MODULATION OF THE EFFECTS OF FLUOROPYRIMIDINES ON TOXICITY AND TUMOR INHIBITION IN RODENTS BY URIDINE AND THYMIDINE

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(Received 10 March; accepted 25 April 1986)

Uridine and/or thymidine were administered concomitantly with 5-fluorouracil (5-FU) and 5'-deoxy-5-fluorouridine (5'-dFUR) to mice and rats in order to establish whether the physiological nucleosides acting in vitro as antagonists can diminish fluoropyrimidine toxicity in vivo. In addition, the influence of orally co-administered uridine on antitumor activity of 5-FU and 5'-dFUR in mice has been studied. All co-administrations aggravated the general toxicity of both fluoropyrimidines. Tumor inhibition was enhanced by uridine, but the therapeutic ratio was not improved compared to monotherapy with either 5-FU or 5'-dFUR.

Key words: Fluoropyrimidines, Uridine, Toxicity, Tumor inhibition.

INTRODUCTION

Modulation of side-effects in cancer chemotherapy has been the goal of various preclinical and clinical investigations. For treatment with antimetabolites, an improvement of the therapeutic ratio is postulated through counteracting their inherent toxicity by co-administration of the competing physiological enzyme substrate. High-dose methotrexate therapy combined with calcium leucovorin to block severe myelosuppression and mucous membrane toxicity attained clinical relevance. Preclinical investigations have been undertaken with the same intention using combinations of 5-fluorouracil (5-FU) with thymidine and guanosine.1-10

Although both antitumor and toxic effects of 5-fluoropyrimidine combinations were increased in some of these studies, an improved therapeutic margin was claimed. Therefore, clinical phase I studies were instituted combining 5-FU with thymidine11,12 or uridine.13 Almost all toxicity parameters, mainly bone marrow depression and gastrointestinal symptoms, were augmented by co-medication. Antitumor activity was increased and in some cases tumor resistance to 5-FU could be abolished, but the clinical superiority of the combination was never proven.

5'-Deoxy-5-fluorouridine (5'-dFUR, doxifluridine), first synthesized in 1976,14 has been shown to possess a high cytostatic activity against a broad spectrum of experimental tumors and a low toxicity.12-17 Oral administration of 5'-dFUR resulted in some gastrointestinal toxicity in man.18,19 Therefore, a possibility of protecting the gastrointestinal tract of patients by giving concurrently uridine which counteracts 5'-dFUR effects in vitro20 seemed very attractive.

The aim of the present study was to find an oral doxifluridine regimen with better tolerance in rodents by co-administration of thymidine (TdR) and/or uridine (UR). In particular, the possibility of counteracting gastrointestinal toxicity was investigated. Second, we wanted to find out whether modulatory effects of uridine would be specific for doxifluridine or would also be seen for the parent fluoropyrimidine 5-FU. Third, a possible improvement of the therapeutic margin had to be determined.

MATERIALS AND METHODS

5-FU and 5'-dFUR were synthesized in the Roche laboratories. 5-FU was dissolved in water for oral gavage or in sterile saline solution for intravenous administration. 5'-dFUR in the lyophilized form (Ro 21-9738/606, a partial sodium salt of the hydrochloride) was dissolved to a 6% stock solution. It was diluted with water or mixed with appropriate volumes of TdR and UR solutions to a final concentration of 6% (isotonic). UR was dissolved in water, TdR is solvent SV. SV was prepared by
dissolving 5 g sodium carboxymethylcellulose (medium viscosity), 4 ml Tween 80 and 5 ml benzyl alcohol in 1 litre bidistilled water. All ready-for-use preparations were kept frozen until administration.

All animals were from Tierfarm Füllinsdorf, Switzerland, and were kept under controlled conditions. They had free access to normal laboratory chow and tap water.

Toxicity studies

Wistar rats, 5 females of 150 g and 5 males of 200 g were used per treatment group. They were weighed and observed daily, 5 times a week, with particular attention to diarrhoea. Blood was taken retro-orbitally under light ether anesthesia for leukocyte counting. Medications were given 5 times a week in a total volume of 10 ml kg$^{-1}$ body wt by means of a gastric tube.

Female Fü-Albino Swiss mice, 5 per treatment group, weighing 25 g were used in the study comparing routes of administration. Intravenous administration was given via the tail vein, oral solutions were given by means of a stomach tube. Blood from the tail vein was taken for leukocyte counts.

After termination of the toxicity studies, animals from all treatment groups were inspected autopsically for pathologic changes of internal organs.

Transplantable tumors

L1210. Viable cells ($10^5$) from donor ascites were injected intraperitoneally into female C57 B1/6 × DBA/2 F₁ (BDF₁) mice weighing 20 g. Daily oral administration of the drugs was started on the day of implantation and continued 5 times a week until death.

Lewis lung carcinoma (3LL) and Crocker sarcoma S180. Viable cells ($3 \times 10^6$) in a tumor tissue suspension of 0.5 ml were subcutaneously implanted into female BDF₁ (3LL) or Swiss mice (S180). Daily gavage of the test solution was started on the day of implantation and continued 5 times a week until one day before tumor excision. 3LL tumors were excised on day 16, S180 tumors on day 9.

RESULTS

(1) Toxicity of oral 5'-dFUR combination in rats

Previous experiments combining 5'-dFUR admin-

istration with UR and TdR showed that TdR doses
up to 2000 mg kg$^{-1}$ and UR doses up to 4000 mg
kg$^{-1}$ daily were well tolerated for one week (data
not shown).

5'-dFUR alone in a dose of 600 mg kg$^{-1}$ caused
body weight loss and slightly lowered white blood
cell counts, but no further toxic signs, in particular
no diarrhoea and no changes in the gastrointestinal
tract. When 5'-dFUR was combined with UR or
TdR or both, death, diarrhoea and severe
leukopenia occurred (Table 1). Small intestines of
autopsied rats treated with combined regimens were
endarmatous and inflamed. The stomach was
moderately, caecum and colon markedly inflated.
Liver, spleen, kidneys and mesenterium seemed to
be of normal size, colour and structure.

5'-dFUR was given as a single treatment of 800 mg
kg$^{-1}$ per day and in combination with UR at
different doses from 100 to 800 mg kg$^{-1}$ starting with
UR one week before 5'-dFUR (Table 2). Treatment
with 5'-dFUR alone caused a marked decrease of
peripheral leukocytes after 5 doses (day 6),
diarrhoea and body weight loss at day 8, but death
occurred only at days 9 and 10. In the groups treated
with combinations, diarrhoea and myelosuppression
appeared earlier, and body weight loss was aggra-
vated by UR in a dose-dependent manner. The
combination with 800 mg kg$^{-1}$ UR caused the first
deaths at day 5 after 3 5'-dFUR administrations. The
autopsy findings of these animals were identical to
those in the first experiment: combined treatment
resulted in gastrointestinal inflammation. Addition-
ally, blood clots were found in the stomach and small
intestine in some cases. Animals treated with 5'-
dFUR alone showed no gross pathological findings.

(2) Toxicity of 5-FU and 5'-dFUR combined with
UR given intravenously and orally to Swiss mice

When the compounds were given by the intra-
venous route, all combinations of 5'-dFUR or 5-FU
with UR were more toxic than single therapy. Body
weight loss as well as myelosuppression and gastro-
intestinal toxicity demonstrated by the occurrence of
diarrhoea and inflamed small bowel at autopsy were
aggravated (Table 3).

When 5-FU and 5'-dFUR were given orally,
similar observations were made. In combination
therapy, diarrhoea, leukopenia, body weight loss
and the number of deaths were increased, clearly
dependent on the dose of UR (Table 4).