ANTIMETASTATIC ACTIVITY OF THE NOVEL SPIN-LABELED NITROSOUREA DERIVATIVE SLNU ON SPONTANEOUS LUNG METASTASES OF THE LEWIS LUNG CARCINOMA

M. Ilarionova[1], K. Todorov[2], L. Horvath[1], D. K. Todorov[1]
Laboratory of Oncopharmacology, National Oncological Center, 756 Sofia, Bulgaria[1]
Chemotherapy Unit, Sofia Cancer Center, 1784 Sofia, Bulgaria[2]

ABSTRACT

The antimetastatic activity of newly synthetized original spin-labeled nitrosourea derivative SLNU in comparison with clinically used nitrosourea antitumor drug lomustin on spontaneous lung metastases of Lewis lung carcinoma (LLC) in BDF1 hybrid mice has been studied. The established effects were "dose-dependent" and "schedule-dependent" and correlated well with the optimal clinical schedule of administration of nitrosoureas. At the used experimental design with intermittent administration both drugs inhibited significantly the growth of LLC metastases (maximal TMI % for SLNU was 100%, and for CCNU – 85.8%), but with insignificant effect on the growth of the primary tumor. The newly synthetized original nitrosourea SLNU showed some advantages in its antimetastatic potential in comparison with the clinically used drug lomustin. Further complex studies in this field are in progress.

Introduction

Clinically detectable metastases have been observed in approximately 30% of patients at the time of initial diagnosis. At the same time most neoplasms consist of different cell populations, with diverse biologic characteristics, including drug sensitivity and widely divergent metastatic potential. Invasion and metastasizing are very complicated multi-step processes involving multiple host-tumor interactions as uncontrolled proliferation, angiogenesis, circulating tumor cell arrest and extra-vascularization, colony formation at the secondary site, evasion of host defenses and resistance to therapy (1).

The aim of our experimental studies was to compare the antimetastatic activity of the novel nitrosourea derivative SLNU with the clinically used nitrosourea drug lomustin.

Materials and Methods

Drugs. SLNU [1-(2-chloroethil)-1-nitroso-3-(2,2,6,6-tetrmethyl-1-oxyl-piperidine-4-il)-urea] is a newly synthetized spin-labeled nitrosourea derivative, prepared by the working team of Prof. Dr. Z. Raikov (Dept. of Chemistry and Biochemistry, Medical Faculty, Tracian University, St. Zagora, Bulgaria). SLNU has been kindly provided for our experiments by this team.

Lomustin (CCNU, Bristol Meyers Squibb) is a clinically used antitumor nitrosourea drug with alkylating and carbamoilating mechanism of action and wide spectrum of therapeutic activity in cancer patients (2, 3).

The both drugs have been administered intraperitoneally on days 3, 7, 11, 15 and 19 after tumor transplantation (day 0) – course treatment and on day 1 – single treatment,

as a suspension in saline with solubilizer Tween 80, in a volume of 0,01 mL/kg.

Animals. BDF1 hybrid mice (C57BL/6 x BDA/2) with a weight range of 18-22 g., male, in groups of 12 to 20 animals were used. Animals were kept in standard laboratory conditions of the Animal rooms in the National Oncological Center, with food and water given *ad libitum*.

Tumor model. The Lewis lung carcinoma (LLC) is maintaining in our laboratory by serial subcutaneous transplantations on inbred C57BL/6 mice. Using tumor material taken by these tumor-bearing mice transplanted in hybrid BDF1 mice, intramuscular solid form of LLC was selected as a model for spontaneously metastasizing tumor in this study.

LLC aroused spontaneously in 1951 as carcinoma of the lung in a C57BL/6 mouse. The subcutaneously or intramuscularly transplanted tumor metastasizes mainly in the lungs of the animals. The following biologic features characterized LLC: multiple metastases in the lung with an even distribution in the lung tissue, progressing almost simultaneously and causing a grouped lethality of animals.

In our experiments LLC was transplanted intramuscularly by tumor cell suspension, containing 1.10^6 tumor cells in 0.2 mL saline, that was prepared by filtration of tumor tissue fragments. (4)

Detection of pulmonary metastases. For the better offset of the lung metastases and their continuous assessment modified method of Wexler was used (5). At the final day of the experiment the pulmonary alveoli were filled up under pressure with an ink solution as described in the original paper. The contrasted in this way lung was fixed in Fekete's solution. The pulmonary metastases could be distinguished as albescent burls on the background of the dark colored lung, which allowed their visualization and counting.

Biometric criteria. The criterion "tumor growth inhibition" (TGI%) for estimation of the antitumor activity on the primary tumor was used. By using the formula: $TGI = (T_{exp} - T_{con}) \times 100 : T_{con}$), where T_{exp} is the mean weight of the tumors (g)in the experimental group ànd T_{con} is the mean weight of the tumors (g) in the control group, was defined the antitumor activity (4).

The criterion "metastatic growth inhibition" (MGI%) for estimation of the antimetastatic activity was used as defined by the formula:

$MGI = (M_{exp} - M_{con}) \times 100 : M_{con}$), where M_{exp} is the mean count of metastases in the experimental group and M_{con} is the mean count of metastases of the control group.

The values of ÒGI% and MGI% equal or higher than 50% were accepted for minimal criteria for statistically significant activity by international standards.

Results and Discussion

Effect of SLNU and lomustin on the primary tumor and metastases of LLC.

According to the used administration regimen of SLNU in our experimental design (days 3, 7, 11, 15 and 19), the highest values of the criterion TGI% achieved 59.4% at dose 23.7 mg/kg x 5, on day 20 after the transplantation of tumor. The higher doses of SLNU were established as toxic (see Materials and Methods).

It was established that SLNU suppressed the growth of the pulmonary metastases of LLC. At dose of 15.8 mg/kg x 5 the criterion TMI% was equal to 94.7% and at dose of 23.7 mg/kg x 5 it was 100%. At the last dose pulmonary metastases could not be established macroscopically as detected by the routine procedure of Wexler (complete antimetastatic effect).

Our studies on antitumor and antimetastatic

activity of ***lomustin*** on LLC established that TGI% for lomustin was similar to the described for SLNU (59.8%).
The maximal criterion MGI% for lomustin (85.8%) was smaller than that for SLNU (100%) at dose of 23.7 mg/kg for both agents.

Effect of single, early administration of SLNU and lomustin on the primary tumor of LLC. In other series of our experiments on LLC a different schedule - single, early administration (on day 1) of both agents was used. It was established that in wide range of doses, the antitumor activity on day 14 was extremely high, with complete tumor growth inhibition (TGI=100%). This maximal effect was reached by using a smaller dose of SLNU in comparison with lomustin. A good correlation "dose-effect" was observed in these studies. At this term of assessment of the antitumor effect was impossible to determine the antimetastatic activity by using the Wexler method due to the early end of experiments (on day 14).
In our other experimental series of investigation on the antitumor activity of the originally synthesized spin-labeled nitrosourea SLNU and lomustin was used experimental design ***with intermittent schedule of administration***. It was found that the inhibition of primary tumor growth of LLC on the day 20 after the transplantation was slightly above the minimal criterion for activity.
In contrast with this slight antitumor activity, at the same schedule the antimetastatic activity was high. For SLNU MGI% was established as 100% and macroscopically pulmonary metastases could not be detected at optimal dose. For lomustin maximal MGI% was 85.8% at optimal dose.
On the other hand, the results of other our experiments with SLNU and lomustin ***in high doses at early, single administration*** showed a high antitumor activity (complete tumor growth inhibition). These experimental data correlated well with the known clinical optimal schedule for nitrosourea drugs - the single administration in high doses, with interval as long as to allow the restoration of hemopoiesis.
Our results showed that antitumor and antimetastatic effects of nitosourea drugs are schedule-dependent and are expressed at different their doses. This fact found in our studies correlated well with the known varieties in the biological behavior of cell clones of the primary and the metastatic tumor.
The newly synthetized spin-labeled nitosourea SLNU showed some advantages in its antitumor and antimetastatic potential in comparison with the clinically used antitumor drug lomustin. The detailed preclinical studies of SLNU as a potential novel nitrosourea antitumor drug are in progress in the Laboratory of Oncopharmacology, National Oncological Center in Sofia and in other institutes.
This work is a part of our extended systemic investigations on the preclinical oncopharmacology of novel and known nitrosourea derivatives, including new approaches to improvement of their antitumor and antimetastatic effects, with possible clinical transfer (6, 7, 8, 9, 10).

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