***IHSA-BBD Complex Affinity for Malignant Neoplasms: A New Potentially Diagnostic and Therapeutic Radiopharmaceutical[†]**

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The previous animal experiments showed that a bovine bile derivative (BBD) (90~95% taurocholic acid) combined with rat globulin localized preferentially in thyroid, stomach as well as in the transplanted Walker 256 carcinosarcoma.

*The objective of the present study is to evaluate the clinical applicability of the BBD-*IHSA complex. The BBD-*IHSA complex, ranging from 130 μ Ci to 1 mCi, was used on 21 inoperable, histologically verified cancer bearing patients for evaluation of the diagnostic potential. Fifteen cases out of twenty-one were positive, showing increased activity over tumor areas on the scintigram. The scintigram revealed the radioisotopes concentrated not only in tumor but in the thyroid, cardiac area, liver and stomach as well.*

*Seven positive cases, six of which were considered terminal, were selected for therapeutic uses of the BBD-*IHSA complex. Clinical results are too early to be evaluated, but two cases have distinctly shown the clinical improvement. The results of preliminary therapeutic trials, though inconclusive, indicate a promising approach to cancer therapy.*

The use of ¹³¹I labeled human serum proteins for the delineation of malignant neoplasms by scintigraphy has been found successful in many ways. Hisada, et al.¹⁾ reported positive results "in thin part(s) of the body, namely the extremities, head and neck". At a later date²⁾ he found that in four cases with bronchogenic carcinoma, using ¹³¹I (human) albumin was not helpful for tumorscanning. Spar, et al.³⁾ have reported that ¹³¹I labeled antibodies to human fibrinogen were preferentially localized in tumors.

In previous communications^{4~5)}, it was shown that *IHSA, when combined with a bovine bile derivative (BBD) (90~95% taurocholic acid), concentrated preferentially in a variety of malignant neoplasms. Similarly, ¹³¹I labeled rat globulin combined with BBD was found to localize preferentially in the thyroid, stomach as well as in the transplanted Walker 256 carcinosarcoma. The ratio of concentration in tumor to the whole body was 6:1 in terms of specific activity.

The use of BBD stems from a concept postulating the formation of endogenous carcinogens (of steroid origin) as etiological factors in some cancers⁶⁾. The assumption was amply confirmed by Kennaway⁷⁾ and Hieger⁸⁾. The acceptance of this hypothesis would explain the highest concentration of these endogenous carcinogens in the liver and in the small intestine, and the most frequent cancer incidences in these sites. The rarity of primary cancer in these organs suggested the presence in bile of a factor or factors possessing anti-tumor properties.

The objective of our present study was to evaluate further the clinical applicability of the BBD-*IHSA complex.

Materials and Methods

The BBD was prepared as previously de-

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scribed⁹⁾. Briefly stated, fresh bovine bile was hydrolyzed with pepsin and HCl, pH3, at 45°~50°C for 3~5 days. The bile[pigments were removed by N butanol. The butanol free aqueous phase was treated with NaCl to saturation. The resultant precipitate was the biological product employed. It should be noted, parenthetically, that we processed 10 liters of fresh bile in our laboratory. The infusion was prepared as follows: All equipment and solutions, except the BBD, were sterilized. Strict asepsis was observed during the procedure. The complex was prepared by mixing the BBD with *IHSA using a magnetic stirrer. Normal saline, 5~10 ml, was added as needed. Stirring was continued for about 30 minutes. The solution was sterilized by a Millipore filter, 0.45 μ , and 5~10 ml of human plasma was added to the filtered solution to minimize autoradiolysis. After another 30 minute stirring the solution was transferred to a rubber-stoppered bottle.

For the test BBD 5 g+*IHSA 1 mCi was used. Five hundred μ Ci of this complex was mixed with about 250 ml of Ringer's solution and administered intravenously at the rate of about 50 drops per minute. For therapy, BBD 12 g+*IHSA 30 mCi was prepared per 60 kg individual, diluted with 1,000 ml of Ringer's solution and administered in the same way as stated above. Twenty drops of Lugol's solution were given with milk to all patients in the evening and the following morning before the radioiodine complex was administered. Patients under therapy continually received the Lugol's solution every morning for 9~12 days after the infusion. Except a transitory diarrhea, controlled by an opiate, we have encountered no complication. Complete blood counts, including blood chemistry, were carried out following the routine procedure. Whole body scintiscans were made on the 1st, 3rd, 5th, 7th and 9th days. The instrument employed was a whole-body Hitachi II A Scintiscanner and a 5-inch NaI crystal, 85 hole collimator. The ratio of radioactive concentration in the tumor to the rest of the body was estimated according to the method of Arimizu and Morris¹⁰⁾.

Case Histories

Case 1

F.I., Hosp. #13237, Diag: Reticulum cell sarcoma. This was an emaciated, 48 year old female, with bilateral cervical, axillary and inguinal adenopathies. Temporary tumor regression followed onconin therapy. On May 24, 1969, P. received 1 mCi *IHSA+5 g BBD. Scintiscan was positive (Fig. 1). On June 13, 1969, P. received an infusion of *IHSA-BBD complex (23 mCi+10 g). Treatment was repeated on July 12, 1969 (*IHSA 15 mCi+BBD 7 g), and on July 26, 1969 (*IHSA 25 mCi+BBD 10 g). Size of tumors gradually increased. No beneficial results were observed. P. expired on August 22, 1969. Cause of death: generalized sarcomatosis.

Case 2

Y.U., Hosp. #13183, Diag: Recurrent carcinoma of stomach. This is a 47 year old male who had a gastric resection (Bilbroth #1) in Nov. 1965. On March 26, 1969, P. was reoperated on for an obstruction. The tumor found at the gastroduodenal juncture was not resectable and the obstruction was relieved by gastrojejunostomy. On May 14, 1969, P. received *IHSA 1 mCi+BBD 5 g. The scintiscan showed a pronounced activity over the tumor area (Fig. 2). On May 22, 1969, P. received *IHSA 30 mCi+BBD 10 g. Patient remained relatively symptom free until June 18, 1969. He was discharged at his request on condition that he would return within 2 weeks for reexamination. In the interim an acute obstruction reoccurred, which was relieved by a colostomy. At present P. is up and about. He is being considered for the further use of *IHSA-BBD therapy.

Case 3

T.I., Hosp. #13096, Diag: Bronchogenic carcinoma with brain and liver metastases. This was a 39 year old male, whose chief complaints were headaches, blurring vision, cough and a l. hemiparalysis. X-ray picture of chest disclosed an extensive abnormal shadow over left upper lung field. On June 24, 1969, P. was given *IHSA 1 mCi+BBD 5 g. The scintiscan was distinctly positive show-

ing increased activity over the l. pulmonary area. An infusion of *IHSA 23 mCi+BBD 14 g was administered on June 27, 1969, and repeated on July 12, 1969, (*IHSA 27 mCi+BBD 14 g). (Fig. 3 A,B) Within about ten days following the last treatment P. was able to move his l. arm and to flex his l. leg upon his abdomen, although the headaches, blurred vision and cough persisted. Unfortunately, upon the insistence of his family, the treatment was discontinued. P. expired on Sept. 7, 1969.

Case 4

A.I., Hosp. #12167, Diag: Malignant hepatoma. This is a 58 year old female, who was first operated on Oct. 8, 1968, for suspected ovarian tumor. On Nov. 5, 1968, she was reoperated for a partial resection of a malignant hepatoma. P. was on endoxan therapy for six months with no appreciable beneficial results. On July 22, 1969, P. was readmitted for a recurrence of the tumor. A recent scintiscan of the liver with ^{198}Au showed an extensive space occupying lesion over right lobe. On July 23, 1969, P. was given *IHSA 130 μCi +BBD 1 g. A regional scan showed a markedly increased activity over the space occupying lesion. On August 11, 1969, P. was given an infusion of *IHSA 30 mCi+BBD 12 g. (Fig. 4-A and B) The treatment (*IHSA 24 mCi+BBD 12 g) was repeated on Aug. 25, 1969, and Sept. 19, 1969. There have been no reactions following the infusions and no suppression of peripheral blood elements. P. has been up and about, pain free and with only minimum discomfort. Size of the tumor has remained about the same. Icteric index has been within normal limits.

Case 5

G.M., Hosp. #13785, Diag: Adeno-carcinoma of l. kidney. This is a 63 year old female, who was first operated on for a mass in the l. lumbar area in 1947. At that time the tumor and l. kidney were removed. P. was reoperated in Dec. 1960 for a recurrent neoplasm. Tumor recurred about the end of 1965. P. became incapacitated by the pain over the l. lumbar and abdominal regions. Movement of l. leg became extremely difficult. In Nov. 1965 P. was explored. Surgery

revealed an inoperable condition, the tumor having involved the abdominal aorta and l. iliac vessels. P. was referred for BBD-*IHSA therapy in Dec. 1965 (IHSA 10 mCi+BBD 10 g). Following the infusion pain was reduced to a minimum and locomotion markedly improved. In July 1966 P. received another treatment of *IHSA 10 mCi+BBD 10 g. At the end of 1966 P. was re-examined as a follow-up. She was found relatively symptom free and tumor was smaller. About March 1969, P. began to experience frequent pains over l. lumbar, l. abdominal and inguinal areas, and pain radiating down l. leg. The pains grew in severity. Her physician then referred her for another course of treatment. P. was admitted to the Chiba University Hospital, Japan, on Sept 2, 1969. General physical examination was non-contributory. Left side of abdomen, however, revealed a diffused mass extending approximately from the l. lower costal margin well into the l. inguinal area. The mass, whose boundaries could be discerned, was found fixed and tender to pressure. On Sept. 9, 1969, P. received *IHSA 20 mCi+12 g of BBD. On Sept. 20, 1969, another infusion consisting of *IHSA 25 mCi+12 g of BBD was administered. On Oct. 3, 1969, P. received another infusion of *IHSA 5 mCi+BBD 6 g. There has been no reaction. Repeated scintiscans distinctly delineated the tumor (Fig. 5). Oct. 11, 1969, the condition on discharge: P. is up and about, tumor measurably smaller, less sensitive to pressure, but firmly fixed posteriorly. There is an occasional unpredictable "shooting" pain radiating along left leg.

Case 6

T.H., Hosp. #12467, Diag: Carcinoma of prostate with pelvic and spinal metastases. This is a 64 year old male complaining of severe pains over the lower spine and pelvic areas, aggravated by locomotion. Prostatectomy was done in July 1968 followed by orchietomy in Dec. 1968. This was accompanied by hormone and ^{60}Co therapy up to July 12, 1969. No improvement was noted. July 15, 1969, P. was referred for *IHSA-BBD therapy. General physical examination was non-contributory, except pressure over lower thoracic

spine caused extreme pain, and the pain was also elicited by compression of the iliac crests. Inguinal glands were markedly enlarged bilaterally and gave pain by being touched. X-rays of spine and pelvis revealed osteolytic involvement of 12th thoracic vertebra and numerous multidimensional osteolytic foci scattered throughout the pelvis. P. received the following course of treatments July 19, 1969, *IHSA 30 mCi+BBD 10 g; July 26, 1969, *IHSA 25 mCi+BBD 10 g; Aug. 18, 1969, *IHSA 30 mCi+BBD 10 g; Sept. 3, 1969, *IHSA 10 mCi+BBD 10 g. There was no reaction. Following the first infusion the pain was alleviated. At present P. feels slight residual pain only when pressure is applied over the 1. iliac area. Except this pain, P. is relatively symptom free. Locomotion can be made effortlessly and painlessly. Repeated comparative roentgenographic examinations of the pelvis have thus far shown no significant changes.

Case 7

T.K., Hosp. #13141, Diag: Bronchogenic carcinoma (Oat-Cell). This is a 48 year old female complaining of a persistent cough. X-rays disclosed an abnormal shadow over 1. upper lung field. Thoracotomy on May 13, 1969, revealed an extensive inoperable tumor involving the 1. hilar region. Following the surgery P. continued on a rapid downward course. On May 30, 1969, P. received 500 μ Ci *IHSA+BBD 5 g complex. Scintiscan showed increased activity over 1. upper lung field correlated to the roentgenographic shadow (Fig. 6-A, B and C). P. received *IHSA-BBD therapy on June 5, 1969, *IHSA 35 mCi+BBD 12 g. Between July 23, 1969, and August 6, 1969, there was no reaction following infusions. Blood picture showed a transitory depression of the WBC. About Aug. 10, 1969, P. was permitted to visit her home. On Aug. 15, 1969, P. returned in severe shock (B.P. 70/40~50), and expired on Aug. 18, 1969. Results of autopsy pending.

Results and Discussion

Tracer studies for the evaluation of the diagnostic potential of the BBD-*IHSA complex were carried out on 21 inoperable, histologi-

cally verified cancer bearing patients (Table 1). The dose ranged from 130 μ Ci to 1 mCi. Five hundred μ Ci were found most useful. Fifteen cases were positive. The findings were based on scintiscans showing increased activity over tumor areas. Six cases were negative. In this category were included the "doubtful" cases, whose lesions happened to be too close to the cardiac, or bladder regions.

The results shown in Table 1 tend to confirm the previously reported preferential localization of the BBD-*IHSA complex in cancer tissue. The affinity cannot be termed cancer-specific, since, as illustrated by Fig. 7, the scintiscan shows the radioisotope concentrated not only in the tumor, but in the thyroid, liver and stomach as well. The test procedure is relatively simple and devoid of reactions. Most of the difficulties encountered were in the cases where the close proximities of the lesions to the cardiac or pelvic areas made delineation of tumors impossible. Another difficulty was the positive identification of small tumors. It is obvious that the results of tracer scintiscanning for diagnostic purposes depend largely on the selection of cases.

Seven positive cases, six of which were considered terminal, were selected for therapy. Of these three died. Autopsy reports are pending. The four other patients have been under observation. Case 4, the interest in this case is illustrated by comparing the ^{198}Au scan with that of the BBD-*IHSA (Fig. 4-A, and B). The increased activity shown in the latter over the "space occupying lesion" suggests the possibility of differentiating a benign condition from a malignant one. Similar observations were previously reported⁵⁾. Clinical results are too early to be evaluated. Case 6, carcinoma of the prostate, has improved clinically, as manifested by alleviation of pain. Repeated pelvic X-rays have shown no change in the dimensions or densities of the osteolytic foci. Continued follow-up should evaluate the future course of current carcinoma. Case 5, adeno-carcinoma of 1. kidney, is particularly noteworthy. A résumé of the patient's history indicated a slowly growing tumor. It reached inoperable dimensions,

Fig. 1 Case 1: Reticulum cell sarcoma. Scintiscan 3 days post *IHSA 1 m Ci-BBD infusion.

Fig. 2 Case 2: Carcinoma of Stomach (Recurrent). Scintiscan 3 days post *IHSA 1 m Ci-BBD 5 g infusion.

Fig. 3-A Case 3: Bronchogenic carcinoma 1. lung.

Fig. 3-B Case 3: Bronchogenic carcinoma with cerebral metastases. Increased activity over upper cranium suggests metastatic foci.

Fig. 4-A Case 4: Malignant hepatoma. Scintiscan with ^{185}Au . A space occupying lesion over right lobe is evident.

Fig. 4-B Case 4: Malignant hepatoma. Scintiscan 14 days post *IHSA 30 mCi-BBD infusion. Increased activity selectively over space occupying lesion is diagnostically significant.

Fig. 5 Case 5: Adeno carcinoma of 1. kidney. Scintiscan of therapeutic dose 3 days post *IHSA 25 mCi-BBD infusion.

Fig. 6-A Case 7: Bronchogenic carcinoma. X-ray picture of chest.

Fig. 6-B Case 7: Bronchogenic carcinoma. Scintiscan 3 days post *IHSA 500 μ Ci-BBD infusion.

Fig. 6-C Case 7: Bronchogenic carcinoma. Scintiscan of therapeutic dose 3 days post *IHSA 40mCi-BBD infusion.

Fig. 7 Increased activity over thyroid, liver, stomach and transplanted tumor in 1. hind leg is evident.

Fig. 8 Photomicrograph of a Walker 256 carcinosarcoma showing about 80% necrosis. Animal treated with BBD+radioiodinated rat globulin.

Table 1

No.	Name	Hosp. #	Age	Sex	Diagnosis	+	-
1	T. K.	13141	49	F	Pulmonary oat-cell carcinoma	+	
2	H. I.	13266	62	M	Tumor (Clear Cell) Rt kidney Pul Met.	+	
3	A. I.	12167	58	F	Hepatoma	+	
4	A. T.	13094	49	F	Carcinoma (Anaplastic) Origin ?		-
5	I. S.	13779	69	M	Carcinoma of the neck		-
6	F. I.	13237	48	F	Ret. Cell Sarcoma	+	
7	K. U.	13183	47	M	Gastric cancer	+	
8	T. H.	12467	64	M	Carcinoma Prostate		-
9	L. N.	13125	65	M	Hypernephroma L	+	
10	K. W.	13045	40	M	Tumor (Malignant) Rt. Maxilla	+	
11	A. Y.	10945	37	M	Ret. Cell Sarcoma	+	
12	M. M.	12375	19	M	Genl. Lymphosarcomatosis	+	
13	K. S.	12368	45	F	Carcinoma L. Breast (Recurrent)		-
14	H. S.	13823	60	M	Carcinoma (Sq. Cell) L. Maxilla	+	
15	R. O.	13207	56	M	Gen. Lymphosarcomatosis		-
16	F. N.	13474	60	F	Ut. Carcinoma Pulmonary Met.	+	
17	T. I.	13096	39	M	Bronchogenic Carcinoma	+	
18	H. S.	13448	38	M	Angiosarcoma (Mediastinal)	+	
19	G. M.	13785	63	F	Tumor L. Kidney Recurrent	+	
20	R. Y.	13847	65	M	Bronchogenic Carcinoma (Bilateral)	+	
21	F. S.	13779	77	M	Gastric Carcinoma (Liver Met.)		-

however, when the patient was last explored. One year after BBD-*IHSA therapy a follow-up examination disclosed that the tumor had regressed and the patient was almost symptom free. In the absence of any other medication, these findings may be ascribed to the only therapy administered. This assumption is confirmed to some extent by the favorable progress observed following the second course of treatment: disappearance of pain, and the measureable regression of the tumor within a relatively short time (five weeks). Since the mechanism of this phenomenon is unknown the results of a similar form of therapy employed in the Walker 256 carcinosarcoma experiments may help us to elucidate a possible mode of action. One group of animals was treated with ^{131}I labeled rat globulin combined with, and another group without the BBD. The animals were sacrificed on the 10th day after tumor implantation. Histopathological examination of the tumors showed 80% necrosis in the BBD-radioiodinated globulin treated animals (Fig. 8) and only 40% necrosis

Fig. 9 Total administered dose 22.5 mCi.

in the tumors of animals treated with the radioiodinated globulin alone⁴). These results suggest a synergistic action, which may prove to be a basic property of the complex.

The validity of this hypothesis gains confirmation in a measure by the analysis of case 5. Calculations made on the basis of 22.5 mCi of

administered dose showed that a ratio of specific activity of tumor to the rest of the body was about 6:1 (Fig. 9). It is obvious that even an initial favorable clinical response cannot be ascribed to the specific activity estimated in the tumor. We are planning, therefore, to investigate further this challenging phenomenon.

Summary and Conclusion

1) A new ^{131}I -HSA-BBD complex concentrates preferentially in a variety of malignant neoplasms. The administration of tracer doses ($500\ \mu\text{Ci}$) has given dominantly positive scintiscan results (15:21). The procedure suggests itself as a potential diagnostic tool.

2) The results of preliminary therapeutic trials, though inconclusive, indicate a promising approach to cancer chemotherapy.

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要 旨

^{131}I -HSA-BBD の悪性腫瘍への親和性について

——RI 診断および RI 治療への応用の可能性——

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ウシの胆汁からブタノールを用いて抽出した物質 (BBD と略す) は 90~95% のタウロコール酸を含んでいる。この BBD を ^{131}I -標識人血清アルブミンに結合させて BBD-IHSA を作製したところ、このものは担がん動物の新生物にも高濃度に沈着することが知られている。本研究の目的は各種悪性腫瘍患者の腫瘍部が BBD-IHSA によってシンチグラム上に描写できるか否かを調べることであり、また BBD-IHSA による治療の可能性を論ずることである。方法および結果: $130\ \mu\text{Ci}$

ないし1.0 mCi の BBD-IHSA を静注し、経時的に全身のシンチグラフィを行ない、シンチグラム上に腫瘍を描出できるか否かを調べた。21例の各種のがんおよび肉腫症例についてこの方法を行なったところ15例に陽性描出を得、6例が描出不能であった。このうち陽性描出5例のシンチグラムを例示した。症例の選択方法によっては陽性描出率をあげることも可能と思われる。

BBD-IHSA による治療の可能性を検討するために、数例の進行性がん症例について10 mCi 以上の BBD-IHSA を静注し、そののちの経過を観察した。観察期間が短くまだ効果を十分に検討するに至らないが、症状の緩解にかなり有効であった症例を経験した。