Failure of L-Histidinol to Improve the Therapeutic Efficiency of 5-Fluorouracil against Murine Breast Tumors

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ABSTRACT

It has been reported that L-histidinol, a structural analogue of the essential amino acid L-histidine, can transiently inhibit proliferative cycling in cells with normal phenotype while allowing continued cell cycle transit in tumor cells. Thus, in the presence of L-histidinol, the toxicity of a proliferation-dependent drug such as 5-fluorouracil (FUra) was found to be reduced in normal tissue cells of the DBA/2J mouse, but not in L1210 leukemia cells in the same mouse. Because of the potential clinical significance of this approach to reduce chemotherapy-associated host toxicity, we evaluated the L-histidinol-FUra combination in a nonleukemic, solid murine tumor model, the BALB/c x DBA/8 F1 (hereafter called CD8F1) breast tumor. The results of these studies indicate that the administration of L-histidinol can protect the CD8F1 mouse from FUra-associated leukopenia, body weight loss, and ultimately, from mortality. However, in contrast to results reported in the L1210 leukemia system, L-histidinol also reduced the cytotoxic activity of FUra against CD8F1 breast tumors. Therefore, although the dose of FUra that could be administered with safety was higher in mice receiving L-histidinol, the therapeutic results of the combination of FUra and L-histidinol were not superior to those obtained with FUra alone at a lower dose.

INTRODUCTION

L-Histidinol, a low molecular weight structural analogue of the essential amino acid L-histidine, has been reported to confer significant improvement in the therapeutic index of both ara-C and FUra against i.p. L1210 leukemia cells in DBA/2J mice (1). The rationale for the use of L-histidinol in conjunction with proliferation-dependent chemotherapeutic drugs such as ara-C and FUra stems from the ability of L-histidinol to reversibly arrest normal cells in a Go-like noncycling state while allowing continued cell cycle transit in most of their tumorigenic counterparts (2-7). Thus in the presence of L-histidinol, the toxicities of proliferation-dependent anticancer drugs such as ara-C and FUra are reduced in a variety of normal cell lines, but not in a number of tumorigenic lines (2, 3, 5). Because of the protective effect of L-histidinol in normal host tissues, FUra could be administered in conjunction with L-histidinol in vivo in DBA/2J mice bearing i.p. L1210 leukemia at approximately 7 times the dose that was tolerated when FUra was administered alone, and the combination of FUra and L-histidinol (at the elevated dose of FUra) produced a statistically significant increase in survival time compared to that which was obtained when FUra alone was administered at maximal tolerated dose (1). These results were extended in a second publication where intrafemoral L1210 leukemia was treated with L-histidinol plus either ara-C or FUra (8). In these later studies, it was demonstrated that the administration of L-histidinol evoked a transient, spontaneously reversible, and selective proliferation arrest in normal murine bone marrow cells in vivo and hence reduced the toxicity of ara-C or of FUra for these normal cells without diminishing the cytotoxicity of these drugs towards intrafemoral L1210 leukemia cells in the same mice (8).

Because of the potential clinical significance of these findings, we were interested in evaluating this approach in a nonleukemic, solid murine tumor model. In the studies presented here, L-histidinol was administered at a dose and schedule suggested in the report cited above (1) to mice bearing advanced first-passage transplants of spontaneous BALB/c x DBA/8 F1 (hereafter called CD8F1) breast tumors. FUra, which is known to be active in this murine breast tumor model, was selected as the proliferation-dependent chemotherapeutic drug. Bone marrow toxicity ensuing from the administration of FUra was ameliorated in CD8F1 mice treated with L-histidinol, confirming similar results reported in DBA/2J mice (1, 8). In addition, we found a previously unreported protection from FUra-associated body weight loss in mice that received L-histidinol. However, L-histidinol caused a diminution of the antitumor activity of FUra in this solid tumor system, and even though higher doses of FUra could be administered safely, there was no selective advantage for the combination of L-histidinol and FUra over FUra alone at its maximally tolerated dose.

MATERIALS AND METHODS

Murine Tumor System. For each experiment, 3-4-month-old CD8F1 mice bearing a first-generation spontaneous syngeneic tumor transplant were distributed among experimental groups so that mice bearing tumors of approximately equal weight were represented in each group. The mice were weighed before each of three weekly courses of treatment (described in text). Tumor measurements were recorded on the day of initiation of treatment and again at 5 or 6 days after each weekly course of treatment. Normal, 3-4-month-old tumor-free CD8F1 mice were used for initial toxicity measurements.

Drugs. FUra, purchased from Sigma Chemical Co. (St. Louis, MO) was dissolved immediately before use in 0.85% NaCl solution. PALA, obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, was dissolved in 0.85% NaCl solution, and the pH was adjusted to 7.2 with 1 N NaOH before adjustment to final volume. Both drugs were administered so that the desired dose was contained in 0.1 ml/10 g of body weight. L-Histidinol dihydrochloride (Sigma) was dissolved in water, the pH was adjusted to 7.3, and the final concentration was adjusted to 100 mg/ml. This stock solution was filter-sterilized and stored frozen until needed.

Toxicity Measurements. Mice were weighed at least twice per week for measurement of body weight changes. In preliminary experiments (not shown) where body weight measurements (and WBC) were performed more frequently, it was determined that the administration of L-histidinol together with FUra did not alter the kinetics of weight loss or WBC depression seen with FUra alone (although, as will be described, it did alter the magnitude of these changes). Student's t test was used to determine the statistical significance of differences in body weight loss between groups. Peripheral WBC levels were measured in tail vein blood with an electronic hematology analyzer. In experiments in which WBC levels were measured, mice were distributed into experimental groups according to their pretreatment WBC count so that mice with approximately equal WBC counts were represented in each group.
group. WBC then were measured at timed intervals after initiation of treatment. Statistical significance of differences in mean WBC levels between groups was determined with Student's t test. Animals were observed for mortality during and for a period of 30 days after treatment. The significance of differences in mortality between groups was determined by χ² analysis.

**RESULTS**

Effect of L-Histidinol on the Toxic Manifestations of FUra in Normal CD8F1 Mice. Two groups of seven normal CD8F1 mice each were treated with a known toxic regimen of FUra, 130 mg/kg/week for four courses. In addition, one of the groups was treated with l-histidinol, 7 mg/mouse, administered at -2, 0, 2, 4, and 6 h in relation to each weekly course of FUra. WBC levels in both groups of mice on day 20 (i.e., the day before the last course of treatment, and before any mice had died) are shown in Table 1. As expected, leukopenia developed in mice treated with this toxic regimen of FUra. The WBC dropped in mice treated with FUra alone from a pretreatment level of 12,764/mm³ to 3,083/mm³ on day 20. In contrast, mice treated with the same regimen of FUra in conjunction with l-histidinol had a WBC of 10,886/mm³ on day 20, which was significantly different from that in mice treated with FUra alone (P ≤ 0.005) and not significantly different from pretreatment levels. In addition, mice treated with FUra alone had lost an average 29% of their body weight by day 20 and three of the seven mice went on to die during the 30-day observation period following the last course of treatment. In contrast, mice treated with FUra plus l-histidinol lost only 3% of their body weight (P ≤ 0.005, compared to mice treated with FUra alone) and none of these mice died during the observation period.

Effect of L-Histidinol on the LD₅₀ of FUra in Normal CD8F1 Mice. To gain some quantitative information concerning the magnitude of protection afforded by l-histidinol treatment, FUra was titrated as a single bolus dose, alone, and in conjunction with l-histidinol, 7 mg/mouse (administered at -2, 0, 2, 4, and 6 h in relation to FUra) in groups of six normal CD8F1 mice. The mice were observed for mortality for 30 days after treatment, and the LD₅₀ for mice treated with FUra alone and for mice treated with FUra plus l-histidinol were calculated from mortality statistics according to the method of Weil (10). The LD₅₀ for FUra alone in these mice was found to be 283 mg/kg (with 95% confidence limits of 225-356 mg/kg), or 78% above the LD₅₀ of FUra alone. This magnitude of protection from FUra-induced mortality in l-histidinol-treated mice is considered meaningful in light of the known steep toxicity-dose relationship of FUra in these mice (11).

Therapeutic Evaluation of L-Histidinol in Conjunction With High Dose FUra. It was obvious from the results of the preceding experiments that the dose of FUra administered to these mice could be escalated with safety when given in conjunction with l-histidinol. Therefore, we proceeded to evaluate the therapeutic efficacy of a high-dose FUra plus l-histidinol regimen of treatment in the same strain of mice (CD8F1) bearing advanced (average, 135 mg) first-passage transplants of spontaneous CD8F1 breast tumors. Mice-bearing mice from a single transplant were separated into groups of 10 mice each so that mice with approximately equal size tumors were represented in each group. Each group was treated with one of various doses of FUra alone or with one of various doses of FUra together with l-histidinol at 7 mg/mouse administered at -2, 0, 2, 4, and 6 h in relation to FUra. A final group was treated with saline in place of drug and served as control for untreated tumor growth. Treatment was administered on day zero (i.e., the day that tumors were measured and the mice were assigned to groups) and again on days 7 and 14. The mean tumor weight as well as the mortality in each of these groups after the last course of treatment are shown in Table 2.

In agreement with the results obtained in the previous experiments in normal, tumor-free mice, significantly higher doses of FUra were tolerated in tumor-bearing CD8F1 mice when the FUra was administered in conjunction with l-histidinol. Specifically, FUra alone at 100 mg/kg/week (group 2) proved to be the maximal tolerated dose, and a higher dose of 125 mg/kg/week (group 4) resulted in an unacceptable mortality rate of 40%. In contrast, treatment with this elevated FUra dose of 125 mg/kg/week in conjunction with l-histidinol (group 5) resulted in no drug mortality; in fact, an even higher FUra dose of 150 mg/kg/week in conjunction with l-histidinol (group 6) appeared to be the maximally tolerated dose, resulting in only 10% mortality. However, despite the ability of the l-histidinol-treated mice to tolerate the higher doses of FUra, tumors in mice treated with l-histidinol plus FUra at 150 mg/kg/week (i.e., the maximal tolerated dose of this combination in this weekly schedule) were not inhibited to any greater extent (807 mg, group 6) than were tumors in mice treated with the maximal tolerated dose of FUra alone, 100 mg/kg (601 mg, group 2). Further, it is obvious from the results in Table 2 that l-histidinol diminished the antitumor activity produced by any

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor wt (mg)</th>
<th>Dead/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUra</td>
<td>4,830 ± 349</td>
<td>3/10</td>
</tr>
<tr>
<td>FUra</td>
<td>601 ± 96</td>
<td>0/10</td>
</tr>
<tr>
<td>FUra + l-histidinol</td>
<td>1,113 ± 209</td>
<td>0/10</td>
</tr>
<tr>
<td>FUra + l-histidinol</td>
<td>483 ± 111</td>
<td>4/10</td>
</tr>
<tr>
<td>FUra + l-histidinol</td>
<td>915 ± 107</td>
<td>0/10</td>
</tr>
<tr>
<td>FUra + l-histidinol</td>
<td>807 ± 227</td>
<td>1/10</td>
</tr>
</tbody>
</table>

* Tumor weight was calculated from measurements taken at 5 days after the third course of treatment.
given dose of FUra. For example, at a FUra dose of 100 mg/
kg/week tumors were significantly larger (1113 mg) in mice treated with L-histidinol (group 3) than in mice that received
the same dose of FUra alone (601 mg, group 2), P ≤ 0.05. The same
pattern was observed at a FUra dose of 125 mg/kg/week
where mice treated with L-histidinol had significantly larger
tumors (915 mg, group 5) than mice treated with the same dose of
FUra alone (483 mg, group 4), P ≤ 0.05. Essentially the same
results were obtained subsequently in four separate experi-
ments, each from a different first-passage CD8F, breast tumor
transplant.

Therapeutic Evaluation of L-Histidinol in Conjunction with a
Therapeutically Synergistic FUra-containing Drug Combination.
We have previously demonstrated that the antitumor activity
of FUra can be modulated selectively by the administration of
a low dose of the pyrimidine antagonist, PALA, approximately
17 h in advance of the administration of FUra (12). At the low
dose, 100 mg/kg, PALA has no measurable antitumor activity
by itself against the CD8F, breast tumor; however, it produces
a greater depression in uridine nucleotide pools in tumor tissue
in vivo than in normal intestinal mucosa or in bone marrow.
Consequently, when FUra is administered after this low dose
of PALA, it becomes incorporated more efficiently into RNA
in place of the uridine nucleotide in tumor tissue, but not in
intestine or in bone marrow (13). Since, in this tumor system,
the incorporation of FUra into RNA has been correlated with
cytotoxicity (14-18), the enhanced incorporation of FUra into
RNA of tumor tissue as opposed to normal target tissues is the
reason for the observed therapeutic synergism between the two
drugs (i.e., PALA and FUra).

Because of the increased antitumor specificity of the PALA
plus FUra combination (over that which can be obtained with
FUra alone), we were interested in determining whether or not
L-histidinol could protect the host against increased toxicity
obtained when the dose of FUra was elevated in the PALA-
modulated combination, and whether or not this would result
in increased antitumor activity in mice bearing first-passage
CD8F, breast tumor transplants. In these experiments, low-
dose PALA (100 mg/kg) was administered 17 h before FUra
at 100 mg/kg (the maximal tolerated dose of the combination)
or FUra at 125 mg/kg (a known toxic dose of the combination).
Additional groups were treated with PALA at 100 mg/kg 17 h
before FUra at 125 mg/kg, or FUra at 150 mg/kg, together
with L-histidinol, 7 mg/mouse administered at -2, 0, 2, 4, and
6 h in relation to FUra. All groups received three courses of
the indicated treatment with a 1-week interval between courses.
Tumor weight, body weight, and mortality were recorded 6 days
after the second course of treatment, and again at 6 days after
the third course of treatment (Table 3).

After two courses of treatment there was no mortality in any
of the groups. However, there was a dose-dependent increase
in body weight loss in animals treated with PALA and FUra.
The difference in weight loss between the two groups receiving
the same regimen of chemotherapy (PALA plus FUra at 125
mg/kg) with and without L-histidinol was statistically signifi-
cant (P ≤ 0.01). At a still higher dose of FUra (150 mg/kg) in
conjunction with L-histidinol, body weight loss was higher
(16%) and close to the body weight loss observed in mice treated
with PALA and the much lower dose of FUra, 100 mg/kg.
However, in addition to host protection, it appears that the L-
histidinol was causing a significant reversal of the antitumor
activity of the chemotherapy. A tumor weight of 199 mg was
recorded in mice treated with PALA and FUra at 125 mg/kg,
whereas a tumor weight of 514 mg was measured in tumors
treated with the same regimen of chemotherapy in conjunction
with L-histidinol (this difference was statistically significant, P
≤ 0.05). Even at a FUra dose of 150 mg/kg in conjunction with
L-histidinol, the mean tumor weight after two courses of treat-
ment, 278 mg, was not significantly smaller than that obtained
in mice treated with the PALA-FUra combination with FUra
at 100 mg/kg.

Focusing on the results obtained 6 days after three courses
of treatment (also shown in Table 3), it becomes obvious that
FUra at 100 mg/kg is the maximally tolerated dose in the
PALA-FUra combination, since mice treated with the next
higher dose (PALA-FUra at 125 mg/kg) exhibited a 78%
mortality rate. In contrast, mice that received L-histidinol in
conjunction with PALA-FUra at 125 mg/kg showed statisti-
cally significant protection from mortality (P ≤ 0.01). However,
where the FUra dose was raised further, to 150 mg/kg, even in
conjunction with L-histidinol, on unacceptable mortality rate
ensued. Therefore, in order to evaluate antitumor activity at
approximately equal toxic doses, we can compare the group
treated with PALA-FUra at 100 mg/kg (mean tumor weight,
374 mg) with the group treated with PALA-FUra at 125 mg/
kg plus L-histidinol (mean tumor weight, 676 mg). From these
results (as in the previous results with FUra alone), it is obvious
that L-histidinol did not improve the therapeutic results ob-
tained in mice treated with PALA-FUra, even though the
administration of L-histidinol did permit the safe escalation
of the dose of FUra administered. It seems reasonable to conclud-
that the therapeutic failure can be ascribed to the ability of the
L-histidinol to protect the tumor from the cytotoxicity of the
PALA-FUra combination just as it protects the normal tissues
of the host.

DISCUSSION

Results of the studies reported here indicate that the admin-
istration of an appropriate regimen of L-histidinol can protect

Table 3 Therapeutic comparison of the drug combination PALA-FUra, administered with and without L-histidinol in CD8F, mice bearing a first-passage breast tumor transplant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor wt (mg)</th>
<th>Body wt change (%)</th>
<th>Dead/total</th>
<th>Tumor wt (mg)</th>
<th>Dead/total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 days after 2 courses of treatment</td>
<td>6 days after 3 courses of treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline control</td>
<td>3,282</td>
<td>-2</td>
<td>0/9</td>
<td>7,506</td>
<td>0/9</td>
</tr>
<tr>
<td>PALA100-FUra100</td>
<td>345</td>
<td>-13</td>
<td>0/9</td>
<td>374</td>
<td>1/9</td>
</tr>
<tr>
<td>PALA125-FUra125</td>
<td>199</td>
<td>-34</td>
<td>0/9</td>
<td>Toxic</td>
<td>7/9</td>
</tr>
<tr>
<td>PALA100-FUra125 + L-histidinol</td>
<td>514</td>
<td>0/9</td>
<td>676</td>
<td>0/9</td>
<td></td>
</tr>
<tr>
<td>PALA100-FUra125 + L-histidinol</td>
<td>278</td>
<td>-16</td>
<td>0/10</td>
<td>Toxic</td>
<td>5/10</td>
</tr>
</tbody>
</table>
the CD8F, mouse from leukopenia and eventual mortality resulting from the administration of toxic levels of the proliferation-dependent drug, FUra. These results confirm previously reported work in a different mouse strain (DBA/2J) in which L-histidinol treatment was associated with protection of femoral colony forming units/culture and prevention of mortality following treatment with toxic doses of FUra (8). In addition, we observed a previously unreported protection from FUra-associated body weight loss in mice treated with L-histidinol. Mice treated with FUra alone had lost nearly 29% of their body weight by six days after the third weekly course of FUra at 130 mg/kg, whereas mice treated with the same regimen of FUra in conjunction with L-histidinol had lost only 3% of body weight, \( P < 0.005 \) (Table 1).

In order to quantitate L-histidinol protection against FUra-induced mortality, FUra was titrated in two groups of CD8F, mice, one of which received L-histidinol. The LD50 for FUra alone was found to be 283 mg/kg which was in close agreement with the previously reported value in these mice (11). In L-histidinol-treated mice the LD50 of FUra was increased 78%, to 504 mg/kg. This may be considered to be a significant degree of protection against a drug with as steep a dose-activity response as FUra (11). However, the magnitude of protection obtained with L-histidinol in these experiments in CD8F, mice was less profound than that reported in DBA/2J mice (1). Although FUra was not quantitatively titrated in the previously published studies (1), they reported complete protection in L-histidinol-treated mice from a dose of 30 mg of FUra in 20-25-g mice, equivalent to an average dose of 1350 mg/kg. This would represent approximately 7 times the LD50 of FUra alone that was reported as averaging approximately 203 mg/kg in the DBA/2J mice (1). Therefore, the DBA/2J mice appear to be slightly more sensitive to the toxic action of FUra, and also more sensitive to the protective activity of L-histidinol than the CD8F, mice used here. Nevertheless, despite the apparent quantitative differences between the two mouse strains, a qualitatively similar protection against FUra toxicity was obtained with L-histidinol in both strains.

The potential therapeutic utility of L-histidinol in conjunction with proliferation-dependent chemotherapy depends upon the achievement of selective, differential activity in normal host tissues versus tumor tissue. Such a differential was observed in studies with L1210 leukemia in DBA/2J mice, where the host was protected from the toxic action of FUra by L-histidinol treatment, while the cytotoxicity of FUra against the leukemia cells was not diminished (and, in fact, was increased) (1). To determine if the same relationship applies in the solid murine CD8F, breast tumor, mice bearing advanced first-passage spontaneous CD8F, breast tumor transplants were treated with FUra alone, or with FUra in conjunction with L-histidinol. Since this is a slow-growing solid tumor, the mice received treatment at weekly intervals for a total of three courses, and the tumors were measured 5 days after the last course of treatment. From the data (Table 2), it can be concluded that when administered alone, a FUra dose of 100 mg/kg/week was the maximal tolerated dose in this schedule, and that at dose, FUra produced highly significant tumor growth inhibition in comparison to saline-treated controls. In mice treated with the same dose of FUra (100 mg/kg/week) in conjunction with L-histidinol, tumor size was significantly larger (1113 mg) than in mice receiving FUra alone at 100 mg/kg/week (601 mg), \( P < 0.05 \). A significant reversal of antitumor activity also was observed at the next higher dose of FUra (125 mg/kg/week) in mice treated with L-histidinol as compared to mice treated with FUra alone. As in the tumor-free mice, the FUra dose could be raised safely in tumor-bearing CD8F, mice treated with L-histidinol, and in this experiment a dose of 150 mg/kg/week was found to be the maximally tolerated dose (i.e., the dose resulting in approximately 10% mortality). But even at this high FUra dose (50% more FUra per week for each of 3 weeks) in conjunction with L-histidinol, the antitumor activity was not better (or significantly worse) than that obtained with the lower dose of FUra (100 mg/kg/week) when administered without L-histidinol (807 versus 601 mg).

A similar result was obtained when L-histidinol was administered in conjunction with the synergistic two-drug combination, PALA-FUra. Without L-histidinol at a PALA dose of 100 mg/kg/week, the maximal tolerated dose of FUra was 100 mg/kg/week (Table 3). However, in conjunction with L-histidinol, FUra at 125 mg/kg could be administered safely with PALA at 100 mg/kg. Nevertheless, despite the ability to administer a higher dose of FUra in the PALA-FUra combination when given in conjunction with L-histidinol, the antitumor activity was not improved over that which was obtained with the lower dose of the chemotherapeutic combination in the absence of L-histidinol. As in the experiment with FUra as a single agent, L-histidinol caused a statistically significant reversal of antitumor activity associated with the PALA-FUra combination (Table 3).

On balance, the administration of L-histidinol together with FUra (or together with a PALA-FUra drug combination) did not result in an improved therapeutic index in CD8F, mice bearing first-passage transplants of the spontaneous breast tumor. Whereas L-histidinol was found to increase the toxic activity of FUra against L1210 leukemia cells (1), we found here that L-histidinol decreased the toxic activity of FUra against the CD8F, breast tumor. Therefore, although a higher dose of FUra could be administered to mice protected with L-histidinol, the tumor also was protected, and therefore, this did not result in an improvement in therapeutic efficacy in the CD8F, breast tumor system. The reason for the difference in the action of L-histidinol vis à vis FUra chemotherapy in L1210 leukemia as compared to CD8F, breast tumor is not known at present, but in the light of the potential clinical significance of this approach to protect the host during cytotoxic chemotherapy, it would appear to be worthwhile to discover the relevant difference in the action of L-histidinol in these two murine tumors.

We have not yet examined the effect of L-histidinol on cell cycle progression in CD8F, breast tumor cells. It is possible that unlike other experimental tumor lines that have been tested, CD8F, breast tumor cells may be sensitive to L-histidinol-mediated inhibition of cell cycle progression. However, other possible biochemical explanations seem equally feasible. L-Histidinol is a competitive inhibitor of histidyl-RNA synthetase (19, 20) and an inhibitor of protein and RNA synthesis in eukaryotic cells (21, 22). Since the cytotoxicity of FUra in the CD8F, breast tumor is known to be correlated with the extent of incorporation of FUra in RNA (14-18), L-histidinol-mediated inhibition of RNA synthesis might be expected to result in decreased incorporation of FUra into RNA with a consequent decrease in FUra cytotoxicity. If, in contrast, FUra cytotoxicity is mediated predominantly via inhibition of thymidylate synthetase in L1210 leukemic cells, L-histidinol-mediated inhibition of RNA synthesis would not interfere with FUra cytotoxicity in this tumor. These and other potential biochemical differences between L1210 leukemia cells and CD8F, breast
tumor, which may be responsible for differential action of L-histidinol are amenable to experimental scrutiny.

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