THE *IN VIVO* ASSESSMENT OF THE PRECLINICAL ONCOCHEMOTHERAPEUTIC EFFECTIVNESS OF SOME FUROSTANOLIC GLYCOSIDES

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Abstract: The significance of the antitumoral action of some bio- or semiynthesis furostanolic-glycoside preparations upon the development of diverse experimental tumoral lines has been appreciated in relation to the therapeutic effect of different doses on carcinogenesis, as well as the experimental oncostatic activity of some standard cytostatics of clinical use (methotrexate, cyclophosfamide, melphalan and 5-fluorouracil). The laboratory treatment with the bioactive agent in various doses – superior and inferior to that which has conditioned the expression of those antitumoral actions upon Guerin T-8 lymphotropic epithelioma and Walker 256 carcinosarcoma – has highlighted the antineoplastic effectiveness` dependence of these agents of the therapeutic dose. The comparative analysis of the evaluation indices values of the antitumoral pharmacodynamic effect – registerd by us in the experimental therapy with the glycoside extracts and with the reference cytostatic drugs – has revealed that the antitumoral potential of the new autochthonous products is higher, equal or near to those of the standard oncochemotherapeutics. The possibility of optimization of the antitumoral efficiency by experimental manipulation of the therapeutic doses – which proves the existence of a dose-response relationship as well as the antineoplastic effectiveness are relevant for the characterization of the ENLE and D_2ENLE as potential oncochemotherapeutic agents. The quantitative preclinical evaluation of the specific pharmacodynamic effect will be completed by the investigation of the new furostanolic-glycoside extracts in tumors with various degree of development.

INTRODUCTION

In the fight against cancer – a real scourge of contemporary times – identification of new, more effective antineoplastic agents, as well as of new ways to decrease cancer cells resistance to cytostatics represent topical and major objectives (Weinstein,2001; Bronchud, 2000; Abrams, 2003; Lodish et al, 2003;DiPirio et al, 2005).

The identification of new pharmacological agents with antineoplastic activity represents a major, topical and very important concern of oncobiological research and of medical practice, which pursues the improvement of antitumoral chemotherapy effectiveness. The discovery of an anticancerous agent – preferentially targeting the tumoral cells and to a lesser extent the normal cells of the host – and its introduction into human chemotherapy are the result of preclinical and clinical complex pharmacological investigations in appropriate experimental models using different biological testing systems. Chemotherapeutic programs of multistage preclinical screening, designed to identify new antitumoral substances, require: successive and interdependent research steps; appropriate experimental models; evaluation indices of the specific pharmacodynamic effect; qualitative and quantitative assessment criteria of the induced antitumoral action (Leiter et al., 1965; Boyd, 1989; Borenfreund&Babich, 1990; Phillips et al., 1990, 1991; Bissery&Chabot, 1991; DeVita, 2001; Cook, 2002; Seethala&Prabhavathi, 2001; Figg&McLeod. 2004).

In this context, our preliminary investigations have revealed, on one hand, the *in vitro* cytostatic and cytotoxic activity, upon the HeLa and HEp-2p tumoral cell cultures, of a new furostanolic-glycoside agents, and on the other hand, the *in vivo* antineoplastic impact of these biosynthesis and semisynthesis preparations upon several experimental tumoral systems and its reproducibility (Gherghel et al, 2005; Rotinberg et al., 2009). To complete the experimental data necessary for the preclinical characterization of the furostanolic-glycoside preparations as antitneoplastic agents it is required a further quantitative pharmacological evaluation of their experimental antineoplastic potential, in the other stages of the preclinical trial on animals with tumors.

Thus, the present work includes the results obtained in the *in vivo* comparative testing of the antitumoral activity of various doses of ENLE and D_2ENLE, as well as of some standard oncochemotherapeutic agents of clinical use on rats with various experimental tumor lines. Therefore, the purpose of our research has been to appreciate the preclinical antineoplastic effectiveness of these biosynthesis and semisynthesis agents. We studied the possibility to increase their antitumoral potential by the experimental modulation of the therapeutical doses, and we also compared them efficiency with the impact of some standard cytostatics upon the tumor development process.

MATERIALS AND METHODS

The active cytostatic compounds of furostanolic glycoside nature which were used in the *in vivo* experiments have been the following: ENLE, a biosynthesis product, specifically extracted and purified from seeds of *Lycopersicum*

esculentum (tomato), and D_2ENLE, which is a semisynthesis derivative, performed from the former by partial oxidation until to obtaining of some aldehydic groups.

Because the semisynthesis agent is not hydrosoluble we included in our screening the solution of 50% ethylic alcohol, which is the vehiculating agent for the D_2ENLE. The standard cytostatics, included in the reference experimental antitumoral therapy, were: methotrexate (MTX), cyclophosphamide (CFS), melphalan (MLF) and 5-fluorouracil (5-FU).

The *in vivo* tests were performed in appropriate experimental models, using white, female Wistar rats – pure line – of 125-150 g b.w, with either Guérin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma. The animals were housed in individual cages having free access to water and standard food in a normal light/dark cycle and at 22^0 C ambient temperature. The tumoral transplant was performed by the subcutaneous injecting of 0.2 ml of cell suspension, obtained by homogenizing cancerous tissue in saline solution containing streptomycin and penicillin (Pollack&Fidler, 1982).

The experimental antitumoral treatment was initiated 24 hours after the tumoral transplant and was continued for 16 and 19 days, respectively, in the case of Guérin tumor and Walker 256 line, respectively. It consisted in daily intraperitoneal (i.p.) administration of the bioactive agents in various doses. Thus, the injected doses have been either higher or lower than the dose which conditioned the expression of them antitumoral action (40 mg/kg. body weight = b.w.). The doses of the standard oncochemotherapeutics were established in relation to that used in clinical antineoplastic therapy. The values of the therapeutical doses, expressed in mg/kg. b. w., are presented in the tables that also include the results for each of the experimental models. An equal volume of saline solution was injected to the control animals.

The oncostatic impact was assessed by determining the rate of mean tumor regression (%M.T.R.), as well as by calculating the T/C ratio (where T = M.T.W. for the treated groups and C = M.T.W. for the control group) and the statistic significance by means of Student's "t" test (Leiter et al, 1965; Jungstad et al, 1971; Davey&Tudhope, 1983; Boyd, 1989; Motulsk, 1995).

The evaluation of the anticancerous effect was performed by comparing the mean tumor weight (M.T.W.) of the treated and, respectively, control rats after slaughtering. The appreciations of the antitumoral therapeutic effectiveness of the ENLE and D_2ENLE also required the comparative analysis of the evaluation indices values we obtained with those set by the selection criteria of antineoplastic substances established in the preclinical screening programs of the Microbiology and Experimental Therapy Institute of Germany (Leiter et al, 1965; Jungstad et al, 1971) and of the National Cancer Institute of the USA (Leiter et al, 1965) for this phase of the preclinical trial.

RESULTS AND DISCUSSIONS

The interference of daily antitumoral therapy, performed by administration of ENLE or D_2ENLE different doses, with the development process of Guérin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma, can be followed from Table 1 and 2.

One can observe that the dose differentiated treatment was followed, in the case of lymphotropic epithelioma, by: a moderate decrease of M.T.W in the case of the group treated with 20 mg/kg b.w., the M.T.R. calculated for this group was 32.73%, and the T/C value was 0.67; a significant antitumoral activity ($p<0.05$), illustrated by the M.T.R. (40.61%) and T/C value (0.59), estimated in the animals treated with 30 mg/kg b.w.; an important cytostatic action in the case of the group treated with 40 mg/kg b.w., which is represented by the M.T.R. (46.67%), T/C (0.53), it having statistical significance ($p<0.02$); a higher antineoplastic impact (M.T.R. of 51.52 and T/C value of 0.48) in the case of animals treated with a dose of 50 mg/kg b.w.; a maximum tumorsupression effect under therapy with 60 mg/kg b.w, in this case the M.T.R. value was 56.97%, the T/C ratio was 0.43, and the statistical significance was 0.01.

Similar results were recorded on rats with Walker carcinosarcoma given daily therapy with various doses of ENLE (Table 1 and Fig. 1). The progressive increase of the dose was also correlated with an optimization of the antitumoral effectiveness in comparison with the untreated, control group. At the dose of 20 mg/kg b.w, the decrease of the mean tumoral weight was only with 21.57%, allowing calculation of a T/C ratio of 0.78. The increasing of the dose to 30 mg/kg b.w has conducted to a M.T.R of 35.78% and a T/C of 0.64, values which are a little bit higher than the standard ones. A dose of 40 mg/kg b.w has determined a decrease of the M.T.W. with 41.67%, the calculated T/C value in this case being of 0.58. The doses of 50 mg/kg b.w and

respectively 60 mg/kg b.w have induced a reduction of the MTW with 47.55% and respectively 50.00%, T/C values of 0.52 and respectively 0.50

Table 1. Experimental oncochemotherapeutic potential of various doses of ENLE biopreparation (mg/kg b.w./day) upon Guérin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma. Figures in brackets indicate the number of experimental animals.

Experimental group	M.T.W.(g)	T/C value	p
Guérin T-8 lymphotropic epithelioma			
Control	16.5 ± 2.6 (15)	–	-
20 mg	11.1 ± 2.1 (10)	0.67	NS
30 mg	9.8 ± 1.3 (10)	0.59	<0.05
40 mg	8.8 ± 1.5 (10)	0.53	<0.02
50 mg	8.0 ± 1.2 (10)	0.48	<0.01
60 mg	7.1 ± 1.3 (10)	0.43	<0.01
Walker 256 carcinosarcoma			
Control	20.4 ± 3.1 (15)	–	-
20 mg	16.0 ± 2.5 (10)	0.78	NS
30 mg	13.1 ± 1.8 (10)	0.64	<0.05
40 mg	11.9 ± 1.5 (10)	0.58	<0.02
50 mg	10.7 ± 1.7 (10)	0.52	<0.01
60 mg	10.2 ± 1.3 (10)	0.50	<0.01

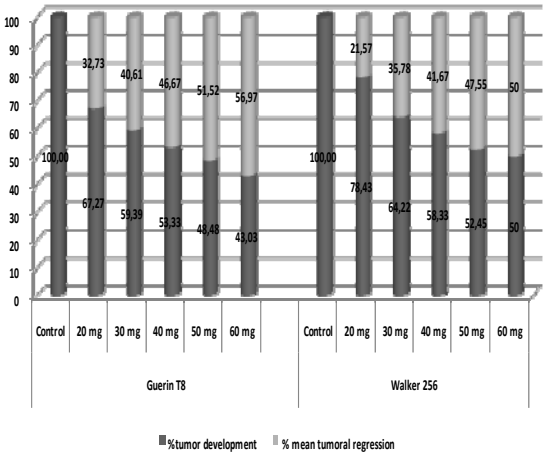

Fig. 1 The effect of the daily i.p. antitumoral treatment with different doses of ENLE upon the development of the Guerin T-8 lymphotropic epithelioma and Walker 256 carcinosarcoma

The second agent used in our experimental model was the semisynthesis product D_2ENLE, its impact upon the carcinogenetic process of the animals bearing Guerin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma could be followed in Table 2 and Figure 2. Also, we looked up at the effect of the 50% hydroalcohol solution, the vehiculating agent of the D_2ENLE, upon the carcinogenesis.

Thus, in the case of the Guerin T-8 lymphotropic epithelioma was observed a significant cytostatic effect at the minimum dose of 10 mg/kg b.w. (M.T.R. of 59.89% and T/C value of 0.40). When the dose was increased to 20 mg/kg b.w., there occurred a decrease of M.T.W. which resulted in a M.T.R. of 81.36% and a T/C ratio of 0.19. These values of the evaluation indices point to an important inhibitory action upon tumoral development of the lymphotropic epithelioma. Therapeutic dosage increase to 40 and 60 mg/kg b.w., respectively, was correlated with a decrease of the antitumoral potential, which still is cytostatistically and statistically significant. The M.T.R. values calculated in this case were 77.97% and 50.85%, respectively, and T/C ratios were 0.22 and 0.49, respectively. In the case of the animals which were subjected to the hydroalcoholic treatment, the tumor development was with 29.38% over the control group (100%).

A similar impact was also registered in the case of the rats bearing the Walker 256 carcinosarcoma. Thus: at the dose of 10 mg/kg b.w has been registered a moderate decrease of M.T.W , the M.T.R. T/C and p values, calculated for this group as compared to the control one, being of 53.29%, 0.46 and <0.01; a significant antitumoral activity ($p<0.001$), illustrated by the estimated M.T.R. (77.63%) and T/C values (0.22), at the animals treated with 20 mg/kg b.w.; an important and statistical significance *in vivo* cytostatic action – a little bit smaller then that from

Table 2. Antineoplastic effectiveness of the daily therapy with semisynthesis extract D_2ENLE in different doses (mg/kg b.w.) upon Guerin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma. Figures in brackets indicate the number of experimental animals.

Experimental group	M.T.W.(g)	T/C value	p
Guérin T-8 lymphotropic epithelioma			
Control	17,7 ± 2,9 (15)	–	
10 mg	7,1 ± 1,1 (10)	0,40	NS
20 mg	3,3 ± 0,8 (10)	0,19	<0,001
40 mg	3,9 ± 0,9 (10)	0,22	<0,001
60 mg	8,7 ± 1,6 (10)	0,49	<0,001
HA 50%	22,9 ± 1,3 (10)	1,29	<0,02
Walker 256 carcinosarcoma			
Control	15,2 ± 2,3 (15)	–	
10 mg	7,1 ± 1,0 (10)	0,46	<0,01
20 mg	3,4 ± 0,7 (10)	0,22	<0,001
40 mg	5,8 ± 1,1 (10)	0,38	<0,002
60 mg	8,6 ± 1,7 (10)	0,56	<0,05
HA 50%	19,4 ± 3,2 (10)	1,28	NS

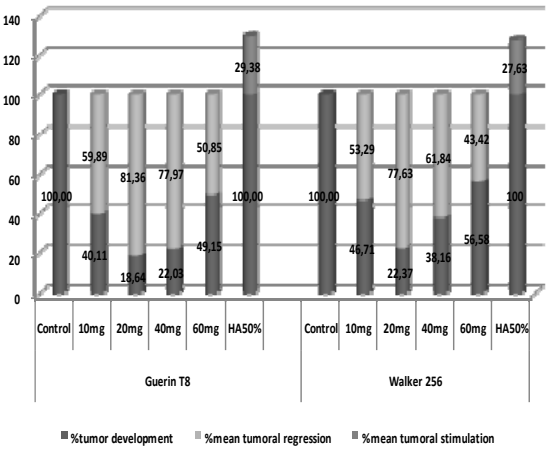

Fig. 2 The development degree of the experimental tumoral systems after the ip. daily treatment with different doses of D_2ENLE and with HA50%.

dose of 20 mg – in the case of the group treated with 40 mg/kg b.w., which is expressed by the M.T.R. (61.84%), T/C (0.38), and p values (<0.002); a smaller antineoplastic impact (M.T.R. of 43.42 and T/C value of 0.56) in the case of animals treated with a dose of 60 mg/kg b.w. The administration of the HA 50% has determined a stimulation of the carcinogenesis, the tumoral mass being increased with 27.63% over the control group and the corresponding T/C ratio being of 1.28.

Therefore, we may conclude that the effectiveness of the anticancerous furostanolic-glycoside treatment is positivelly correlated with the used doses.

The apreciation of the experimental antitumoral efficiency of ENLE and of its semisynthetic derivate D_2ENLE has also required the comparative analysis with the therapeutic potential of some standard oncochemotherapeutic agents, in laboratory conditions.

Table 3 includes the evaluation indices values of the antitumoral impact induced by ENLE, D_2ENLE, methotrexate, cyclophosphamide, melphalan and 5-fluorouracil, respectively, on the development of the solid Guérin T-8 and Walker 256 tumors.

Here are the effects of the antitumoral treatment of the animals with lymphotropic epithelioma, compared with the data in the control group:

– ENLE extract has significantly inhibited ($p<0.01$) the evolution of lymphotropic epithelioma; this effect being expressed by a decrease of M.T.W., by the M.T.R. value (48.15%) and by the T/C ratio (0.52);

– D_2ENLE agent has determined a profound diminution of the mean tumoral weight, the calculated M.T.R., T/C ratio and statistical significance being of 76.30%, 0.23 and <0.001;

– methotrexate has significantly diminished the M.T.W., it allowing the estimation of a M.T.R. rate of 80.2% and a T/C value of 0.20;

– cyclophosphamide has induced a significant cancerostatic effect, expressed by M.T.R. (69.6%)

and T/C (0.30);

– melphalan and respectively 5-fluoruracil have determined a significant antineoplastic impact, which is correlated with a mean tumoral regression of 48.5% and respectively 52.1%, and a T/C value of 0.51 and respectively 0.48.

Table 3. The antitumoral effect of the experimental glycosidic treatment upon Guérin T-8 lymphotropic epithelioma and Walker 256 carcinosarcoma, compared with that of some standard cytostatics. Figures in brackets indicate the therapeutic doses (mg/kg b.w./daily) and the number of experimental animals, respectively.

Experimental group	M.T.W.(g)	T/C value	p
Guérin T-8 lymphotropic epithelioma			
Control	13.5 ± 1.7 (15)	-	-
ENLE (40,0)	7.0 ± 0.8 (10)	0.52	<0.01
D_2ENLE (40,0)	3.2 ± 0.5 (10)	0.23	<0.001
MTX (0.075)	2.7 ± 0.6 (10)	0.20	<0.001
CFS (1.0)	3.1 ± 0.9 (10)	0.23	<0.001
MLF (0.075)	6.9 ± 1.2 (10)	0.51	<0.01
5-FU (5.0)	6.5 ± 1.0 (10)	0.48	<0.002
Walker 256 carcinosarcoma			
Control	14.8 ± 2.0 (15)	-	-
ENLE (40,0)	8.5 ± 1.8 (10)	0.57	<0.05
D_2ENLE (40,0)	5.9 ± 1.1 (10)	0.40	<0.002
MTX (0.075)	3.7 ± 0.9 (10)	0.25	<0.001
CFS (1.0)	4.5 ± 1.1 (10)	0.30	<0.001
MLF (0.075)	8.7 ± 1.6 (10)	0.59	<0.05
5-FU (5.0)	7.8 ± 1.4 (10)	0.53	<0.01

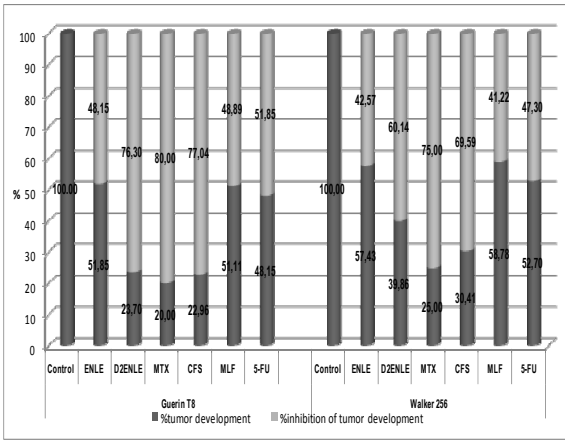

Fig. 3 Anticarcinogenic impact of the furostanolic-glycoside products and standard cytostatic agents.

The comparative testing of the antitumoral effect of the biosynthesis and semisynthesis furostanolic-glycoside agents and of the standard cytostatics was also performed in Walker 256 tumoral system. The experimental results are also presented in Table 3 and figure 3.

Once again, the evaluation indices values of the ENLE and D_2ENLE pharmacodynamic action (M.T.R. of 42.57% and 60.14%; T/C ratio of 0.57 and 0,40) have indicated – in comparison with the control group – a significant ($p<0.05$ and $p<0.002$) inhibitory effect upon Walker 256 carcinosarcoma development. The M.T.R. as well as T/C values of 75.0% and respectively 69.6% as well as 0.25 and respectively 0.30 – recorded on rats given methotrexate and respectively cyclophosphamide treatment – have revealed the high and significant ($p<0.001$ and respectively $p<0.001$) antitumoral potential of these standard agents.

The experimental therapy with melphalan and 5-fluorouracil, respectively, has been correlated with an estimated M.T.R. of 41.22% and 47.30%, respectively, as well as with T/C ratios of 0.59 and 0.53, respectively. These values of the evaluation indices – significant in relation to controls – have proved that these reference antitumoral drugs are characterized by a moderate to high therapeutic effectiveness on the Walker 256 carcinosarcoma for the doses and treatment scheme used by us.

The results obtained in this preclinical screening stage enable the assessment of the antitumoral effectiveness of glycosidic agents in comparison with that of the standard cytostatics.

The preclinical characterization of a substance as an antineoplastic agent is conditioned by the qualitative and quantitative evaluation of the specific pharmacological effect upon several experimental tumoral systems. For this purpose, the methodology – established by the national and international chemotherapeutic programs of preclinical screening in diverse and adequate experimental models – requires evidence of the agent antitumoral action and of the reproducibility of this pharmacodynamic effect, as objectives of the qualitative pharmacological evaluation. It also imposes the assessment of the antineoplastic pharmacotherapeutical effectiveness of the new agent according to the criteria of quantitative pharmacological evaluation: the demonstration of the existence of a dose-response relationship; the comparative analysis of its antitumoral effect with that of some standard cytostatics of clinical use (Leiter et al, 1965; Jungstand et al, 1971; Figg&McLeod, 2004).

Our preliminary results (Gherghel D. et al, 2005; Rotinberg P. et al., 2009) met the criteria of the qualitative evaluation of the ENLE and D_2ENLE furostanolic-glycoside products antitumoral action. We have then embarked upon additional investigations in order to provide a quantitative evaluation of the antineoplastic activity of these original biosynthesis and semisynthesis furostanolic-glycoside agents. Among other things, our research focused on the relation between their antitumoral effectiveness and their therapeutic doses which were used in the experimental treatment of the Guérin T-8 lymphotropic epithelioma and Walker 256 carcinosarcoma. This experimental aspect, representing one of the objectives of the present study, has revealed different intensities of the antitumoral effect, as an expression of the treatment with various doses, both lower and higher that the one that has conditioned the manifestation of the pharmacodynamic action of the biosynthesis and semisynthesis extracts. Thus, the progressive increase of the ENLE therapeutic dose from 20.0 to 30.0, 40.0, 50.0 and 60.0 mg/kg b.w., respectively, was correlated with a corresponding and progressive intensification of the tumorsuppression effect, estimated on the basis of the consecutive M.T.R. values (32.73%, 40.61%, 46.67%, 51.52% and respectively 56.97%, in the case of Guérin T-8 tumor, as well as 21.57%, 35.78%, 41.67%, 47.55% and respectively 50.00% in the case of Walker 256 tumor). This effect was also illustrated by the dynamics of the recorded T/C ratios, which points to their concomitant decrease (0.67, 0.59, 0.53, 0.48 and 0.43, for the epithelioma, as well as 0.78, 0.64, 0.58, 0.52 and 0.50, for the carcinosarcoma). The treatment of the animals bearing the Guerin T-8 lymphotropic epithelioma with increasing doses (10, 20, 40 and 60 mg/kg b.w.) of D_2ENLE was correlated with an enhancement of the antineoplastic impact for the first three doses (M.T.R. values of 59.89%, 81.36% and 77.97%, the corresponding T/C ratios being of 0.46, 0.22 and 0.38), meanwhile the last used dose, probably due to a toxic effect, has induced a smaller antineoplastic impact (the MTR value and T/C ratio being of 50.85% and 0.56). In the case of the rats bearing the Walker 256 carcinosarcoma, the administration of increasing doses of D_2ENLE has determined similar effects as on the former experimental tumoral system. Thus, in the case of the first three doses, the calculated M.T.R. values were of 53.29%, 77.63% and respectively 61.84%, while the corresponding T/C ratios were of 0.46, 0.22 and respectively 0.38. In the case of the last dose (60 mg/kg b.w.) the antitumoral impact was smaller than that of the first doses (MTR value of 43.42% and T/C ratio of 0.56)

The administration of the vehiculating agent HA50% to the rats bearing either Guerin T-8 lympotropic epithelioma, or Walker 256 carcinosarcoma, has as result a stimulation of the carcinogenetic process with 29.38% or 27.63%, probably due to its toxic effects. From this perspective, the impact of the selected semisynthesis furostanolic-glycoside agent could be higher in the conditions of selecting an appropriate vehiculating agent, with a lesser toxicity.

Appreciation of the results, obtained in the first stage of the quantitative evaluation of the antitumoral effect – meant to establish the existence of a dose-response relationship as a criterion for the estimation of the therapeutical effectiveness of the studied agents – requires their analysis according to the stipulation of the reference screening programs imposed for this preclinical investigation stage. According to the German and American programs, the dose-response relationship is confirmed if: M.T.R. values have progressively increased in relation to the raising of the therapeutic dose; at least one of the T/C ratios, obtained after the dose differentiated treatment, is within the limits of the admitted range (0.42-0.54).

In the light of the above criteria, our evaluation indices values of the antitumoral action of the biosynthesis and semisynthesis preparations highlights the existence of a positive relationship between the therapeutical dose and the intensity of the pharmacodynamic effect. The possibility of preclinical optimization of antineoplastic effectiveness of these furostanolic-glycoside agents by therapeutic dose manipulation enables us to define the preclinical range of the therapeutic doses. In the case of the semisynthesis agent, namely D_2ENLE, the higher dose do not determined an amplification of the antineoplastic impact, probably due to an enhancement of its toxic effect. But, the lower doses (especially the dose of 30 mg/kg. body weight), than the dose which conditioned the expression of its antitumoral action (40 mg/kg. body weight), have determined a higher negative impact upon the tumor development. We remind the fact that the vehiculating agent of this semisynthetic preparate is a concentrated solution of hydroalcohol (50%), which has a procarcinogenic effect. Thus, the real antineoplastic impact of the D_2ENLE is shadowed, attenuated by the toxic effect of the HA50%. Therefore, it is necessary the identification of a new vehiculating agent, with a smaller toxicity.

The existence of dose-response relationship has required a thoroughgoing study of the preclinical quantitative evaluation, of the pharmacotherapeutical antitumoral efficiency in a further stage, by comparing the antineoplastic potential of our bioactive agents with that one of some standard cytostatics of clinical use, in the conditions of laboratory experiments.

Consequently, the treatment of rats bearing Guérin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma with the ENLE and D_2ENLE compounds and also with the reference oncochemotherapeutic agents was correlated with the induction of some characteristic antitumoral effects. These were quantitatively expressed by the M:T.R. values of 48.15% or 42.57% (ENLE), 76.30% or 60.14% (D_2ENLE), 80.00% or 75.00% (methotrexate), 77.04% or 69.59% (cyclophosphamide), 48.89% or 41.22% (melphalan), 51.85% or 47.30% (5-fluorouracil) and by corresponding T/C ratios. According with the reference preclinical screening programs, a substance is considered as a possible oncochemotherapeutic agent if it has produced a M.T.R. value of at least 35% (Leiter et al, 1965), or if the induced M.T.W. decrease has resulted in a T/C ratio of 0.54–0.64 (Leiter, 1965) in at least one solid tumoral system out of three tested systems. From this viewpoint, the antitumoral action of the ENLE and D_2ENLE has been reconfirmed. The comparative analysis of evaluation indices values of the anticancerous activity reveals a significant experimental therapeutic effectiveness of the autochthonous furostanolic-glycoside agents. The biosynthetic agent ENLE has an antineoplastic impact smaller (in comparasion with methotrexate and cyclophosphamide), similar (in comparison with the 5-fluorouracil and melphalan) than the antitumoral potential of the reference agents. D_2ENLE has induced a similar effect with that of the methotrexate and cyclophosfamide, and even higher comparatively with melphalan and 5-fluorouracil.

In the light of the above results, one can appreciate that the autochthonous furostanolic-glycoside agents are characterized by a significant antitumoral therapeutic efficiency – in

comparison with the one of the reference cytostatics – in our experimental conditions (for the doses and the tumoral systems used in our screening).

The possibility to improve the cytostatic effectiveness by manipulating the therapeutical doses, as well as the antitumoral potential significance of the ENLE and D_2ENLE agent, established by comparison with the standard oncostatic drugs, have partially made possible the quantitative evaluation of them antineoplastic pharmacodynamic effect.

CONCLUSIONS

1. Testing of the glycosidic products effect upon the Guérin T-8 lymphotropic epithelioma and Walker 256 carcinosarcoma development has revealed a directly proportional correlation between the therapeutical dose and the antineoplastic potential of the ENLE and D_2ENLE agents.

2. The comparative analysis of the experimental antitumoral impact of the furostanolic-glycoside compounds and of some standard cytostatics, respectively, was relevant for the appreciation of a significant oncostatic effectiveness of those bioactive agents.

3. The positive answers obtained to these major problems of the preclinical quantitative pharmacodynamics evaluation require further investigation of the therapeutic efficiency of original furostanolic-glycoside agents upon tumors with different degrees of development.

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