FLUORESCENCE OF HUMAN LYMPHATIC AND CANCER TISSUES FOLLOWING HIGH DOSES OF INTRAVENOUS HEMATOPORPHYRIN

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IT HAS been demonstrated that porphyrins have a tendency to accumulate in neoplastic, embryonic, and traumatized regenerating tissues of several species of animals.[1, 2, 3] It has also been shown that hematoporphyrin accumulates in lymph nodes and lymphatic tissues and tissues with a high mitotic index in general.[5] Attempts to utilize these findings in a practical way in three human subjects undergoing operations for head and neck cancer were unsuccessful.[5] One patient had received 30 mg. of hematoporphyrin, another received 60 mg., and a third received 120 mg. of hematoporphyrin intravenously. With these dosages neither the tumors nor the lymph nodes exhibited a sufficient degree of red fluorescence to be detectable.

In view of the spectacular results in animals, this observation was not understood. A careful study of the history of these patients revealed that the head and neck regions had been irradiated with roentgen rays prior to the porphyrin injections. It was postulated that such radiation procedures might have suppressed mitotic activity in these tissues and thus destroyed their affinity for porphyrin. Hematoporphyrin, however, accumulated in all lymph nodes of animals after partial body irradiation. The dosages of hematoporphyrin used in the case of animals were, however, proportionately much higher than the 120 mg. used on the human subjects. It was therefore thought that it might be feasible to demonstrate lymph nodes and cancer tissues in human subjects if the dosage could be increased to 500 to 1000 mg. Even though relatively high doses of porphyrins have been employed in dogs, rabbits, mice, rats, monkeys, and guinea pigs, it seemed desirable to proceed with caution in elevating the dose in human subjects, because of the reports of the toxicity of hematoporphyrin that appear in the literature.[4] The primary purpose of this investigation was to ascertain if the dosage of hematoporphyrin could be increased in human subjects to a level sufficiently high to be practical for demonstration of lymphatic and neoplastic tissues. It will be shown that it is indeed possible to give as high as 1 gm. of hematoporphyrin by the intravenous-drip method over a twenty-four-hour period. It was also possible to demonstrate that, when sufficiently high doses of hematoporphyrin are administered, the lymphatic and cancer tissues of human subjects fluoresce a brilliant red as compared with normal tissues of other types. Thus it has been possible to utilize this method to demonstrate and delimit lymphatic and cancer tissue in human subjects.

MATERIALS AND METHODS

Eleven human patients, whose ages ranged from 46 to 76 years were studied in this series. The criteria for selecting a patient were as follows: (1) nonsensitivity to hematoporphyrin as demonstrated by an intradermal skin test, and (2) suspected or definitely known carcinoma.

The intravenous route of administration was employed in all cases. Forearm veins were chosen because of the relatively long administration period. This interfered least with the patients' feeding and excretory problems. A no. 20 needle was introduced into the vein, and the drip rate was set between fifteen and twenty drops per minute. On the first few patients to receive the hematoporphyrin, the blood pressure and pulse were noted every fif-

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teen minutes during the administration. Both pressure and pulse remained stable, and it was further noted that there was no temperature aberration during or after the infusion.

The chemical used in all cases was hematoporphyrin hydrochloride (recrystallized). A unit for injection was prepared by dissolving the desired amount of hematoporphyrin in 600 cc. of 0.166 *M* sodium lactate, to which had been added 1 gm. of sodium bicarbonate. The hematoporphyrin was found to be readily soluble in this alkaline medium (*p*H 7.6). It should be noted that the solution used in these experiments contained no phenol and thus differs from commercially available hematoporphyrin solutions. The first unit contained 300 mg. of hematoporphyrin, whereas all other units contained 500 mg. of the drug. The resulting solution was doubly filtered (through filter paper) and bottled. The unit was then sterilized in a standard autoclave for fifteen minutes at 250° C.

A standard blood administration set was used to introduce the solution intravenously. The time of administration varied from three to ten hours with an average of six hours. Because it was sometimes impossible to know of the planned surgery more than twenty-four hours beforehand, some cases received their hematoporphyrin on the afternoon or evening prior to surgery. Others, because of cancellation of cases for various reasons, received their hematoporphyrin as much as seventy-two hours prior to surgery.

In order to demonstrate fluorescence, a near-ultraviolet spotlight was employed. The light was generated by a General Electric reflector spot quartz mercury-arc light, medical unit. A bright red fluorescence was indicative of a relatively high concentration of hematoporphyrin. Patients were examined under this spotlight prior to receiving hematoporphyrin in order to detect any naturally occurring red fluorescent areas. Some typical porphyrin red fluorescence was most commonly noted at the nasolabial folds (sebaceous secretions) and on the posterior portion of the tongue.

At the time of operation, the near-ultraviolet spot beam was directed at the lesion and its adjacent areas in the operative field. As the specimen was removed, it was more carefully examined and dissected under the spot beam in a completely darkened room. It was then possible to record pictorally the red fluorescence by employing a special photographic technique as follows:

TABLE 1

SUMMARY OF CASES STUDIED

Case no.	Postoperative diagnosis	Hematoporphyrin dosage, mg.	Red fluorescence
1	Squamous-cell ca. of tongue	300	+
2	Squamous-cell ca. of tongue	500	+
3	Adenoca. of prostate	500	−
4	Squamous-cell ca. of penis	500	+
5	Adenoca. of signoid colon with extension to urinary bladder	500	+
6	Ependymoma of cervical cord	500	−
7	Fibrotic breast abscess	500	−
8	Olfactory-groove meningioma	500	+
9	Adenoca. of rectum	1000	++
10	Adenoca. of ascending colon	1000	++
11	Ca. of breast	1000	++

1. *Specimen Photography.* Specimens were photographed in a darkroom using two near-ultraviolet spotlights as the exciting source. A 5-in. lens set at *f*/4.7 with a 7 in. bellows extension was employed. The system contained a 2A filter, and the film was Ektachrome daylight. Exposures varied from one to two minutes depending upon the amount of fluorescence.

2. *Patient Photography.* This technique was developed to record those cases in which relatively long exposure time was impossible. A 5-in. lens set at *f*/4.7 with a 7 in. bellows extension was again employed. The 2A filter was present as before. However, this time the exciting light was generated by a no. 50 flash bulb. A Corning filter no. 5970 covered the flash lamp. Exposure was made by open flash.

Successive increments of hematoporphyrin were used in order to observe tolerance cautiously. The first patient received 300 mg., the next seven patients received 500 mg., and the last three patients received 1000 mg. of hematoporphyrin (Table 1).

SUMMARY OF CASES STUDIED

There were two reasons for increasing the dosages in these patients: (1) no toxicity was noted when the smaller doses were given; and (2) the 300 mg. and 500 mg. dosages produced encouraging results, but it was felt that these could be enhanced with larger doses.

Case 4. This case was diagnosed as a carcinoma of the penis and afforded us the following information. Because of a technical error, this patient received his 500 mg. of hematoporphyrin in three hours instead of six. At the conclusion of this procedure, a near-ultraviolet black light was directed at the lesion, at which time a bright-red fluorescence was noted in the involved area. This indicated that there was a very prompt uptake of hematoporphyrin by the neoplastic tissue, and it further revealed

that a fairly rapid administration was without untoward side effects.

Cases 5, 8, and 1 received 300 to 500 mg. and were all successful in that the lesion fluoresced red under near-ultraviolet light, but lymphatic systems were poorly visualized in cases 5 and 1.

The carcinoma of the prostate (case 3) did not visibly fluoresce red either at the time of operation or when the specimen was taken to the darkroom. Grossly the tumor did not appear malignant. The pathological report, however, revealed a "small adenocarcinoma with invasion of the capsule and hyperplasia." Further studies will be necessary to determine whether any or all types of prostatic neoplasms have any tendency to accumulate hematoporphyrin.

The ependymoma of the cervical cord (case 6) did not become red fluorescent within twenty-four hours after the administration of 500 mg. of hematoporphyrin. Why this tumor failed to fluoresce is not understood.

The only other tumor that did not fluoresce red was that of case 7. This had been diagnosed preoperatively as a carcinoma of the breast, and therefore it was expected that it would be red fluorescent. However, no red fluorescence was observed, and the lesion was finally diagnosed as a fibrotic breast abscess.

The following cases received 1000 mg. of hematoporphyrin.

Case 11. M. L., J. H. H. 665882, was a 51-year-old colored woman. Seven months prior to admission, this patient noted a firm, nontender mass in the left breast about the size of a walnut. Because the mass continued to grow in size, the patient consulted her physician, who referred her to the hospital. She had also noted a lump in her axillary region. The patient was admitted to the hospital with the presumptive diagnosis of carcinoma of the left breast.

On March 8, 1954, the patient was tested intradermally with hematoporphyrin solution and found to be nonsensitive. Following this procedure, the patient was given 500 mg. of hematoporphyrin dissolved in 600 cc. of 0.166 *M* sodium lactate containing 1 gm. of sodium bicarbonate. This solution was administered by slow intravenous drip over an eight-hour period. The following morning this procedure was repeated, again using 500 mg. of hematoporphyrin. On the afternoon of the same day, the patient was taken to the general operating room. After routine preparation of the patient, the operating room was made dark, and the near-ultraviolet spotlight was directed on the exposed left breast. It was possible at this time to see beneath the skin the surface limitations of the underlying malignant mass that showed dull red and had a delineating serrated border. At this point the lights were turned back on and a small incision was made in the lower outer quadrant for the purpose of obtaining a confirmatory frozen section. The tissue removed was noted to contain hematoporphyrin as evidenced by red fluorescence under ultraviolet light. A radical mastectomy was then performed. Two nodes fluoresced in the axilla, and we were able to see lymph vessels clearly showing bright red fluorescence under ultraviolet light. Following the block dissection of the lesion, the concentration of hematoporphyrin, as evidenced by red fluorescence, was noted to be high. Definite red fluorescence was observed throughout the entire tumor. Color photographs of the fluorescing phenomena were taken. No toxicity to the increased dosage of hematoporphyrin was noted.

Case 10. W. S., J. H. H. 582122, was a 49-year-old colored man. Five months prior to admission, this patient noted the gradual onset of soreness in the right side of his abdomen, unrelated to meals or activity. Concurrent with this symptomatology, the patient developed anorexia and weight loss. Because of the persistence of these symptoms, the patient presented himself to the accident room, where roentgen-rays and later a barium enema revealed a right colon filling defect. The patient had had no bowel irregularities, melena, or indigestion. His weight loss in five months was 16 lb.

On March 1, 1954, the patient was given 500 mg. of hematoporphyrin solution intravenously by slow drip over an eight-hour period. On March 2, 1954, this procedure was repeated with a five-hour-administration period. On March 3, 1954, the scheduled day of surgery, the case was cancelled, but on the following day the patient was taken to the general operating room. As the peritoneum was incised and the right colon exposed, the room was made dark and an ultraviolet spotlight was directed at the ascending colon. It was possible to fluoresce the lesion through the bowel wall; the lesion was dull red at this time. Later, when the free specimen of bowel was longitudinally incised, it was possible to view the fungating cauliflower lesion in toto. When the ultraviolet light beam was directed at this lesion, it was intensely red fluorescent. The surrounding tissues did not fluoresce red. Again no toxic effects of this high dosage were noted.

Case 9. H. D., J. H. H. 662139, was a 76-year-

old colored man. Six months prior to admission to the hospital, the patient had an episode of loose stools tinged with blood. The patient also stated that he had been losing some weight. The diarrhea cleared with conservative treatment, and the patient described his general health as good. Two weeks prior to admission, his physician palpated an abdominal mass and subsequently referred him to the hospital. Here a rectal mass was also found. It was biopsied and proved to be adenocarcinoma.

On February 24, 1954, the patient received 500 mg. of hematoporphyrin intravenously in the usual manner over a six-hour period. On the morning of February 25, 1954, this procedure was repeated with an additional 500 mg. of hematoporphyrin and on the same day the patient was taken to the general operating room where an abdominal incision was made for exploratory purposes. After the peritoneum was incised, a loop of bowel was held up with its attached mesentery. The ultraviolet light was directed toward a large nodule in the mesentery. The nodule showed bright-red fluorescence and was later confirmed as being metastatic carcinoma. It was interesting to note in this case that the lymph vessels leading to this lesion also stood out as bright-red strands. The surrounding tissues did not fluoresce. No toxicity was observed in this patient.

SUMMARY AND CONCLUSIONS

A series of eleven patients have been given injections of hematoporphyrin intravenously in dosages varying from 300 mg. to 1000 mg. These dosages are far in excess of those previously thought to be toxic, but there has been no evidence of toxicity in any of our cases. The first case studied received 300 mg., the next seven cases received 500 mg. each, and the last three cases received 1000 mg. of hematoporphyrin each. In those cases receiving less than 1000 mg. of hematoporphyrin, the ability to demonstrate red fluorescence in lymphatic and cancer tissues was good but was not considered optimal. In those cases in which 1000 mg. of hematoporphyrin was given, the demonstration of red fluorescence in lymphatic and cancer tissues was considered excellent. In one case, direct visualization of the cancer tissue occurred through the skin and in another through the bowel wall. It now appears to be possible to utilize the red fluorescence of hematoporphyrin and its tendency to concentrate in tumors to assist the surgeon to visualize and delineate neoplastic tissue during operations. The detection of small or obscure lymph nodes may also be facilitated by these methods.

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