Beneficial Effects of a Lupeol-Cyclodextrin Complex in a Murine Model of Photochemical Skin Carcinoma

DALIANA MINDA1*, IOANA ZINUCA PAVEL1,2#, FLORIN BORCAN3, DORINA CORICOVAC1, IULIA PINZARU1, FLORINA ANDRICA1, CLAUDIU MORGOVAN3*, LUCIAN DUMITRU NITA1, CODRUTA SOICA1, DANINA MUNTEAN1, CLAUDIA CRINA TOMA1
1*Victor Babes* University of Medicine and Pharmacy Timisoara, Faculty of Medicine, Department of Pathophysiology, 2 Eftimie Murgu Sq., 300041 Timisoara, Romania
2 #  Authors with equal contribution to this paper.

Lupeol is a natural compound with lupan skeleton found in several fruits and medicinal plants comprehensively investigated for its anti-inflammatory and antitumoral properties. In this study we showed that lupeol, formulated as a complex with cyclodextrin and topically administered in a mouse model of photochemically-induced skin carcinoma, was associated with an important reduction of the tumor mass and inflammatory reaction, respectively. The lupeol compound also significantly improved the physiological parameters (transepidermal waterloss, erythema, skin hydration, sebum) of the skin measured by means of non-invasive method.

Keywords: murine photochemical cancer, lupeol, cyclodextrin, transepidermal waterloss

Lupeol (lup-20(29)-an-3β-ol) is a compound with lupan skeleton that belongs to the pentacyclic triterpenes class and presents important pharmacological properties including anti-inflammatory and antitumor activity (fig. 1). The efficiency of its anti-inflammatory activity was unequivocally demonstrated in different pathologies such as chronic inflammation and arthritis. The potency of the anti-inflammatory effect of lupeol is comparable with that of a 100 mg/kg acetylsalicylic acid [1-3]. There were described some similar properties with acetylsalicylic acid, but no ulcerous damages were detected. Regarding its toxicity, it can be considered very low, a characteristic of the majority of pentacyclic triterpenes. It was shown that after the administration of a total dose of 2g/kg body weight to rats no damaging or lethal effects were recorded. As mechanism of action of lupeol it was stated that this compound induces the release of chemotactic pro-inflammatory factors [3, 4]. Current reports mention the fact that lupeol is a chemoprotective agent which can suppress skin toxicity induced by the benzoyl peroxide administration [1, 3, 5, 6]. This active agent reduces promotion of skin cancer induced by benzoyl peroxide by enhancing the activity of antioxidant enzymes with the subsequent inhibition of the benzoyl peroxide-related oxidative stress related [6]. The aforementioned data offer new perspectives regarding the use of lupeol as therapy for diseases associated with oxidative stress.

For this study, we propose a chemically-induced skin carcinoma animal model accelerated by ultraviolet type B (UVB) radiation because is a relative fast and easy reproducible model that involves both carcinogenic processes and inflammation. UVB is considered to play a significant role in the promotion of skin carcinoma and development of inflammation [7]. Previous studies proposed lupeol as a therapeutic solution in a two-stage model of chemical carcinogenesis [8]. Lupeol significantly blocked the activity of ornithine decarboxylase, an enzyme which is an important biomarker in tumor promotion [9].

The aim of the present study was to assess non-invasively the effects of topical application of lupeol formulated as a complex with hydroxy-propil-gamma cyclodextrin 1:1 ratio in a murine model of photochemical skin carcinoma animal model.

Experimental part

Materials and methods
Lupeol was purchased from Sigma Aldrich (Germany) and hydroxy-propil-gamma cyclodextrin (HPGCD) from Cyclolab (Hungary). All the other reagents used in the study were obtained from Chimopar Bucharest (Romania) and were of analytical purity.

Complex preparation
Preparation of complexes was described in our previous work [10, 11]. In brief, lupeol and HPGCD mixture 1:1 was kneaded with 50% ethanol solution. The final product obtained by kneading procedure and evaporation of the solvent was a solid compound capable of increased dissolution in water phases.

* email: claudiu.morgovan@yahoo.com

Fig. 1. Chemical structure of lupeol
Animals

In this study were used SKH1 male mice that were purchased from Sulzfeld, Germany. The mice were divided in 3 groups (n=6 mice/group): group 1 – control group (no application of the carcinogens or UVB, but only the cyclodextrin saline solution), group 2 – mice exposed to UVB 2 times/week 5 min after the chemical carcinogen application and treated with cyclodextrin saline solution 30 min before UVB exposure and group 3 - similar interventions with the ones described for group 2, but the mice were topically treated 30 min before exposure with a 5% saline solution of lupeol complex. The experiment was developed for 6 weeks. Animals were fed ad libitum and kept under standard conditions (constant temperature and humidity of 22.5 ± 2° C and 55 ± 5%, 12-h light/dark cycle). All experimental procedures were approved by UMFVBT Bioethical Committee.

Skin carcinoma animal model

A solution of 7,12-dimethylbenz(a)anthracene (DMBA) (390 nmol/0.1 mL acetone) was topically applied on the back of the mice (a single application in the first week of experiment) before irradiation. The mice were exposed to UVB radiation using Vilber VL-6.M/6W lamp according to the following protocol: 2 times/week for 5 min reaching a total dose of around 200 J/m². Lupeol formulated as a complex with cyclodextrin was applied starting from the first week of experiment for 6 weeks. The maximum volume used per application was 0.2 mL of solution on exposed area, 30 min before carcinogens exposure [4].

Erythema measurement (hemoglobin)

The hemoglobin measurements were performed using a research device from Courage Khazaka endowed with a Mexameter MX18 probe (Courage&Khazaka Electronics, Cologne, Germany). The maximum units for Mexameter are 999 (interval 0-999) and the measurement is based on the absorption/reflexion. The time of measuring was continuous, for 20s. The protocol indicated melanin evolution and haemoglobin status (pigmentation and erythema). The device was applied on affected/analyzed areas. The data were registered by the specific soft of the Mexameter MX18 device and then expressed as units. All data were processed as initial and final measurements values on the same area.

Physiological changes of skin

In order to verify the physiological changes of skin after exposure to toxic agents and to lupeol treatment, we used specific devices that describe the water and lipid content, pH, such as TEWL-meter, pH meter, Sebumeter and Corneometer – multiprobe adapter from Courage Khazaka, Germany. The measurements of these parameters respected the same protocol as the one used for the determination of melanin and erythema.

Statistics

All data were analyzed using paired Student’s t tests or One-way Anova followed by Bonferroni’s post-tests in order to establish the statistical difference between experimental and control groups; *, ** and *** indicate p < 0.05, p < 0.01 and p < 0.001. A 0.05 level of probability was taken as level of significance.

Results and discussions

Macroscopic evaluation of lupeol treatment effects

According to the macroscopic images taken during the first weeks of experiment it can be observed that in the control group (group 1) no damage was detected after the application of the cyclodextrin solution. The noxious effects
of the two agents, DMBA and UVB radiation, were well defined in group 2 and the cyclodextrin saline solution had no therapeutic effect. As compared to the lesions observed in group 2, the skin of group 3 (mice treated with lupeol) presented a similar aspect to the one observed in the control group, characterized by a small degree of injury. Control group (group 1) was used in this study in order to verify if cyclodextrin alone induced toxicity at skin level after topical application.

At the end of the experiment (after 6 weeks) the mice from group 2 and 3 developed tumors. The treated group (group 3) showed a significant decrease in tumors volume and number as compared to group 2 (mice treated with cyclodextrin, fig. 2).

The tumor size (mm) was measured with a caliper, and the tumor growth expressed as tumor volume was estimated using the formula: \[\text{length} \times \text{width}^2 / 2\] [12]. Group 3 treated with lupeol complex presents lower values of tumor growth (around 180 mm\(^3\) after 6 weeks) whereas for the mice from group 2, the tumor volume was around 330 mm\(^3\) at the end of the experiment.

**Non-invasive measurements**

In order to assert our results resulted from the macroscopic evaluation, there were measured different skin physiological parameters, such as: melanin, erythema, transepidermal waterloss (TEWL), skin pH, skin hydration and sebum content. The measurements started in the first week of experiment and were realized for all period of 6 weeks.

**Transepidermal waterloss (TEWL) measurements**

TEWL initial values were measured for each mouse in the first day of experiment and there were between 1.5-1.8 g/cm\(^2\)/h. There can be observed that the mice treated with the cyclodextrin solution (group 2) presented increased TEWL values to a maximum difference of 1.5 units after six weeks, while in the group of mice treated with lupeol complex (group 3) the difference between the final and initial values was kept at around 1.0 unit at the end of the experiment (fig. 3).

**Skin pH measurement**

The initial values of skin pH were measured for each mouse in the first day of the experiment and there were between 6.25-6.60 units. The pH of the mouse skin does not change too much in this experiment; there were obtained modification around 0.50 units for Group 2 and 3 while the control group presents variations around 0.10 units (fig. 4).

**Sebum evaluation**

Another physiological skin parameter evaluated in this study was the sebum content. At the beginning of the experiment the values of sebum content were between 7-13 units. During the experiment it was observed a significant decline of sebum content in group 2 as compared to control group (group 1) and the group treated with lupeol (fig. 5).

**Melanin measurement**

SKH-1 mice generally present low values of melanin (between 93-115 arbitrary units). Our results indicated that exposure to UVB radiation led to an increase of melanin values in both groups of mice exposed (groups 2 and 3) what can be observed as a mild skin pigmentation (around 20 units / 6 weeks), the increase being more marked in group 2 that was treated only with cyclodextrin solution.

![Fig. 4. Skin pH measurement: Group 1 - control group (only cyclodextrin solution), Group 2 – mice exposed to UVB after the chemical carcinogen (DMBA) application and treated with cyclodextrin saline solution 30 minutes before irradiation, and Group 3 (treated group) same protocol as for group 2, but treated with lupeol complex (data are expressed as differences ± SE).](image)

![Fig. 5. Sebum evaluation: Group 1 - control group (only cyclodextrin solution), Group 2 – mice exposed to UVB after the chemical carcinogen (DMBA) application and treated with cyclodextrin saline solution 30 minutes before irradiation, and Group 3 (treated group) same protocol as for group 2, but treated with lupeol complex (data are expressed as differences ± SE).](image)
(fig. 6). It is important to mention that the change of melanin content was not important for this mice strain (the scale of Courage-Khazaka Mexameter probe is between 0-999 arbitrary units).

**Erythema evaluation**

The absolute values of erythema measured in the first day of experiment were between 29-38 arbitrary units. An important increase of erythema was observed in group 2 (over 30 units / 6 weeks), while in the case of group 3 the increase was much lower due to the applications of lupeol complex (fig. 7).

**Hydration of stratum corneum**

Hydration of stratum corneum was measured for each mouse in the first day of experiment and there were obtained values between 6.7-9.1 arbitrary units. Our results registered significant changes of skin hydration values in the groups of study. A marked decrease of this parameter was observed in the group that was treated only with cyclodextrin in comparison with the control group (group 1 – no exposure to carcinogens) and with the group treated with lupeol (group 3), what indicates a protective effect of lupeol against DMBA and UVB toxic effects. The treated group (group 3) presented also a reduction of the skin hydration as compared to control group (fig. 8).

It is known that both DMBA and UVB contribute to skin carcinoma and are considered useful tools in the development of experimental models of this type of cancer [4, 5]. The macroscopic images taken in the first weeks of experiment showed that the mice treated with lupeol complex (group 3) presented a reduced degree of injury and inflammation at skin level as compared to the mice from group 2 that were treated only with the solution of cyclodextrin, what indicates the effectiveness of lupeol as an anti-inflammatory agent. Similar results were obtained by Saleem et al., when used lupeol as treatment in a mouse model of skin carcinoma induced by benzoyl peroxide [6].
The results obtained by non-invasive method completed these observations. Lupeol induced an improvement of all skin physiological parameters (TEWL, sebum, melanin, erythema, skin hydration) evaluated in the present study as compared to the non-treated group (group 2).

The data obtained for the control group indicated that the cyclodextrin solution had no toxic effects at skin level and did not influence the skin parameters. Melanin is not well define as parameter because were used hairless white mice (SKH1). Lupeol exhibits an important high wound healing potential in a dead space wound healing mice (SKH1). Lupeol prevents DMBA-induced DNA and skin damages at low healing potential in a dead space wound healing mice (SKH1). Lupeol exhibits an important high wound healing potential in a dead space wound healing mice (SKH1). Lupeol protects DNA and skin damages at low doses (200 μg/mouse). According to our results lupeol developed an important chemopreventive activity on skin level. Our observations showed that lupeol treatment was effective after topical application in a mouse model of skin carcinoma chemically-induced (by DMBA) and accelerated with UVB irradiation. It is known that UVB exposure changes skin quality and induces ROS species generation [7, 13]. Because of its antioxidant potential lupeol participates positively in skin quality assurance [14]. An important observation was that lupeol improved the quality of skin by reducing transepidermal waterloss (TEWL). This aspect is related to the important anti-inflammatory activity via selective inhibition of cyclooxygenase [6, 14, 15]. In addition, we showed that is an agent that maintains the balance between the hydrophilic/lipophylic content. pH is maintained under the same proper conditions (not important variance) during all experimental period.

Conclusions
Lupeol is an active agent at skin level with a relevant anti-inflammatory and chemopreventive activity. When topically applied as prophylactic agent, lupeol reduced noxious processes like irritation, inflammation and skin degradation. Lupeol also acted as a protective compound against UVB skin exposure. The observations strongly recommend topical administration of lupeol as a protective and regenerative strategy in pathologic conditions associated with skin inflammation and damage.

Acknowledgement: The research was funded by POSDRU grant no. 159/1.5/S/136893 grant with title: “Partenerial strategic pentru cresterea calitati cercetarii stiintifice din universitatile medicale prin acordarea de burse doctorale si postdoctorale - DocMed.Net 2.0”

References

Manuscript received: 17.02.2014