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# SYNTHESIS AND ANTITUMOR ACTIVITY OF A POLYMERIC DERIVATIVE OF COLCHICINE

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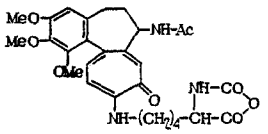
UDC 615.277.3:547.944.6].012.07

Colchicine drugs arrest mitosis at the metaphase by damage to the spindles. They are effective by either local or oral administration [2]. However, the high toxicity of colchicine prevents its use in clinical oncology.

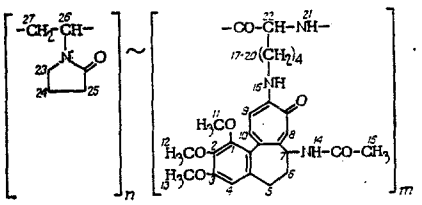
With a view to reducing the toxicity and improving the therapeutic activity of this alkaloid, we have synthesized a block-copolymer (CLVP) from N-vinylpyrrolidone (VP) and colchicidyl-L-lysine N-carboxyanhydride (CL-CA) [4]. CLVP consists of the water soluble carbon chain and polypeptide blocks bearing colchicine in the side chains. CLVP is therefore of interest as a novel biodegradable physiologically active polymer. This opens up the possibility of changing the lengths of the polymer blocks in this molecule to control its properties in the body, including its ability to undergo enzymic cleavage.




CL-CA

CL-CA was obtained by phosgenating colchicidyl-L-lysine in suspension in a dry inert solvent.

CLVP was synthesized by anionic polymerization of CL-CA using a polymeric initiator obtained from VP.




CLVP

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 0091-150X/88/2210-0744$12.50 © 1989 Plenum Publishing Corporation

TABLE 1. Effects of CLVP on the Growth of Transplanted Tumors

| Tumor strain | Test compound | Dose, mg/kg | Mean tumor mass, g | Inhibition of tumor growth, % | 1 − P to control |
|---|---|---|---|---|---|
| Walker's carcinoma | CLVP | 200 | 24,5 | 25,0 | 0,740 |
| | Control I | 0,3 | 17,0 | 46,0 | 0,808 |
| | Control II | | 32,1 | — | — |
| Sarcoma 45 | CLVP | 200 | 7,5 | 15,0 | 0,668 |
| | Control I | 0,3 | 6,0 | 30,0 | 0,668 |
| | Control II | | 8,5 | — | — |

TABLE 2. Effects of CLVP and Colchicine on Body Weight in Tumor-bearing Animals

| Tumor strain | Test compound | Change in body wt. of the animals after the test, g | 1 − P to control I |
|---|---|---|---|
| Walker's carcinoma | CLVP | −19 (−27) | 0,999 |
| | Control I | −38 (−27) | 0,999 |
| Sarcoma 45 | CLVP | −10 (−11,5) | 0,640 |
| | Control I | −38 (−27) | 0,999 |

Note. Change in body weight in control II animals given in brackets.

The structure of the CLBP was confirmed by comparing its UV and PMR spectra with those of the starting materials. The UV spectrum of CLVP shows characteristic absorption at 355.6 and 413.2 nm for the colchicine moiety [6]. The PMR spectrum of CLVP contains the following proton signals (δ, ppm):

1,82 (24-$CH_2$, $CH_2CH_2CH_2CH_2$), 2,14 (15-$CH_3$, 23-$CH_2$), 2,50 (25-$CH_2$, 5-$CH_2$), 3,48 (27-$CH_2$, 6-$CH_2$), 3,68 (11-$OCH_3$), 4,00 (12- и 13-$OCH_3$), 4,40 (22-CH), 6,86 (10-CH), 7,28 (9-CH), 7,60 (4-$CH_3$), 8,02 (8-CH).

## EXPERIMENTAL (CHEMISTRY)

UV spectra were obtained on a Specord M40 spectrophotometer (East Germany) in water. PMR spectra were obtained on a Varian XL-100 (100 MHz, USA), in solution in $D_2O$, internal standard HMDS. The average molecular mass was measured on a Hitachi Perkin-Elmer-115 (Japan). The polymeric initiator was poly-N-vinylpyrrolidone with a terminal amino group, obtained as described in [1]. The average molecular mass of the polymeric initiator was 11,100.

Colchicidyl-L-lysine N-Carboxyanhydride (CL-CA). Gaseous phosgene was passed into a stirred solution of 1 g of colchicidyl-L-lysine in 50 ml of dioxane at 25°C for 1 h. The temperature rose gradually to 40°C, and the mixture was kept at this temperature for a further 3 h, until bright red crystals of the monomer began to separate. The product was collected at the filter, purified from impurities by reprecipitation from DMF with ether, and dried in vacuo to constant weight to give 0.8 g (77%) of CL-CA, mp 109-111°C. Found %: N 7.81. $C_{28}H_{33}N_3O_8$. Calculated %: N 7.79.

Polymerization. A solution of 1 g of CL-CA (1.7 mmole) and 0.8 g of the polymeric initiator (7 mmole) in 25 ml of DMF was kept under nitrogen at 25°C for 100 h. The resulting block-copolymer was isolated by precipitation with acetone to give 1 g (55.5%) of product, mp 243-245°C. The average molecular mass was 15,000, and the colchicidyl-L-lysine units constituted 58 wt. %. The composition of the block-copolymer was determined by UV spectroscopy, using a calibration plot of optical density at 355.6 nm versus colchicidyl-L-lysine content.

## EXPERIMENTAL (BIOLOGY)

The acute toxicity, and effects on tumor growth and body weight of CLVP in tumor-carriers were determined. The maximum tolerated dose was found by daily administration over a period of ten days to the tumor-bearing experimental animals. The CLVP was given in the maximum tolerated dose. The controls used were colchicine (control I) and physiological saline (II).

The animals were weighed before and after the test, with deduction of the mass of the tumor, and the tumor mass was measured at the end of the test. The acute toxicity and antitumor activity were assessed by standard methods [5]. The median lethal dose ($LD_{50}$) for CLVP by the intraperitoneal route was 800 mg/kg and the maximum tolerated dose 200 mg/kg, the values for colchicine being 1.6 and 0.3 mg/kg respectively. CLVP is therefore less toxic by a factor of 500 than colchicine.

The test results were evaluated statistically by the Student–Fisher and M. D. Mashkovskii method [3]. The results were regarded as significant when $1 - P = 0.950$ or more.

The tests were carried out with 110 mongrel white rats weighing 110-130 g. The antitumor activity of CLVP was examined on Walker carcinosarcoma 45 and sarcoma 45, transplanted into rats. The test compound was administered at day 3 to the animals with the Walker carcinosarcoma 45, and at day 7 with sarcoma 45, following transplantation of the tumor, for ten days intraperitoneally. The animals were killed under ether narcosis three days after the last dose of the compound.

The effects of CLVP on tumor growth with colchicine and physiological saline as controls are shown in Table 1, from which it will be seen that the test compounds (CLVP and colchicine) do not have statistically significant growth inhibitory activity.

Examination of the effects of CLVP and colchicine on the body weight of the experimental animals (Table 2) showed that in all the tests CLVP statistically significantly prevented weight loss, whereas colchicine under these conditions significantly reduced body weight in the tumor-bearing animals.

Hence, the block copolymer of colchicidyl-L-lysine with poly-N-vinylpyrrolidone is less toxic than colchicine, which shows that a search for antitumor drugs in this area holds promise.

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