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Conventional and novel strategies in the treatment of adrenocortical cancer

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Abstract

Adrenocortical carcinoma is a highly malignant neoplasm with an incidence of two per million people per year. Several treatment strategies have resulted in temporary or partial tumor regression but very few cases have attained long survival. Surgical resection of the primary tumor and metastases is most effective. Several chemotherapeutic protocols have been employed with variable success. Mitotane (o,p'-DDD) is an adrenalytic drug effective in inducing a tumor response in 33% of patients treated. Mitotane requires metabolic transformation for therapeutic action. Tumors may vary in their ability to metabolize mitotane and the ability of tumors to transform mitotane may predict the clinical response to the drug. Preliminary data show a possible correlation between metabolic activity of neoplastic adrenocortical tissue and response to mitotane. We have attempted to develop mitotane analogs with enhanced adrenalytic effect. Compared to mitotane, a di-chloro compound, the bromo-chloro and di-bromo analogs appear to have a greater effect. Future approaches to the treatment of adrenocortical carcinoma are likely to be based on blocking or reversing the biological mechanisms of tumorigenesis. Angiogenic and chemotactic mechanisms may play a role in adrenal tumor growth and inhibition of these mechanisms may result in inhibition of tumor growth. New mitotane analogs with greater adrenalytic potential could be a promising approach to developing more effective and selective therapies for adrenal cancer. Alternative approaches should attempt to suppress tumor growth by means of compounds with anti-angiogenic and anti-chemotactic activity.

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Adrenocortical carcinoma is a rare, highly malignant neoplasm which occurs with an incidence of two per million population per year. It represents 0.2% of all cases of cancer. Several treatment strategies have resulted in temporary or partial tumor regression but very few cases have attained long survival (1). More effective therapy is needed and it is likely to come from a better understanding of tumor biology; specifically, the oncogenic and tumorigenic processes that govern early cell mutation and the growth and dissemination of an established tumor.

The difficulty in assessing the effectiveness of published treatment protocols stems from the fact that most series are limited in the number of patients studied. There is great variability in the drugs used, the stage and extent of the tumor, and the malignancy grade. In addition, there is lack of a uniform defini-

tion of response, the duration of response is unclear and multiple treatments are given in variable sequences.

Several of the larger series indicate that the most effective treatment is the surgical resection of the primary tumor and metastases, with 56% of patients showing extended survival. Abdominal irradiation is associated with tumor response in 15% and systemic chemotherapy in less than 10%. Several chemotherapeutic protocols have been employed with variable success. Combinations of adriamycin, vincristin, cisplatin and etoposide have been used in escalating doses with some success. Other drugs such as taxol, suramin and gossipol have been used with equivocal results.

Mitotane (o,p'-DDD) is an adrenalytic drug with selective activity on the adrenal cortex which has been found to be effective in inducing a tumor response in 33% of patients treated. The duration of response has been quite variable, ranging from 1 to 204 months. The use of mitotane as adjuvant therapy in patients with stage I and II adrenocortical carcinoma is controversial because of lack of convincing data that the drug can prevent tumor recurrence and the significant toxicity associated with its administration.

A better understanding of the mechanism of action of mitotane could be useful in explaining its antitumor effect and in developing a rationale for the design of more effective and less toxic compounds. Mitotane belongs to the class of drugs that requires metabolic transformation for therapeutic action. As a result of this transformation, active metabolites are produced that cause toxicity either through covalent binding to specific targets within the cells or by oxygen activation with superoxide formation. The concept that mitotane requires metabolic transformation for activity stems from observations of its variable activity in different animal species. The dog adrenal, the most responsive to mitotane, is also the most capable of metabolite formation and covalent binding. In contrast, the human adrenal is less capable of both transformation and binding and is less responsive (2).

We proposed a pathway of mitotane metabolism that follows the well-known process by which chloramphenicol causes toxicity. Mitotane is hydroxylated at the ß-carbon and quickly transformed by dehydrochlorination into an acyl-chloride. The acyl-chloride either covalently binds to bionucleophiles in the target cells or, by losing water, is transformed to the acetic acid derivative (DDA) for renal excretion. The initial hydroxylation step is carried out in the mitochondria and is catalyzed through a P-450 enzyme. The importance of this metabolic transformation can be tested by the introduction of a methyl group at the ß-carbon of o,p'-DDD, a procedure that blocks the metabolic transformation to the acyl-chloride. Whereas dogs treated with mitotane show prompt suppression of cortisol secretion and increases in serum ACTH levels, treatment with the methylated analog (mitometh) lacks this effect. Similarly, while mitotane causes necrosis of the adrenal cortex, mitometh has no significant effect (3).

The pathway of metabolic transformation of mitotane has been extensively studied in our laboratories (4). Formation of hydroxylated derivatives can be shown by incubation of dog adrenal homogenates with radiolabeled o,p'-DDD. HPLC analysis of the homogenate after incubation shows the appearance of hydroxylated metabolites and the production of DDA. We have characterized the adrenal enzymes involved in mitotane metabolism. The metabolic reaction is dependent on O_2 and NADPH, and is inhibited by 68% with a mixture of O_2-CO (20:80). The metabolic transformation is inhibited by ketoconazole, but not by other specific enzyme inhibitors such as aminoglutethimide and metyrapone, or substrates such as cholesterol, 11-deoxycorticosterone, 11-deoxycortisol, corticosterone and androstenedione. It is possible that the mitotane-metabolizing

enzyme is a novel non-steroidogenic P-450 present in the adrenal cortex and active in the metabolism of xenobiotics.

We have also investigated the cellular target to which mitotane metabolites are covalently bound. Incubation of normal adrenal and adrenal tumor homogenates with a radiolabeled analog of mitotane show that most of the radioactivity is associated with proteins with molecular weights of 49.5 and 11.5 kDa. The sequence and structure of these proteins have not been worked out.

Another possible mechanism mediating the adrenalytic effect of mitotane is oxidative damage through production of free radicals. We have tested the effect of tocopherol acetate on the antiproliferative activity of mitotane and shown that this activity is reversed by the addition of this antioxidant to NCI-H295 adrenal cancer cell cultures.

Thus, metabolic transformation and free radical formation are mechanisms involved in the cytotoxicity of mitotane. Tumors vary in their ability to metabolize mitotane and the ability of tumors to transform mitotane may predict the clinical response to the drug. Preliminary data show a possible correlation between metabolic activity of neoplastic adrenocortical tissue and response to mitotane. We have developed a tritium release assay to test the ability of adrenal tumors to metabolize mitotane (5). Tritiated mitotane is incubated with adrenal homogenates or cell suspensions. The unreacted substrate is removed and the amount of tritium released into the aqueous media is determined. The amount of tritium released correlates with the metabolic transformation into the acyl-chloride. Results from the tritium release assay correlate with data obtained using ^{14}C-labeled compound. This assay has potential clinical applications for selecting patients who are responsive to mitotane from those who are not likely to respond.

Based on the mechanism of action of mitotane described, we have attempted to develop mitotane analogs with enhanced adrenalytic effect. We postulated that replacement of chlorine with more reactive halides such as bromine might increase the transformation of substrate to the acyl-chloride. Two compounds, bromo-chloro and di-bromo analogs were synthesized. When these analogs were added to NCI-H295 adrenal cancer cell cultures, there was a dose-dependent suppression of cell growth and cortisol production. Compared to mitotane, the bromo-chloro and di-bromo analogs appear to have a greater effect. These effects were also shown *in vivo* when dogs were treated with equimolar doses of the analogs. Atrophy of the zona fasciculata and reticularis was greater with the brominated analogs than with mitotane.

Future approaches to the treatment of adrenocortical carcinoma are likely to be based on blocking or reversing the biological mechanisms of tumorigenesis. For example, angiogenic and chemotactic mechanisms may play a role in adrenal tumor growth. Inhibition of these mechanisms may result in inhibition of tumor growth.

We have recently described a CXC chemokine-producing adrenocortical carcinoma. The patient, a 74-year-old man, presented with intermittent fever, marked leukocytosis and elevated acute phase reactants. A cell line developed from this tumor actively produced chemokines *in vitro*, including interleukin-8 and epithelial neutrophil activating protein-78 (ENA-78), potent angiogenic and chemotactic chemokines. These cells were transplanted subcutaneously into SCID mice and an animal model of this tumor was developed. The tumors were infiltrated with neutrophils and a similar neutrophil infiltration was seen around the central veins in the mouse liver. Preliminary studies with passive immunization against ENA-78 have shown 50% suppression of tumor growth that was associated with suppression of endothelial cell and leukocyte markers in the tumor.

In summary, the development of new

mitotane analogs with greater adrenalytic potential could be a promising approach to developing more effective and selective therapies for adrenal cancer. Alternative approaches should attempt to suppress tumor growth by means of compounds with anti-angiogenic and anti-chemotactic activity.

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