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ENHANCEMENT OF HEPATIC TYROSINE AMINOTRANSFERASE INDUCTION IN THE RAT BY 5-FLUOROURACIL*

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Abstract—5-Fluorouracil (5-FU) treatment with large doses in adrenal-intact rats inhibited glucocorticoid induction of tryptophan oxygenase (TPO, EC 1.13.1.12) while simultaneously enhancing induction of hepatic tyrosine aminotransferase (TAT, EC 2.6.1.5). 5-FU treatment alone stimulated hepatic TAT several-fold in intact rats, and less so in adrenalectomized rats, while enhanced induction of TAT by 5-FU with glucocorticoids occurred equally well in adrenalectomized rats. Enhanced induction of TAT in 5-FU treated rats was not obtained if the primary inducer of TAT was insulin.

The normal apparent half-life *in vivo* of glucocorticoid-induced TAT was 1·9 hr; this was increased to 3·9 hr by 5-FU cotreatment. These data support the likelihood that 5-FU supports TAT induction by altering the turnover *in vivo* of glucocorticoid-induced TAT.

5-FLUOROURACIL (5-FU), given as such or as its metabolic precursor 5-fluoro-orotic acid, has been shown to inhibit the induction *in vivo* of certain enzymes in mammalian tissue in response to appropriate inducing agents. This effect of 5-FU has been described in rat liver with tryptophan oxygenase[1,2] and with threonine dehydrase and ornithine transaminase.[3] Inhibition of enzyme induction by 5-FU has been widely reported in bacterial systems and has been ascribed to its incorporation into messenger RNA thereby producing translational errors in messenger RNA read-out.[4] However, some inducible enzyme systems in mammalian cells are not inhibited by 5-FU under conditions whereby inhibition could be obtained with actinomycin D.[5–7] A further anomalous action of 5-FU is described in this report: the drug was found to enhance rather than to inhibit tyrosine aminotransferase (TAT) induction in rat liver by glucocorticoids under conditions whereby tryptophan oxygenase (TPO) induction was effectively inhibited.

MATERIALS AND METHODS

Male Sprague–Dawley rats (from Zivic–Miller Laboratories, Allison Park, Pa.) were used throughout and were maintained over a Sani-Cel (Paxton Processing Co., Paxton, Ill.) bedding with tap water and Purina Laboratory Chow. Adrenalectomized rats (prepared under ether anesthesia) were used approximately 2–3 days following surgery with supplementation of 0·9% NaCl in their drinking water. All drug treatments were scheduled to ensure that time of day would not affect enzyme activity values.[8,9]

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Following appropriate drug treatments, rats were stunned by a blow on the neck, then perfused *in situ* via the hepatic portal vein with ice-cold 0·9% NaCl to remove excess hepatic blood. The livers were quickly excised, blotted free of excess NaCl solution and immediately frozen on dry ice. Livers were homogenized in 3 vol. of ice-cold 0·15 M KCl containing 1 mM EDTA and 50 mM tris HCl (pH 7·4 at 4°). TPO was assayed by the Knox–Feigelson method[10] in supernatant fractions obtained by centrifugation at 40,000 g_{max} for 10 min. TAT was assayed in 40,000 g_{max} (20 min) supernatant fractions by the method described by Diamondstone,[11] but with incubation at 30°. One unit of either enzyme is that amount which catalyzed the formation of 1 μmole of product during the period of incubation.

RESULTS

The experiments described in this report were conducted in order to examine further an anomalous finding in this laboratory that 5-FU enhanced the hepatic TAT stimulating action of CCl_4 treatment.* This stimulating action of CCl_4 on hepatic TAT was adrenal-dependent and was blocked by actinomycin-D.* Reserpine also enhances hepatic TAT (and TPO) activity via an adrenal glucocorticoid mechanism.[12,13] For purposes of comparison with CCl_4, therefore, reserpine was selected as an adrenal-dependent inducer of hepatic TPO and TAT.

TABLE 1. ENHANCED INDUCTION OF HEPATIC TYROSINE AMINOTRANSFERASE (TAT) WITH RESERPINE BY 5-FLUOROURACIL*

Treatments	Experiment 1†		Experiment 2‡	
	TPO	TAT	TPO	TAT
	Enzyme units/g + S.E.			
Controls (sham injected)	*3·3* ± 0·4	68 ± 6	*3·2* ± 0·6	22 ± 2
Reserpine (5 mg/kg, s.c.; 6 hr).	8·1 ± 0·7	204 ± 24	7·6 ± 0·6	*154* ± 22
5-Fluorouracil	*4·0* ± 0·6	85 ± 6	*2·5* ± 0·4	*162* ± 25
5-FU plus reserpine	*4·7* ± 0·3	417 ± 12	*3·8* ± 0·4	309 ± 31

*Any mean values italicized do not differ significantly ($P > 0·05$) from any other such means within the same column. Each mean was computed from six rats in each treatment group; Student's *t*-test was used in all tables for calculation of statistical significance.

†Adrenal-intact rats 300 g; 5-FU dosage: 250 mg/kg at zero time and 125 mg/kg at 4 hr (total = 375 mg/kg, i.p.). Animals were killed at the 6-hr point.

‡Adrenal-intact rats 180 g; 5-FU dosage: 250 mg/kg every 2 hr for three doses (total = 750 mg/kg, i.p.). Animals were killed at the 6-hr point.

5-FU was given to adrenal-intact rats alone or simultaneously with reserpine. The experiments described in Table 1 indicate that 5-FU itself stimulated hepatic TAT, and that such enhancement of hepatic TAT was not accompanied by a similar increase in TPO. These data also illustrate that while TPO enhancement by reserpine was effectively blocked in both experiments by 5-FU coadministration as would be

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expected,[1,2] TAT activity in rats receiving both drugs was approximately double that obtained with reserpine alone.

In order to establish whether this peculiar action of 5-FU was due simply to the additive effects of either drug, a similar experiment was performed but in adrenalectomized rats. It was first found that adrenalectomy did not abolish the dose-related intrinsic TAT stimulating action of 5-FU, although it reduced the extent of this action considerably (Fig. 1). Thus, a large total 5-FU dose of 750 mg/kg given to adrenalectomized rats increased TAT activity (over controls) by approximately 50 enzyme units per g liver (Fig. 1). In another experiment a 750 mg/kg injection of 5-FU in two divided doses increased enzyme activity in the adrenal-intact rats by 140 units/g liver (Table 1, experiment 2).

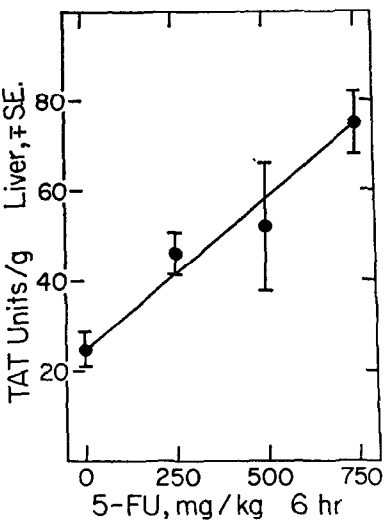

FIG. 1. Dose–response relationship between hepatic tyrosine aminotransferase (TAT) activity and 5-fluorouracil (5-FU) dosage in adrenalectomized rats. 5-FU was given, for a 6-hr period, in single injections of the doses indicated. The average weight of the animals used was 190 g.

TABLE 2. ENHANCED INDUCTION OF HEPATIC TYROSINE AMINOTRANSFERASE (TAT) WITH HYDROCORTISONE OR CORTICOSTERONE BY 5-FLUOROURACIL IN ADRENALECTOMIZED RATS

Treatments	TAT units/g ± S.E.	
Controls (sham injected)	43 ± 14	
5-Fluorouracil (250 mg/kg i.p., 6 hr)	85 ± 9	
Hydrocortisone (30 mg/kg i.p. in propylene glycol [2 ml/kg], 6 hr)	215 ± 20	*
Corticosterone (29 mg/kg i.p. in propylene glycol [2 ml/kg], 6 hr)	181 ± 20	
Hydrocortisone plus 5-FU	303 ± 33	*
Corticosterone plus 5-FU	299 ± 41	

*Any two mean values joined by a vertical line do not differ significantly ($P > 0.05$). Each mean is representative of six rats.

After this study, adrenalectomized rats were treated as described under Table 2, i.e. with 5-FU alone or combined with equimolar doses of hydrocortisone or corticosterone in place of reserpine. Table 2 illustrates that 5-FU retained its ability to enhance hepatic TAT in adrenalectomized rats given the glucocorticoids as primary inducers. With either hormone, the increase obtained with 5-FU coadministration was greater than could be accounted for by a simple additive effect. Enhancement of TAT by 5-FU was also obtained in adrenalectomized rats receiving maximally effective doses (100 mg/kg of the sodium succinate ester) of hydrocortisone along with

TABLE 3. EFFECT OF 5-FLUOROURACIL ON HEPATIC TYROSINE AMINOTRANSFERASE (TAT) UNDERGOING "MAXIMAL INDUCTION" BY HYDROCORTISONE IN ADRENALECTOMIZED RATS*

Treatments	TAT units/g ± S.E.
None	28 ± 6
5-FU (250 mg/kg, i.p.)	54 ± 8
Hydrocortisone† (100 mg/kg, i.p., 6 hr)	148 ± 20
Hydrocortisone plus 5-FU	294 ± 25

* Each treatment group was comprised of six rats of average weight 210 g. All mean values differ from each other at a $P < 0{\cdot}05$.

† Hydrocortisone succinate (Solu-cortef, Upjohn).

5-FU (Table 3), suggesting that 5-FU was not acting merely by altering the metabolic disposition of the glucocorticoid hormones.[14,15] This is supported also by the data in Fig. 1, which indicate that 5-FU increased TAT in 2–3 day adrenalectomized rats, i.e. in the absence of a source of endogenous or exogenous glucocorticoid hormones.

In addition to adrenal steroids, possible physiologic hormone regulators of TAT turnover *in vivo* in the rat include growth hormone,[16] insulin and glucagon,[17] and adenosine 3′,5′-cyclic monophosphate.[18] Insulin and glucagon induce TAT by a mechanism similar to but not identical with adrenal steroids.[17] We therefore investigated the ability of 5-FU to enhance TAT induction in the presence of insulin as the primary TAT inducer in adrenalectomized rats. The data in Table 4 indicate that some specificity in the 5-FU effect exists, namely that the drug was not able to enhance significantly the TAT-inducing action of insulin. However, since insulin induction of TAT is a short-term effect of the hormone,[17] the lack of 5-FU to demonstrate any significant activity on TAT in adrenalectomized rats in this experiment may be related to the shorter duration (3 hr) of 5-FU treatment.

An increase in the apparent concentration of enzyme activity in an organ can be due to a change in the kinetic properties of the enzyme or can result from enhanced rate of enzyme synthesis due, for example, to enhanced messenger RNA synthesis,[19] or via a decreased rate of enzyme degradation *in vivo*.[20] While enzyme turnover rates have been measured with elegant immunologic and double-isotope labeling techniques,[20–23] an estimate to changes in the apparent half-life *in vivo* of an induced enzyme can be made simply by measurement of the exponential decay rate of induced enzymic activity.[24] Such an experiment is illustrated in Fig. 2.

TABLE 4. EFFECT OF 5-FLUOROURACIL ON INSULIN INDUCTION OF HEPATIC TYROSINE AMINOTRANSFERASE (TAT) IN ADRENALECTOMIZED RATS*

Treatments	TAT units/g ± S.E.
Controls (sham injected)	20 ± 2
5-Fluorouracil (250 mg/kg, 3 hr i.p.)	31 ± 6
Insulin (8 units/kg, 3 hr i.p.	50 ± 6
Insulin (8 units/kg, 3 hr i.p.) plus 5-FU (250 mg/kg, 3 hr i.p.)	63 ± 5

* Each group contained five adrenalectomized rats of an average weight of 300 g. Groups receiving insulin were given 2 ml of 10% glucose solution (i.p.) each hr. Any two means joined by the same vertical line are not significantly different ($P > 0.05$).

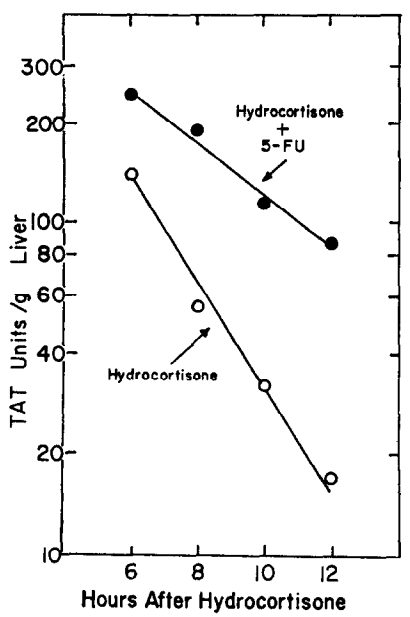

FIG. 2. Effect of 5-fluorouracil (5-FU) cotreatment with hydrocortisone on apparent decay rates *in vivo* of hepatic tyrosine aminotransferase (TAT). Two groups of adrenalectomized rats (average weight, 190 g) were given either hydrocortisone only (30 mg/kg in propylene glycol) or hydrocortisone together with 250 mg/kg 5-FU, all injections via the i.p. route. At 6 hr and every 2 hr thereafter animals were killed and prepared for TAT assay. The apparent $T_{1/2}$ for the control (hydrocortisone only) group was 1·9 hr; the $T_{1/2}$ of the treated (hydrocortisone + 5-FU) group was 3·9 hr.

It can be seen that the hydrocortisone-induced enzyme decayed with an apparent $T_{1/2}$ of 1·9 hr (in agreement with previous values).[25] 5-FU cotreatment increased the apparent $T_{1/2}$ to 3·9 hr. These data suggest that 5-FU is either promoting the continuation of TAT synthesis in response to hydrocortisone or is inhibiting the degradation of TAT *in vivo*, or both.

DISCUSSION

The major significant biologic actions of 5-FU result from the following effects: (a) inhibition of thymidylate synthetase[26] after conversion to deoxyfluorouridylic acid[27] with the subsequent inhibition of DNA synthesis;[28] (b) interference with orotate and uracil utilization; and (c) incorporation into cellular RNA,[4,28] and thereby producing effects ascribable to miscoding.[29,30] It is difficult to resolve the enigma described in the present report on the basis of the available information. 5-FU appropriately inhibited TPO induction by glucocorticoid and simultaneously enhanced TAT induction. The effect on TPO is a classical illustration of 5-FU action;[31] the effect on TAT is not. That 5-FU did not inhibit TAT induction is indeed surprising but not without precedent, since induction of some other inducible enzymes is not at all affected by 5-FU[5,6] including glucocorticoid induction of TAT in cell culture.[7] The 5-FU enhancement of TAT induction by glucocorticoids suggests that TAT represents a unique class of inducible enzyme(s) in rat liver and that studies of its regulation must be performed with this in mind.

Tomkins *et al.*[32,33] have proposed a translational regulatory mechanism for TAT, based largely on superinduction of glucocorticoid-preinduced TAT by actinomycin D and 5-FU.[34] This mechanism invokes a regulatory role for a postulated cytoplasmic TAT repressor and its short-lived (relative to TAT messenger) messenger RNA. 5-FU could become rapidly incorporated into mRNA coding for this repressor, thereby coding for a nonfunctional repressor and removing the postulated translational inhibition of TAT synthesis. However, we must also reconcile the strong likelihood that 5-FU given with glucocorticoid in the present studies would be incorporated also into TAT mRNA and thereby inhibit synthesis of both TAT and repressor. Alternatively, if TAT synthesis was regulated purely (however unlikely) by a post-transcriptional process, the 5-FU effect quite adequately fits the translational repressor model proposed by Tomkins *et al.*[32,33]

Kenney has demonstrated that cycloheximide[22] and actinomycin D[23] cause an apparent inhibition of TAT degradation, ostensibly because of inhibition of the accumulation of proteins which are required for the degradative process or because of the accumulation of cellular inhibitors of enzyme degradation. Levitan and Webb also have shown that 8-azaguanine[35] and 5-azacytidine[36]—both of which cause extensive hepatic polysome breakdown in the rat[36,37]—similarly appear to stabilize TAT *in vivo*. Furthermore, we have observed that carbon tetrachloride treatment enhances TAT induction* (and inhibits TPO induction)[10] by hydrocortisone in the face of massive polysome breakdown.[38] Primaquine also superinduces hepatic TAT in the rat by an unknown mechanism.[39]

There thus exists a potpourri of data suggesting an unusual regulatory process(es) for TAT in rat liver and in cell cultures derived therefrom. We now can include 5-FU in this pharmacopeia of chemical agents which would ordinarily be expected to inhibit, if anything, TAT induction. The major common relationship between this widely diverse group of drugs is that they disturb some aspect of gene synthesis and/or function (including protein synthesis).

However, it is felt that the apparent alteration of TAT turnover by 5-FU described in this paper could be explained by a simple miscoding phenomenon at the level of

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messenger RNA translation. It is possible that 5-FU could direct the insertion of a "wrong" amino acid in certain key regions of the TAT molecule provided that the FU is not inserted at a degenerate codon. Such a "wrong" amino acid could alter the enzyme's affinity (stability) to the degradative process without noticeably altering (or indeed, enhancing) the catalytic properties since this is a testable hypothesis. It remains to be shown that certain properties (immunologic, heat stability, etc.) of the enzyme are detectibly altered by 5-FU treatment.

That 5-FU enhancement of TAT induction by insulin was not observed remains a subject of future experimentation in this phenomenon. While this finding may contradict our miscoding proposal, it has been shown that the mechanisms of TAT induction by glucocorticoids and insulin are not identical.[17]

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