A comparison regarding antiproliferative action between soy total extract and genistein

CORINA TIULEA¹), CAMELIA PEEV¹), DIANA BREZOVAN²), CRISTINA DEHELEAN³), A. MOTOC⁴)

¹)Department of Pharmacognosy, Faculty of Pharmacy, "Victor Babeş" University of Medicine and Pharmacy, Timisoara
²)Department of Cell Biology–Histology–Embryology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Banat, Timisoara
³)Department of Toxicology, Faculty of Pharmacy
⁴)Department of Anatomy, Faculty of Medicine "Victor Babeş" University of Medicine and Pharmacy, Timisoara

Abstract

The aim of this study is to make a comparison between the action of genistein and total soy extract regarding anticancer action on two different in vivo models: phytobiological test and animal model, and to see which of the two tested samples present a greater antiproliferative effect. Soybean seeds were grounded and a solvent formed of DMSO–ethanol–water in rapport 5–70–25 v/v/v was prepared. The extraction was made using an ultrasonic bath (Falc LCD Series) for 30 minutes, 59 kHz. The solvent was evaporated with a rotary evaporator at 50°C. Genistein was acquired from Extrasynthèse (France), hydroxypropyl-γ-cyclodextrin (HPGCD) from Cyclolab Hungary, 7,12-dimethylbenz[a]anthracene (DMBA), dimethylsulfoxide (DMSO), and 12-O-tetradecanoylphorbol-13-acetate (TPA) from Sigma Aldrich, Germany. Because of the poor water solubility, genistein was prepared in a complex with hydroxypropyl-γ-cyclodextrin in a molar ratio 1:2 by kneading method and total soy extract in a mass ratio 1:4 also by kneading method. Phytobiological test indicated an inhibition index over 50% in case of solutions of concentration between 8–33% in both samples, suggesting a possible antiproliferative action at a superior level. Study on C57BL/6J mice was made on which it was induced cancer with physical agents like DMBA, and it was promoted with TPA. Mice where divided in four groups: Group A – blank group, Group B – mice who received total soy extract, Group C – mice who received genistein, Group D – untreated mice. Results on animal model show that both soy total extract and genistein inhibited the initiation and promotion of chemically-induced skin tumorigenesis, but genistein had a greater success in recovering skin lesions type experimental malignant melanoma.

Keywords: soy extract, genistein, phytobiological test, mouse, DMBA, antiproliferative.

Introduction

It has been shown that lifestyle and dietary habits play an important role in the reduction of cancer risk [1]. A remarkable amount of research regarding the healthy effects of soy consumption was carried out during the past 20 years, and the conclusions were that in most of the cases positive effects can be attributed to the presence of isoflavones, a class of organic compounds produced almost exclusively by the members of the Fabaceae / Leguminosae (bean) family [2].

Genistein is an isoflavone, phytooestrogenic compound found in high levels in soy product. In vitro and in vivo studies have shown that genistein (4',5,7-trihydroxyflavone) has been demonstrated to have weak estrogenic and antiestrogenic properties, to inhibit topoisomerase II and angiogenesis, to have antioxidant properties and to induce cell differentiation. So, it has been associated with beneficial effects in treatment of osteoporosis, cardiovascular diseases, high blood pressure and different types of cancer (breast, skin, prostate) [3]. But, soybeans and soy-based foods have hundreds of phytochemical compounds. In recent years, accumulating evidence has suggested that the isoflavones or soy proteins stripped of phytochemicals only reflect certain aspects of health effects associated with soy consumption. The benefice effect of soy may be attributed also to other phytochemicals, either alone or in combination with isoflavones or soy protein [4].

It is known the fact that the vegetal product used in the pharmaceutical and food area is the soybean, Sojae semen, with a composition formed of proteins 35–40%, lipids 15–20%, sugars 15–35%, isoflavones 0.12–0.3% [5].

In order to compare the antiproliferative action between soy total extract and genistein in vivo phytobiological test and study on C57BL/6 mice were made. Phytobiological test generally provides information for determining the synergistic relationship between the complexity of the chemical composition of plant extracts and possible biological action. Phytobiological test can provide important data of natural chemical compounds regarding the inhibitory action of cellular...
activity, cytostatic, mitodepressive action or regarding the stimulatory cellular activity [6].

Another in vivo model for the analysis of potential anticancer action is the animal model. For this study, we chose C57BL/6 mice on which we induced cancer with physical agents: repeated application of a carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) with subsequent application of a tumor promoter: 12-O-tetradecanoylphorbol-13-acetate (TPA) which can markedly enhance the development of carcinogen induced melanoma [7].

The aim of this study is to make a comparison between the action of genistein and total soy extract regarding anticancer action on two different in vivo models: phytobiological test and animal model and to see which of the two tested samples present a greater antiproliferative effect.

Materials and Methods

Soy seeds were kindly provided from Department of Plant Culture, University of Agricultural Sciences and Veterinary Medicine, Timișoara, Romania. In the previous study, seeds were considered for quantitative analysis of total lipids (Soxhlet), proteins (Kjeldahl), polyphenols (Folin-Ciocalteu) and isoflavones – daidzin, genistin, daidzein and genistein (HPLC). Analyze showed 21.4% total lipids/100 g sample, 39.40% proteins, 303 mg total polyphenols/100 g sample, heterosides: daidzin 819 mg/g and genistin 905.6 mg/g, and aglycones: daidzein 91 mg/g and genistein 119 mg/g. Values fit literature data [8].

Soybean seeds were grounded and a solvent formed of DMSO-ethanol-water in rapport 5–70–25 v/v/v was prepared [9]. The extraction was made at room temperature using an ultrasonic bath (Falc LCD Series) for 30 minutes, 59 kHz. The solvent was evaporated with a rotary evaporator at 50°C.

Genistein was acquired from Extrasynthese (France), hydroxypropyl-γ-cyclodextrin (HPGCD) from Cyclolab Hungary, 7,12-dimethylbenz[a]anthracene (DMBA), dimethyl sulphoxide (DMSO) and 12-O-tetradecanoylphorbol-13-acetate (TPA) from Sigma Aldrich, Germany.

Because it has a poor water solubility, genistein was extracted in a complex with hydroxypropyl-γ-cyclodextrin in a molar ratio 1:2 by kneading method and total soy extract in a mass ratio 1:4 also by kneading method [10].

For the determination of antiproliferative action by phytobiological test study of seeds germination of garden cress (Lepidium sativum) was made. L. sativum seeds were put to germinate in Petri dishes, 10 cm in diameter, on filter paper, soaked with water in darkness at a temperature of 25°C. After 24 hours, the radicles reach 1–2 mm in length. Water was removed and was added 12 mL of solution to be analyzed in different concentrations ranging from 2 to 33%. Seeds were kept 24 hours in this solution, after which were listed in a preservative solution consisting of salicylic acid 2 g, sodium phenolate 2 g, zinc sulphate 2 g, in 100 mL distilled water. Sample analyzed were solutions of different concentrations (2–33%) of soy total extract/genistein incorporated in hydroxypropyl-γ-cyclodextrin. The control samples were treated only with water. After 24–48 hours, the radicles were measured on millimeter paper both from the grains treated with extractive solution of different concentrations and those treated with water as control samples. For each sample, we performed five measurements of 10 seedlings. The inhibition rate (I [%]) is calculated with the formula:

\[ I = \frac{L_m}{L_t} \times 100 \]

where

- \( L_m \) – is the average length of radicles from grains treated with water [mm];
- \( L_t \) – is the average length of radicles from grains treated with extractive solutions [mm].

Values higher than 50% for the inhibition rate indicate a potential antiproliferative effect, the extractive solutions for which the results show this kind of activity, must be considered for further analyzes in a superior level of screening process [11].

Animal studies were conducted on C57BL/6J female mice of 6–7 weeks. Mice were purchased from Charles River (Germany). The work protocol followed all NIAH (National Institute of Animal Health) rules – animals were maintained during the experiment in standard conditions: 12 hours light-dark cycle, food and water ad libitum, temperature 24°C, humidity above 55%. The number of mice taken into study was twenty.

For testing genistein and total soy extract efficacy, the dorsal hair was shaved by surgical clippers and the twenty mouse were divided in four groups, each group formed of five mouse. Group A was the blank group. Group B received for one week 15 µmol of total soy extract, included in HPGCD, applied on the back of the mice and after that, initiation was made by using 10 nmol DMBA. Mice were then treated with twice-weekly 4 mg TPA, a tumor promoter. Group C received 15 µmol of genistein, included in HPGCD, applied on the back of the mice and after that initiation was made by using 10 nmol DMBA. Mice were then treated with twice-weekly 4 mg TPA. Group D received no treatment only the initiation with 10 nmol of DMBA and promotion using 4 mg TPA. All reagents were topically applied to mouse skin in 0.2 mL acetone [1].

Mice were sacrificed in week 20 after the initiation of skin carcinogenesis with DMBA. Macroscopic examination of skin and visceral organs was made.

For the histological analysis, tissue samples (skin) were fixed in 10% formalin solution and were embedded in paraffin and cut at 4 µm. Finally after deparaffinized the samples were stained with Hematoxylin–Eosin (HE) and microscopically analyzed.

Results

Inhibition index values for the aqueous solution of genistein/soy total extract incorporated in hydroxypropyl-γ-cyclodextrin are present in Figure 1.

Concentrations were chosen according to data presented in literature regarding other total vegetal extracts/pure active phytochemicals [6]. One can
observe an inhibition index over 50% in case of solutions of concentration between 8–33% in both samples suggesting a possible antiproliferative action at a superior level.

On macroscopic level, we can observe that after inducing the two-stage skin carcinoma there is no specific target organ affected, but rather a general alteration of skin with macroscopic appearance of the tumor (Figures 2–5). It can be noticed the difference in the incidence and diameter of tumors between Group A – blank group (Figure 2), Group D – untreated mice (Figure 5), Group B – mice who received total soy extract (Figure 3), Group C – mice who received genistein (Figure 4). The highest incidence was observed in Group D – untreated mice. Regarding treated mice, we can observe that mice who received genistein presented a lower incidence of tumors than mice who received total soy extract.

On microscopic examination of histological sections made through the skin of mice in Group A – blank group (Figure 6) it can be observed the normal structure of the skin with the three layers: the epidermis, dermis and hypodermis.

Microscopic study of histological sections made from fragments of skin taken from mice in Group B showed the presence of a junctional melanocytoma characterized by the presence of large nests of melanocytes in the epidermis, near dermo-epidermal junction. Also, in the election place was observed a clear thickening of the epidermis (Figure 7). In terms of cytological aspect, the melanocytoma is pleomorphic, showing epithelioid, spindle or mixed cell type, less pigmented.

Microscopic examination of samples of skin taken from mice in Group C also showed a thickening of the skin in place of choice (Figure 8). But, compared with the histological findings of skin in Group B, the proliferating melanocytes phenomena have been greatly reduced, small nests were seen, formed from three to ten cells, of polygonal or spherical shape, slightly pigmented, located only in the epidermis, without exceeding the basal membrane.
Figure 8 – Histological sections made through the skin of mice in Group C: the attenuated proliferative phenomena at the level of junctional melanocytoma (HE stain, 100×).

Microscopic study of histological sections made from fragments of skin taken from mice in Group D showed, in addition to significant thickening of the epidermis, the presence of proliferative phenomena in the epidermis and also an alterative phenomena in the dermis, more pronounced compared to the histological skin found in Group B (Figure 9). This analysis corresponds to the group of mice who received 10 nmol DMBA as an initiator and 4 mg TPA for the promotion of skin carcinogenesis but not received any treatment.

Figure 9 – Histological sections made through the skin of mice in Group D: proliferative events in the epidermis, alterative phenomena in the dermis (HE stain, 100×).

Discussion

Data from the phytobiological test suggest that solutions of genistein/soy total extract of concentration between 8–33% present an inhibition index over 50%, direct proportional with the concentration of the solutions. Results show that genistein and soy total extract can be taken in a superior screening stage for the antiproliferative action. Lower concentrations are not enough for this kind of action. As presented data show, solutions of concentration between 2% and 4% have an inhibition index under 50%. One can notice that inhibition index values for the two types of extractive solutions are similar with the observation that in case of the solution containing genistein slightly elevated values are noticed. This remark can lead to the idea that isoflavonic structures like genistein and daidzein can be responsible for the antiproliferative action.

On macroscopic level, we can observe that after inducing the two-stage skin carcinoma there is no specific target organ affected but rather a general alteration of skin with macroscopic appearance of the tumor. It can be noticed the difference in the incidence and diameter of tumors between Group A – blank group (Figure 2), Group D – untreated mice (Figure 5), Group B – mice who received total soy extract (Figure 3), Group C – mice who received genistein (Figure 4). The highest incidence was observed in Group D – untreated mice. Regarding treated mice, we can observe that mice who received genistein presented a lower incidence of tumors than mice who received total soy extract. This observation can support the idea that both the total extract and the pure isoflavone genistein present antiproliferative action, but it seems that genistein alone is a more powerful agent than the total soy extract.

From present study could also be noticed that no significant difference in body weight and food consumption were observed between soy extract/genistein-treated mice and blank group, which implies that topical administration of soy extract and genistein had no significant toxicities.

On microscopic examination of histological section made through the skin of mice in Group A – blank group (Figure 6) it can be observed the normal structure of the skin with the three layers: the epidermis, dermis and hypodermis. The epidermis represents a stratified keratinized squamous epithelium, or corneum well-structured. Dermis, the second layer of skin has well shown, the two layers: the papillary layer and reticular layer, glandular structures and epidermal cornified (hairs). Hypodermis, the deep layer of skin is rich in adipocytes and has a well-represented fibrous layer.

Microscopic study of histological sections made from fragments of skin taken from mice in Group B showed the presence of a junctional melanocytoma characterized by the presence of large nests of melanocytes in the epidermis, near dermo-epidermal junction. Also, in the election place was observed a clear thickening of the epidermis (Figure 7). In terms of cytological aspect, the melanocytoma is pleomorphic, showing epithelioid, spindle or mixed cell type, less pigmented (Figure 8). Is important to note that the cell type has no relation to the degree of malignancy. This is the case of mice pretreated with soy extract before the induction of two-stages skin carcinogenesis. The histological analysis shows difference between normal skin (mice Group A) and affected and untreated skin (mice Group D) underlining the positive effect of pretreatment with soy extract. The protective effect of soy extract, due to its chemical composition: lipids, proteins, polyphenols, and isoflavones – daidzin,
Microscopic examination of samples of skin taken from mice in Group C also showed a thickening of the skin in place of choice (Figure 9). But, compared with the histological findings of skin in Group B, the proliferating melanocytes phenomena have been greatly reduced, small nests were seen formed from three to ten cells of polygonal or spherical shape, slightly pigmented, located only in the epidermis, without exceeding the basal membrane. This is the case of mice pretreated with genistein before the induction of two-stages skin carcinogenesis. The histological analysis shows difference between normal skin (mice Group A) and affected and untreated skin (mice Group D) underlining that genistein, the aglycone from genistin, the active isoflavonoid from soybean has a better action than soy total extract regarding the recovery of skin lesions type experimental malignant melanoma. Action of genistein on melanoma was also studied by Chan HY and Leung LK [13] and they explained the similar results obtained as a consequence to the mechanism of that DMBA–DNA inhibition of flavonoids, witch they have studied on MCF-7 viable cells. Wei H et al. [1] concluded that at a dose of 10 mmol, genistein almost completely diminished DMBA-induced bulky DNA adducts, which suggests a possible anti-initiation action of genistein, and also inhibited edema induction by TPA.

Microscopic study of histological sections made from fragments of skin taken from mice in Group D, showed, in addition to significant thickening of the epidermis, the presence of proliferative phenomena in the epidermis and also an alterative phenomena in the dermis, more pronounced compared to the histological skin found in Group B. This analyze correspond to the group of mice who received 10 nmol DMBA as an initiator and 4 mg TPA for the promotion of skin carcinogenesis but not received any treatment. It is important to underline that although DMBA is a carcinogen, this “initiating” single-dose does not cause skin tumors in the mice. Tumor development requires subsequent repetitive treatment with a tumor promoter. One week after carcinogen exposure, the mice receive twice-weekly treatments with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate, TPA [14]. Tumors aroused in 19 weeks.

Conclusions

Phytobiological test indicated genistein and soy total extract as possible anti proliferative agents. Results on animal model show that both soy total extract and genistein inhibited the initiation and promotion of chemically-induced skin tumorigenesis, but genistein had a greater success in recovering skin lesions type experimental malignant melanoma.

References


Corresponding author
Corina Tiulea, Department of Pharmacognosy, Faculty of Pharmacy, “Victor Babeș” University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041 Timișoara, Romania; Phone +40744–648 855, e-mail: corina_tiulea@yahoo.com

Received: February 14th, 2011
Accepted: November 2nd, 2011
