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RESEARCH ARTICLE

Differentiation of the DMBA Signaling Pathway on the Development of Skin Tumor Based on Histopathological Changing

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ABSTRACT

Because they develop into carcinomas and manifest in invasive and metastatic cancers, studies applying animal models have been performed continually over the years. Due to the long time needed to conduct its use and the high mortality rate of experimental animals, it frequently becomes an obstacle to efficient research. In this study, mice were administered 100 mg of DMBA (7,12-dimethylbenz [a] anthracene) twice daily to rub on the skin following it was dissolved in 0.5 ml of acetone. Methods: Two observations were carried out for the study; the first received 100 ug of DMBA twice daily for three weeks (50 ug in the morning and 50 ug in the afternoon), and the second used the same dose for 5 weeks. Results: Squamous cell carcinoma and malignant skin tumors had been identified by histological examination 3 weeks after DMBA intervention, while the same results were found 5 weeks afterwards. In this study, a malignant skin tumor in the form of squamous cell carcinoma became apparent in three and five weeks following applying the 100 ug of DMBA twice daily. Conclusion: After 3 weeks, a malignant skin tumor in the form of squamous cell carcinoma was found in an animal model applying DMBA at a dose of 100 ug twice a day.

INTRODUCTION

Skin tumours are tumours that can occur from keratinocyte cells, melanocytes, skin adnexa, or cysts. Malignant tumours are local cell proliferation that shows growth with abnormal cell differentiation and atypical cells [1]. Skin cancer is the most common form of cancer. It is divided into two subcategories: non-melanoma skin cancer (NMSC) and melanoma skin cancer (MSC). NMSC consists of Basal Cell carcinoma (BCC) and Squamous Cell Carcinoma (SCC) [2,3]. The three most common histological subtypes include cutaneous basal cell carcinomas (cBCCs), cutaneous squamous cell carcinomas (cSCCs), and cutaneous malignant melanomas (cMMs) where the skin has 16% of the total body weight, and is arranged in two main layers: epidermis and dermis. The histological subtype, cMM is the most common in Western countries, while non-melanosis skin cancer is more

common cMM in Asia [4]. In the HDI (Human Development Index) Incidence, basal cell carcinoma accounts for 80% of non-melanoma skin cancers. Of all types of cancer, skin cancer is the most common in the world; Basal Cell Carcinoma (BSK) and Squamous Cell Carcinoma (SSK) account for around 99% of all NMSK, where BSK is 3 to 5 times more common than KSS [5]. Non-melanoma malignant skin tumours It has several different characteristics, both in terms of various forms of behaviour and causal factors, growth and metastatic capabilities. In cases of BSK and SSK, if detected at an early stage, the prognosis is good [6]. It has been reported that the nodular subtype is the most common type of BCC, followed by the superficial and infiltrative subtypes. The nodular subtype is the most common, second place is the ulcerative subtype, followed by the infiltrative and superficial subtypes. On the face and head, BCC is more often localized in the nasal area, while SCC is in the earlobe. It has been demonstrated that the older the patient, the greater the vertical dimension of the tumour [7]. The ageing of the global population is becoming more serious, which may be the main reason for the increase in BCC ASIR worldwide. 12 In addition, an unexpected and significant increase in BCC ASIR occurred in East Asia, where efforts to reduce BCC prevention and screening programs must be carried out to reduce healthcare costs and morbidity [8]. In contrast to other regions of the world, the death rate and DALYs due to SCC in Southern Sub-Saharan Africa in 2019 were still very high compared to 2019. incidence. Many patients suffer from SCC and other cancers in Sub-Saharan Africa. Depletion of ozone in the stratosphere causes an increase in the ultraviolet-B (or UVB) component of solar UVR reaching the Earth's surface [9]. As a consequence, there is a major impact on the incidence and burden of skin cancer. Currently available for malignant skin tumours are synthetic drugs, but most treatments have limitations because in inducing malignant tumours in animals, they can cause serious side effects such as neurotoxicity, myocardial infarction and thromboembolism [10]. Experiments on animals are an important bridge between cell experiments and experiments. clinical. Under certain conditions, the emergence and development of animal diseases are similar to humans, and animals have anatomy, physiology and heredity that are similar to humans. Therefore, animal models are often used to study human diseases. In cancer research, the use of animal models can help us understand it [11].

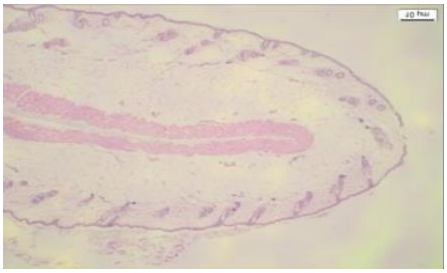
The genetic basis of cancer and the role of specific genes and gene mutations in the occurrence and development of cancer, also facilitate the development and testing of antineoplastic drugs. invasive and metastatic carcinoma.11 DMBA (7,12-dimethylbenz [α] anthracene), is a widely studied Polycyclic Aromatic. Hydrocarbon that has long been known to cause cancer including skin tumors in humans. It has been found that DMBA is phototoxic in bacteria as well as in animal or human cells and photo-mutagenic in strains of Salmonella typhimurium. Light irradiation can change several photoproducts including benz(a)anthracene 7,12-dione, 7-hydroxy-12-ketone-7-methylbenz(a)anthracene [12]. The DMBA compound is very cytotoxic and can cause apoptosis in A20.1 lymphoma cells Murine B [13]. DMBA is a compound that can cause death. Clinical symptoms that can be caused by this compound include disturbances in the digestive and respiratory processes and can cause irritation to the skin, eyes and gastrointestinal tract [14]. According to research by Wongso H., death in mice occurs due to the toxic nature of DMBA. Toxic levels that are too high will cause the rat's resistance to decrease and ultimately cause death. In general, the animal model is used for studies of cancer in mice. Likewise, our research used BalC mice weighing 20-35g. 4 weeks of age, which is expected to be effective in forming carcinogens, and how long does it take for DMBA application to reach or form skin malignancies?

MATERIALS AND METHODS

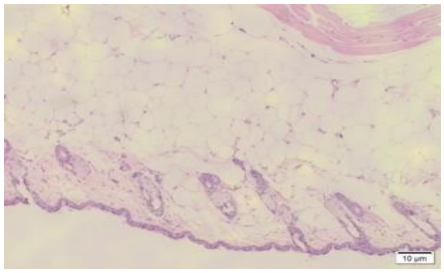
All BalC mice taken homogeneously, with the same weight, were smeared with 100μg DMBA in 0.5ul acetone, where acetone was the solvent. This acetone did not change the structure of DMBA and was not significantly different from previous studies with controls that did not use acetone. This is also proven by previous research which proved that the results were not significantly different between the normal control group and the acetone control group. 16,17,18Mice were divided into 6 groups

according to the number of days applied, The mice were sacrificed every 1 week and examined histopathologically, every week until the fifth week, especially in the fifth week we divided into two groups on the 33rd and 35th days. Mice were smeared for 5 weeks, week I group (days 1-7), 2 mice (IA and IB) were smeared with DMBA on the back every morning and evening, where first the mice were shaved on the back, then smeared with DMBA, on the 7th day a biopsy was performed to see histopathological changing. Group II consisted of 2 mice (IIA and IIB) also applied daily 2 times a day from day 8 to 14. At the end of the second week, 2 mice were sacrificed on day 14 and a histopathological examination was performed to see the growth of skin tumors. Group III consisted of 2 mice (IIIA and IIIB) which were applied daily 2 times a day, from the 15th day to the twenty-first day., On the 22nd day, the mice were biopsied. Then histopathological examination was carried out to see the development of the tumour. The 3rd-week mice were sacrificed as well as the 4,5, groups, the same thing was done. Group IV consisted of 2 mice (IVA and IVB) which were smeared every day 2 times a day, from day 22 to day twenty-eight, and day 29 the mice were biopsied, and then a histopathological examination was carried out to see the development of the tumour. The 4th-week mice were sacrificed for histopathological examination. The examination was carried out at the Histopathological Department of the Faculty of Medicine, University of Hasanuddin, Indonesia. Group V consists of 2: group 1: from day to day 33 and group2; to day 35, each examination was 2 mice (33 and 35 days) which were smeared every day, On

RESULTS



1a. normal

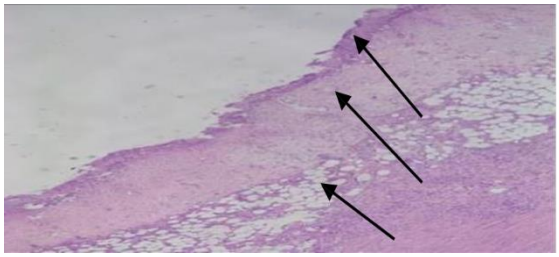


1b. normal

Figure 1: The serial histopathological changes before and after DMBA intervention

In the form of curved tissue wholly covered by skin with epidermis and dermis within normal limits. Before administering DMBA.picture 1b.

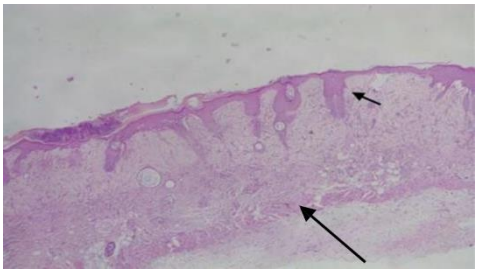
a. There is no hyperplasia; the thickness of the epidermis is around 1- 4 cell layers
b. Adnexa within normal limits
c. Inflammatory cells/mononuclear lymphocytes 1-2 were found
d. There is a distribution of microbes, round/rod/coccus in shape purplish blue in the dermis
e. No dysplasia/neoplastic cells were found



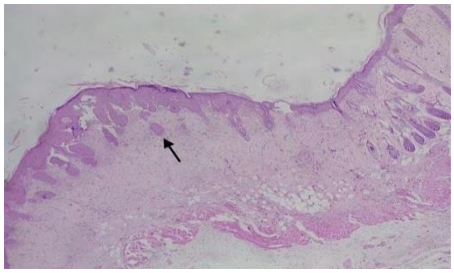
1c.

Skin tissue, in the form of inflammation in the epidermis and dermis, is in normal condition after administering DMBA for one week.picture 1c.

a. The epidermis appears inflamed.
b. Dermis appears normal, subcutis normal
c. No hyperplasia found



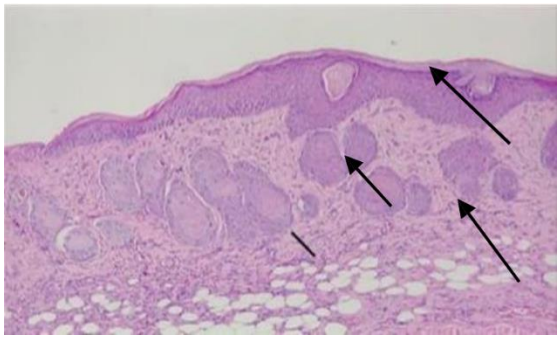
1d.



1d.

Skin tissue with epidermis and dermis in an abnormal condition, After administering DMBA on day 14. picture 1d.

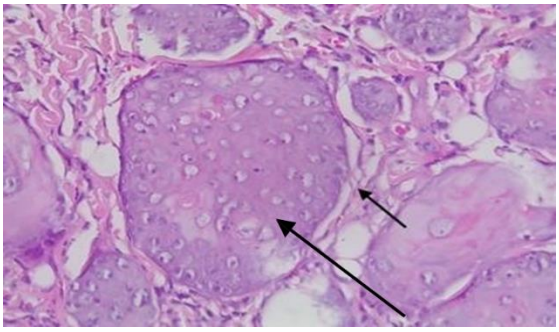
a. Epidermis appears with slight inflammation
b. Hyperplasia of the epidermis layer was found to penetrate the dermis
c. Subcutis below the dermis



1e.

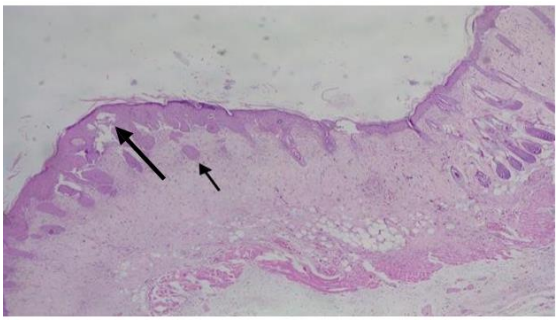
Skin tissue in the form of thickened epidermis,. visible dermis leading into the dermis after administration of DMBA on day 21.picture 1e

a. Epidermal hyperplasia
b. There are many tumor islands in the dermis
c. Subcutis below the dermis, which begins to widen



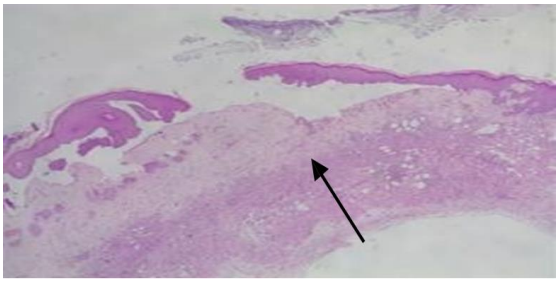
1f.

Keratin masses were visible between the squamous epithelial tumour nests on DMBA administration on day 21.pictture 1f. Atypical, pleomorphic tumour cell nuclei, prominent nucleoli, neoplastic cells The cell nucleus is next to the pink cytoplasm



1g.

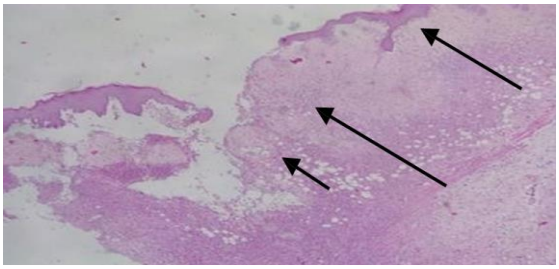
The epidermis layer of skin tissue appears to thicken after administering DMBA on day 29



1h.

Epidemic layer, thickened dermis, leading epidermal hyperplasia into the dermis. The epidermis layer is starting to break down and lift upwards.

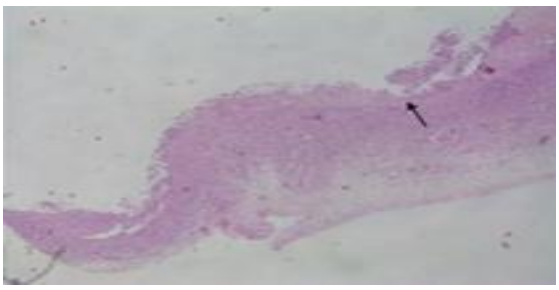
a. Part of the dermis filled with tumour cells does not appear in the subcutis
b. Still visible squamous cell tumour.



1i.

The epidermis and dermis skin tissue appear to be lifted from the skin Subcutaneous tissue after administration of DMBA on day 33.

a. Part of the epidermis and dermis has been separated.
b. Ulcus leading to the bottom
c. Hyperplasia is still visible



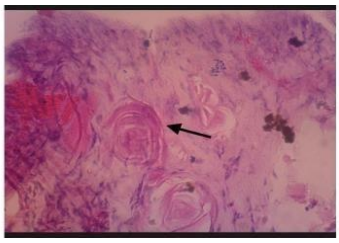
1j.

Epidermal skin tissue detaches from the dermis, necrosis of the dermis. After administration of DMBA on day 35

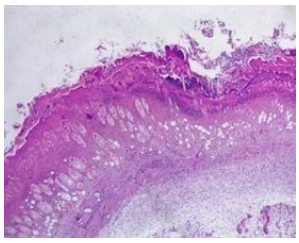
a. Tissue damage to the dermis, and ulcus.
b. The dermis does not show cutaneous adnexa
c. Subcutis is not visible

Figure 2: Histopathological changing after 3 weeks of DMBA intervention

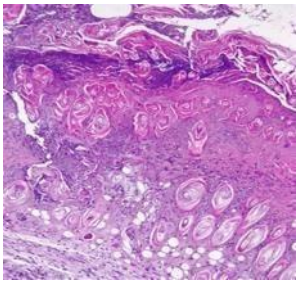
In group A we did not do the PA examination on day 20, group A mice died, and group B survived on day 22, we biopsy and divided 2 tissues in different PA laboratories. In group B on day 22 of the clinical picture of mice, skin lesions were seen with lesions covered with thick crusts. done in the Lab. Anatomical Pathology RS. Koja found that the epidermis had extensive ulcers and necrosis in the epidermis, but no malignant skin tumors were found. Laboratory examinations at Hasanuddin University Animal Hospital found keratin pearl (Horn Pearl).



a.
Horn Pearl,
After DMBA administration on day 21



b.
Picture. Histologically after 3 weeks of DMBA administration, ulcers and part of the epidermis appeared, and inflammation and thickening occurred towards the dermis.



c.
Picture. Histologically after 3 weeks of DMBA administration, the epidermis and dermis tissue were inflamed. The layers of the epidermis and dermis were detached, and ulcers appeared. Part of the dermis was destroyed.

d.
Picture. Mice skin after 3 weeks of DMBA administration

DISCUSSION

7,12-dimethylbenz(a)anthracene (DMBA) is a polycyclic aromatic hydrocarbon (PAH) found in the tar fraction of cigarette smoke, as well as in automobile and furnace exhaust gases. DMBA is responsible, through interactions with the aryl hydrocarbon receptor (AhR) [19]. The AhR complex is translocated to the nucleus through protein binding, the AhR nuclear translocator is(ARNT).In signalling in the nucleus, the AhR/ARNT complex regulates gene transcription by binding to DNA at the dioxin-responsive element (DRE). DMBA is also in the nucleus activating predominantly and induces a number of genes for phase 1 enzymes (cytochrome P-450) such as CYP1A1, 1A2 1B1, and several other genes. The phase 1 enzyme, CYP1A1, which is responsible for the production of aryl hydrocarbon hydrolase (AHH) and causes oxidative metabolism of the AhR ligand, if increased will have the effect of being a carcinogenic compound. Phase 1 enzymes also increase the production of reactive oxygen species (ROS), which have been shown to be associated with lipid peroxidation, oxidative DNA damage, and cause pathological effects, these enzymes mediate free radicals, such as superoxide, hydrocarbon oxide, nitrogen oxide, resulting in DNA addiction mutagenic through covalent bonds with exocyclic purine amino groups or oxidative stress which induces casino genesis. [19,20] In Jung Kwon-Yeo's research, the molecular mechanism of carcinogenesis caused by DMBA was discovered. The existence of intracellular signalling related to cell proliferation, migration and invasion revealed the effects of DMBA on Wnt/b-catenin signalling and the epithelial-mesenchymal transition (EMT) process [20].

Histopathological examination at the Veterinary Hospital, found signs of malignancy in the form of Horn Pearls found in Squamous Cell Carcinoma. To assess a malignant tumour, an examination is carried out by a pathologist, one or several pathologists, who assess whether the malignant tumour cells are keratinized, or without keratinization, also with a stromal ratio of only 1 or more than 1 stroma, whether the infiltrate is well differentiated or not. assessing the presence of squamous cell carcinoma with pearl horn sign, histopathological examination showed a well-differentiated tumour infiltrate [21,22]. As for the death of one of the mice because every time you apply the fur on the mice, clean the area around it so that the toxic substances are more quickly absorbed into the skin and around the skin lesions. This results in faster tissue damage whereas the picture is only extensive tissue destruction and necrosis, causing the death of one of the mice. Another study by Martha (2013) used DMBA+TPA at a dose of 100 µg, the results showed SSK at week 13 and a dose of 50 µg 4 times exposure every week. The frequency of DMBA application affects the occurrence of dysplasia and squamous cell carcinoma. From this study it was also said that there was no effect of TPA administration on dysplasia and SSK, what was influential was that the dose and frequency of DMBA application influenced the occurrence of dysplasia and squamous cell carcinoma.

The more frequently DMBA is given, the faster the growth of squamous cell carcinoma [23]. Muchsin.D. 2016 using a dose of 100 µg DMBA in 5ul acetone without TPA. obtained. The more frequently DMBA is given, the faster the growth of squamous cell carcinoma. , This study used a single 100 µg DMBA in 5 µl acetone, twice a week for eight weeks. In the study, tumour growth occurred in the group with DMBA exposure at a frequency of twelve and sixteen times, twice-a-week intervals. Malignant tumours appeared in the sixth week [24]. Zhang Wen, et al. 2017, In a DMBA study with hamsters weighing 100-110 g l, aged 8-10 weeks, duration of administration 14 weeks, applying 50 ugs of DMBA hamsters to the right buccal pouch 3 times a week, to induce carcinoma and there were results of Squamous Cell Carcinoma, hyperplasia and mild to severe dysplasia at Week 14 [25]. Study (Martines2020) with DMBA Administration (0.5% DMBA with buccal staining 3 times a week) there was no macroscopic evidence of tumour at 12 weeks in In the first experiment, no mucosa was exposed to DMBA. The epithelium and basement membrane were well differentiated, with polyhedral cells and a hyperkeratinized outer layer. After 14 weeks The sac was exposed to a carcinogenic agent, showing low hyperplasia and signs of lymphocytic infiltration in response to chronic exposure to the carcinogenic DMBA after using an immunosuppressive agent after 4 weeks of Dexamethasone + DMBA, necrosis and dysplasia were found microscopically while proving macroscopic signs of fibrosis, indicating malignancy [26]. Other studies have shown that metabolically formed DMBA-induced tumour genesis will result in syn- and anti-DMBADE forms and that syn- and anti-DMBADE have tumour-promoting activity, and all tumours are generated through the initiation of A- to T transversion (-CAA- to -CTA-).[27] Wongso with a dose of 17.5 mg/kg BW aged 4 weeks, which is 100%. The fastest cancer incidence rate resulted from giving DMBA 20 mg/kg BW to mice aged 4 weeks and 6 weeks, with 3 repetitions. Meanwhile, the mortality rate occurred in mice induced by DMBA of 25 mg/kg BW with mice aged 4 weeks, namely by 75%. The fastest incidence of cancer occurred when 20 mg/kg BW DMBA was given for 4.5 weeks in 4-week-old mice. Meanwhile, the longest time to induce cancer occurred when giving DMBA 17.5 mg/kg BW to mice aged 5 weeks, namely 8.5 weeks after induction [15].

CONCLUSION

From the research above, we conclude the frequency of administration of DMBA dissolved in 0.5ul acetone, with a dose of 100ug applied in the morning and evening, 50ug each. For 3 weeks, it can induce the occurrence of malignant skin tumours, which can be proven on histological examination in both trial 1 and trial 2. Where malignant skin tumours in the form of Squamous Cell Carcinoma and malignant skin tumours (Pearl Horn) were found. Previous research to induce tumour cells required quite a long time, between 8-24 weeks. The frequency and time of DMBA exposure and the age of the animal also determine the formation of malignant skin tumours.

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Author's contributions: CR, MH, FT, DKS, RS conceived and designed the study, conducted research, provided materials, and collected and organized data. CR, MH, FT, DKS, and RS drafted the manuscript. CR, MH, RD, DKS, and FT analyzed the data and interpreted data. CR, MH, RS, FT, and DKS wrote initial and final draft article, and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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