exhibit a selectively toxic action in short-term organ cultures of human colonic tumour tissue compared to normal tissue from the same patients. In addition, this compound is cytotoxic in vitro to both cell lines and xenografts derived from human colonic tumours. Based on our studies, we suggest that 1-naphthol and related derivatives may be of potential benefit in cancer chemotherapy.

In short-term organ cultures, human colonic tumour tissue was markedly more sensitive than normal tissue, from the same patient, to inhibition of protein synthesis by 1-naphthol (Figure 1). A half maximal inhibitory effect was evident at 0.76 mM for tumours but was not reached until 2.1mM in normal tissue. Selective toxicity towards the tumour has been apparent in 19 out of 24 patients so far studied. The limited value of 5-fluorouracil in

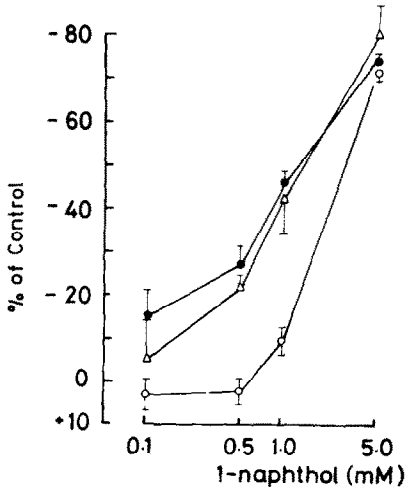

Fig. 1. Effect of 1-naphthol on protein synthesis of short-term organ cultures of human colorectal tumours and corresponding normal tissue from the same patient and of human colorectal tumour xenografts. Tissue taken at surgery was set up in short-term organ culture, according to the method Autrup et al,[4] as were three human colonic adenocarcinoma xenografts passaged in nude mice. Cytotoxicity was assessed by measuring inhibition of protein synthesis using a 2 hour pulse of ^{3}H-leucine at the end of a 24 hour treatment period. This graph shows the means and s.e.m. for 19 colorectal tumours (●—●) and normal tissue (o—o) and the three xenografts (▲—▲). Values for control cultures in normal tissue varied from 10,000 to 75,000 dpm/mg protein whilst tumour tissue varied from 4,500 to 45,000 dpm/mg protein.

the chemotherapy of colorectal carcinoma was reflected by its lack of selectivity in our culture system (results not shown). Short-term organ cultures of human colonic tumour xenografts also showed a dose-dependent inhibition of protein synthesis with 1-napththol (Figure 1) similar to that observed with colonic tumours cultured immediately after removal at surgery. Although these concentrations of 1-naphthol may seem rather high, the compound has been given to humans at doses of 6 grams per day for 3 successive days in the treatment of hookworm disease with very little toxicity.[3]

In order to further investigate the potential antitumour activity of 1-naphthol, its action on a human colonic adenocarcinoma cell line was also studied. LoVo cells showed a dose-dependent inhibition of protein synthesis with 1-naphthol (Figure 2). This inhibition of protein synthesis correlated with cytotoxicity assessed by trypan blue exclusion (results not shown).

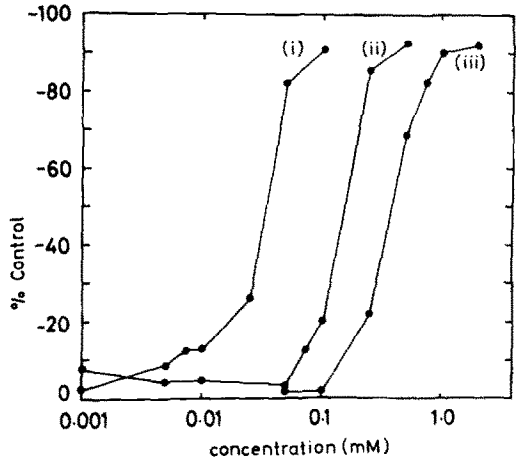

Fig. 2. Effect of 1-naphthol and naphthoquinones on protein synthesis in LoVo cells. LoVo cells, a human colonic adenocarcinoma cell line,[5] were grown as monolayers in Hams F-12 medium containing 10% foetal bovine serum in a humidified 5% CO_2 atmosphere in air at 37°C. Cytotoxicity was assessed by monitoring protein synthesis inhibition, as described in the legend to Fig. 1, of cells seeded into microtitre plates. The graph shows the percent change compared to control cultures for six replicates at each dose of (i) 1,4-naphthoquinone, (ii) 1,2-naphthoquinone and (iii) 1-naphthol.

In the above studies 1-naphthol exhibits both a marked inhibitory effect on protein synthesis in a variety of different model tumour systems and also an apparent selective toxicity to short-term organ cultures of human colorectal tumour tissue compared to normal tissue. The mechanisms for this selective toxicity are not yet clear. Our previous studies showed that short-term organ cultures of human lung squamous cell tumours metabolised 1-naphthol (used in our earlier study as a model phenolic substrate) predominantly to the glucuronic acid conjugate, whilst normal lung formed the sulphate ester conjugate.[6] A similar, but not as dramatic, difference was evident between normal colon and colonic tumours.[7] It is conceivable that the greater production of glucuronic acid conjugates in tumour tissue may selectively deplete this tissue of UTP, as UDP-glucuronic acid is an essential co-factor for glucuronic acid conjugation.

Selective uptake of 1-naphthol into tumour tissue, as was found for a structurally related compound, Synkavit, (2-methyl, 1,4-naphthaquinol bis-disodium phosphate)[8] could explain the differential toxicity between normal and tumour tissue. However, the uptake of Synkavit into tumour cells was due to higher levels of alkaline phosphatase on tumour cell membranes.[9] As yet we have no evidence for the selective uptake of 1-naphthol in our systems.

A more likely explanation for the selective toxicity is the formation of cytotoxic quinones. These could be formed by hydroxylation of 1-naphthol to 1,2- or 1,4-dihydroxy-naphthalene, which may then be subsequently oxidised to their respective quinones. Several other known anticancer agents, such as adriamycin, daunorubicin, mitomycin C, streptonigrin and lapachol, possess quinone groups and their toxicity may be mediated by formation of semiquinone radicals and active oxygen species or by quinone binding to critical cellular macromolecules.[10]

Some support for the involvement of 1,2- and or 1,4-naphthoquinone in mediating the toxic effects of 1-naphthol has been suggested by (i) their greater toxicity in our culture systems (Figure 2), (ii) a marked potentiation of the toxic effects of 1-naphthol by dicoumarol, an inhibitor of NAD(P)H quinone reductase,[11] an enzyme known to protect against the cytotoxic effects of quinones[12] (results not shown). In addition, the tumour tissue may be more susceptible to the cytotoxicity of the quinones as certain tumours are deficient in

superoxide dismutase,[13] glutathione peroxidase[14] and catalase.[15]

Direct covalent binding of naphthol or metabolites to cellular macromolecules is another possible cause of toxicity. Using radiolabelled naphthol we have shown a dose dependent increase in covalent binding in LoVo cells which correlates with an increased toxicity. It was also noted that the tumour tissue always bound more naphthol than the normal tissue. These results suggest that both covalent binding and production of active oxygen species may be involved in naphthol toxicity.

Although the mechanism of toxicity of 1-naphthol remains unclear, it is apparent from the selectively toxic action described in this study, as well as its toxicity to a variety of different model tumour systems in vitro, that 1-naphthol and related analogues may be of value in cancer chemotherapy.

Acknowledgements

This work was supported by a grant from the Cancer Research Campaign of Great Britain. We thank the Department of Pathology, St. Marks Hospital, for the supply of colorectal tissue. We acknowledge Dr. B. Drewinko for permission to utilise the LoVo cell line and thank Dr. B. Hill (ICRF) for supplying the cell lines. We are grateful to Mr. M. Jones, Institute of Cancer Research, for supplying the human colonic tumour xenografts. We are indebted to Miss C. E. Arthur for the preparation of this manuscript.

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