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Short communication

Comparative antitumor activity of ruthenium derivatives with 5'-deoxy-5-fluorouridine in chemically induced colorectal tumors in SD rats

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Summary. The activity of the newly synthesized ruthenium derivative imidazolium-bis(imidazole)tetrachlororuthenate (III) [ImH($RuIm_2Cl_4$)] was compared with that of 5'-deoxy-5-fluorouridine (5'dFUR) in autochthonous acetoxymethyl-methylnitrosamine (AMMN)-induced colorectal cancer in SD rats. Following coloscopic diagnosis of colorectal tumors treatment was administered twice weekly for a 10-week period. ImH($RuIm_2Cl_4$) exhibited considerable antitumoral efficacy compared with 5'dFUR (20 T/C % and 60 T/C %, respectively) against the growth of AMMN-induced colorectal adenocarcinoma in SD rats. The mortality rates with ImH($RuIm_2Cl_4$) were dose-related, but its efficacy did not vary in all doses administered.

Introduction

Colorectal cancer is one of the most frequent malignancies in the western world [4], with rising incidence and mortality rates [18]. The results reported for chemotherapy of this tumor remain disappointing [6, 13, 21].

Acetoxymethyl-methylnitrosamine (AMMN, Fig. 1) -induced autochthonous colorectal cancer in SD rats appears to be a more reliable model than transplanted tumors for prediction of the efficacy of new compounds against gastrointestinal cancer [23]. The majority of tumors are well-differentiated adenocarcinomas with a relatively long tumor volume-doubling time. Furthermore, the chemoresistance of these tumors makes this model more similar to the human situation [17, 20].

5-Fluorouracil is one of the classical agents used in chemotherapy of colorectal cancer [19]; its prodrug 5'-deoxy-5-fluorouridine (5'dFUR) has shown a better therapeutic index than 5-fluorouracil in several murine tumors [3, 7]. Moreover, 5'dFUR has shown a 26% response in a phase II clinical study on advanced colorectal adenocarcinoma [1]. Considerable effort is being devoted, to the development of new agents for the treatment of colorectal adenocarcinomas.

In recent experiments, diethoxybis(1-phenylbutane-1,3-dionato)titanium (IV) (budotitane), which represents a new class of tumor inhibiting metal complexes, has shown promising results in this animal model [2, 9]. Guided by the efficacy of this metal complex, a new class of water-soluble heterocyclic coordinated ruthenium complexes

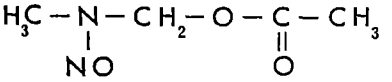

Fig. 1. Structrure of acetoxymethyl-methylnitrosamine (AMMN)

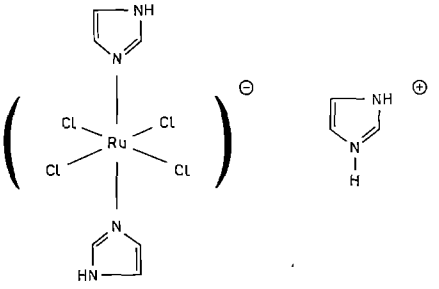

Fig. 2. Structure of imidazolium bis-(imidazole)tetrachlororuthenate (III), ImH($RuIm_2Cl_4$)

was synthesized and evaluated preclinically. One of these compounds imidazolium-bis(imidazole)tetrachlororuthenate (III), ImH($RuIm_2Cl_4$); (Fig. 2), showed remarkable activity against leukemias P388 and L1210 and against the subcutaneously transplanted B16 melanoma [11]. Moreover, the efficacy of this new compound was compared with that of the relatively new fluoropyrimidine 5'dFUR for activity against the tumor growth of autochthonous AMMN-induced colorectal cancer in SD rats.

Material and methods

Animals and tumor induction. The animals, 84 male Sprague-Dawley rats (Charles River Bredding, Sulzfeld, FRG), were purchased at a weight of 140–160 g and thereafter kept under conventional conditions: 2 rats per Macrolon III cage, tap water and Altromin pellets ad libitum. Colorectal carcinomas were induced using fresh 0.2% solutions of acetoxymethyl-methylnitrosamine (AMMN) [15, 22] in physiological saline: 2 mg/kg was administered intrarectally at weekly intervals for 10 weeks by means of a rectal tube, the tip of which was inserted as far as the colonic flexure.

Diagnosis of tumors and evaluation of treatment. At the beginning of the 5th week after completion of the 10-week induction period, the animals were anesthesized using chloral hydrate (3 g/kg i.p., diluted in physiological sa-

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line). A careful endoscopic examination of the colon was performed using a pediatric bronchoscope (Olympus BF, Type 4C2, Olympus Optical Co. Tokyo) [12, 14], and those animals that showed evidence of tumors were randomly allocated for treatment and control groups. Treatment started immediately thereafter and continued for 10 weeks.

All animals were treated twice weekly via the tail vein using freshly prepared $ImH(RuIm_2Cl_4)$ (0.4%) diluted in 0.9% NaCl solution. The doses administered were chosen with reference to previous experiments [11]. 5'DFUR was administered i.p. twice a week, diluted (16.7%) in 0.9% NaCl solution. The animals were sacrificed after 10 weeks of treatment; those seen to be in a moribund state were killed prematurely. They were dissected and the last 20 cm of the gut was removed, opened and weighed. The volume of each tumor was estimated by measuring three diameters according to the formula axbxc/2. For statistical analysis a multiple rank sum test according to Dunn was performed [5].

Results

The antitumor activity of 5'dFUR and that of $ImH(RuIm_2Cl_4)$ after 10 weeks of treatment are given in Table 1. 5'DFUR caused only a 37% tumor growth inhibition compared with untreated controls ($T/C \times 100 = 63$), whereas regular administration of $ImH(RuIm_2Cl_4)$ caused a significant reduction ($P < 0.05$) of the median tumor volume at all dosages (80% tumor growth inhibition compared with controls or $T/C \times 100 = 20$, Fig. 3). Interestingly, the dose schedule of $ImH(RuIm_2Cl_4)$ was related to the toxicity in terms of deaths, but did not modify the efficacy of this compound.

Discussion

Metal complexes derived from cisplatin are useful anticancer agents against several human tumors, but are not effective against colorectal cancer [16]. More recently developed new metal complexes, such as derivatives of titanium [8–10], have shown considerable anticancer activity in this tumor model [2]. Furthermore, ruthenium derivatives such as $ImH(RuIm_2Cl_4)$ have produced encouraging results in primary screening models [11].

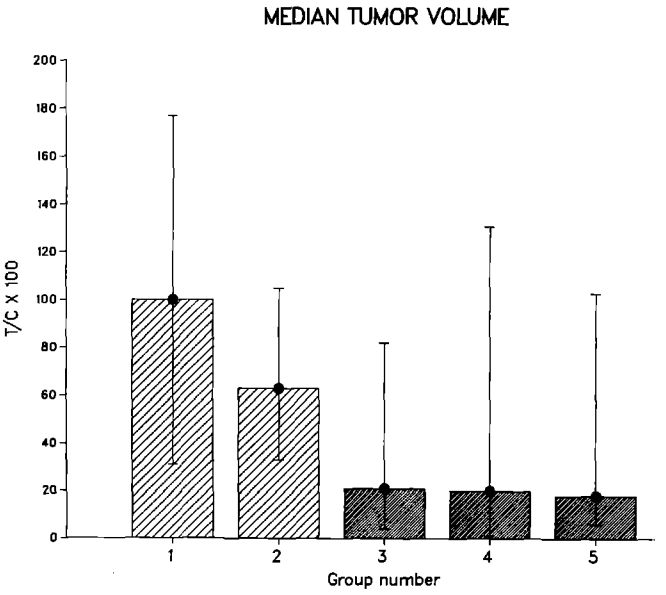

Fig. 3. $T/C \times 100$: quotient of the median tumor volumes of treated and control group $\times 100$. *1*, control; *2*, 2×300 mg/kg 5'dFUR; *3*, 2×14 mg/kg $ImH(RuCl_4Im_2)$; *4*, 2×12 mg/kg $ImH(RuCl_4Im_2)$; *5*, 2×7 mg/kg $ImH(RuCl_4Im_2)$, ▨ $P < 0.05$; ▨ NS

Since 5'dFUR is one of the current new fluoropyrimidines in clinical use with response rates around 30% [1], we have compared the efficacy of this compound with that of $ImH(RuIm_2Cl_4)$ against acetoxymethyl-methynitrosamine (AMMN)-induced colorectal cancer. A significant reduction of the median tumor volume was found when $ImH(RuIm_2Cl_4)$ 7 mg/kg was given, compared with a 37% reduction ($T/C \times 100 = 63$) following the administration of 5'dFUR. Since $ImH(RuIm_2Cl_4)$ 12 and 14 mg/kg were related with higher toxicity in terms of deaths but did not increase the efficacy, it appears that 7 mg/kg is a suitable dose, and this will be used as a reference dose in future experiments.

More studies on this ruthenium derivative are under way, to confirm the observed high antitumor activity. These results have prompted the planning of a phase I

Table 1. Effect of imidazolium-bis(imidazole)tetrachlororuthenate (III), $ImH(RuIm_2Cl_4)$ in acetoxymethyl-methylnitrosamine (AMMN)-induced colorectal rat adenocarcinoma

Group no.	No. of animals	Treatment[a] schedule	Median tumor volume per rat (95% confidence limits)	T/C X 100	Mortality (%)
1	20	Control	386 (120–683)	100	0 (0)
2	22	2×300 mg/kg 5' dFUR i.p.	243 (126–404)	63	1 (5)
3	20	2×14 mg/kg ImH i.v. ($RuCl_4Im_2$)	80* (14–315)	21	9 (45)
4	12	2×12 mg/kg ImH i.v. ($RuCl_4Im_2$)	78* (4–506)	20	1 (8)
5	10	2×7 mg/kg ImH i.v. ($RuCl_4Im_2$)	69* (24–401)	18	0 (0)

[a] Treatment was given twice weekly for 10 weeks and started following endoscopic diagnosis of the tumors (week 15)
* $P < 0.05$

clinical study. It is hoped that these new compounds might make it possible to improve the poor results of chemotherapy in slowly growing tumors such as colorectal cancer.

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