Tumor Res. **15**, 1-10 1980

Porphyrins as Tumor Localizing Agents and Their Possible Use in Photochemotherapy of Cancer

A Review

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Chemicals with a selective affinity for neoplastic tissues may be taken advantage of in diagnosis as well as in therapy of cancer. One of the most promising classes of such compounds are the prophyrins. These compounds exhibit a characteristic red fluorescence, which is a useful property for their detection *in vivo*. Furthermore, the porphyrins have a strong photodynamic activity. Thus, biomolecules and cells are damaged when irradiated with light in the presence of porphyrins and oxygen. This makes photochemotherapy of certain forms of cancer possible. It has also been reported that porphyrins may sensitize tumors to ionizing radiations, and that porphyrin derivatives may be used as anticancer drugs.

Porphyrin accumulation in tumors

The first observation of porphyrin fluorescence from neoplastic tissue was made by Policard in 1924 (52). He reported that experimental rat sarcomas exhibited red fluorescence when they were excited with light. This was attributed to porphyrins formed endogeneously by bacteria infecting the tumors. Körbler (36, 37) detected a similar red fluorescence in excised breast carcinomas as well as in a number of superficially situated tumors, but found no evidence that bacteria were involved. Later a number of investigators have reported a preferential accumulation of porphyrins and porphyrin precursors in neoplastic tissues (54, 55, 70). It was shown that this accumulation is not due to an enhanced activity of the enzymes involved in the formation of heme (55), but reflects the affinity of tumors for porphyrins.

Auler and Banzer (4) seem to be the first to observe the accumulation of injected porphyrins in tumors. They found that hematoporphyrin injected in rats accumulated in primary as well as in metastatic tumors, but also to some extent in lymph nodes.

Figge and coworkers showed that a number of porphyrins has a selective

* This work was supported by the Norwegian Cancer Society.

affinity for neoplastic, embryonic and regenerating tissues in rodents (23). Mice with tumors tolerated higher doses of ^{65}Zn hematoporphyrin than did mice without tumors (42). This indicates that the tumors accumulated the porphyrin and thus protected critical organs from radioactive damage.

A number of investigations have been carried out by Lipson and coworkers to elucidate the possible aid of porphyrins in the detection of cancer (for references, see see (47)). They introduced the acetic acid — sulfuric acid derivative of hematoporphyrin hydrochloride (termed Hpd) and examined a number of cancer patients. Porphyrin fluorescence was observed in every case in which a malignant lesion could be reached by the activating light. Later studies including larger numbers of patients were similarly promising.

Gregorie and coworkers studied 226 cancer patients with the technique of Lipson (26). 76% of the malignant tumors showed fluorescence, while about 23% of the benign lesions also showed a faint fluorescence. They concluded that the method might be useful in locating the obscure foci of primary and metastatic cancer, notably squamous cell carcinomas. Recently Gomer and Dougherty injected ^{3}H- and ^{14}C-labelled Hpd in mice carrying tumors, and found that the tumors accumulated more Hpd and retained it for a longer time than did skin and muscle. However, the concentration of Hpd was higher in liver, kidney and spleen than in the tumors.

Winkelman (65) introduced tetraphenylporphine-sulfonate (TPPS) and claimed that it had a better tumor-localizing ability than Hpd. TPPS may be easily differentiated from and measured in the presence of endogeneous porphyrin (67). According to Winkelman (65, 67, 69) the tumor to tissue ratio of TPPS concentrations was significantly larger than unity for normal tissues, including liver, kidney and spleen. Recently Carrano *et al.* (8) studied the localization of highly purified TPPS in virus-induced murine sarcomas. Their work confirmed the tumor-localizing ability of TPPS, but the concentration in the kidneys was higher than found by Winkelman. TPPS is removed from the body at slower rates than Hpd. This may limit the clinical usefulness of this compound.

The attempts to couple radioactive elements to porphyrins, for scintillation scanning and radiotherapeutic purposes, have met with limited success. Altman and Salomon (3) reported that parenterally given bis (2-iodoethyl-^{131}I) deuteroporphyrin localized selectively in spontaneous and transplanted carcinomas in mice. The incorporation of metals in the porphyrins reduces their tumorlocalizing ability (66, 68). This probably explains the redults obtained by Bases *et al.* (7) who were unable to detect preferential uptake of ^{64}Cu-labelled porphyrins in human tumors.

The binding of porphyrins to tissue requires that two or more of the

side groups of the tetrapyrrole nucleus are capable of forming hydrogen or ionic bonds (6). The electron configuration of the hydrophobic nucleus itself also influences the binding properties. A free base porphyrin is flat and planar while a metalloporphyrin has a dz^2 metal orbital perpendicular to the molecular plane. This may be the reason why metalloporphyrins have a low tumor-localizing ability. Porphyrins with the radioactive label in their ring structure have binding properties similar to unlabelled porphyrins (25, 69). This is probably the key for future preparations of radioactively labelled porphyrins for external scanning purposes.

Recently a number of optical systems have been designed for the purpose of tumor detection by fluorescence excitation (16, 34, 40). By use of one of these devices the authors claim to be able to discern a Hpd mass density of 10 pg/cm^2 (16). Clinical trials showed that the system was capable of imaging small squamous carcinomas (2 mm diameter by 1 mm thick) 65 hours after injection of 2 mg/kg Hpd. It should be remarked that the porphyrin fluorescence is partly quenched in darkly pigmented tissues such as liver, kidney and spleen (25). Thus, when possible, radioactively labelled porphyrins should be used in quantitative measurements.

Summarizing the results cited above, nearly all investigators have observed that porphyrins accumulate in tumors. Some authors report that injected porphyrins concentrate in liver, kidney and spleen to a higher degree than in tumors, while the results of others disagree with this. The reason for the discrepancy seems to be that different porphyrins and probably different degrees of purity have been used. Porphyrins are difficult to purify, and this is a major obstacle in this research.

Why do porphyrins accumulate in tumors?

Generally, injected porphyrins bind to serum proteins and circulate in the blood until they are eliminated through the liver and/or through the kidneys. Cellular uptake of porphyrins is at least partly due to pinocytotic activity. This is indicated by the fact that a number of dyes, among them some porphyrins, are associated with lysosomes (1, 2, 35, 60). However, the intracellular localization of porphyrins does not follow a simple pattern. Uroprophyrin I is taken up by lysosomes (2), hematoporphyrin is evenly distributed in the cytoplasm (64) and TPPS associates mainly with the nucleolus (5). Therefore, even though it has been shown that malignant cells *in vitro* may take up larger amounts of porphyrins than normal cells (9, 47), it is likely that extracellular conditions in cancerous tissue are more important:

Kosaki and coworkers found some extracellular "bodies" in tumors (38, 39). These "bodies" had a high affinity for protoporphyrin, and it was

claimed that they contained a tumor specific phospholipid called "malignolipin". Some investigators have doubted this work (28, 51), while others have obtained results which partly confirm it as regards the presence of "malignolipin" in tumors and its porphyrin binding properties (30, 57). In correspondence with this is also the recent finding that the vascular stroma in tumors contain particularly high levels of Hpd after an injection (17).

Musser *et al.* (48, 49) have studied the distribution of TPPS in tumor-bearing mice and its binding to fibrin matrices. It has been shown that certain animal tumor cells when transplanted into a host become invested within a fibrin-gel-matrix, and the authors suggest that fibrin act as a binding parameter in porphyrin localization in tumors (49).

Finally, the fact that the pH of the interstital fluid in tumors is lower than the pH of the blood afferent to the tumors (27) influences on porphyrin binding in the tumor tissue. We have shown that the amount of hematoporphyrin associated with human cells of cancerous origin after 40 min incubation at 37°C was 30 and 50 per cent lower at pH 7.2 and 7.8, respectively, than at pH 6.8. Hematoporphyrin binding may also explain why this compound inhibits the respiration of liver homogenate at pH 6.5~6.6 but not at pH 7.0~8.9 (46).

Porphyrins in photochemotherapy of cancer

The attempts to use porphyrins in the treatment of cancer have proceeded along three main lines: a) exploring the potentiality of chemically modified porphyrins as anticancer drugs, b) determining whether or not they sensitize tissues to ionizing radiations and c) trying to use their properties as photosensitizers in combination with visible light.

Promising results from clinical trials with two porphyrins as anticancer drugs have been reported (50, 61). The compounds were diaquacobalt protoporphyrin (COPP) and mercury (II) hematoporphyrin disodium salt (Merphyrin). To our knowledge few such experiments have been carried out. A further evaluation of this must await a more thorough study.

A number of investigators have looked for a possible radiosensitizing effect of porphyrins *in vivo* (13, 41 and references cited in 13) and *in vitro* (21, 63). However, the results are partly conflicting and difficult to interprete. Thus, one must await more experimental results to evaluate the potentialities of porphyrins as radiation modifying agents.

The most promising aspect of porphyrins in cancer research is the development of photochemotherapy, taking advantage of the photosensitizing effect of the porphyrins as well as their tumorlocalizing ability. The fact that biomolecules and cells are destroyed by exposure to light in the presence of porphyrins has been known for more than half a century. (See review

by Spikes (60)). Red light, which excites porphyrins, penetrates relatively deeply into tumors (19). The light intensity 2 cm below the tumor surface is about 10% of the incident light intensity.

The first attempt to treat human skin cancer with fluorescing dyes and light was carried out shortly after the introduction of x-rays in cancer therapy (29). Auler and Banzer were the first ones to treat animal tumors with light after injection of porphyrins (4). The next attempt came nearly three decades later, with the work of Diamond and coworkers (15). They treated glioma cells both *in vitro* and transplanted s. c. in rats with administration of hematoporphyrin followed by irradiation with light and found that the glioma cells were killed. It should be remarked that hematoporphyrin does not cross the blood-brain barrier (63). Kelly and coworkers (31, 32) studied photodynamic destruction of human bladder carcinomas with Hpd as sensitizer. Hpd preferentially localized in malignant and premalignant epithelium, and irradiation with light through a quartz light guide inserted into the bladder caused tumor destruction. Human bladder tissues were transplanted to immunosuppressed mice and treated with Hpd and light. Marked response of the tumor but little response of normal bladder tissues resulted. Sery (59) found that retinoblastoma cells were sensitive to treatment with hematoporphyrin and light. Retinoblastoma is a cancer of the photoreceptor elements of the retina and is therefore well suited for phototherapy.

The most extensive work with phototherapy of cancer has been carried out by Dougherty and coworkers (18, 19, 20). Long-time cures were observed for four types of mouse and rat tumors exposed to light ($\lambda \approx 600$ nm) 24 or 48 hours after i. p. injections of Hpd (2.5～5.0 mg/kg body weight) (18). The report from the first clinical trial showed that 111 of 113 cutaneous or s. c. malignant lesions of different types responded to the photochemotherapy (19). A high therapeutic ratio between tumor and skin was obtained by allowing at least 3 days between Hpd injection and light exposure. Similarly promising results were obtained in another clinical trial, where recurrent breast carcinomas were treated in the same way (20). Several complete cures (3～4 years) have been observed in the work of this group. At present a number of research institutes and cancer clinics in many countries are involved in research aimed at phototherapy of cancer (see ref. 53). Modern laser- and fiberoptic technology will probably contribute significantly to this research.

Basic research on the mechanisms of sensitized inactivation of cells and tissues is of great importance at the present stage of development, and the information of such research may be taken advantage of in the clinical trials. An example of this is our findings that hematoporphyrin sensitized pho-

toinactivation of human cells *in vitro* becomes more efficient when the irradiation temperature is reduced below 37°C, and that a given total dose becomes more efficient when it is split (43). The explanation of the former observation may be that repair processes are more efficient at 37°C than at lower temperatures. The latter observation is due to the fact that the first part of a split dose gives a sublethal damage to the cells, mainly to their outer membrane. Thus the cells become "leaky", take up more porphyrin and become more vulnerable to the second part of the split dose. Transfer of damage between cells in close contact has been observed *in vitro* (11). Such a cooperative effect may indicate intercellular transport of lytic enzymes leaking out of damaged lysosomes. In fact, Allison *et al.* (1) showed that uroporphyrin I is localized in the lysosomes of monkey kidney cells and macrophages, and irradiation with light results in release of lysosomal enzymes. Furthermore, these authors demonstrate similar morphological changes of the treated cells as we do: swelling and development of cytoplasmic blebs (44). This observation may explain the efficiency of photodynamic cancer treatment. Parts of a tumor that receive too small light doses for direct inactivation may become indirectly inactivated by lytic enzymes or toxic products released from the inactivated cells. However, since Hpd is concentrated in the vascular stroma, it is also possible that damage to blood vessels contributes to tumor destruction.

The light induced reactions taking place in a cancer cell sensitized with a porphyrin have probably similarities with the reactions in a red cell of a patient with erythropoietic protoporphyria (EPP). These cells have an elevated concentration of protoporphyrin, due to a disorder in the porphyrin metabolism of the body, and are lysed when exposed to light. A number of authors have studied this phenomenon (for references, see (24)) and it seems that peroxidation of membrane lipids and crosslinking of membrane proteins play major roles in the lytic process. The ageresponse curve for NHIK 3025 cells treated with hematoporphyrin and light (10, 12) parallells the expected variation in membrane fluidity through the cell cycle (14). Kessel (33) has found a greatly disturbed permeability of the cell membrane after treatment with various porphyrins and light. Thus, a number of independent observations indicate that damage to membranes, either to lysosome membranes or to the outer cell membrane, is a factor of major importance in porphyrin mediated photodynamic inactivation of cells. The inactivating damage seems to be caused by excited oxygen molecules (singlet oxygen) arising from energy transfer from the photoexcited porphyrin molecules (44, 60). However, other photoproducts have been postulated to be of importance; such as hydroxyl radicals, porphyrin radicals, solvated electrons

and hydrogen peroxide. This will probably be elucidated in the near future. One should especially pay attention to the fact that the oxygen concentration is usually low in tumors, and therefore explore the reactions taking place under hypoxic conditions.

The main negative effect of porphyrin injections are the skin reactions caused by exposure to sunlight. The sensitivity to sunlight usually lasts for a few weeks after porphyrin injection. One should also be aware of the possible carcinogenic effect of porphyrin and light treatment (56). Hematoporphyrin does not bind to DNA but still induces strand breaks in DNA (45). At a given level of survival the frequency of single strand breaks is higher after x-irradiation than after photodynamic treatment. The same is true for the induction of sister chromatid exchanges (45). This indicates that the carcinogenic risk is larger after conventional x-irradiation than after photochemotherapy. However, other test systems for carcinogenicity should be applied in the future to test the validity of this statement.

In conclusion a number of research groups have shown that several types of tumors, even radioresistant ones, respond to photochemotherapy. Complete eradication of lesions has been observed. One may hope that testing of new purified porphyrins and improvements of irradiation equipment will make photochemotherapy an efficient mode of cancer treatment.

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