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Pressure Sensitive Adhesive for 6-Mercaptopurine Transdermal Delivery for Solid Tumor in Mice

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The aim of the present study was to develop and characterize the physiochemical properties of transdermal patches of 6-Mercaptopturine (6-MP) and evaluate the cytotoxicity, antitumor activity in Balb/c mice using Dalton's lymphoma ascites (DLA) as model cell lines, skin irritation and sensitization in mice and human subjects. 6-MP was loaded into the patches according to pharmacokinetics properties of 6-MP. Cytotoxicity was measured by exposing cell suspension to an increased concentration of drug from 50-1000 ng/ml and the viable cell count was measured by the tryphan blue exclusion method. Results confirmed that 1000 ng/ml of 6-MP was cytotoxic, and there was an increase in the life span (%ILS) by 71.0% with maximum survival time of 36 $\pm$ 1days for 6-MP transdermal patches, results were statistically significant ($p < 0.05$) compared to untreated control, and anti-tumor activity was very effective compared to the oral route. Patches did not show any sign of erythema, vesiculations or bullaous reaction, the mean cumulative skin irritation and adherence scoring for both animals and humans proved no irritation sensitization reaction scores were 0 and less than 1, good adherence was seen with score = 0, with complete adherence to skin, without leaving any adhesive on skin with score = 0 in human subjects. Transdermal patches showed 100% flatness, thickness 150 $\pm$ 0.03 mm, good content uniformity, folding endurance (>500 foldings), tensile strength was 2.1 to 7.2 Newton's, elongation was 3–5%, patches were stylish, smooth, transparent, and flexible in appearance. The thumb tack test showed good adhesion, Velcro® protection jackets were suitable for study and served our purpose for licking, scratched and rubbing of applied patches.

Keywords: 6-Mercaptopuirne; transdermal; Dalton's lymphoma ascites; cytotoxicity

1 Introduction

Pressure sensitive adhesives (PSA) are used extensively in the pharmaceutical industry as an adhesive layer to adhere to drug delivery devices, both as active and passive patches, to the stratum corneum (1). PSA are used as adhesives in these systems due to their desirable properties of good initial and long-term adhesion, clean removability, drug compatibility and highly viscoelastic properties which fulfill the prerequisites for attachment to soft tissue. PSA are important components of transdermal drug delivery systems (TDDS), because they ensure intimate contact between the drug-releasing area of TDDS and skin surface, which is critical for controlled release of the drug (2). Choosing a suitable PSA for a TDDS is not simple because the requirements are more demanding than those for as a simple medical tape. PSA are mainly examined for their potential to produce skin irritation or sensitization, another requirement of PSA is that it must not leave residues when peeled off from either the release liner or the skin (3). Furthermore, the TDDS has produced many successful commercial products over the years, which possess several advantages over more traditional methods. These include avoidance of first-pass intestinal and hepatic metabolism, avoidance of variable rates of absorption and metabolism inherent with oral treatment, continuous, non-invasive infusion of drugs which have short biological half-lives, avoidance of the risks and the inconvenience associated with peritoneal treatment, and elimination of gastrointestinal irritation resulting from pharmaceutically active and inactive ingredients, so these must be easy to remove from the release liner and from the skin, without causing pain that might discourage patients from using these products (4). The above described characteristics are strongly dependent on the mechanical properties of the PSA. The properties of the PSA layer in a TDDS depend on the incorporated drug, the components of the TDDS (e.g. backing film, release liner), the excipients (e.g. penetration enhancers, solubilizers and tackifying agents), and the chemical composition of the PSA. The Single-layer

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Drug-in-Adhesive system, one approach in which there is inclusion of drug directly within the adhesive. The objective of this study is to prepare a Single-layer Drug-in-Adhesive transdermal patch of PSA for 6-MP, evaluate the physicochemical properties of the same, cytotoxicity, anti-tumor activity of 6-MP in Dalton's lymphoma ascites (DLA) tumor induced in mice, and cumulative skin irritation and sensitization and patch adherence studies in humans and animals.

2 Experimental

2.1 Materials

6-Mercaptopurine and d-limonene was purchased from Sigma-Aldrich, Polyethylene glycol, Sodium hydroxide was purchased from Rankem Chemicals, Mumbai, DuroTak® 387-2082 (Poly vinyl acrylate) was received as a gift sample from National Starch and Chemical Company (Netherlands) CoTran (9726-Ethylene Vinyl acetate (EVA) Film), Release liner 3M Scotch (9742 Release Liner-Fluoropolymer Coated Polyester films) samples were received as a gift sample from (3M St. Paul). All the remaining chemicals and reagents were used without further purification. All the human experiments were conducted according to the rules and conditions of the declarations of Helsinki for humans. The Institutional Human Ethical Committee (IHEC), Government of India approved the study and informed consent was obtained from each volunteer.

2.2 Animals

Balb/c mice (6–8 weeks old) of 25–30 gm body weight were used in the study, and the study was conducted in the Amala Cancer Research Center, Amalanagar, Thrissur, Kerala The animals were kept in air-controlled rooms, fed with normal mice feed (Sai Feeds, Bangalore, India) with water *ad libitum*. All the animal experiments were performed according to the rules and conditions of the Institutional Animal Ethical Committee (IAEC), Government of India.

2.3 Cell Lines

Dalton's lymphoma ascites (DLA) cells were obtained from the Amala Cancer Research Center, Thrissur, Kerala, India. The cells were maintained as ascites tumor in Swiss albino mice.

2.4 Preparation of Transdermal Patches in Drug in Adhesive Approach

The release liner 3M Scotch (9742 Release Liner-Fluoropolymer Coated Polyester films) was held in place on a flat surface. A sample of each polymeric adhesive mixture was placed across the top edge of the release liner. The mixture was cast onto the release liner by drawing a multiple clearance film applicator AR 5315 (Pacific Scientific, Sliver Spring, MD) the accurate amount of drug per 10 cm^2 was determined by varying adhesive thickness, wet adhesive film was dried at 75° to 30 min. The (backing membrane) CoTran (9726-Ethylene Vinyl acetate (EVA) Film) (polyethylene monolayer film) was placed on the top of the coatings. The transdermal films were cut into circular pieces having a 3.6 cm diameter corresponding to a 10 cm^2 surface area. The drug-in-adhesive patches were made of flexible backing, the accurate amount of the drug per 10 cm^2 was loaded based on the pharmacokinetic parameters of 6-MP such as Volume of distribution (V_d), total body clearance (Cl_t) and therapeutic plasma concentration (or) minimum effective concentration (5). The approximate dose per day = Flux (158.4 $\mu g/cm^2/h \times 24\ h \times$ surface area (10 cm^2) = 38.016~40 mg, desired flux = Clearance {(11 ± 4 ml/min/kg) (for 60 kg = 660 ml/min)} × minimum effective concentration (MEC) (0.04 μg/ml)/surface area (10 cm^2) = 158.4 $\mu g/cm^2$ h.

2.5 Evaluation of the Physicochemical Parameters of Transdermal Patches

2.5.1 Thickness

The thickness of the patch was measured by a Vernier caliper (Mitotoyo, Japan) at three different points on the film.

2.5.2 Drug Content

Drug loaded polymeric films of 1 cm^2 were taken from three different locations of the 10 cm^2 film, dissolved in the 2 ml of methanol and sonicated for 10 min. The volume was made up to 10 ml using 0.1 N NaOH absorbance and was measured at 321 nm using a UV-visible spectrophotometer (UV 1700 Shimadzu, Japan).

2.5.3 Folding Endurance

The folding endurance value can be defined as 'the number of times a film can be folded at the same place without breaking'. This test is an index of the brittleness of the film, the lower the folding endurance value, the more brittle the film. It is an important test to assess the integrity of the film, the folding endurance was determined according to films (6) of (2 × 4 cm^2) and was folded in the center between finger and thumb and then opened. This is called as 'one folding'. The procedure was repeated until the film showed breakage or cracks in the center. The total number of folding operations was termed as the 'folding endurance value'.

2.5.4 Flatness

An ideal transdermal patch should possess a smooth surface and should not constrict, after application to the skin, over time. Therefore, the flatness of the patches was studied by cutting them into strips and placing the strips on square glass molds (2 × 4 cm^2) for 10 min, and then measuring their lengths. Percent flatness was determined as follows.

%Flatness $= L_1 - L_2/L_1 \times 100$, where L_1 and L_2 are the initial length and final length of each strip, respectively.

2.5.5 Elongation

The percent elongation was evaluated by the percentage elongation at break value. Rectangular film of 1 inch width 15 cm length was cut using the blade and a scale marked 2.5 cm from the edges lengthwise. The film was held between the jaws of Instron 4411 (Instron, Germany). One jaw was kept stationary and another was pulled slowly at 10 mm/min until the film just broke. The percent elongation at break was calculated using the below equation. Percent elongation at break $= (I_B - I_A)/I_A \times 100$, Where $I_A =$ Initial length of film (cm), and $I_B =$ length of film (cm) at break.

2.5.6 Thumbtack Test

One week after the preparation of Transdermal patches, the thumb was lightly put into contact of the adhesive for a short time (10 sec) and then withdrawn quickly. The test was also done by varying the pressure and time of contact and there was no difficulty in pulling the thumb from the adhesive. It was possible to perceive how easily, quickly and strongly the adhesive can form a bond with skin. It is a most simple and straightforward test for the evaluation of the adhesive skin bonding. The adhesion properties were expressed by the following value range; good adhesion, poor adhesion and no adhesion.

2.5.7 Evaluation of Adhesive Properties

Several methods have been used to evaluate the adhesive properties of pressure sensitive adhesives, peel adhesion tests are commonly performed to determine the adhesion of a transdermal patch. The adhesive properties of the patches were evaluated using the Instron 4411(Instron, Germany). 90° Dynamic Adhesive Strength Peel Test (90° DASPT) was done according to the American Standard Test Methods (ASTM) (7). The objective of the 90° DASPT is to determine the peel force, in Newton (N/mm^2), needed to remove the TDDS from a standard stainless steel surface using a 90° peel angle with a constant peel rate of 30 cm/min at constant temperature and relative humidity. Higher values indicated greater bond strength. The pulled patch does not leave any residue on the plate, it indicates 'adhesive failure' which is desirable for most applications, especially for transdermal drug delivery system. If some residue is left behind, it suggests "cohesive failure" which often signifies a lack of cohesive strength.

2.5.8 Drug to Polymer Interactions

Differential scanning calorimetry (DSC) was used for characterizing solid-state polymer-polymer and drug-polymer interactions. The mechanical treatment for preparing physical mixtures of polymers and the drug (i.e. simple blending or stressed cogrinding). DSC is used to investigate and predict any physicochemical interactions between the components in the formulations. The thermal properties of drug and their physical mixtures. An interaction, DSC will show changes in melting point, peak shape and area and/or the appearance of a transition. However, there are invariably some changes in transition temperature and area peak shape and area by virtue of mixing two components, and this is not due to any interaction. Chemical interactions are indicated by the appearance of new peaks, or where there is gross broadening or elongation of exo- or endothermic changes. The pure drug, medicated and placebo film were analyzed at the 10°C/min from 50°C up to 300°C (TA Instruments Germany).

2.6 Design of Velcro Protection Jackets

The major problem encountered was during experiments how to protect the applied transdermal patch from being licked, scratched and/or rubbed off once applied to the shaved dorsal surface of mice skin. A Velcro® jacket was designed with small modifications as reported earlier, Su et al. (8). A Velcro® jacket was made to cover the entire trunk of the mice and opening at the top, which was designated for application of the transdermal patch. A Velcro® jacket protected the transdermal patch and allowed for good ventilation, details of the Velcro® protection jackets are illustrated in Figure 1. It serves our purpose quite well and the mice were able to maintain a normal life pattern wearing this jacket.

2.7 Determination In-vitro Cytotoxicity Activity of 6-Mercaptopurine to DLA Cells

DLA cells (1×10^6 cells) were incubated with various concentrations of drug (100–1000 ng/ml) in final volume of 1 ml for 3 h at 37°C, after incubation the viability of cells were determined by the tryphan blue dye exclusion method, Talwar, 1974 (9).

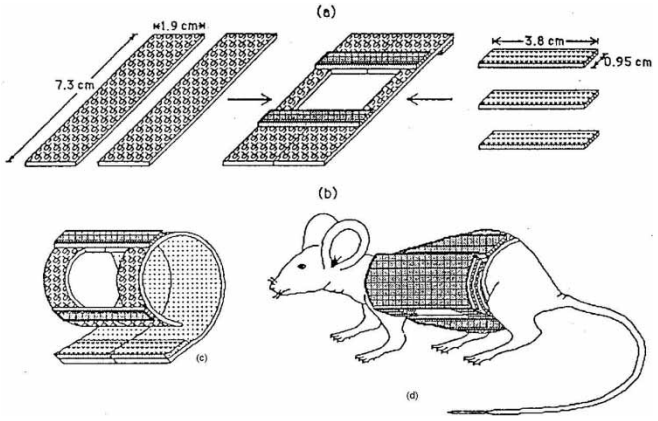

Fig. 1. A schematic illustration of a Velcro® jacket, (a) two pieces of Velcro® together with opening, (b) joined pieces of Velcro®, (c) the finished jacket to wrapped around the trunk of shaved mice, (d) once the patch was applied, another piece of Velcro®, is covered over the opening for protection the transdermal patch.

2.8 Determination of the Effect of Transdermal Patch on Survival Time of DLA Bearing Animals

DLA cells were aspirated, washed, and suspended in phosphate buffer saline (PBS). Balb/c mice were divided into three groups (10 mice/group). All the animals were induced tumor by injecting DLA cells 1×10^6 cells/animal subcutaneously on the peritoneal cavity. Group I was kept as untreated control. Group III was administered with 6-MP through drug in adhesive transdermal patch applied on the dorsal surface of mice for 10 consecutive days. In group II, drug was administered orally for ten consecutive days. The death pattern of animals due to tumor burden was noted and the percentage of increase in life span was calculated using the formula T-C/C $\times$ 100, where 'T' and 'C' represent the number of days the treated and control animals survived, respectively.

2.9 Determination the Effect of Transdermal Patch of 6-MP on Solid Tumor Development in Animals

Three groups (10 mice/group) of mice were induced solid tumor by injecting DLA cells (1×10^6 cells/animal) subcutaneously on the right hind limbs. 6-MP single layer Drug-in-adhesive transdermal patch was applied to the shaved (Whal hair clipper, model 9962, Whal clipper corporation, China) dorsal surface of animal (40 mg/dose/animal) daily for 10 days to IV group of mice. V group animals, 6-MP was administered orally (50 mg/dose/animal.) for 10 consecutive days, VI group was kept as untreated control. The diameter of developing tumor was calculated using the formula, $V = 4/3\ \pi r_1^2\ r_2$, where V is the volume of tumor, r_1 and r_2 is the radii of tumor in different planes. A reading was taken on every 5th day up to 30 days. The results were compared with untreated control.

2.10 Cumulative Skin Irritation Study and Sensitivity in Animals and Human Subjects

Sample size: 30 subjects, exclusion criteria: Dermatologic disease that might interfere with the evaluation of test site reaction, duration of study: 22 days. Study design: A randomized, controlled, repeat patch test study. Each subject applied placebo patches (without drug) to be tested. Patches were applied for 23 h (plus or minus 1 h) daily for 21 days to the same skin site. Sample size of 30 animals was selected for study, study design: A controlled, repeat patch test of test patch i.e., single-layer drug-in-adhesive 6-MP patch was applied on a shaved (Whal hair clipper, model 9962, Whal Clipper Corporation, China) dorsal portion of mice. Patch application: one patch per day to each animal was applied; patches were applied for 23 h (plus or minus 1 h) daily for 21 days to the same skin site. At each patch removal, the site should be evaluated for reaction and the patch reapplied. Application of a test patch should be discontinued at a site if predefined serious reactions occur at the site of repeated applications. Application at a different site may subsequently be initiated. Evaluations: Scoring of skin reactions and patch adherence should be performed by a trained and blinded observer at each patch removal, using an appropriate scale as described below. Skin irritation was planned and done according to the United States Food and Drug Administration (USFDA). All applications were initiated on the right side of the forearm for all subjects, the patches were applied on a clean, dry area of the forearm. All subjects were examined for signs and symptoms of skin irritation, and patch adherence was evaluated immediately prior to the removal of the patches. The primary outcome was the skin irritation score, measured by erythema. Erythema was graded by a trained clinical evaluator using a daylight blue incandescent lamp, on a 5-point scale of 0–4, where 0 = none, 1-slight, just perceptible, 2 = slight, with definitive margins, 3 = moderate, obliteration of margins, 4 = severe, vivid, spreading well beyond margins. Scores of 3 or greater were of an intensity that would most likely have been perceived and noticeable by the patient. Secondary evaluation criteria were patch adherence scores, adhesive residue scores, local skin reaction scores, and sensitivity scores. Patch adherence was evaluated at the end of the wear period, immediately prior to removal of the patch. Scores corresponded to the percentage of the patch surface in contact with the skin according to a 5-point scale of 0–4, where 0 = patch adhered >90% (completely on), 1 = patch adhered 75–90% (edges lifting of or center raised), 2 = 50–75% (half off), 3 = patch adhered <50% (just hanging on), 4 = patch not present on skin. Immediately following removal of the patch, the amount of adhesive remaining at the patch, the amount of adhesive remaining at the patch site was examined and graded on a 4-point scale of 0–3, where 0 = none, 1 = light, 2 = medium and 3 = heavy, sensitivity was scored on a 4-point scale of 0-3, where 0 = not sensitized (no significant clinical sign of symptom), 1 = mild sensitivity (erythema and light oedema), 2 = moderate sensitivity (erythema with infiltration, raised, spreading beyond the borders, with or without vesiculation), 3 = strong sensitivity (large vesiculobullous, vividly red infiltrated plaques).

2.11 Statistical Analysis

The results are expressed as mean $\pm$S.D statistical evaluation of the data was done using student's *t*- test using Sigma Stat.3.0, USA.

3 Results and Discussion

3.1 Determination of the Cytotoxic Concentration of 6-MP on DLA Cells

In-vitro cytotoxicity was observed by the exposure to 6-MP in different concentrations, reduced the ability of DLA cells to survive, this could be observed directly by examining microscopically using haemocytometer, stained by tryphan blue,

cells can be readily identifiable as a dark blue circles, to estimate the activity by counting the cells in the field of view per 100 cells counted, results are shown in Table 1. Similar results were obtained for 6 MP concentration dependent cytotoxicity in Molt F4 cells, cell viability after treated for 48 h with 0, 2, 5 and 10 μM 6-MP was 96.7%, 75.7%, 45.1% and 35.5%, respectively (10). M. Tidd and co-workers (11) have reported Ehrlich ascites carcinoma (EAC) cell proliferation in mice continue to multiply with 6-MP intraperitoneal injection and cell number were lower to 4×10^6 cells/mouse compared to untreated control, EAC cells proliferation was counted by peritoneal risings, from groups of 5mice which had received identical tumor cells. Extensive research has been performed to elucidate the mechanisms of by which 6-MP exerts its cytotoxic effects on the tumor cells. Most attention has been focused on the incorporation of the 6-MP into DNA as thioguanine nucleotides, whereas the methylation pathway of 6-MP metabolism is considered to be a detoxifiying process after low-dose oral maintenance treatment and therefore negatively affecting the therapy. It has no intrinsic cytotoxic activity, but is converted into active metabolites before it exerts its cytotoxic action. The first step in the anabolic pathway of 6-MP is its conversion into the nucleotide, thioinosine monophosphate. This compound is converted into thioguanosine monophosphate, which is cytotoxic after incorporation into DNA and RNA, or into methylthioinosine monophosphate, which is an inhibitor of the purine *de novo* synthesis (12). The purine 'salvage' enzyme hypoxanthine phosphoribosylytransferase (HPRT) catalyzes the initial step in the biological activation of 6-MP. The formation of intracellular thionucleotide metabolites is essential for thiopurine cytotoxicity (13), 6-MP eventually forms a 6-thioguanine nucleotides (TGNs), but a variety of intermediary thionucleotides are formed enroute to the TGNs. The fraudulent nucleotides can potentially produce the cytotoxic and immunosuppressive actions by different mechanisms. Cytotoxicity has been directly linked to the incorporation of drug derived TGNs into DNA (14), but in addition, some thionucleotide metabolites can inhibit *de novo* purine synthesis. 6-MP is an ideal drug candidate for transdermal delivery into systemic circulation that mimics continuous infusion, which bypass first pass metabolism in the liver and will decrease in the toxicities likes neutropenia, dangerous bone marrow suppression and increase the bioavailability of the 6-MP. Due to the controlled release of 6-MP, the multiplication of ascites cells is inhibited, *in-vitro* (data not shown) data can be extrapolated by this minimum effective concentration (MEC) of drug is penetrated and inhibited the de-novo synthesis, leading to cytotoxicity.

Table 1. Cytotoxicity of 6-mercaptopuirne Dalton's lymphoma ascites (DLA) cells

Concentration (ng/ml)	Percentage of cytotoxicity (%)
1000	96
500	73
250	52
100	24
50	13

Daltons lymphoma ascites (DLA) cells were incubated with different concentrations (100–1000 ng/ml) of 6-MP, percentage of dead cells was determined by tryphan blue dye-exclusion.

3.2 Antitumor Activity of 6-Mercaptopuirne through Transdermal Patch

There was a significant reduction in tumor volume in the animals treated with single-layer drug-in-adhesive transdermal patch treated animals compared to untreated control and oral therapy. Tumor volume of control animal was 6.01 cm^3 on the 30TH day, while the 6-MP transdermal patch was only 2.73 cm^3 on the same day, results are shown in Figure 2, the data was statistically significant ($p < 0.005$) compared to control.

3.3 Determination of the Effect of 6-MP through Transdermal Patch on the Survival of DLA Tumor Bearing Animals

The increase in life span (ILS%) was evaluated by comparing the mean survival by comparing the mean survival time of animals in each treated and untreated control group. DLA cells tumor bearing mice treated with 6-MP single-layer drug-in-adhesive transdermal patch was found to be significantly increased in ILS. Control animals survived only 21 ± 1days after the tumor inoculation, while the 6-MP oral therapy and 6-MP transdermal patches, animals survived 34.5 ± 1.29 days and 36 ± 1 days with ILS of 66.66% and 71.0%, data is shown in the Kaplan Meier graph (Figure 3 and Table 2).

3.4 Physicochemical Parameter Evaluation of Transdermal Patch

Patches were made with a drug loading of 2 mg/cm^2. Moreover, knowing the influence of the amount of penetration

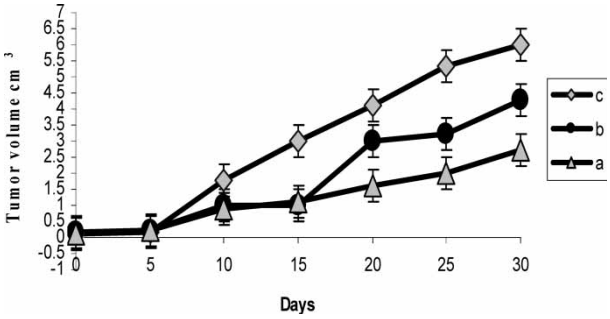

Fig. 2 Anti-tumor effect of (a) 6-MP single-layer drug-in-adhesive transdermal patch, (b) 6-Mercaptopurine oral administration and, (c) untreated control, against DLA induced solid tumor in mice. Drug was administered after 24 h of injecting DLA cells.

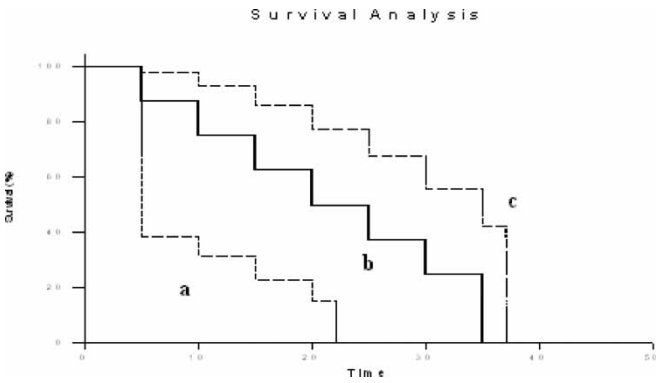

Fig. 3. Kaplan-Meier graph for the animal survival rate of DLA tumor-bearing animals by administration 6-mercaptopurine through (a) untreated control, (b) oral administration and (c) single-layer drug-in-adhesive transdermal patch. Drug was administered after 24 h of injecting DLA cells.

enhancer in determining the flux drug was loaded in adhesive matrix (40 mg/patch) from which the MEC and flux of 6-MP can be achieved with d-limonene 6% w/w (acts as a penetration enhancer) through human skin (data not shown). The values were found to be 2.0 ± 0.1 mg (n = 3). The flatness study indicated a 100% flatness of patch and it has no level of immediate constriction and it can be suitably placed onto the skin's surface where it will remain as such without constriction for a long period of time, one hundred-percent flatness means that there is no constriction. The thickness of the patches was in the average of the three values and it was 150 ± 00.32 mm (n = 3). The folding endurance test is an index of the brittleness of the film which showed more than 500 foldings (n = 3), the films were found to be more elegant, smooth, transparent and flexible which can be attributed to the plasticizer. The adhesive did not leave any residual on the residual plate the PSA Duro-Tak® 87-2196 showed good adhesion, the peel force was between 2.1 to 7.2 Newton's, which was the acceptable limits for TDDS.

Table 2. Effect of single-layer Drug-in-Adhesive (DIA) transdermal patch of 6-mercaptopurine on the life span of Dalton's lymphoma ascites (DLA) tumor in animals

Groups	No. animals with tumors	No. of days survived	Increase in life (%ILS) T-C/C × 100
Control	10/10	21 ± 1	—
6-MP Conventional (50 mg Tablets)	10/10	34.5 ± 1.29	66.66%
6-MP single-layer Drug-in-Adhesive (DIA) transdermal patch	10/10	36 ± 1[a]	71.0%

[a]Values are ± SD (n = 10), $p < 0.001$ (student's *t*-test).
Animals were treated with 10 doses of 6-mercaptopurine conventional route and transdermal patch, after injecting Dalton's lymphoma ascites (DLA) cells (10^6 cells/animal). Survival of the animals were observed for 40 days.

Duro-Tak® 87-2196, which has a carboxylic group, has functional group help in wetting the adherent surface and causes the hydrogen bonding between the PSA and surface of substrate. The thumb tack test is the widely accepted test for testing the tack properties of the adhesive polymer, major drawback of the thumbtack test is subjective and the fact that the data are poorly quantifiable, the studies were simultaneously performed, blind on three samples. The optimized transdermal patches showed good adhesion. Elongation for transdermal patches was between 3–5%. Each mouse wore a Velcro® protection jacket during the entire experimental period and each was individually housed. At the end of each dosing interval, Velcro® protection jacket was temporarily detached, for the treatment, with finite dose formulations which were applied onto the skin of the dorsal surface of animal (i.e., the treatment would start on the day after the tumor inoculation and continue for 10 consecutive days), there was no considerable modification of the melting point of the drug in physical binary mixtures observed, suggesting no interaction between the two (Figure 4). On the other hand, there was no significant shift in the melting endothermic peak of the drug in physical drug-polymer mixtures, this revealed the absence of solid-state interaction between the drug (6-MP) and the present PSA polymer.

3.5 Cumulative Skin Irritation Study, Sensitivity and Adherence in Human Subjects and Animals

At each evaluation during the trial, the sites treated with patches exhibited less irritation, at the end of the study the number of subjects with irritation (scores 1–4) was significantly less. Scores of 1–2 were observed among the two human subjects, moderate and severe erythema (scores 3and 4) was not observed in any human subjects. Animals showed the score = 0 confirmed that there was no skin irritation and sensitivity. Patch adhesion was greater with 96.4%, respectively showing over 75% adhesion (scores 0 and 1) in both human subjects and animals. The percentage of patients with adhesive residue was significantly less with scores of 0. The overall percentages of subjects found to have no sensitization or local skin reactions such as pruritus, scaling and oedema. Patient compliance and effective patch adhesion are essential to the efficacy to the

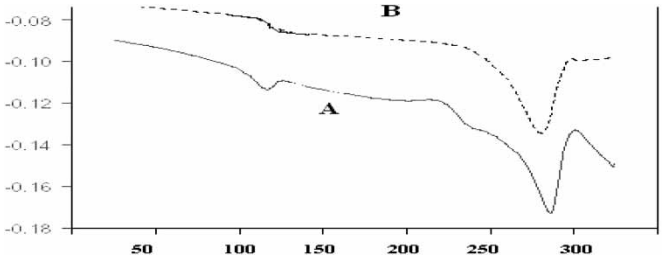

Fig. 4. Differential scanning calorimetry, a) Pure 6-mercaptopurine, b) Binary mixture in 1:1 ratio of 6-Mercaptopurine:polymer.

transdermal delivery; the degree of irritation experienced by patients may be a factor in the degree of patient compliance with the treatment.

4 Conclusions

The present study has demonstrated that 6-MP could reduce the DLA cells solid tumor development significantly and could also enhance the mean survival time of DLA cell tumor bearing mice. Cytotoxic concentration of the drug can delivered through the TDD system. A non-invasive route of TDD could decrease the severe side effects like hepatotoxicity, first pass metabolism in and increase the bioavailability of 6-MP, this could increase patient compliance. The Velcro® protection jacket served our purpose quite well and the mice were able to lead a normal life pattern wearing this jacket.

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