A Potential Antitumor Factor (ATF) Isolated from a Bovine Bile Derivative (BBD) (II)

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Received March 2, 1971

The intraperitoneal administration of a non-dialysable component obtained from a bovine bile derivative (BBD) manifested in the Walker 256 carcinosarcoma various degrees of tumor inhibition. This proved proportional to the dosage employed. Histopathological examination of the specimens clearly corroborated these findings. In the lowest dose group, 1.25mg/100g rat, no difference was found between the treated tumors and those of the controls. In the highest dose group, 10mg/100g rat, the tumors were found extensively and pervasively necrotic. No toxicity was demonstrable in either kidney or liver in any of the control groups.

Introduction

The theoretical consideration for the use of BBD as a chemotherapeutic agent was previously described1). Briefly stated, it was based on the possible formation of endogenous carcinogens, produced in the course of steroid metabolism.2) This was subsequently amply confirmed by Kennaway and Hieger3),4). The rare occurrence of primary malignancies in the liver and small intestine suggested the presence in bile of a factor, or factors possessing anti-tumor properties.

BBD was found to consist of 95% taurocholic acid and a 5% as yet undetermined component. When combined with 131I-labeled rat globulin, it formed a complex (Fig. 1). Scintigraphy proved the BBD complex to concentrate preferentially in tumor tissue (Walker 256 carcinosarcoma)5). In man similar reproducible results were obtained, when BBD, combined with 131I-labeled human albumin, or globulin, concentrated preferentially in a variety of human cancers,5,6) As a potential antitumor agent, however, the material proved equivocal7). This prompted the investigation of the 5% component.

Material and Methods

The BBD was prepared as previously, de-

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ter, and dialysed against distilled water until
the dialysate was negative to chlorides and
negative, or only faintly positive, to a Petten-
kofer reaction. The dialysed solution was
treated with NaCl to saturation, and the precip-
itate dissolved in n-butanol. The solvent was
removed at reduced pressure and the residue
dissolved in pyrogen-free n-saline, to which 1:
10,000 merthiolate was added. The solution
was then passed through a 0.45μ Millipore
filter, and transferred to a rubber-stoppered
bottle. Randombred 100g Wistar male rats
were inoculated with 0.1 ml minced Walker
256 carcinosarcoma, and placed in groups of
6 in. wire cages. An equal number of ani-
mals, but minus tumor inoculation, was simi-
larly segregated. Treatment regimen is given
in Table 1. Animals were housed in a con-
stant temperature room. Regular laboratory
pellets and water were supplied ad libitum.
All treatments were administered ip on day 3
post inoculation and continued for 4 consecu-
tive days. On day 10 the animals were sacri-
ficed. Tumors were resected, weighed and
preserved in 10% formalin. One kidney and
a section of liver from each animal were simi-
larly treated. This procedure was repeated
4 times, except that in the last experiment
the “no-tumor” animals were not sacrificed
and maintained for one month.

| Table 1 |
| Outline of experiment |
| Tumor-bearing group | Non-tumor group |
| Number of animals | Treatment: all dilution in 5ml n-S |
| Dosage group | Initially | At term | Initially | At term |
| 1 | 24 | 22 | 24 | 1.25mg |
| 2 | 24 | 22 | 24 | 2.5 μ |
| 3 | 24 | 16 | 24 | 5 μ |
| 4 | 18 | 11 | 24 | 10 μ |
| 5 | 24 | 24 | 24 | normal sal. |
| Total | 114 | 71 | 120 | 120 |

It should be noted that in one of the
experiments animals were sacrificed on day
14. The resected intact tumors of the 10mg
treated group appeared dimensionally almost
equal to those of the controls. Sectioning of
the former, however, disclosed a thin layer of

Fig. 2 ATF, Control large, plastic tumor
cells infiltrating muscle. (× 53)

Fig. 3 ATF, 5mg/100g·rat. Tumor shows
areas of necrosis with foci of leucocytic
infiltration. (× 21)
Fig. 4 Animals were divided into 5 groups, 6 per group: A) 0 = Controls, (n= saline), B) 1.25mg, C) 2.5mg, D) 5mg, and E) 10 mg/100g rat. Dilutions made up to 5ml and administered i.p. for 4 consecutive days. The curve was plotted as average tumor weight of the respective groups on day 10 post tumor transplantation.

Fig. 5 Relative tumor size of the 10mg treated and control group.

viably tumor surrounding a mass of necrotic tissue. The latter, in contrast, presented a solid tumor with only a small, central necrotic area (Fig. 2 and Fig. 3).

Results and Discussion

In Fig. 4 the graph shows a distinct relationship between dosage and tumor weight. This observation held generally true in all the four experiments. In some of the “10mg” treated animals only traces of tumor could be found. This has not occurred in any of the other groups. The histopathological findings are in complete agreement with the gross observations. Histological examination of the livers and kidneys of the “non tumor” controls showed “no changes that are degenerative either in the liver, or kidney” (Pathologist’s report). Clinical evaluation of ATF in a variety of human cancer is now in progress.

Summary

1) A new potential antitumor factor (ATF), isolated from BBD has shown to inhibit the growth of the Walker 256 carcinosarcoma.

2) The compound has manifested no toxicity in the doses employed.

Acknowledgment

The authors gratefully acknowledge the liberal contribution of BBD supplied by the National Research & Chem. Co. and the California Gland Co. We are also thankful to the following Drs. for their cooperation; Dr. M. Nakano, T. Saegusa, T. Toyama, G. Uchiyama, and N. Yui and our gratitude to the Shionogi Pharmaceutical Co. and Takeda Pharmaceutical Co. for the Walker 256 tumor.

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2) Penn, H. S.: Inoperable Malignancy, Med. Record, 142, 213-7 (1935)


要旨

ウシ胆汁抽出物（BBD）の腫瘍発育抑制作用（ATF）に関する研究（II）

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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は95%のタウロコール酸を含んでいる。透析によりタウロコール酸を除去した成分に³¹I-標識人血清アルブミンを結合法に腫瘍を照射後集まり、また化合物をペーパークロマトグラフィで展開することにより安定であることを確かめた。この物質を Walker 256 がん肉腫ラットの腹腔内に注射したところ、腫瘍の発育抑制効果を認めた。投与量と効果はほぼ比例関係にあり、また組織病理学的な検査でも同じことが示された。しかし最少投与群1.25mg/100g（体重）と対照群との間に差異は認められなかった。最大投与群10mg/100g（体重）では腫瘍に広範な壞死がみられた。毒性を投与群と对照群とについて調べたところ肝と腎について有意の差は認められなかった。