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RESEARCH ARTICLE

PREPARATION OF A LOCAL ANTI-WART AND ANTI-INFLAMMATORY RUB OF HYDROXY PROPYL METHYL CELLULOSE AND POLYGLYCIDOL BASED SATURATED LIPID, DI-DODECYL OXYPROPANE-2-POLYGLYCIDOL CONTAINING CURCUMIN AS DERMAL MEDICAMENT

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ABSTRACT

Background: Curcumin is a non-toxic neutracical, which possesses/exhibits an antioxidant activity and inhibits intracellular factors such as Nf-κB, cyclooxygenase-2 (COX-2), lipooxygenase (LOX), and inducible nitric oxide synthase (iNOS). However, it is poorly absorbed and has a limited bioavailability. If curcumin could be delivered directly into the cell, it might be a potential therapeutic agent.
Objective: The aim is to prepare a medicament to deliver curcumin directly through the cutaneous membrane and to find out the potentiality as a topical therapeutic.
Method: We synthesized a polyglycidol-derived lipid, DDP-PG (M_w = 2650), which was incorporated into hydroxypropyl methyl cellulose, HPMC (7%), to prepare a rub containing curcumin. Another formulation containing monoolein rac glycerol, MO (a reported skin penetration enhancer) with DDP-PG was incorporated as a control. Subsequently, curcumin-containing DDP-PG was evaluated directly for therapeutic purposes treating local apoptotic skin, inflammatory site and benign tumor mass (wart).
Results: The diffusion of curcumin was evaluated *in vitro* using a biomembrane (fish swim bladder). The diffusion of curcumin form DDP-PG based formulations did not differ from that of MO, which indicated their suitability as a transdermal vehicle. Accordingly, after application in a few dermal disorders, it showed skin cured very promisingly from apoptotic keratinocytes, clearing the inflammation perfectly and dramatically reduced the size of the wart (a benign tumor) as well.
Conclusion: Curcumin can be delivered directly with a *du novo* skin penetration enhancer, DDP-PG in HPMC based rub. A "Bench to Bed Technology" was approached for controlling local inflammation, apoptotic keratinocytes, and benign tumor like wart, studied here.

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INTRODUCTION

Curcumin, a component of turmeric *Curcuma Longa*, a solid gold of India and its' subcontinents has been shown to be non-toxic nutraceutical, to have antioxidant activity, and to inhibit such mediators of inflammation as NFκB, cyclooxygenase-2 (COX-2), lipooxygenase (LOX), and inducible nitric oxide synthase (iNOS) [i, ii, iii, iv]. Significant preventive and/or curative effects have been observed in experimental animal models of a number of diseases, including arteriosclerosis, cancer, diabetes, respiratory, hepatic, pancreatic, intestinal and gastric diseases, neurodegenerative and eye diseases[4].

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However, it is very poorly absorbed while taken orally and its bioavailability, is so limited that to date it is not the choice of drug given orally for an acceptable therapeutic range. In such a case a transdermal formulation is suitable especially when skin is targeted. Hence, if curcumin can be delivered directly inside the cell, it could control genetic irregularity and might be potential tool as therapeutic. In order to achieve this goal, here we are looking for a topical dosage form to deliver curcumin inside cell directly as a transdermal therapeutic agent. A transdermal delivery is superior to that of oral and other routes, especially when a particular part of skin is targeted[v,vi,vii]. To fulfil a fruitful transdermal dosage form or medicament, a skin penetration enhancer is essential. Many kinds of skin penetration enhancers such as sulfoxides (dimethyl sulfoxide, DMSO), Azones (eg. Laurocapram), Pyrrolidones(2-

pyrollidone), alcohols and alkanols (ethanol, or decanol), glycols, surfactants and tarpenes have been reported.[8]Glycerol monooleate (Peceol) and polyglyceryl-3 dioleate (PlurolOleiqueCC) are two suitable skin-compatible oleins used in pharmaceutical formulations as penetration enhancers[viii]. However, still there are options to introduce more exciting enhancers to be used in transdermal drug delivery system TDDS formulations. Hyperbranchedpolyglycidol is a water-soluble polyether with numerous terminal hydroxyl groups, which has structural similarities with poly(ethylene glycol), PEG[ix]. Whereas PEG is suffering with the PEG-dilemma[x] problem which limits its use in biological application, polyglycidol and its derivatives with lipid moiety have attracted research interest for its safety, biocompatability, non-immunogenicity and long circulation properties[xi, xii, xiii, xiv].

Hyperbranchedpolygylcidol self-assembled monolayers are as protein resistant as PEG self-assembled monolayers, and are also thermally and oxidatively more stable, suggesting that hyperbranchedpolyglycidol is a promising biomaterial[xv].Here, we introduce a novel non-phospholipid conjugate that is composed of a linear polyglycidol chain linked to a strongly hydrophobic lipid-mimetic residue, DDP-PG (Figure 1) as a skin penetration enhancer, which is incorporated with hydroxypropyl methyl cellulose (HPMC) to prepare a dosage form rub and chased against a few skin pathological conditions like apoptotic keratinocytes originated from psoriasis, topical inflammation and wart type benign tumors on skin.

MATERIALS AND METHODS

Materials

All solvents (methanol, dichloromethane) as well as ethyl vinyl ether (99%, Aldrich) were purified by distillation. Glycidol (96%, Aldrich) was distilled under reduced pressure. Ethyl vinyl ether (99%, Aldrich), AlCl3.6H2O (99%, Aldrich) were used as received. Ethoxyethylglycidyl ether (EEGE) was obtained by a reaction of glycidol and ethyl vinyl ether as described elsewhere[xvi]. Fractions of EEGE of purity exceeding 98.5%, determined by gas chromatography, were used for polymerizations.

AlCl3. 6H2O (99%, Aldrich), KOH (Merck), 1-dodecanol (Aldrich), 1,6-diphenyl-1,3,5-hexatriene (Aldrich), 5(6)-carboxyfuorescein (Aldrich), and dodecyl/tetradecylglycidyl ether (Aldrich) were used without purification. HPMC and MO were purchased from the Sigma-Aldrich co. Fish swim-bladder was separated freshly from the local market and instantly used in the experiment. Human volunteer model of skin disorders were selected, informed consent was obtained and they were treated in accordance with the ethical institutional regulations of the Medical University of Sofia (Committee of ethic in scientific research to the Medical University –Sofia CESRMUS).

Methodology

Synthesis of DDP-PG

DDP-PG was synthesized according to a procedure described elsewhere[xvii] with some modifications. First the starting materials dodecyl alcohol (1-dodecanol) and epibromohydrin reaction to synthesize dodecyl glycidyl ether in presence of NaH with continuous argon gas and at moderate temperature of 50 °C, a kind of anionic polymerization, we also reported previously for various kind DDP-PG[xviii] (Fig. 1). In the second step we synthesized didodecyloxypropane 2-ol by reacting dodecyl glycidyl ether and dodecyl alcohol in presence of $SnCl_4$ and argon gas with high temperature around 120 °C. In third stage, the product was vacuum hydrolyzed with KOH in presence of benzene to produce respective DDP-KO. In fourth stage, one more ring opening anionic polymerization of DDP-KO and protected glycidol (ethoxy ethyl glycidyl ether, EEGE) to produce DDP-EEGE in the presence of argon gas only. After completion of polymerization reaction the protected DDP-EEGE was confirmed by NMR. And finally, a deprotection reaction was performed in presence of $AlCl_3.6H_2O$ in methanol to produce expected DDP-PG (MW around 2700). The reaction mixture product was filtered by Hylfo super gel and the solvent was evaporated under reduced pressure.

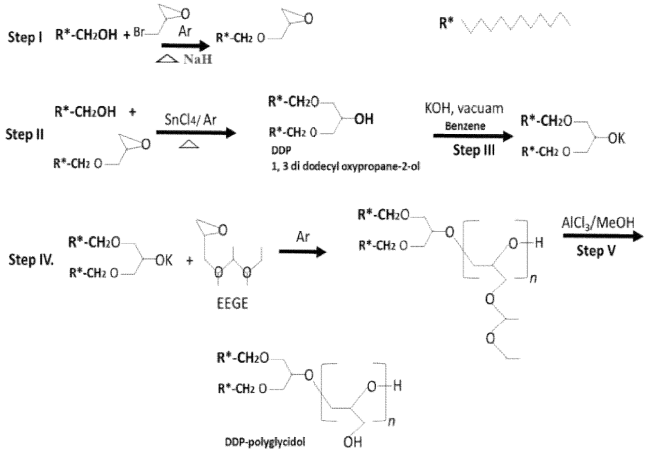

Fig 1. Schematic diagram of the synthesis of DDP-PG DDP-PG was synthesized from the precursor di-dodecanols and with protective glycidol, ethoxy ethyl glycidyl ether (EEGE) followed by a terminal hydrolysis of methanolic $AlCl_3$. Chemical structure of the polyglycidol-derivatized lipid, DDP-PG, used in this study. Molecular weight, Mw = 2650, degree of polymerization of the polyglycidol chain, n = 30.

Preparation of HPMC/DDP-PG based rub and curcumin loading

All the materials for a rub preparation were followed as like solvent like solute rule under optimum temperature. In step I, 30 of mg DDP-PG (6%) was dissolved in 200µl of water and curcumin (100 µg) was dissolved in 100µl ethanol. The two solutions were mixed to prepare an emulsion. In step II, HPMC 35 mg (7%) was dissolved in water and in step III, monoolein (MO) 30mg (6%) was dissolved in ethanol (Table 1).

Thereafter, the emulsions from step I and step II were mixed at step IV, and then mixture was mixed with step III at step V. The final mixture was subjected to at least 3-5 freezing and thawing cycles (freeze at -30°C for 15h and then thawing at RT for 15 min). At least 3-5 cycles were performed to make the rub (Table 1 and Fig. 2).

Table 1. Formulations of the TDS-patch gels containing curcumin

FM-C	FM-1	FM-2	FM-3	FM-4
HPMC 7% Control patch	HPMC 7% CR 100µg	HPMC 7% CR 100µg DDP-PG 6%	HPMC 7% CR 100µg DDP-PG 6% MO 6%	HPMC 7% CR 100µg DDP-PG 12%

permeation was performed under in phosphate buffered saline at pH 6.8 and a temperature of 37 °C. A rotator of 20 mm in length and 10 mm in diameter was used with a rotation speed of 500 rpm. Rub-loaded bladder was set in the extraction beaker to a way of up-side down of the bladder under sink condition (Fig. 3).

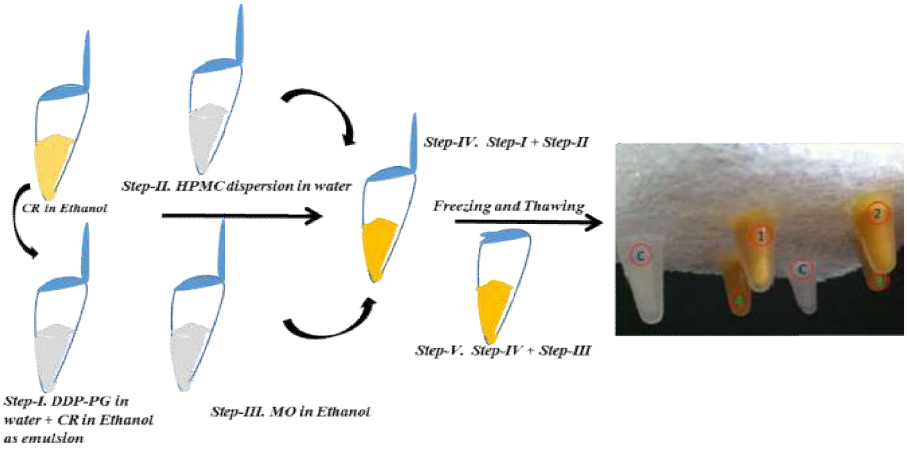

Fig 2. Preparation of pharmaceutical rub of HPMC/DDP-PG and HPMC/DDP-PG/MO, containing curcumin

Four formulations were prepared: (1) FM-C control of only HPMC 7% (w/v); (2) DDP-PG 6% (w/v) + HPMC 7% (w/v) with CR 100µg; (3) DDP-PG 6% (w/v) + HPMC 7% (w/v) + MO (6%) with CR 100µg; (4) DDP-PG 12% (w/v) + HPMC 7% (w/v) with CR 100µg.

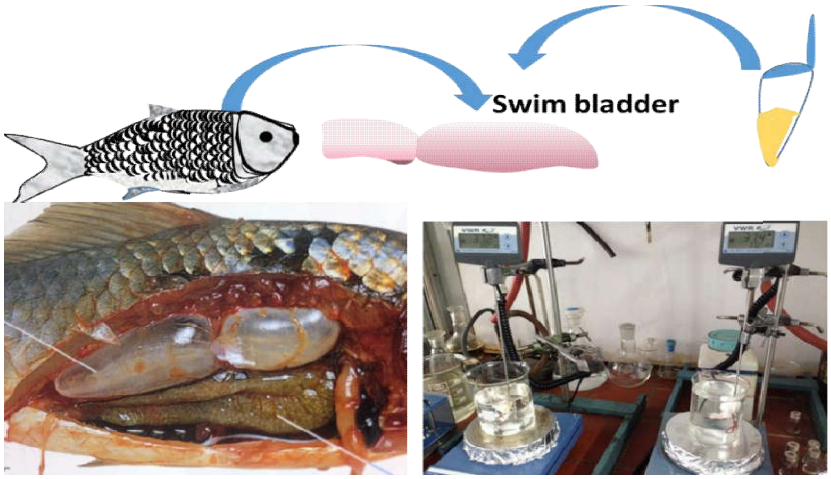

Fig 3. **Swim-bladder dissection and curcumin extraction studies**

A carp fish was freshly dissected carefully and both upper and bottom parts of the bladder were used for the permeation kinetic studies of curcumin from the formulation of DDP-PG 6% (w/v) + HPMC 7% (w/v) + CR. A modified beaker method was followed for kinetic analysis.

Dissection of a fish swim bladder, rub loading and curcumin extraction studies

In vitro transdermal penetration studies were performed taking a bio-membrane, a fish swim bladder, which has got many tight junctions resemblesto stratum corneum of the skin[xix, xx]. A swim bladder was dissected carefully from a carp fish and kept in ice box. Rub containing curcumin was loaded in two parts of swim bladder, one was DDP-PG rub and another one was DDP-PG/MO. Thereafter the loaded bladder was closed tightly and set with a stand in a beaker containing PBS, following a modified beaker method drug extraction studies. Curcumin

Thereafter at predetermined time intervals 5 ml sample was withdrawn from theacceptor phase and examined for the concentration of curcumin at 450 nm by a UV-visible spectrophotometer, Beckman Coulter DU 800. A standardcurve was prepared using various concentration of curcumin in ethanol at the same wavelength and used to measure the curcumin concentration for unknown sample, withdrawn from the extraction sample.

Application of Rub (HPMC/DDP-PG), containing curcumin in apoptotic skin (apoptotic keratinocytes) originated from atopic dermatitis/Dyshidrotic eczema/myxoid cyst, topical inflammation and in benign tumor like wart.

One case history of a volunteer ambulatory patient of apoptotic skin was found out to apply the rub topically. This is stated in the materials section. The volunteer subject was advised neither to use any topical drugs for inflammation nor to take any oral medication of local apoptotic skin. The subject was advised to apply rub 2-3 times *qs* to the affected area at least for one week. After one week, the diameter of lesion was measured.

Another case history of local inflammatory skin was found out and the subject was advised as previously not to take any medication. The patient was advised to apply the rub in the same way to the site of inflammation. After one week the site was monitored. An ambulatory human volunteer with wart was advised not to take any anti-wart medications at least one or two day before the application of the rub. The rub was applied 2-3 times daily and the subject was monitored for a week. After one week, the size of the wart was measured. Another ambulatory subject with wart on hand rather than leg was advised to apply the rub. And the size of the wart evaluated in the same way. All the volunteers were treated in accordance with the ethical institutional regulations of the Medical University of Sofia (Committee of ethic in scientific researches to the Medical University –Sofia)

RESULTS

Here we synthesized a polymer DDP-PG reported elsewhere [18, 19] with some modification and a topical pharmaceutical dosage form, rub, was prepared based on HPMC/DDP-PG loaded with curcumin (Fig. 1 and Fig. 2). Kinetic profiles of curcumin were depicted from Figure 4 and the calulated kinetic parameters were described in the Table 2. The diffusion exponent, n, and kinetic constant, *k*, were determined according to the equation $M_t/M_\infty = kt^{n}$ [xxi, xxii], where M_t/M_∞ is the fraction of extracted curcumin from the patch gel through the swim-bladder (Fig. 3 and Fig. 4, Table 2).

Table 2. Kinetic parameters of transdermal permeationsimulated with a bio-membrane (a carp fish swim-bladder)

TDS-patch formulation	Fraction permeation	Kinetic constant	Diffusion exponent
HPMC-DDP-PG	71%	0.27	0.21
HPMC-DDP-PG/MO	52%	0.23	0.17

As shown in Figure 4, after one and half hour of incubation, there was around 70% curcumin permeation in the case of HPMC/DDP-PG rub and approximately 50% in the case of HPMC/DDP-PG/MO preparation.

Both, the kinetic constant and diffusion exponents are higher for the HPMC/DDP-PG rub (Table 2). MO was reported as skin permeation enhancer. Accordingly, we compared the kinetic profiles of DDP-PG with the MO and thereby designed this rub for direct therapeutic application.After an application of one week in apoptotic skin, the watery exudation has been dramatically cleared **(**Fig. 5a and 5b).

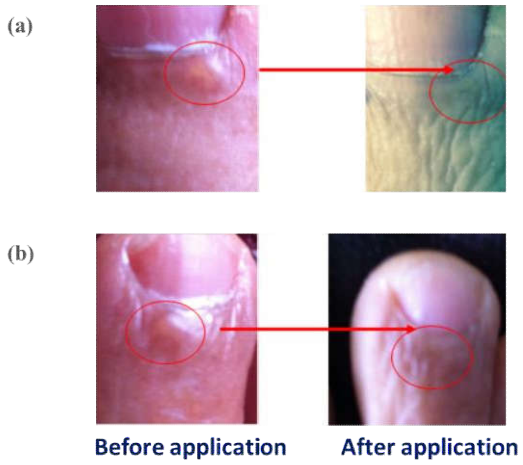

Figure 5. Rub application to the apoptotic skin (apoptotic keratinocytes) on leg fingers

a) A mixoid cyst type watery exudation (apoptotic keratinocytes) on great toe, b) apoptosis on long toe. The lesions disappeared after one week application of the rub.

In another volunteer subject of topical inflammatory skin, the application of the HPMC/DDP-PG rub results in complete skin healing after 5 days (Fig. 6). In Fig. 7, two more volunteer subjects having benign tumors like wart, one in leg and the other in point finger, are shown. In subject I, after an application of one week 2-3 times a day, the size of the wart was dramatically reduced to one third of total size. The same application was performed in the subject II, having the wart in point finger, for a period of two weeks 2-3 times a day.

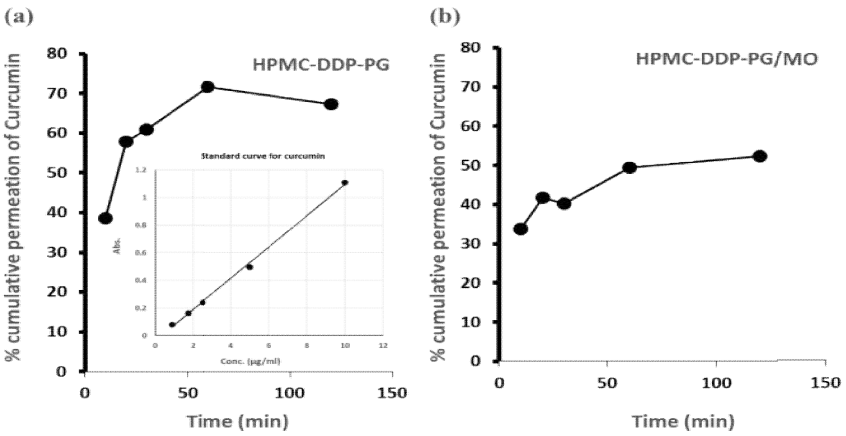

Fig 4. Kinetic profiles of curcumin permeation through the swim bladder

% cumulative permeation vs. time was plotted to analyze the kinetics. A standard curve was also prepared to determine the concentration of curcumin in the withdrawn samples. The sample represents as (a) for FM-2: DDP-PG 6% (w/v) + HPMC 7% (w/v) + CR, and (b) for FM-3: DDP-PG 6% (w/v) + HPMC 7% (w/v) + MO (6%) + CR. Mean of the three independent experiments were plotted against the time.

Noteworthy, the wart was reduced after one week and disappeared at all after two weeks of the application, indicating the HPMC/DDP-PG rub containing curcumin as a potential medicament against the wart (Table 3).

Table 3. Therapeutic application of rub (HPMC/DDP-PG) containing curcumin

Type of skin disorder	Before application	After application
Apoptotic keratinocytes	+++	-
Local inflammation	+++	-
Benign tumor (Wart) Subject-I	+++	+
Benign tumor (Wart) Subject-II	+++	-

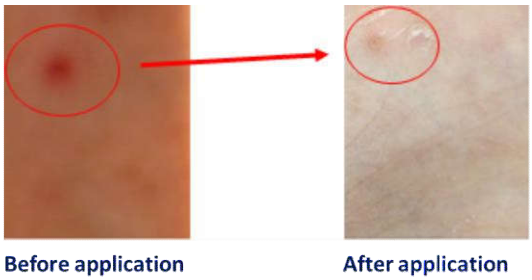

Figure 6. Rub application to the local inflammatory skin Rub was applied to the locally inflammatory skin near footpad. After one week application, the inflammatory skin changed to normal skin.

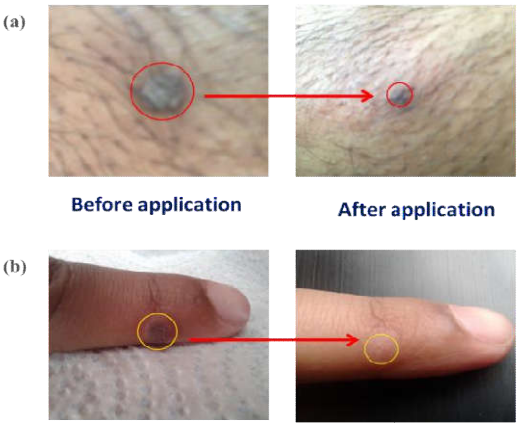

Figure 7. Rub application to the senile wart, kind of benign tumor mass

a) Wart on lower part of leg of one individual applied the rub for one week and interestingly, the size reduced to almost one third of the original size, b) Wart on point finger of another volunteer individual, just disappeared after two week of application.

DISCUSSION

Curcumin delivery through liposomal cargo *in vivo* as well as oral delivery have been reported to date. However, transdermal effective delivery of curcumin is still an area to be reported, especially in topical dermal disorders. Here, a topical rub containing curcumin, introducing a newly synthesized polyglycidol-derivatized lipid, DDP-PG, as a skin penetration enhancer is reported and it is compared to glycerol monooleate, MO – a known skin penetration enhancer. The kinetic constants and diffusion exponents, which were extracted from the kinetic profiles, can be parameters of chemical engineering to compare MO and DDP-PG (Table 2).

Kinetic constant depends on the macromolecular network system of the swim-bladder and also the patch-gel composition [25, 26] and it was comparable between DDP-PG patch and MO patch. The diffusion exponent, which is dependent on the mechanism of the membrane permeation, is slightly larger for the DDP-PG based patch gel than that of the MO based patch-gel. Neither of the patch systems are Fickian but an irregular Fickian diffusion. It can be a logical similarity with that of MO based patch gel system and could be used in skin preparation. Apoptotic skin is the apoptotic keratinocytes that may result from atopic dermatitis or Dyshidrotic eczema or myxoid cyst/digital mucous cyst. A major role in pathology of above mentioned diseases is ascribed to Fas-induced apoptosis and moreover to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL).[xxiii, xxiv, xxv] Curcumin has got a potential effect to block the transcription factors like NFκB, TNFα[1, xxvi] and might be a promising pathway to cure local apoptotic skin (Fig. 5). The latter is sometimes too difficult to control if not treated in time. Usually,steroids are given to control this condition but the major drawbacks of the therapy are the immune suppression and aggesive return of symptoms. Recent data have expanded the concept that inflammation is a critical component of tumor progression. Many cancers arise from sites of infection, chronic irritation and inflammation. It is now becoming clear that the tumor microenvironment is largely orchestrated by inflammatory cells[xxvii]. Curcumin suppresses the proinflammatory transcription factors nuclear factor-kappa B (NfκB) and IL-6[xxviii]. There was no more sign of inflammation after the application of rub containing curcumin, where DDP-PG functioned as a membrane permeation enhancer for stratum corneum (Fig. 6, Table 3).

Local wart (Benign tumor) is also another dermal disordered extrusion, called also *Seborrhoeic keratosis*, Senile wart (http://medical.cdn.patient.co.uk/pdf/4327.pdf). Without treatment, they usually continue to grow and can become darker and more crusty[xxix]. Usually liquid nitrogen and surgery are applied to control these[xxx]. However, these are bit inconvenience in many cases. In addition, if it appears in face, it would be unpleasant and looks odd and should be treated cosmetically. In Fig. 7, after application of one week, the wart mass was reduced more than two third of the total mass.

In another subject the wart almost disappeared after the application of 2 weeks of the rub. It has been reported that *in vitro* treatment of the human Ben-Men-1 meningioma cell line and of a series of 21 primary human meningioma cell cultures with curcumin (1-20 μM) strongly reduced the proliferation in all cases in a dose dependent manner[xxxi]. Moreover, Fluorescence-activated cell sorting (FACS) analysis demonstrated curcumin-induced cell cycle arrest at G2/M and had been shown to act anti-tumorigenic in different types of tumors[xxxii]. Here consistently curcumin loaded rub blocked the further growth of tumor mass though it is benign, and terminally in one case removed totally, depending on the duration of usage (Fig. 7b, Table 3). However, further intensive investigation would be carried out under a clinical trial.

Conclusion

Here, we have introduced a top-down approach like a 'bench to bed technology' to manage local apoptotic and inflammatory skin as well as benign tumor mass (senile wart). We introduced a *du novo* skin permeation enhancer DDP-PG, so that curcumin like nutraceutical can go inside directly into the cutaneous layer. In such a way we can control dermatological disorders in the days ahead.

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