A STUDY ON SONODYNAMIC THERAPY-ANTITUMOR EFFECT OF NOVEL SONODYNAMIC COMPOUNDS UNDER ULTRASOUND

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Abstract ——— Diacetylhematoporphyrin-mitomycin C conjugate (diAc-Hp-MMC) (**1**) prepared by a condensation of diacetylhematoporphyrin with MMC in the presence of *N, N*- dimethylaminopyridine and dicyclohexylcarbodiimide, and two of the pyridocarbazole derivatives (**8**) and (**9**) were provided for sonodynamic compounds and these compounds were found to show excellent cell-killing effect, comparing to those of the known sonodynamc compound hematoporphyrin, Hp 2HCl (**2**), Di-Ac-Hp (**3**), Hp-Co (**4**), Hp-Fe (**5**), Acrinol (**6**) and MMC (**7**).

Hematoporphyrin (Hp) has been known to show antitumor effect owing to selective accumulation to malignant tumor and an ability to generate locally singlet oxygen under visible light irradiation.[1~4] Application of this photodynamic therapy has been limited to a surface treatment of a living tissue because of a poor ability of penetration of visible light. To overcome this problem, new method utilizing cavitation induced by ultrasound, sonodynamic therapy has several merits in terms of strong ability of penetration of the ultrasound to tissue and accurate focus on target region. It is known that use of Hp in sonodynamic study has showed remarkable antitumor effect by generating singlet oxygen, cell oxidizing agent, as well as its effect under visible light.[5~8] In this context , we have chosen several compounds such as diacetylhematoporphyrin-mitomycin C conjugate (diAc-Hp-MMC) (1)[9] and two of the pyridocarbazole derivatives (**8** and **9**)[10] as the candidates for sonodynamic compounds and their antitumor effectson Sarcoma 180 were compared with those of Hp-Co(**4**), Hp-Fe complexes (**5**),[11] acrinol (**6**), MMC (**7**), Hp (**2**) and Hp-diAc (**3**) under various ranges of the ultrasound power induced from the ultrasound irradiation system as shown in Figure 1[12] and the results were shown in Figure 2.[13] As found in Figure 2, both diAc-Hp-MMC (**1**) and pyridocarbazole derivatives (**8** and **9**) showed cell-killing effect (20 % for **1**, 30 % for **8** and 25 % for **9**) under 2.5 w, whereas the other compounds (**2, 3, 4, 5, 6** and **7**) did not show any effect. As the power is increased, cell-killing effect became stronger as their cell- killing rates being 58 % for **1**, 65 % for **8** and 70 % for **9** under 3.0 w, while 27~50 % for other compounds, respectively. Cell-killing rates were increased up to 72 % under 3.5 w for all of the compounds. It is worth while to describe that the compounds (**1, 8** and **9**) showed remarkable cell-killing effect even in a lower ultrasound intensity. These results suggest that compounds (**1** , **8** and **9**) effectively absorb ultrasonic energy and generate activated oxygen[14] which expresses cell-killing effect as well as that of Hp derivatives in photodynamic therapy. Allowing for the side effect of ultrasound to living body in sonodynamic therapy, high susceptibility of sonodynamic compounds to ultrasound power is important since prolong and high power irradiation of ultrasound result in indiscriminate collapse of normal cells and malignant cell.[6] Enhancement of sonodynamic effect under ultrasound irradiation with the compounds (**1, 8** and **9**) which were developed by us indicates that these compounds can be useful not only for the therapy of the superficial tumors, but also nonsupeficial tumors.

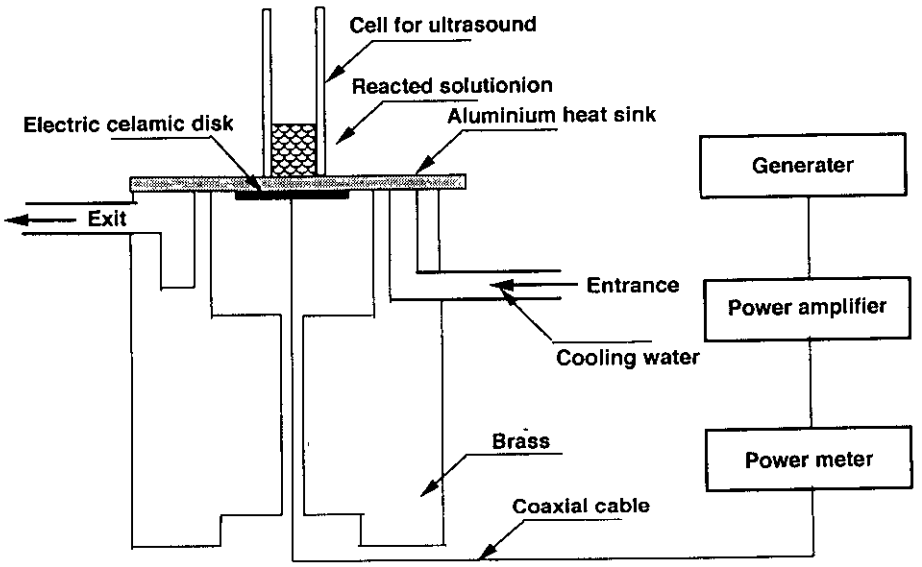
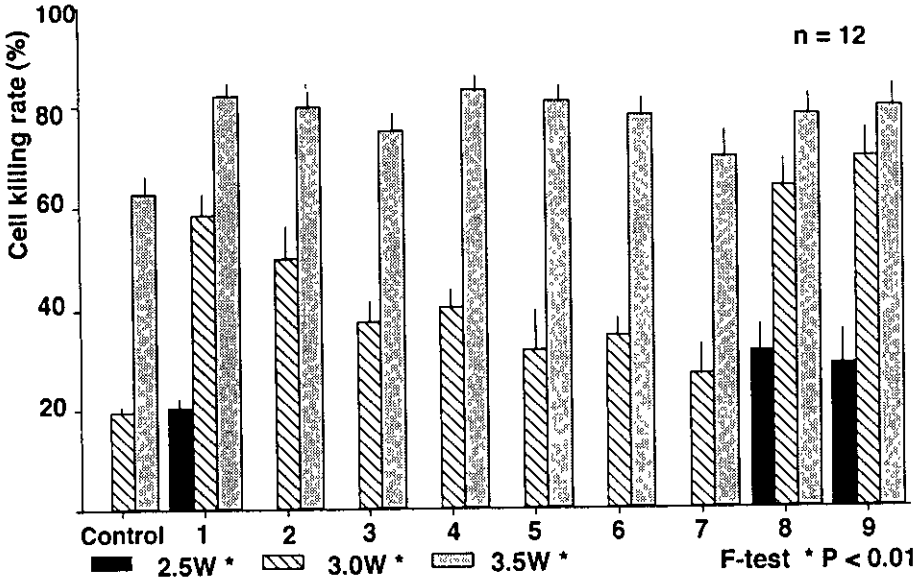


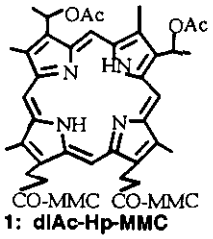
Figure 1. in vitro Insonation System



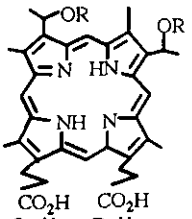
Control ; 1%DMSO in PBS 1; diAc-Hp-MMC 2; Hp·2HCl 3; diAc-Hp
4; Hp-Co 5; Hp-Fe 6; Acrinol 7; MMC 8; Pyridocarbazole-1
9; Pyridocarbazole-2

(All of the tested compounds were dissolved in 1%DMSO-PBS)

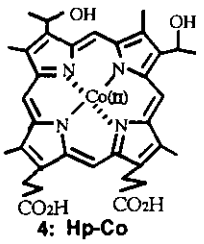
Figure 2. Effects of Ultrasound with all of the Tested Compounds on Isolated Sarcoma180 Cells



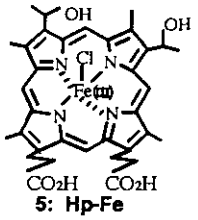
1: diAc-Hp-MMC



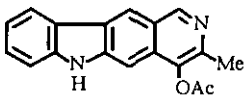
2: Hp, R=H
3: diAc-Hp, R=Ac



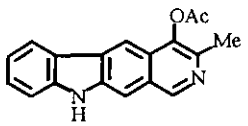
4: Hp-Co



5: Hp-Fe



8: Pyridocarbazole-1



9: Pyridocarbazole-2

REFERENCE AND NOTES

1. K. R. Weishaupt, C. J. Gomer, and T. J. Dougherty, *Cancer Res.*, 1976, **36**, 2330.
2. T. J. Dougherty, J. E. Kaufman, A. Goldfarb, K. R. Weishaupt, D. Boyle, and A. Mittlem, *ibid*., 1978, **38**, 2628.
3. M. Das, R. Dixit, H. Mukhtar, and D. R. Bickers, *ibid*., 1985, **45**, 608.
4. E. R. Bodaness and P .C. Chan, *J. Biol. Chem.*, 1977, **252**, 8554.
5. N. Yumita, R. Nishigaki, K. Umemura, and S. Umemura, *Jpn. J . Cancer Res.*, 1989, **80**, 219.
6. S. Umemura, N. Yumita, R. Nishigaki, and K. Umemura, *ibid*., 1990, **81**, 962.
7. N. Yumita., R. Nishigaki, K. Umemura, and S. Umemura, *ibid*., 1990, **81**, 304.
8. N. Umita, A. Okamura, R. Nishigaki, K. Umemura, and S. Umemura, *Jpn. J. Hyperthermic Oncol.*, 1987, **3**, 175.
9. E. Sato, T. Suzuki, M. Nishimura, K. Unno, T. Kato, and H. Mori, *The 41th Annual Meeting of Japanese Cancer Association* (OSAKA), August, 1982, p.406.
   DiAc-Hp-MMC was prepared as follows: Dicyclohexylcarbodiimide (228 mg, 1.11 mmol) was added portionwise to a solution of diAc-Hp (300 mg, 0.44 mmol), MMC (294 mg, 0.88 mmol) and 4-dimethylaminopyridine (135 mg, 1.11 mmol) in dry CH_2Cl_2 (100 ml) and the mixture was stirred for 6 h at room temperature under N_2 in the dark and the reaction mixture was chromatographed on silica gel (15% MeOH in $CHCl_3$) to provide diAc-Hp-MMC (255 mg, 0.19 mmol) in 44 % yield as dark brown powder. Ir ($CHCl_3$) 3500, 3380 ($CONH_2$), 1734, 1690, 1650 (C=O), 1600, 1570 (C=C) cm^{-1}; ^{1}H-nmr (270 MHz, $CDCl_3$) 1.58 (6H, *s* , MMC-CH_3), 2.29, 2.30 (6H, each *s* , $COCH_3$), 2.31~2.38 (6H, *m* , $CH_3CHOCOCH_3$ x 2), 2.92, 2.99 (each 3H, *s* , OCH_3), 3.58, 3.63, 3.76, 3.78 (each 3H, *s* , ring methyls), 7.48 ~ 7.60 (2H, m, $CH_3CHOCOCH_3$ x 2) 9.96, 10.09, 10.40, 10.46 (each 1H , *s* , meso); ms (FAB) *m/z* 1315 (*M*+1).
10. T. Kametani, Y. Ichikawa, T. Suzuki, and K. Fukumoto, *Tetrahrdron* , 1974, **30**, 3713.
11. R. Bonnet and M. J. Dimsdale, *J. Chem. Soc.,Perkin Trans. 1*, 1972, 2540.
12. Sarcoma180 cells (2 x 10^6) and each tested compound (25 μg) containing dimethyl sulfoxide to improve solubility were suspended in phosphate buffer solution (1 ml) saturated with oxygen. The output acoustic power in a standing wave from generator and power amplifier to ultrasound transducer with a resonant frequency of 2.26 MHz were calibrated in the power meter. Cell-killing rate was respectively measured in 60 sec after the insonation started and cell damage by insonation were detected by staining of the cells with trypan-blue dye, respectively.
13. Sarcoma180 in PBS were kept at 0°C during experiment.
14. This was certified by dramatical decrease of the cell-killing rates of the tested compounds in the presence of histidine and mannitol known as the activated oxygen scavenger.

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