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SYNTHESIS AND TUMORICIDAL ACTIVITY OF WATER SOLUBLE PORPHYRINYL-THYMIDINES AND RELATED PORPHYRINS

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ABSTRACT

The new cobalt(II)meso-5,10-di(N-methyl-4-pyridinium)-15,20-di(p-phenylene-5'-O-thymidine)porphyrin suppressed the growth of human malignant melanoma cells (M21-HPB) by 95 percent when applied as a 2.5 x 10^{-5}M solution in tris for 3 days without exposure to light. It was the most effective of the investigated porphyrinyl-nucleosides.

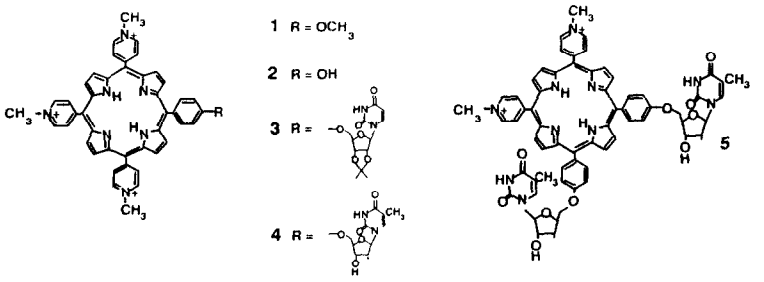
The synthesis of porphyrinyl-nucleosides in our laboratory[1] raised the question of their biological activity. The first representatives of this new class of compounds were insoluble in water which could greatly reduce their bioapplicability. This obstacle had been overcome[2,3] by obtaining the water soluble porphyrinyl-uridines containing N-methyl 4-pyridinium groups in the meso-positions not occupied by the nucleoside units. However, the problem of the synthesis of water-soluble porphyrinyl-thymidines still remained open.

We now want to report[4] the achievement of this goal and revealing of the antitumor activity of the cobalt(II) derivative of a particular water soluble porphyrinyl-thymidine.

Porphyrins[5-8] and nucleosides[9-11], i.e. the structures representing the components of the authors' porphyrinyl-nucleosides, are currently the subject of vigorous research. It concerns tumoricidal effects, antiviral activity, and inhibition of HIV. However, the bioactivity of porphyrinyl-nucleosides has not yet been studied.

The synthesized porphyrins include a porphyrin core which contains at least two N-methyl-4-pyridinium meso-substituents and a p-phenylene link to OCH_3, OH, 5'-O-2',3'-isopropylideneuridine or 5'-O-thymidine, as shown for 1-5. These compounds were then utilized as the free base or were metallated by Co(II).

Condensation of p-anisaldehyde, 4-pyridinecarboxaldehyde and pyrrole in propionic acid[12,13] gave in addition to other porphyrins and polymers the meso-(4-pyridyl)$_{4-n}$-(p-methoxyphenyl)$_n$porphyrins, n=1,2. They were separated by column chromatography on silica gel using $CHCl_3$/MeOH as an eluent. Demethylation with pyridine hydrochloride at 220°C gave the respective p-hydroxyphenyl derivatives. To prepare porphyrinyl-uridine **3** and porphyrinyl-thymidines **4** and **5**, the proper p-hydroxyphenylorphyrin was condensed with either 5'-O-tosyl-2',3'-O-isopropylideneuridine or 5'-O-tosylthymidine[14] in a DMF solution containing Cs_2CO_3 or NaH.



Metallation with Co(II) and N-methylation of 4-pyridyl substituents were achieved as previously described.[3] The products were characterized by elemental analysis and spectroscopic methods.[15] The compounds were investigated as solutions in tris buffer at 10^{-6}, 10^{-5} and 2.5 x 10^{-5}M concentrations.[16]

The free base porphyrin **2** containing the p-hydroxyphenyl substituent instead of nucleoside did not show any significant growth suppression of the malignant melanoma cells, see Figure 1. Metallation with Co(II) resulted in a small increase in the activity of **2** which was independent of concentration. However, the replacement of the p-hydroxyphenyl substituent by p-methoxyphenyl **1** resulted, in the case of free base porphyrin at 2.5 x 10^{-5}M, in the increase of growth suppression to 66% of the control, the phenomenom being concentration dependent. The metallation of **1** with Co(II) increased the growth suppression to 43% of the control. The free base porphyrinyl-nucleosides containing uridine **3**, and thymidine **4** and **5**, differed in their effect on the growth of the malignant melanoma cells depending on the type of nucleoside and the applied concentration. Porphyrinyl-uridine **3** at the lowest concentration (10^{-6}M), acted as a growth suppressant, 66% of control. It practically ceased that activity at 10^{-5}M and at 2.5 x 10^{-5}M and instead began to stimulate the growth of the malignant cells, 123% of control. Metallation with Co(II) reversed this trend in activity; an increase in suppression of the malignant cells took place with the increase of concentration, from 80% to 71% of the control, for the extreme concentrations. The free base porphyrinyl-thymidines **4** and **5**, contrary to porphyrinyl-uridine **3**, showed steady, although limited, enhancement with the increase of concentration, which for **4** reached 70% of the control at 2.5 x 10^{-5}M. The metallation with Co(II) of porphyrinyl-thymidine containing one thymidine unit, **4Co**, did not influence the mentioned activity much; however, the cobalt(II) derivative of porphyrinyl-dithymidine, **5Co**, showed strong concentration-dependent suppression of malignant cells: 53% of the control at 10^{-5}M and 5% of the control at 2.5 x 10^{-5}M.

The results achieved at this step of the investigation point out remarkable changes connected with (i) the presence of a particular nucleoside substituent, and (ii) metallation with Co(II). In the absence of cobalt in the porphyrin center, the porphyrinyl-nucleosides in question are either tumor growth suppressing or stimulating agents. This depends on the type of nucleoside and concentration, while after metallation they always behave as suppressants. The suppression of the growth of malignant melanoma

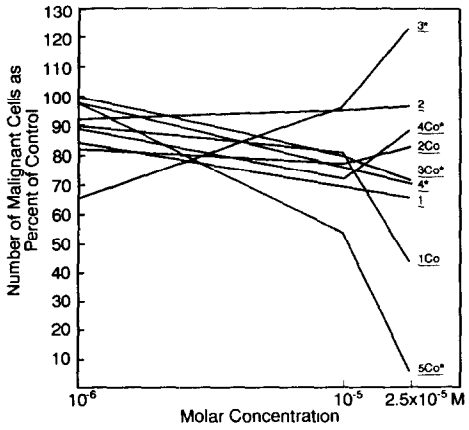


Figure 1. The number of malignant melanoma cells as percent of control as acted upon by the investigated porphyrins in tris buffer.

cells to 5% of the control by **5Co** makes this compound a proper candidate for a large scale investigation of its toxic activity. Although Co(II) ions themselves behave as malignant cell suppressants (to 80% of control for 2.5 x 10^{-5}M), the substantial differences in the activity of all cobalt-metallated porphyrins in question point to a predominant influence of the nature of the porphyrin system.

Meunier et al[6a] have found recently that a specific cytotoxicity of meso-metalloporphyrins containing N-methylpyridinium substituents and the p-nitro-, p-amino- and p-hydroxyfunctions depended for a particular central atom on the number of pyridinium groups. Porphyrins with three such groups were much more active toward murine leukemia cells L1210 in vitro than those containing two groups.[6b] The present results show that the suppression of the growth of malignant melanoma cells by cobalto-porphyrinyl-dithymidine **5Co** which contained two pyridinium groups was much stronger than that caused by cobalto-porphyrinyl-monothymidine containing three pyridinium groups. This points to much more specific influence of thymidine substituents than those of pyridinium substituents. In view of many previous reports[6c] it is reasonable to expect an interaction of the authors' compounds with DNA of both the N-methylpyridiniumporphyrin and thymidine (or uridine) units. The presence in the molecule **5Co** of two "terminal" thymidine units can alter the interaction with DNA as compared to one thymidine unit in **4Co**. One of the reasons for this can be different accessibility to the polynucleotide chain of the N-methylpyridiniumporphyrin structural fragment, which is hindered differently by the nucleoside unit(s) in **4Co** and **5Co**. Another reason is the possibility of the interaction of **4Co** and **5Co** via thymidine nucleobase with one or two polynucleotide chains, respectively. This can be important considering the fact that the DNA affinity is the key factor for the biological activity.[17]

References and Notes

1. Kus, P.; Knerr, G. and Czuchajowski, L., *Tetrahedron Lett.*, **1990**, *31*, 5133.
2. Czuchajowski, L., Habdas, J., Niedbala, H. and Wandrekar, V., *Tetrahedron Lett.*, **1991**, *32*, 7511.
3. Czuchajowski, L.; Habdas, J.; Niedbala, H. and Wandrekar, V., *J. Heterocyclic Chem.*, **1992**, *29*, 479.
4. Czuchajowski, L.; Niedbala, H.; Wandrekar, V.; Shultz, T. and Seaman, W., 204th Annual ACS Meeting, Washington D.C., Aug 23-28, **1992**, ORGN 274.
5. Pandey, R.K.; Smith, K.M. and Dougherty, T.J., *J. Med. Chem.*, **1990**, *33*, 2032.
6. (a) Ding, L.; Casas, C.; Etemad-Moghadam, G.; Meunier, B. and Cros, S., *New J Chem.*, **1990**, *14*, 421. (b) ID_{50} (μM) 0.7 vs 2.3 for Mn(III) derivatives; (0.8 for 5Co). (c) See: 6(a), refs. 4-11.
7. Huang, S.Q.; Jiand, Z.C.; Li, L.Z. and Yang, J., *Acta Pharm. Sinica*, **1991**, *26*, 415.
8. Dixon, D.W.; Schinazi, R. and Marzilli, L.G., *Ann. N.Y. Acad. Sci.*, **1990**, *616*, 511.
9. Yang, C.O.; Wu, H.Y.; Fraser-Smith, E.B. and Walker, K.A.M , *Tetrahedron Lett.*, **1992**, *33*, 37.
10. Sahlberg, C., *Tetrahedron Lett.*, **1992**, *33*, 679.
11. Iigo, M.; Nishikata, K-I.; Nakajima, Y.; Szinai, I.; Veres, Z.; Szboles, A. and DeClerq, E., *Biochem. Pharm.*, **1990**, *33*, 1247.
12. Salmon, L.; Verlhac, J-B.; Bied-Charreton, C.; Verchere-Beaur, C.; Gaudemer, A. and Pasternack, R.F., *Tetrahedron Lett.*, **1990**, *31*, 519.
13. Czuchajowski, L.; Bennett, J.E.; Goszczynski, S.; Wheeler, D.E.; Wisor, A K and Malinski, T., *J. Am. Chem. Soc.*, **1989**, *111*, 607.
14. E.g., a solution of meso-5,10-di(4-pyridyl)-15,20-di(p-hydroxyphenyl)porphyrin (255 mg, 0.39 mmol) and 5'-0-tosylthymidine (618 mg, 1.56 mmol) in DMF (150 mL) was added dropwise over a period of 2 h at 65°C to a solution of NaH (60%) (187 mg, 4.68 mmol) and Ce_2CO_3 (254 mg, 0.78 mmol) in DMF (100 mL). After stirring at that temperature for 24 h the mixture was cooled to room temperature and filtered The filtrate was added to water (500 mL) with methanol (150 mL) and extracted with chloroform (3 x 100 mL). The combined chloroform layers were washed with 5% NH_3 aq., then with water and dried. After evaporation of the solvent, the residue was chromatographed on silica gel column, $CHCl_3$/MeOH 20:1 applied as an eluent. The product appeared as the third fraction, yield 4%.
15. meso-Tri(N-pyridyl)-p-phenylene-5'-O-thymidineporphyrin: FAB-MS, $(M+1)^+$ 858m/z, 1H NMR(DMSO): 11.41(s, 1H, N[3]H), 9.04 (d,5.0Hz, 6Hpyrid), 8.89(s,8H,β-pyr), 8.26 (d,4.7Hz, 6Hpyrid), 8.15 and 7.44 (dd, 7.9Hz, 4Hphenylene), 7.71 (s,1H,H-6), 6.40-6.33 (m,1H,H-1'), 5.59 (d,4.1Hz,1H,H-3'), 4.57-4.28(m,3H,H-4',H-5'), 2.29(m,2H,H-2'),1.84(s,3H,C[5]CH_3),-3.00(s,2H,NHporph). N-methyl derivative, **4**: 1H NMR(DMSO) 11.44 (1H,N[3]H), 9.50 (d,6.5Hz, 6Hpyrid), 9.18 (m,3Hpyrid), 9.07-9.00 (11H, 3Hpyrid, 8Hβ-pyr), 8.20 and 7.53 (dd, 8.5Hz, 4Hphenylene), 7.74 (s, 1H, H-6), 6.39(tr,1H,H-1'), 5.60 (m,1H,H-3'), 4.74 (s,9H, N-CH_3), 4.57 and 4.32(m,3H,H-4',H-5'), 2.33(m,2H,H-2'), 1.86(s,3H,C[5]CH_3),-2.99(s,2H,NHporph); UV-vis(water)223,261,426(S),521,565,585,643; ($CHCl_3$) 259,419(S),549,587,646. meso-5,10-Di-15,20-(4-pyridyl)-15,20-di-(p-phenylene-5'-O-thymidine)porphyrin· FAB-MS, $(M+1)^+$ 1096 m/z 1H NMR(DMSO) 11.41 (s,2H,N[3]H), 9.04(d,5.3Hz,4Hpyrid), 8.93(s8Hβ-pyr), 8.26(d,5.4Hz, 4Hpyrid),8.16 and 7.45(dd,8.4Hz,4Hphenylene), 7.72(s,2H,H-6), 8.02 and 7.22(dd,8.2Hz,4Hphenylene), 6.36(tr,2H,H-1'), 5.59(m,2H,H-3'), 4.57-4.28(m,6H,H-4',H-5'), 2.29(4H,H-2'), 1.84(m,6H,C[5]CH_3,-2.95(s,2H,NHporph). N-methyl derivative, **5**: 1H NMR(DMSO) 9.37(m,4Hpyrid), 8.92(m,10H,2Hpyrid,8Hβ-pyr), 8.06 (m,4H,2Hpyrid,2Hphenylene), 7.94(d,8Hz,2Hphenylene), 7.64(s,2H,H-6), 7.40(d,2Hphenylene), 7.18(d,8Hz,2Hphenylene)6.28(tr,2H,H-1'), 5.58(m,2H,H-3'), 4.74(s,6H,N-CH_3), 4.50-4.41(m,6H,H-4',H-5'), 2.30(m,4H,H-2'),1.88(s,6H,C[5]CH_3),-2.98(s,2Hporph). **5Co**: UV-vis (water,316,436(S), 548.
16. Human malignant melanoma cells (M21-HPB) cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum, 50μg/mL gentamicine, 10μg/mL amphotericin, 50μM HEPES buffer, incubation at 35°C; pH of the medium maintained at 7.2-7.4. Initially 10^6 cells were plated in flasks maintained in the culture medium for 24 h. The medium was then replaced with medium alone or medium supplemented with the porphyrin in tris at 0 (control), 10^{-6}, 10^{-5} and 2.5 x 10^{-5}M. The control flasks contained anion concentration equivalent to that found in each porphyrin Cells were harvested following 3 days of incubation using phosphate-buffered saline containing 0.25% trypsin. Cell viability was determined using the trypan blue exclusion method. The cells were counted when the growth in the corresponding control flasks was 55-60% confluent and contained 12-14 x 10^6 cells
17. Ding, L.; Etemad-Moghadam, G., Cros, S.; Auclair, C. and Meunier, B., *J. Chem. Soc., Chem. Commun* ,**1989**, *1711.*