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Short communication

Treatment of chemically induced autochthonous rat mammary and colorectal carcinomas with interleukin-2

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Summary. The antineoplastic efficacy of human interleukin-2 (IL-2) in autochthonous methylnitrosourea-induced mammary carcinoma and in acetoxymethly-methylnitrosamine-induced colorectal carcinoma of Sprague Dawley rats has been investigated. Under the conditions applied, IL-2 was non-toxic. In the mammary carcinoma IL-2 was therapeutically inactive. In the colorectal carcinoma, 1200 U IL-2/day exhibited significant antitumour activity in established tumours as well as in tumours treated "prophylactically" before their manifestation ($P < 0.05$). The effect of IL-2 seemed to be more pronounced when given before manifestation of colorectal tumours ($T/C = 8.7\%$ vs 17.8% in established tumours). The differential sensitivity of the autochthonous mammary and colorectal carcinoma may be explained by differences in their proliferation rates and differences in volumes at the beginning of IL-2 therapy. IL-2 seems to be preferentially active in small tumours with a low proliferation rate, a feature typical of colon tumours

Key words: Rat mammary carcinomas – Colorectal carcinoma – Interleukin-2

Introduction

Interleukin-2 (IL-2) is a biological response modifier. It can augment various T cell functions including the activation and proliferation of natural killer cells and the induction of secondary cytokines [20]. Although severe side-effects have been reported, IL-2 has been found useful in the treatment of certain cancer types such as malignant melanoma and kidney cancer. In these tumours, 10%–25% remissions can be achieved. Other, more common neoplasms such as cancers of the breast, lung or colon generally show no or sometimes a weak response to IL-2 therapy [20]. On the other hand, IL-2 has exhibited significant antitumour activity in the majority of transplantable tumour systems studied [2, 13, 17, 18, 21, 24, 25, 28], although in some murine tumour models IL-2 alone did not reveal any antineoplastic effect [1, 8, 11, 14, 22, 29]. In most of these tumours in which IL-2 by itself was inactive, this agent has been found to act synergistically when combined with the alkylating agent cyclophosphamide [8, 11, 16, 21, 22].

In retrospect, a discrepancy has emerged between promising preclinical results of IL-2, obtained with various transplanted tumours, and the observed lack of antineoplastic benefit in many common human cancers [20]. In view of the low predictivity of the experimental systems used so far, our group has focussed on the use of autochthonous animal models in the preclinical evaluation of new cytostatic compounds or biological response modifiers for many years [4–6, 9, 27]. These primary tumours seem to be more comparable to the human situation with regard to biological and histological properties than are transplanted tumours, although their handling is considerably more difficult and expensive [10, 12]. In particular the tumour/host interactions are thought to be more relevant to the human situation [19].

Two studies using autochthonous tumour models have been detailed so far. One of these revealed a significant antineoplastic effect of IL-2 when given "prophylactically" during the manifestation period of a colorectal tumour [9]. In the other, following the surgical removal of the primary methylcholanthrene-induced sarcoma, pulmonary metastases and local recurrences were not influenced by IL-2 [12]. Since established primary tumours had not been treated with IL-2 until the start of this study, these experiments have been performed in gross lesions of the autochthonous methylnitrosourea-induced mammary and acetoxymethly-methylnitrosamine-induced colorectal carcinomas of the rat.

In addition, IL-2 was coadministered with mafosfamide, a relatively new oxazaphosphorine derivative, which has been previously shown to augment host antitumour activity when given at low doses [23].

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Materials and methods

Drugs. Crystalline *N*-methyl-*N*-nitrosourea (MNU) and acetoxymethylmethylnitrosamine (AMMN) were synthesized by Prof. M. Wiessler (Institute of Toxicology and Chemotherapy, German Cancer Research Center, Heidelberg, FRG). MNU was dissolved at 1% in Sörensen buffer (pH 6) and distilled water (20/80, v/v).

Highly purified human interleukin-2 (IL-2), derived from peripheral blood lymphocytes, was provided by Biotest Pharma, Dreieich, FRG. IL-2 was diluted in 0.1% v/v rat albumin solution {10^5 or 10^6 BRMP units IL-2/ml; for biological response modifier programme (BRMP) reference see [26]}.

Mafosfamide was kindly provided by Asta Werke (Bielefeld, FRG). A 2% solution in physiological saline was used.

Mammary carcinomas. Fifty female Sprague Dawley rats (Zentralinstitut für Versuchstierzucht, Hannover, FRG) were purchased aged 40+1 days and kept under conventional conditions (3 rats per size-III Makrolon cage during the tumour induction time and subsequently 1 animal per size-II Makrolon cage during therapy; temperature 22 ±2° C; relative humidity 55 ± 10%). They received Altromin pellets (diet 1320) and tap water ad libitum.

Mammary carcinomas were induced by three i.v. injections of 50 mg/kg MNU into the tail vein, on days 50, 71, and 92 of life [3]. Beginning 4 weeks after the first injection of MNU, rats were weighed and palpated twice weekly throughout the experimental period to record tumour manifestation.

Individual tumour volumes were estimated as the product of two vertical axes ($a \times b^2/2$) as measured by vernier calipers. The total tumour volume per animal was calculated as the sum of all individual tumours. Rats with a total tumour volume of more than 0.8 cm^3 were randomly allocated to experimental groups. Therapy started immediately thereafter (week 1). For administration of IL-2, osmotic minipumps (Alzet) were implanted s.c., which release a constant flow rate for 14 days. After 2 weeks they were replaced by fresh implants. Therapy lasted 4 weeks. At the beginning of week 5, animals were killed and dissected and mammary tumours were excised, weighed, and examined histologically.

Colorectal tumours. Sprague-Dawley rats (125 male animals; Charles River Breeding, Sulzfeld, FRG) were obtained at a weight of 140–160 g and thereafter kept under conventional conditions: 2 rats per Makrolon III cage, tap water and Altromin pellets ad libitum. Colorectal carcinomas were induced using fresh 0.2% solutions of AMMN 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 [9].

At the beginning of the 5th week after completion of the 10-week induction period, the animals were anaesthesized using chloral hydrate (3 g/kg i.p., diluted in physiological saline). A careful endoscopic examination of the colon was performed using a pediatric bronchoscope (Olympus BF, Type 4C2, Olympus Optical Co., Tokyo). Animals that showed evidence of tumours were randomly allocated for treatment and control groups (groups A). Rats that showed no evidence of tumour were separated and randomized to groups B. Treatment started immediately thereafter by s.c. implantation of osmotic minipumps as described above. This procedure was repeated five times.

All animals were killed after 10 weeks of treatment. They were dissected and the last 20 cm of the gut removed and opened. After weighing, the volume of each tumour was calculated according to the formula $a \times b \times c/2$. The complete number of tumours and their localization within each colon were also determined.

Statistics. Therapeutic efficacy was measured on the basis of the median total tumour volumes of treated groups versus the respective control ($100 \times T/C$). For analysis of repeated tumour volume measurements the nonparametric multivariate test, as described by Koziol and Donna [15], was used. Tumour volumes at the end of the experiment were compared using the method of multiple rank sums according to Dunn [7].

Results

In established MNU-induced mammary carcinoma, continuous administration of 1200 BRMP units or 12000 BRMP units IL-2 per rat each day for 4 weeks did not produce significant toxicity or therapeutic efficacy. The combined treatment with 12000 BRMP units IL-2 per rat and day and a single dose of mafosfamide was also ineffective (Table 1).

Table 2 shows the antitumour effects of IL-2 administered either to animals with established AMMN-induced colorectal carcinomas (group A) or to animals that at the same time were tumour-free according to endoscopical examination (group B). Also in these experiments, IL-2 did not exhibit significant toxicity as documented by mortality rates and body weight gain of the IL-2-treated animals. In all treated groups, significant antineoplastic effects were observed ($P < 0.05$). In group A, treatment with 1200 BRMP units IL-2 resulted in a similar antitumour effect to

Table 1. Efficacy of interleukin-2 and low-dose mafosfamide in methylnitrosourea-induced mammary carcinoma

Group no.	Treatment	No. of animals	Median tumour volume per rat (cm^3) (95% confidence limits)			Median tumour number per rat (95% confidence limits)			Mortality, *n* (%)	Body weight difference[a] (%)
			Week 1	Week 3	Week 5	Week 1	Week 3	Week 5		
I	Control	20	1.5 (1.3–1.9)	26.3 (22.3–35.5)	49.2 (39.4–71.1)	3.0 (2–3)	7.0 (6–7)	7.0 (7– 9)	7 (35)	+15.3
II	1200 U[d] IL-2[b]	10	1.3 (1.1–2.0)	13.8 (8.1–31.0)	41.2 (29.7–53.4)	2.0 (1–5)	6.5 (4–9)	8.5 (7–10)	0 (0)	+19.5
III	12000 U[d] IL-2[b]	10	1.4 (1.0–2.0)	20.1 (13.7–31.2)	46.7 (31.1–55.7)	1.0 (1.0–3.0)	7.0 (5.0–9.0)	7.5 (6.0–10.0)	0 (0)	+19.5
IV	12000 U[d] IL-2[b]+ mafosfamide[c]	10	1.5 (1.0–2.6)	22.1 (7.4–35.9)	49.9 (21.4–69.9)	2.0 (1.0–3.0)	6.0 (3.0–7.0)	6.5 (5.0–8.0)	0 (0)	+22.1

[a] Median body weight per group at the end of therapy (week 5) minus initial body weight (week 1) as a percentage of initial body weight
[b] Units/day, continuously for 4 weeks, administered by s.c. implanted osmotic minipumps
[c] Day 1, 10 mg/kg, i.p.
[d] BRMP units

Table 2. Efficacy of interleukin-2 and low-dose mafosfamide in acetoxymethyl-methylnitrosamine-induced colorectal carcinoma

Group no.	Treatment	No. of animals	Median tumour volume per rat (mm^3) (95% confidence limits)	$T/C \times 100$	Median tumour number per rat (95% confidence limits)	Mortality, n (%)	Body weight difference[a] (%)
A control	–	20	67.5 (29–86)	100	3 (3–4)	2 (10)	+12.2
AI	1200 Ug IL-2[b, c]	15	12.0 (4–69)[d]	17.8	2 (2–3)[d]	0 (0)	+12.0
AII	1200 Ug IL-2[b, c] + mafosfamide[e]	15	25.5 (8–38)[d]	37.8	2 (1–4)[d]	1 (7)	+ 8.9
AIII	2400 Ug IL-2[b, c]	15	16.0 (8–69)[d]	23.7	2 (2–3)[d]	0 (0)	+11.1
B control	–	20	68.8 (31–152)	100	3 (3–3)	0 (0)	+ 8.9
BI	1200 Ug IL-2[c, f]	20	6.0 (1–12)[d]	8.7	2 (1–2)[d]	0 (0)	+ 5.6
BII	1200 Ug IL-2[c, f] + mafosfamide[e]	20	11.5 (5–16)[d]	16.7	2 (1–2)[d]	1 (5)	+ 8.8

[a] Median body weight per group at the end of therapy (week 10) minus initial body weight (week 1) as a percentage of initial body weight
[b] Treatment started following endoscopical diagnosis of tumours (median tumour volume: 10 mm^3)
[c] Units/day, continuously for 10 weeks, administered by s.c. implanted osmotic minipumps
[d] Significantly different from controls ($P < 0.05$) according to Dunn [7]
[e] Day 1, 10 mg/kg, i.p.
[f] Treatment started before manifestation of the tumours
[g] BRMP units

that produced by 2400 BRMP units IL-2 (T/C = 17.8% vs T/C = 23.7%, respectively). When mafosfamide was coadministered with 1200 BRMP units IL-2, no improvement in anticancer efficacy was observed (T/C = 37.8%).

When 1200 BRMP units IL-2 was given before the manifestation of the tumours, a T/C of 8.7% was observed. Again, mafosfamide did not enhance the activity of IL-2 alone (T/C = 16.7%).

Discussion

IL-2 has been found active in a series of transplantable tumour systems [2, 13, 17, 18, 21, 24, 25, 28]. This study on the role of IL-2 in established autochthonous mammary and colorectal cancers was intended to investigate its efficacy in models more relevant to the human situation [10, 12, 19]. Both models have been characterized by a number of chemotherapeutic studies and have been considered useful for the preclinical evaluation of new cytostatic agents [3–5, 27] as well as biological response modifier [6, 19]. Differences between the clinical and the experimental situation described, however, should be noted: patients usually undergo surgical intervention, and subsequent therapy is given to treat metastatic disease, whereas the IL-2 treatment in this study was given to treat primary tumours.

Both the MNU-induced mammary carcinoma and the AMMN-induced colorectal carcinoma are relatively slowly growing tumours with tumour-volume doubling times of 8 days and 18.5 days respectively [4]. The lack of IL-2 activity in the mammary carcinoma studied may be attributed to its relatively faster proliferation rate as compared to the colorectal cancer in which IL-2 was effective. Similar observations have been made when IL-2 activity was investigated in four transplanted mammary tumours with different growth rates. While the two faster-growing tumours did not respond to IL-2, the more slowly proliferating tumours were affected by this agent [29]. Furthermore, the varying tumour volumes at the beginning of therapy probably have contributed to the observed differential response. The initial tumour volume was more than 100-fold higher in mammary tumours as compared to colorectal cancers (1.5 cm^3 versus 10 mm^3 respectively; Tables 1 and 2). It is well known that the initial tumour burden is an important prognostic factor for the effectiveness of any anticancer therapy. This was confirmed by the present experiment. Even when comparing the treatment results of animals bearing small established tumours with that of animals without macroscopically visible carcinomas, a trend towards a higher efficacy in favour of the latter group was discernible (Table 2).

Another factor that might have influenced the therapeutic outcome is the sex of the animals. Whether or not females respond less favourably than males to IL-2 treatment, is unclear from the present study results and has to be clarified in future experiments. Mafosfamide was supposed to be particularly suited for combination with IL-2 because of its capacity to augment host antitumour immune responses by activating T cells and natural killer cells [23]. However, in none of the three experiments did mafosfamide increase the antineoplastic effect of IL-2 alone. The reasons for this failure remain speculative. To date, the immunogenicity of both autochthonous tumour models has not been investigated and the role of immune functions on tumour growth is unknown. A hint at an immune-mediated antitumour effect might be, however, that the tumour re-

sponse was not enhanced by doubling the IL-2 dose (group AI and group AIII, Table 2).

In additional experiments to assess the immune response to IL-2 in spleen cells of AMMN-induced rats (data not shown) no clear correlation with therapy results was obtained in a natural killer cell assay and from lymphoproliferation induced with phytohaemagglutinin, concanavalin A, pokeweed mitogen or IL-2. The latter result is in accord with a previous study [9]. Therefore, other parameters of response to IL-2 have to be investigated in a forthcoming study, to obtain a better correlation.

In summary, IL-2 activity was found to be dependent on the growth rate and the size of autochthonous carcinomas. IL-2 seems to be preferentially active in small tumours with a slow proliferation rate.

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