REPRODUCIBILITY AND DOSE DEPENDENCY OF THE ANTITUMORAL PHARMACODYNAMIC EFFECT OF SOME AUTOCHTHONOUS POLYSACCHARIDIC OR POLYPHENOLIC BIOPREPARATIONS OF FUNGAL AND VEGETAL ORIGIN

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Abstract: The impact of polysaccharidic or polyphenolic biopreparations – characterized *in vitro* as cytotoxic and cytostatic agents – upon the carcinogenesis process has been investigated by *in vivo* successive testing of their effects on the development of Guérin T-8 lymphotropic epitelioma and Walker 256 carcinosarcoma. The similarity of mean tumoral regressions, of the T/C ratios (between mean tumoral weights of the treated and control groups), of the T/C values products of the successive retests – registered after the polysaccharidic or polyphenolic treatment - with the evaluation indices standard values, as well as the including of the retests T/C x 100 values between the limits of the admissible variability range have highlighted the reproducibility of their antineoplastic effect. Also, the significance of the antitumoral action of the studied biopreparations upon the rats bearing diverse experimental tumoral lines has been appreciated in relation with the therapeutic effect of different doses on carcinogenesis, as well as from point of view of the experimental oncostatic activity of some standard cytostatics of clinical use (methotrexate, cyclophosfamide, melphalan and 5-fluorouracil). The laboratory treatment with the polyphenolic agents in various doses – superior and inferior to that which has conditioned the expression of their antitumoral effect upon Guerin T-8 lymphotropic epithelioma and Walker 256 carcinosarcoma – has demonstrated the antineoplastic effectiveness` dependence of these biopreparations on 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 polyphenolic extracts and with the reference cytostatic drugs – has revealed that the antitumoral potential of the new autochthonous biopreparations is higher, equal or near to those of the standard oncochemotherapeutics. The reproducibility of the antitumoral impact and possibility of optimization of the antitumoral efficiency by experimental manipulation of the therapeutic doses are relevant for the characterization of the polyphenolic extracts as potential oncochemotherapeutic agents.

INTRODUCTION

At present, the antineoplastic chemotherapy holds a priority in the fight against cancer disease, a real scourge of contemporary times. Although there has been continuous progress in cancer diagnosis and treatment as a result of recent discoveries in cellular, subcellular and molecular oncobiology, anticancerous chemotherapy is still of little effectiveness, because its negative impact on the normal cells of the organism under neoplasm aggression, and the development of resistance phenomenon of the malignant cells to the cytostatic drugs action (Stroescu, 1995; Bronchud, 2000; DeVita, 2001; Abrams, 2003; Lodish et al., 2003; DiPirio et al, 2005;).

In the fight against cancer , the discovery of new chemical pharmacological agents with antineoplastic activity, as well as of new ways to decrease cancer cells resistance to cytostatics represent a very important concern of chemopharmaceutical and oncobiological research and of medical practice, which pursues the improvement of antitumoral chemotherapy effectiveness (Pollak&Fidler, 1982; Boyd, 1989; Valeriote et al, 1984; Weinstein, 2001; Wong, 2002; Workman&Kaye, 2002; Abrams, 2003; Figg&McLeod. 2004; DiPirio et al, 2005;).

The characterization of an anticancerous agent – 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 some preclinical and clinical complex pharmacological investigations on 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; qualitative and quantitative assessment criteria of the induced antitumoral action; evaluation indices of the specific pharmacodynamic effect and their standard values (Leiter et al., 1965; Boyd, 1989; Borenfreund&Babich, 1990; Phillips et al., 1990, 1991; Bissery&Chabot, 1991; Stroescu, 1995; Miron, 2000; DeVita, 2001; Cook, 2002; Kuimelis, 2001; Seethala&Prabhavathi, 2001; Workman&Kaye, 2002; Figg&McLeod. 2004).

In this context, our preliminary investigations have revealed the *in vitro* cytostatic and cytotoxic activity, upon the HeLa and HEp-2p tumoral cell cultures, of some new polysaccharidic and polyphenolic extracts, as well as the *in vivo* antineoplastic impact of these fungal or vegetable products upon several experimental tumoral systems (Rotinberg et al,

2007). To prove reproducibility of these autochthonous biopreparations – another objectiv of the qualitative pharmacological evaluation - and to appreciate the preclinical antineoplastic effectiveness of the polyphenolic biopreparations by the experimental modulation of the therapeutical doses and by comparing their efficiency with the impact of standard cytostatics upon the tumor development process – objectives of the quantitative pharmacological evaluation - we appealed to adequate *in vivo* models represented by rats with various experimental tumor lines.

The present paper describes and examines the results of successive retests and, also, of the comparative tests of the antitumoral activities of various doses of studied agents, as well as of some standard oncochemotherapeutic agents of clinical use, in order to the qualitative and quantitative pharmacodynamic assessment of the tumorsuppressor effect of the active bioproducts.

MATERIALS AND METHODS

The bioactive products of polysaccharidic or polyphenolic nature, namely EPzAgsp or EPfRc and EPfHr, were specifically extracted from *Agaricus sp.* (EPzAgsp), through an aqueous extraction, or *Rosa canina* (EPfRc) and *Hippophae rhamnoides* (EPfHr), obtained from the bark's shrubs through a hydroalcoholic extraction. Also, in the case of the quantitative pharmacodynamic assessment, the standard cytostatics, included in the reference experimental antitumoral therapy, were: methotrexate, cyclophosphamide, melphalan and 5-fluorouracil.

In vivo testing of the EPzAgsp, EPfRc and EPfHr antitumoral activity has been performed on Wistar, white female rats of 125-150 g, either with Guérin T-8 lymphotropic epithelioma or with Walker 256 carcinosarcoma. Both these experimental tumoral lines are of solid type. The animals were housed in individual cages, having free access to water and standard food, in a normal light/dark cycle and a 22^{o} C ambient temperature.

Twenty-four hours after the subcutaneous tumoral transplant, with 0.2 ml suspension of cancerous cells (Pollak&Fidler, 1982), the antimalign treatment was initiated and continued for 16 days in the case of Guérin T-8 tumour and for 19 days in the case of Walker 256 tumour. It was applied through intraperitoneal (i.p.) daily injection of the polysaccharidic biopreparation, in dose of 50 mg/kg body weight, and of the polyphenolic extracts, the dose in this case being of 20 mg/kg body weight. An equivalent volume of saline solution was administered to the control animals.

Three successive tests were also performed in the same experimental conditions as in the preliminary investigations which have highlighted the antitumoral pharmacodynamic action of the studied biopreparations (the same experimental tumoral systems; the same therapeutical dose; the same treatment program and pattern). This experimental model was necessary for the confirmation of their antitumoral impact.

In the case of the appreciation of the preclinical antineoplastic effectiveness of the polyphenolic extracts, the treatment consisted in daily intraperitoneal (i.p.) administration of the bioactive agent in various doses. Thus, the injected doses of natural polyphenolic agents have been either higher or lower than the dose which conditioned the expression of their antitumoral action (20 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 therapeutically doses, expressed in mg/kg. b. w., are presented in the tables that also include the results for each of the experimental models.

The assessment of the antineoplastic effect was based on the comparison of the mean tumour weight (M.T.W.) in the treated and respectively, control animals after sacrifice.

The evaluation of the antitumoral activity was made by determining the mean tumour regression (% M.T.R) and by the calculation of the T/C value (where T = M.T.W. in the treated groups and C = M.T.W. in the control groups), as well as of 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 demonstration of the pharmacodynamic effect reproducibility has also involved the assessment of some specific indices: T/C x 100 value of the retests; the superior and inferior limits of the admissible variability range, established on the basis of the formulas T/C x 100 x 1.82 and T/C x 100 / 1.82 (the T/C x 100 value corresponds to the first test); the products of the T/C values obtained in the first two test and in all tests.

The appreciations of the antitumoral therapeutic effect reproducibility and effectiveness of the studied agents also required the comparative analysis of the evaluation indices values we obtained with those set by the selection criteria of antineoplastic agents 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; Dold, 1978) for these steps of the preclinical trial.

RESULTS AND DISCUSSIONS

In a first stage, we have studied, on rats with various experimental solid tumoral systems, the influence of therapy with EPzAgsp, EPfRc and EPfHr polysaccharidic or polyphenolic agents upon the development of Guérin T-8 lymphotropic epithelioma and Walker 256 carcinosarcoma in order to demonstrate the reproducibility of their antitumoral pharmacodynamic effect.

It can be seen – from the results included in Table I – that the i.p. daily therapeutical administration of the bioactive agents to the rats bearing one of the solid tumours induced a significant decrease of the mean tumoral weights by comparison with the control group.

Thus, in the initial experiment, M.T.W, M.T.R. and T/C ratio values point to the existence of an inhibitory effect upon the tumorgenesis process in comparison with the control groups, its intensity varying in relation to the type of experimental tumor and to the bioactive agent used. The induced antitumoral effect – expressed by a decrease of the M.T.W. in comparison with the corresponding control groups - has been illustrated by the M.T.R. of 28.57% (EPzAgsp), of 41.27% (EPfRc) or of 39.70% (EPfHr), in the case of lymphotropic epithelioma, and respectively of 30.23% (EPzAgsp), of 40.41% (EPfRc) or of 42.47% (EPfHr), in the case of the carcinosarcoma, as well as by T/C values of 0.71, 0.59 or 0.60 and respectively 0.70, 0.60 or 0.58.

The T/C x 100 values of the opening test were in the case of the lymphotropic epithelioma, of 71% (EPzAgsp), 59% (EPfRc) or 60% (EPfHr), and, in the case of the Walker 256 carcinosarcoma, of 70%, 60% or 58%. These values were necessary in order to define the admissible variability range. For the polysaccharidic biopreparation, the upper and lower limits are 129.2% and 39.0%, for the Guerin T-8, and of 127.4 and 38.5%, for the Walker 256. In the case of the EPfRc and respectively EPfHr, these limits were: 107.4% and 32.4% and respectively 109.2% and 32.9%, in the case of the lymphotropic epithelioma, as well as, of 109.2% and 32.9% and respectively 105.6% and 31.9%, in the case of the carcinosarcoma.

Table I. Successive testing of the daily antitumoral therapy with EPzAgsp, EPfRc and EPfHr on rats bearing Guérin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma. Figures in brackets indicate the number of experimental animals.

Group / Treatment	M.T.W. (g) X ± E.S.	%M.T.R.	T/C value	p	M.T.W. (g) X ± E.S.	%M.T.R.	T/C value	p
	Guerin T8 lymphotropic epithelioma				Walker 256 carcinosarcoma			
Control	14.70±1.78 (15)	-	-	-	12.90±1.90 (15)	-	-	-
EPzAgsp	10.50±3.30 (10)	28.57	0.71	NS	9.00±3.90 (10)	30.23	0.70	NS
Control	18.10±1.60 (15)	-	-	-	15.80±1.75 (15)	-	-	-
EPzAgsp	12.40±3.01 (10)	31.49	0.69	NS	10.40±3.15 (10)	34.18	0.66	NS
Control	11.90±1.50 (15)	-	-	-	10.30±1.70 (15)	-	-	-
EPzAgsp	8.82±2.70 (10)	25.88	0.74	NS	8.00±1.80 (10)	22.33	0.78	NS
Control	16.55±1.94 (15)	-	-	-	14.60±1.87 (15)	-	-	-
EPfRc	9.72±2.11 (10)	41.27	0.59	<0.05	8.70±2.10 (10)	40.41	0.60	<0.05
EPfHr	9.98±2.25 (10)	39.70	0.60	<0.05	8.40±2.30 (10)	42.47	0.58	<0.05
Control	19.40±1.70 (15)	-	-	-	17.30±2.10 (15)	-	-	-
EPfRc	10.82±2.00 (10)	44.23	0.56	<0.01	9.31±1.90 (10)	46.18	0.54	<0.01
EPfHr	10.24±2.30 (10)	47.22	0.53	<0.01	8.72±2.15 (10)	49.60	0.50	<0.01
Control	13.60±2.10 (15)	-	-	-	13.40±2.10 (15)	-	-	-
EPfRc	8.47±1.90 (10)	37.72	0.62	NS	8.35±1.80 (10)	37.69	0.62	NS
EPfHr	8.80±1.7 (10)	35.29	0.65	NS	8.25±1.7 (10)	38.43	0.62	NS

The values of evaluation indices in the first retesting confirm the nonsignificant or significant antitumoral potential of EpzAgsp or of EPfRc and EPfHr, their T/C x 100 values being:

-in the case of the lymphotropic epithelioma, of 69%, 56% and 53%. The T/C values of the first two experiments allow us to estimate products of 0.49, 0.33 and 0.32;

-in the case of the carcinosarcoma, of 66%, 60% and 58%. The T/C values of the first two

experiments allow us to estimate products of 0.46, 0.32 and 0.29.

Finally, the second retesting has also pointed to the anticancerous action of EPzAgsp, EPfRc and EPfHr bioactive agents, the evaluation indices being close to those in the previous experiments. In that case the T/C x 100 values were 74% (EPzAgsp), 62% (EPfRc) and 65% (EPfHr), for the Guerin T-8,as well as, of 78% (EPzAgsp), 62% (EPfRc) and 62% (EPfHr), in the case of Walker 256 carcinosarcoma. The products of the T/C values in the three successive tests were, for lymphotropic epithelioma, 0.35, 0.20 and 0.21, and for carcinosarcoma, 0.36, 0.20 and 0.18.

The interference of daily antitumoral therapy, performed by administration of EPfRc and EPfHr in different doses, with the development process of Guérin T-8 lymphotropic epithelioma, can be followed from Figure 1.

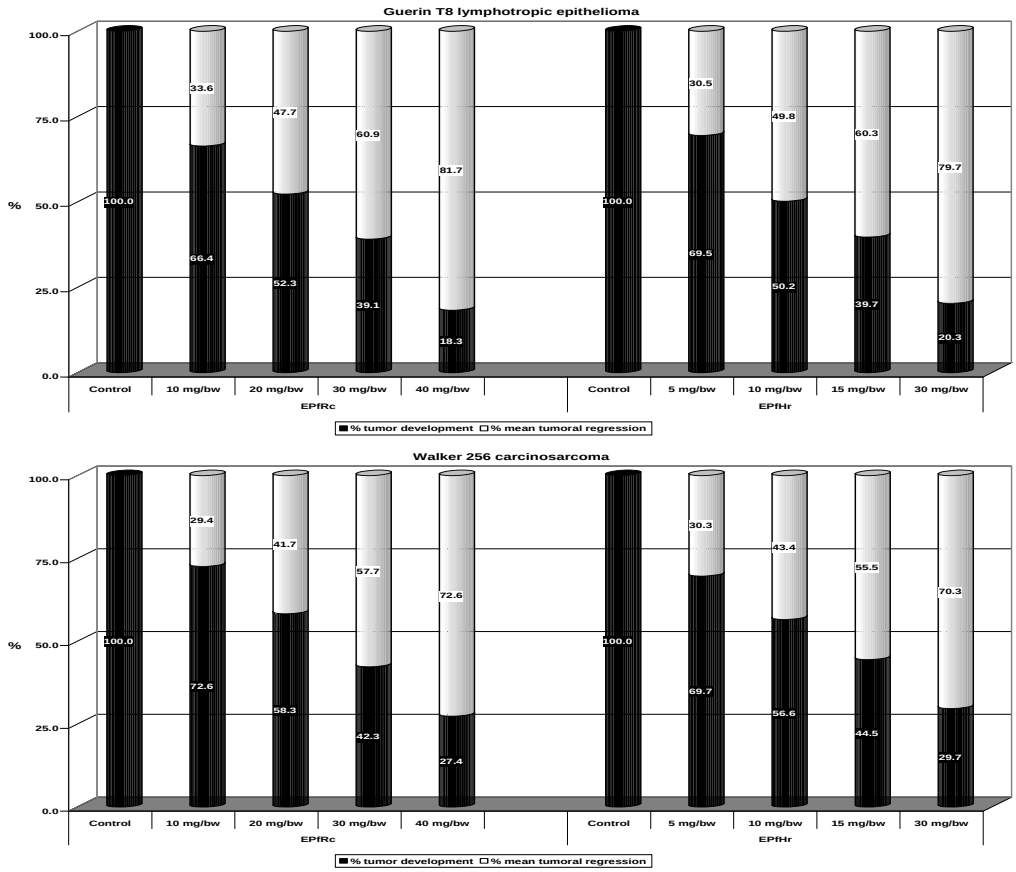

Figure 1. Experimental oncochemotherapeutic potential of various doses of EPfRc or EPfHr biopreparation (mg/kg b.w./day) upon Guérin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma

It can be observed that the differentiated treatment was followed by: a moderate decrease of M.T.W in the case of the groups treated with EPfRc (10 mg/kg b.w.) or EPfHr (5 mg/kg b.w.). The corresponding M.T.R. were 33.6% (EPfRC) and 30.5 (EPfHr), and the T/C values were 0.66 and 0.69; a significant antitumoral activity ($p<0.01$), illustrated by the M.T.R. (47.7% and

49.8%) and T/C value (0.52 and 0.50), estimated in the animals treated with 20 and 10 mg/kg b.w. of EPfRc and EPfHr; an important cytostatic action in the case of the groups treated with 30 and 15 mg/kg b.w., which is represented by the M.T.R. (60.9% and 60.3%), T/C (0.39 and 0.39) values, it having statistical significance ($p<0.001$); a maximum tumorsupressor effect (M.T.R. of 81.7% and 79.7% as well as T/C values of 0.18 and 0.20) in the case of animals treated with a dose of 40 mg/kg b.w. (EPfRc) or 30 mg / kg b.w. (EPfHr), the statistical significance being 0.001 in both cases.

Similar results were recorded on rats with Walker carcinosarcoma submitted to daily therapy with various doses of EPfRc and EPfHr (Figure 1). The progressive increase of the dose was also correlated with an optimization of the antitumoral effectiveness in comparison with the untreated, control group.

Thus, a nonsignificant cytostatic effect was observed for the minimum doses of EPfRc (10 mg/kg b.w.) or EPfHr (5 mg/kg b.w), the M.T.R. values being of 29.4% or 30.3 and T/C values being of 0.72 or 0.70). When the dose was increased to 20 or 10 mg/kg b.w., there occurred a decrease of M.T.W. which resulted in a M.T.R. of 41.7% (EPfRc) or 43.4% (EPfHr) and a T/C ratio of 0.58 or 0.56. These values of the evaluation indices point to an important inhibitory action upon tumoral development. Therapeutic dosage increase to 30 and 40 mg/kg b.w. or 15 and 30 mg/kg b.w., was correlated with a significant enhancement of the antitumoral potential: the M.T.R. values were 57.7% and 72.6%, in the case of EPfRc or of the 55.5% and 70.3%, in the case of EPfHr. The corresponding T/C ratios were 0.42 and 0.27 or 0.44 and 0.30.

Therefore, we may conclude that the polyphenolic treatment has inhibited the development of tumors in relation to the dose employed.

The evaluation of the experimental antitumoral effectiveness of these polyphenolic biopreparations has also required the testing of the effect of some standard oncochemotherapeutic agents upon carcinogenesis in laboratory conditions.

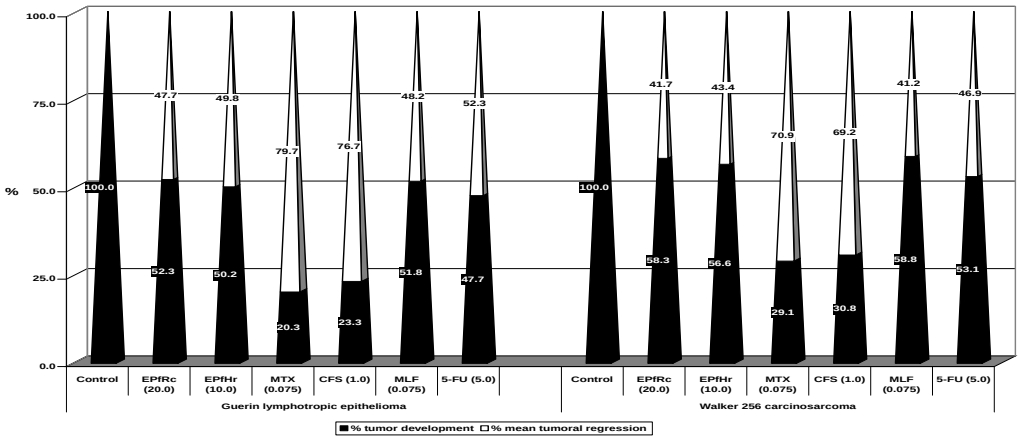

Figure 2. The effectiveness of oncochemotherapy with various doses (mg/kg b.w./daily) of polyphenolic agents or standard cytostatics applied to rats with Guerin T-8 or Walker 256 carcinosarcoma.

Figure 2 shows the evaluation indices values of the antitumoral impact induced by EPfRc and EPfHr, methotrexate, cyclophosphamide, melphalan and 5-fluorouracil, respectively, on the development of the solid Guérin T-8 and Walker 256 tumor.

Here are the effects upon the lymphotropic epithelioma of the antitumoral treatment

compared with the data in the control group:

– EPfRc and EPfHr agents have significantly inhibited ($p<0.01$) the evolution of lymphotropic epithelioma; this effect is expressed by decrease of M.T.W., by the M.T.R. value (47.7% and 49.8%) and by the T/C ratio (0.52 and 0.50);

– methotrexate has significantly diminished the M.T.W., a fact which was correlated with a M.T.R. rate of 79.7% and a T/C value of 0.20, respectively;

– cylophosphamide has induced a significant cancerostatic effect, the M.T.R. and T/C value being 76.7% and 0.23;

– melphalan and respectively 5-fluoruracil have determined a significant antineoplastic impact, which is correlated with a mean tumoral regression of 48.2% and respectively 52.3%, and a T/C values of 0.52 and respectively 0.48.

The comparative testing of the antitumoral impact of the EPfRc and EPfHr, as well as of the standard cytostatics was also performed in Walker 256 tumoral system. The experimental results are, also, presented in Figure 2.

Once again, the evaluation indices values of the EPfRc and EPfHr pharmacodynamic actions (M.T.R. of 41.7% and 46.9%; T/C ratios of 0.58 and 0.56) have indicated – in comparison with the control group – a significant ($p<0.05$) inhibitory effect upon Walker 256 carcinosarcoma development. The M.T.R. as well as T/C values of 70.9% and respectively 69.2% as well as 0.29 and respectively 0.31 – recorded on rats given methotrexate and respectively cyclophosfamide treatment – have revealed the high and significant ($p<0.001$ and respectively $p<0.002$) antitumoral potential of these standard agents.

The experimental therapies with melphalan and 5-fluorouracil, respectively, have resulted in an estimated M.T.R. of 41.2% and 46.7%, respectively, as well as in corresponding T/C ratios of 0.59 and 0.53, respectively. The values of the evaluation indices – significant in relation to controls – have proved that these reference antitumoral drugs are characterized by a moderate to high antitumoral action on the Walker 256 carcinosarcoma for the doses and treatment scheme used by us.

The experimental results obtained in this preclinical screening stage enable the assessment of the antitumoral effectiveness of EPfRc and EPfHr agents in comparison with that of the standard cytostatic agents.

The enormous number, the various nature (biosynthesis, semisynthesis and synthesis) and the structural diversity of the bioactive compounds with putative anticancerous action have made it necessary to include a preliminary phase of *in vitro* testing upon tumoral cell cultures. This ensures – by means of the required assessment criteria – the selection of only the active cytostatic and/or cytotoxic agents, which will then be included in the successive and interdependent steps of the *in vivo* preclinical screening on animals with various experimental tumoral lines. The qualitative and quantitative evaluations of their specific inhibitory effect on the tumor development process may allow the final preclinical pharmacological characterization of such substances as new oncochemoterapeutic agents (Jungstad et al, 1971; Calabresi&Parks, 1985; Boyd, 1989; Kuimelis, 2001; Weinstein, 2001; Abrams, 2003; DiPirio et al, 2005; Ruddon, 2007).

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; Edwards, 1975; Dold, 1978; Figg&McLeod, 2004).

In vitro characterization of the EPzAgsp, EPfRc and EPfHr extracts as potential cytotoxic and cytostatic agents on some tumoral cell cultures of human origin, was a condition for their passing in the complex program of the *in vivo* preclinical screening (Mihai et al., 2007; Rotinberg et al, 2008). The highlighted antitumoral pharmacodynamic effect of the EPzAgsp, EPfRc and EPfHr bioactive agents upon rats bearing tumors, has imposed thoroughgoing research in order to demonstrate the reproducibility and stability of the oncostatic property of these biosynthetic preparations. Thus, we have performed a series of three successive tests in identical experimental conditions with those of the primary testing, which revealed their *in vivo* cytostatic effect. We have then embarked upon additional investigations in order to provide a quantitative evaluation of the antineoplastic activity of polyphenolic extracts, the most selected antitumoral active agents.

Appreciation of the results, obtained in the context of the qualitative and quantitative evaluation of the antitumoral effect – meant to establish the reproducibility of the antitumoral effect, 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 stages. Thus, for assessing the replicability of the induced antitumoral action, the german program requires, for this step, that the successive tests should result in close M.T.R. values, meanwhile the American screening program imposes that the T/C x 100 values of the retests should be between the upper and the lower limits of the admissible variability range. The value obtained by multiplying the T/C ratios of the first two tests as well as of all three tests must be 0.20 – 0.24 and 0.08 – 0.09, respectively. Also, 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 evaluation indices, one can discuss and interpret the results we obtained, in the successive testings of the antitumoral activity of polysaccharidic and polyphenolic extracts on rats bearing either Guérin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma.

The significant values of the M.T.R. – induced in Guérin tumour by antimalignant treatment with EPzAgsp, EPfRc and EPfHr, as well as on Walker tumour – obtained in the successive tests are, with the exception of the polysaccharidic agent, higher than the imposed minimum level (35.0%). At the same time, the T/C x 100 values of the retests – calculated on the basis of the T/C ratios in the experimental groups given oncostatic treatment with the EPzAgsp, EPfRc and EPfHr are between the lower and upper limits of the corresponding admissible variability ranges. Moreover, the values estimated by multiplying the T/C ratios – both of the first two tests, and of all three tests – after the polysaccharidic or polyphenolic antitumoral therapy are close to the standard values established by the reference American program.

The comparative analysis of our values with those stipulated by the preclinical screening programs, for this second step of the qualitative evaluation, certifies the reproducible and stable character of the antineoplastic pharmacodynamic effect induced by polyphenolic extracts. We would also like to add that the results have displayed the antitumoral pharmacotherapeutic

spectrum of the studied preparations highlighting at the same time its greater therapeutic effectiveness on the Guérin T-8 lymphotropic epithelioma.

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. Thus, the progressive increase of the EPfRc and EPfHr therapeutic dose was correlated with a corresponding intensification of the tumorsuppression effect, estimated on the basis of the consecutive M.T.R. values and by the dynamics of the recorded T/C ratios, which points to their concomitant decrease. In the light of the above criteria, our evaluation indices values of the antitumoral action of the polyphenolic extracts highlights the existence of a relationship between the therapeutical dose and the intensity of the pharmacodynamic effect.

The existence of dose-response relationship has required a thoroughgoing study of the preclinical quantitative evaluation of the antitumoral pharmacotherapeutical 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. The comparative analysis of evaluation indices values of the anticancerous activity reveals a significant experimental therapeutic effectiveness of the polyphenolic agents. This is smaller (in comparasion with methotrexate and cyclophosphamide), similar (in comparison with the 5-fluorouracil) and even higher (comparatively with melphalan) than the antitumoral potential of the reference agents.

In the light of the above results, one can appreciate that the natural polyphenolic agents presents 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 by us).

The demonstration of the cytostatic action reproducibility, of the existence of dose-response as well as of the antitumoral potential significance of the polyphenolic extracts completes the qualitative and quantitative evaluation of their pharmacological effect.

CONCLUSIONS

The bulk of our experimental results is relevant for the real, reproducible and stable character of the pharmacodynamic antineoplastic effect of the EPfRc and EPfHr which have been studied.

Testing of the polyphenolic agents effects upon the lymphotropic epithelioma and carcinosarcoma development has revealed a directly proportional correlation between the therapeutical dose and the antineoplastic potential of the EPfRc and EPfHr agents.

The comparative analysis of the experimental antitumoral impact of the polyphenolic extracts and of some standard cytostatics, respectively, was relevant for the appreciation of a significant oncostatic effectiveness of these natural bioactive polyphenolic extracts.

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