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ADMINISTRATION OF QUEUINE TO MICE RELIEVES MODIFIED NUCLEOSIDE QUEUOSINE DEFICIENCY IN EHRLICH ASCITES TUMOR TRNA

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SUMMARY: The tRNA from Ehrlich ascites tumor cells is deficient in the modified nucleoside Q (queuosine). Continuous infusion of Q base (queuine) to tumor-bearing mice reverses the deficiency of Q in Ehrlich ascites tRNA, and coincidently, causes an inhibition of tumor growth.

The modified nucleoside Q (queuosine), 7-{[(cis-4,5-dihydroxy-2-cyclopenten-1-yl)-amino]-methyl}-7-deazaguanosine, occurs exclusively in the first position of the anticodons of $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ [these tRNAs form a set in that they accommodate the codons NA^{U}_{C} (where N=U,C, A, or G)] (1). The occurrence of Q in the anticodon suggests the possibility of Q-dependent translational control; however, functional differences between Q-free tRNA ([Q-]tRNA) and Q-containing tRNA ([Q+]tRNA) have not been detected using protein synthesis systems in vitro (2-5). Unlike other modifications of tRNA, Q is formed in part as the free base and then is incorporated at the posttranscriptional level into tRNA by the enzyme tRNA-guanine transglycosylase (6,7) (Fig. 1). The mammalian enzyme exchanges queuine (the base of Q) for guanine in vitro (6,7). In contrast, the bacterial enzyme exchanges a precursor of queuine into tRNA, and further modification to yield Q occurs at the polynucleotide level (7). The mammalian reaction presented in Fig. 1 is

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presumably physiologically significant, because a factor in bovine amniotic fluid that enables cultured mammalian cells to synthesize [Q+]tRNA has been identified as queuine (8). Normal mammalian tRNA of the NA^{U}_{C} codon set is almost completely substituted with Q or hexose-containing derivatives of Q (9,10); however, Q-deficient tRNA has been observed in (a) certain tumors (2,9-12), (b) fetal and regenerating liver (8,13,14), (c) reticulocytes (4,13,14), and (d) germ-free mice fed a defined diet (16). The present study was designed to determine if the administration of queuine could relieve the deficiency of Q in tumor tRNA in vivo. The results demonstrate that the administration of queuine to tumor-bearing mice reverses the deficiency of Q in Ehrlich ascites tRNA and, coincidentally, causes an inhibition of the growth of these neoplastic cells.

MATERIALS AND METHODS

Queuine was isolated from bovine amniotic fluid (7- to 9-month gestation, Irvine Scientific Co.) by a modification (J.R. Katze, unpublished) of a described procedure (6). Animals were housed in cages containing hardwood chip bedding and were fed standard laboratory chow (Ralston Purina). tRNA was isolated, aminoacylated using a crude aminoacyl-tRNA synthetase preparation from mouse liver, and recovered by DEAE-cellulose chromatography after aminoacylation as described (10). Reversed-phase chromatography employed a column (0.6 x 21 cm) of RPC-5 support developed at 27°C with a 300 ml 0.48 M - 1.0 M NaCl linear gradient in standard buffer, with 1.5 ml fractions collected, and the radioactivity measured as described (10) [^{3}H]His-tRNA was oxidized by periodate as described (16), and then recovered from the reaction by absorption and elution from DEAE-cellulose prior to RPC-5 chromatography.

RESULTS AND DISCUSSION

The data shown in Figure 2 and Table 1 demonstrate that Ehrlich ascites tumor $tRNA^{His}$ is significantly Q-deficient, confirming and expanding other observations (9). The deficiency of Q in $tRNA^{Asp}$ in Ehrlich ascites tumor cells is negligible (data not shown), in keeping with evidence that $tRNA^{Asp}$ species are preferred substrates for tRNA-guanine transglycosylase (ref. 17 and W.R. Farkas and J.R. Katze, unpublished). The side chain of Q contains a cis-diol group which, when oxidized by periodate (1), causes a specific delay in the elution of [Q+]tRNAs from RPC-5 columns (18). The

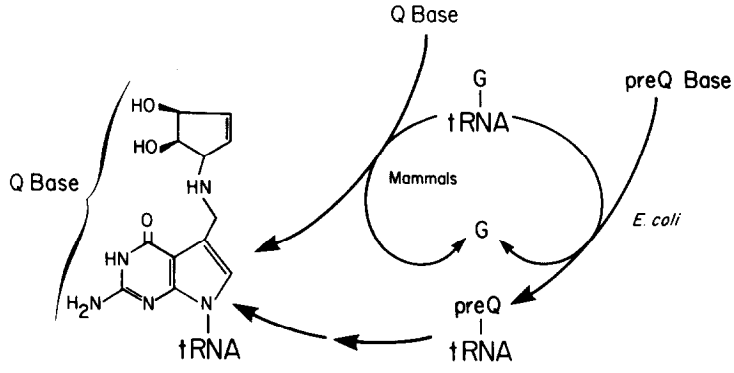

Fig. 1. Comparison of tRNA-Guanine Transglycosylase Catalyzed Steps of Q Synthesis in Mammals and E. coli. The abbreviation preQ base represents 7-(aminomethyl)-7-deazaguanine (see ref. 7). Whether the bacterial pathway also operates in mammals is unknown.

chromatogram in Fig. 2 shows a representative sample of tumor $tRNA^{His}$ before and after treatment with periodate. Only $tRNA_1^{His}$ was affected by periodate and is thus identified as [Q+]; by contrast, $tRNA_2^{His}$ and $tRNA_3^{His}$ were unaffected by periodate and are [Q-]. Mammalian $tRNA_1^{His}$ and $tRNA_2^{His}$ were shown

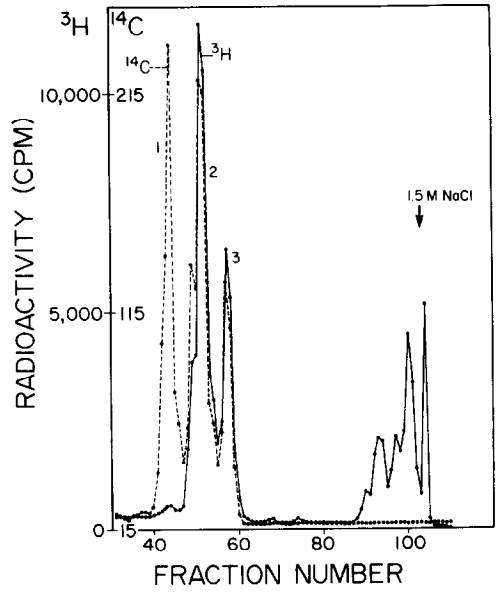

Fig. 2. Co-Chromotographic comparison of Ehrlich ascites tumor $tRNA^{His}$ before and after periodate oxidation. [^{14}C]His-tRNA is from the tumor of one mouse (Table 1, Exp. 2, untreated with queuine). [^{3}H]His-tRNA is from the same tRNA sample, but treated with periodate after aminoacylation. Additional experimental details are found in Materials and Methods.

TABLE 1. Effect of Queuine on $tRNA^{His}$ Isoaccepting Species From Ehrlich Ascites Tumors*

Group	N	Tumor $tRNA^{His}$ Species %1 [Q+]	Tumor $tRNA^{His}$ Species %2 [Q-]	Tumor $tRNA^{His}$ Species %3 [Q-]	Liver $tRNA^{His}$ Species %1 [Q+]	Liver $tRNA^{His}$ Species %2 [Q-]	Liver $tRNA^{His}$ Species %3 [Q-]
				Experiment 1			
Tumor-bearing	3	46 ± 14	42 ± 13	12 ± 1	NT	NT	NT
Tumor-bearing + Queuine (0.05 A_{260} Units/hr)	2	77 ± 1	22 ± 1	0.2 ± 0.2	NT	NT	NT
				Experiment 2			
Tumor-bearing	7	30 ± 10	53 ± 9	17 ± 4	80 ± 4	14 ± 3	6 ± 2
Tumor-bearing + Queuine (0.21 A_{260} Units/hr)	3†	70 ± 4	28 ± 4	1.2 ± 0.4	89 ± 3	11 ± 3	0.6 ± 0.2
Control	4	-	-	-	83 ± 2	14 ± 1	3 ± 1

*Mice (male Swiss Albino, 30-40 grams) were inoculated with 10^6 tumor cells on Day 0 and separated into three groups: (1) infused continuously with saline; (2) infused continuously with queuine; (3) received tumor alone. In Experiment 1, queuine (106 A_{260} units/ml in H_2O) or saline were delivered to the tumor-bearing animals with Alzet osmotic minipumps (Model 1702) at the rate of 0.5 l/hr; and animals were sacrificed on Day 9. In Experiment 2, queuine (214 A_{260} units/ml in H_2O) or saline were delivered with Alzet osmotic minipumps (Model 2001) at the rate of 1 ul/hr; and animals were sacrificed on Day 7. Pumps were implanted subcutaneously on Day 0. $tRNA^{His}$ species were resolved as described in Fig. 2. The several different preparations of tRNA did not differ significantly with respect to amount of $tRNA^{His}$ (data not shown). The data for tumor-bearing animals which received either saline or no treatment were grouped together because no difference was observed between them. Values are means ± standard deviations. NT signifies not tested.

†These tumor $tRNA^{His}$ values are the means from only 2 of the 3 mice treated with queuine because in the third mouse the small amount of cells recovered (0.1 ml packed cell volume) and a significantly different pattern for $tRNA^{His}$ (92% species 1; 8% species 2; 0.1% species 3) indicated that only normal peritoneal cells were detected.

previously to be [Q+] and [Q-], respectively (4,10,15), and the present and prior data (6,11) are consistent with $tRNA_2^{His}$ and $tRNA_3^{His}$ being Q-free precursors of $tRNA_1^{His}$. Table 1 shows that liver $tRNA^{His}$ is 80-90% [Q+] and that tumor $tRNA^{His}$ is less than 50% [Q+]. In two experiments, treatment of tumor-bearing animals with two different doses of queuine shifted the tumor $tRNA^{His}$ species-distribution toward that observed for liver. These data (Table 1 and Fig. 2) are consistent with the incorporation of exogenous queuine into [Q-]tRNA *in vivo*.

It is of interest that four times more queuine was delivered in Experiment 2, than in Experiment 1, yet the tumor $tRNA^{His}$ derived from both groups of treated mice was modified similarly. However, while no effect of queuine on tumor-growth was noted in Experiment 1 (data not shown), the higher dosage of queuine in Experiment 2 produced an apparent antitumor effect (Table 2). The total tumor recovered from the queuine-treated mice in Experiment 2 was less than that recovered from the untreated controls; indeed, no tumor was recovered from one animal. Moreover, the 7-day increase in body weight was prevented as well. Together these observations reflect impaired tumor-growth.

There are several possible explanations for the relative deficiency of Q in the tRNA of tumors, including: a) a defect in the biosynthesis, cell uptake, or insertion of precursor base (queuine or prequeuine) into tRNA; b) excessive catabolism of precursors of Q or removal of Q from tRNA; and c) a

TABLE 2. Effect of Queuine on the Growth of Ehrlich Ascites Tumor Cells in Mice*

Group	No. of Mice	Weight Ratio Days 7/0	Packed Volume of Tumor Cells (ml)
Tumor-bearing	7	1.29 ± .08	1.9 ± 0.6
Tumor-bearing + Queuine	3	1.10 ± .07	0.5 ± 0.5
Control	3	1.00 ± .05	--

*The tumor-bearing groups and nontumor-bearing control group from Experiment 2, Table 1, were weighed daily. One mouse from the control group in Table 1 was not weighed on Day 0 and is not represented here. The values represent the mean ± S.D. for each group.

tumor-elicited metabolite that interferes at some point in the normal metabolism of Q. With regard to these possibilities, tumor tissue has been reported to contain adequate levels of tRNA-guanine transglycosylase (7); however, the de novo synthesis of Q has yet to be demonstrated in mammals and nothing is known about the catabolism of Q. Additionally, the data in Table 1 allow the suggestion that the tumor may alter the metabolism of Q in non-tumorous tissues: a) $tRNA_3^{His}$ increased to a small extent in the livers of the tumor-bearing animals and b) treatment of these animals with queuine led to a decrease in liver $tRNA_3^{His}$ to below the values for control liver.

Finally, it is of interest that deficiences of Q in tRNA, similar to those observed here in Ehrlich ascites tumors, have been observed in the livers of germ-free mice fed a defined diet (16). If otherwise apparently normal germ-free mice can be deficient in Q, the undermodification per se cannot be a sufficient requirement for tumorigenicity. The data at hand, however, are consistent with the possibility that a deficiency of Q favors tumor growth; therefore, the phenomenon reported here has potential significance for the therapy of neoplasia.

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