Available online at www.sciencedirect.com
ScienceDirect
Nuclear Medicine and Biology 35 (2008) 233–237
NUCLEAR MEDICINE — AND — BIOLOGY
www.elsevier.com/locate/nucmedbio

Bioevaluation of ^{32}P patch designed for the treatment of skin diseases

Maria Jimena Salgueiro[a,*], Hebe Duran[c], Monica Palmieri[b], Rosana Pirchio[d], Vanina Medina[a], Ricardo Ughetti[e], Maximo Croci[f], Jorge Nicolini[e], Marcela Zubillaga[a]

[a]*Radioisotope Laboratory, School of Pharmacy and Biochemistry, University of Buenos Aires, 1113 Buenos Aires, Argentina*
[b]*Biodiversity and Experimental Biology Department, School of Exact and Natural Sciences, University of Buenos Aires, 1113 Buenos Aires, Argentina*
[c]*Radiobiology Department, National Energy Atomic Commission, 1150 Buenos Aires, Argentina*
[d]*Dosimetry Department, National Energy Atomic Commission, 1150 Buenos Aires, Argentina*
[e]*Research and Development Department, Laboratorios Bacon SAIC, 1603 Buenos Aires, Argentina*
[f]*Institute of Immunooncology, Dr. E. Crescenti, 1187 Buenos Aires, Argentina*

Received 21 June 2007; received in revised form 6 August 2007; accepted 16 August 2007

Abstract

Objective: The objective of this study was to design and evaluate a ^{32}P patch for the treatment of skin diseases.
Materials and Methods: The patch was prepared from chromic phosphate ^{32}P and silicone. Bioelimination and biodistribution in healthy and treated animals, and the therapeutic efficacy of two treatment schemes (single dose and fractionated dose) in an animal model of skin cancer were studied.
Results: Based on the bioelimination and biodistribution studies, no leakage of ^{32}P from the patch was observed. The treated tumors reduced their mean diameter compared to controls. The single-dose therapeutic scheme showed a higher number of complete and partial remissions compared to the fractionated scheme. These results were confirmed by histopathological analysis of the samples.
Conclusion: The ^{32}P patch was designed and produced according to specifications for the treatment of superficial lesions of the skin. Although the ^{32}P patch is an open source, it behaves like a sealed one for use in brachytherapy treatments.

Keywords: ^{32}P; Brachytherapy; Skin cancer; Patch

1. Introduction

Radiotherapy has been used in the treatment of skin diseases since radiation was discovered [1–7]. The experience in treating ophthalmologic diseases was the basis for brachytherapy treatments of different skin conditions employing both γ- and β-emitters. Some of the brachytherapy devices used for such treatments consist of pearls, catheters and wires containing ^{198}Au, ^{137}Cs and ^{192}Ir, or needles and seeds of ^{90}Y that have to be implanted into the tumor. There are also applicators containing ^{90}Sr/^{90}Y that were developed for contact brachytherapy. In recent years, specially designed patches containing β-emitters such as ^{90}Y, ^{188}Re and ^{166}Ho have been developed for contact brachytherapy of skin lesions [3–7]. The results obtained by these working groups are very encouraging for the promotion of such treatment modality. These kinds of devices may be designed based on the shape of the lesion, and the activity of the radionuclide contained may be variable and strictly related to dosimetric calculations for different types of treatment schemes. Moreover, they also fulfill the advantages of brachytherapy-localized treatments.

In this regard, we designed a ^{32}P patch, as this radioisotope is commercially available in South America. In a previous work [8], we have evaluated some of its radiopharmaceutical characteristics (absence of radioactivity leakage in vitro and homogeneity of the source) as being applicable to brachytherapy treatments. On the other hand, preliminary results of its in vivo therapeutic efficacy using SENCAR mice were promising.

The aim of the present work is to confirm that the ^{32}P patch is an open source that behaves very similarly to a sealed one and thus can be employed in brachytherapy treatments. In this regard, we performed biodistribution and

* Corresponding author. Radioisotope Laboratory, Physics Department, School of Pharmacy and Biochemistry, University of Buenos Aires, 1113 Buenos Aires, Argentina. Tel.: +5411 4964 8202x33; fax: +5411 4964 8202x31.
E-mail address: jsalgueiro@ffyb.uba.ar (M.J. Salgueiro).

0969-8051/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.nucmedbio.2007.08.004

bioelimination studies, as well as therapeutic-effect studies, using single and fractionated doses in SENCAR mice. Bioevaluation of the radiopharmaceutical characteristics of the ^{32}P patch is focused on demonstrating that this contact brachytherapy device is suitable for the treatment of skin diseases.

2. Materials and methods

2.1. Production and control of ^{32}P patch

Each patch was designed by taking into account the size and shape of tumors. Bioelimination and biodistribution studies used these same patches in animals in order to mimic treatments. For experimental purposes, the patches were not shielded. For the production of patches, 10 mCi of ^{32}P was purchased as phosphoric acid ^{32}P [National Energy Atomic Commission (CNEA), Buenos Aires, Argentina], and its radiochemical purity was controlled [9]. The patch was prepared as previously described [8]. Briefly, we used chromic phosphate ^{32}P (30–70 nm) obtained from phosphoric acid ^{32}P (as described by Anghileri [10,11] and Anghileri and Marques [12]) and silicone (Silastic J-White 80, Dow Corning; The Dow Chemical Co. and Corning, Inc., USA). The radiochemical purity of chromic phosphate ^{32}P was evaluated [13] before use. Chromic phosphate ^{32}P was washed with isopropyl alcohol (Anhedra, Argentina), centrifuged to 2000 rpm for 0 min and finally dried at 80°C in order to obtain a fine powder. Afterwards, the chromic phosphate ^{32}P powder was mixed with silicone and dried at room temperature for 4 h.

For the measurement of activity concentration, a sample of the patch was dissolved in 5 ml of hyamine hydroxide (MP Biomedicals, LLC, USA) at room temperature overnight. An exactly measured aliquot was added to a vial containing 3 ml of a complete-phase combining system for liquid scintillation counting (PCS Amersham Biosciences, USA). Activity measurement was performed in a liquid scintillation counter (Wallac 1410 Liquid Scintillation Counter; Pharmacia Wallac, Oy, Finland) in accordance with ^{32}P protocol, with a relative error of <1%. Results were expressed as megabequerels per square centimeter (or mCi/cm^2), taking into account the weight of the sample and the density of the patches. Homogeneity control of ^{32}P distributed in the matrix of the patches was performed by autoradiography using a photographic paper (B/W RC paper Semi Matt, Multicontrast Premium; Agfa, Germany) and by direct exposure of a sample of the patch in a dark room for 4 min. Standard techniques were used to obtain photographs.

2.2. Animals

All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (US National Research Council, 1996). We used 7- to 9-week-old female mice of the SENCAR strain (CNEA). The animals were fed ad libitum with balanced standard food and maintained in stainless-steel cages with 12-h light/12-h dark cycles and controlled room temperature.

Biodistribution and bioelimination studies were performed with control animals, whose backs were carefully shaved, 3 days before patch application using a scheme similar to that used with animals in therapeutic-effect studies.

Therapeutic-effect studies were performed using the classical mouse model of two-stage skin carcinogenesis [14–16]. Briefly, the backs of 7- to 8-week-old female SENCAR mice were shaven with surgical clippers at least 3 days prior to treatment, and only mice in the resting phase of the hair cycle were used. Mice were initiated by a single topical application of 20 nmol of 7,12-dimethylbenz[*a*]anthracene (Sigma Chemical Co., St. Louis, MO, USA) in 0.2 ml of acetone. Afterwards, they were topically treated twice a week with 3.25 nmol of 12-*O*-tetradecanoylphorbol-13-acetate (TPA; Sigma Chemical Co.) in 0.2 ml of acetone, starting 10 days after initiation and over a period of 16 weeks.

2.3. Bioelimination studies

After patch application, 10 animals were placed (for 7 days) in stainless-steel metabolic cages (Model T 304; La Técnica) that allow the separation of urine and feces. Samples of urine and feces were collected daily, weighed, homogenized and dissolved in 5 ml of hyamine hydroxide for 24 h at room temperature. Then a 500-μl aliquot was mixed with 3 ml of the system for liquid scintillation counting and then measured as described above.

2.4. Biodistribution studies

After the bioelimination study, the animals were sacrificed and the following were extracted: heart, lungs, liver, spleen, femur, stomach, kidneys, intestine, skin and blood. A 50-mg (exactly weighed) sample of each organ was processed for liquid scintillation counting as follows: mixed with 2 ml of hyamine hydroxide for desegregation, 200 μl of H_2O_2 for discoloration and 200 μl of 1 N HCl for acidification. Then a 500-μl aliquot was mixed with 3 ml of the system for liquid scintillation counting and then measured as described above.

The same protocol was followed to process the organs of animals in therapeutic-effect studies in order to investigate the biodistribution of ^{32}P in treated mice.

2.5. Therapeutic effect study

Fourteen animals with similar tumor initial sizes were randomized in two groups. Physical dose and time exposition were determined based on dosimetric calculations described below. Group 1 comprised 14 control tumors and 14 treated tumors, and used a single-dose scheme of 40 Gy for 13.5 h of exposition. After patch application, seven control and seven treated tumors continued receiving TPA, and the rest received acetone during the follow-up period. Group 2 comprised 13 control tumors and 9 treated tumors,

and used a fractionated scheme. These animals received 5 Gy on Days 1 and 4 of treatment for 3.1 and 3.61 h, respectively, and 10 Gy on Days 2, 5 and 8 for 6.58, 7.63 and 8.42 h, respectively. After the application of the patch, TPA was again administered to all animals at the same frequency during the follow-up period. The patches were applied by direct contact with lesions and further affixed firmly with a hypoallergenic adhesive tape to prevent displacement. The follow-up period was 36 and 40 days for Groups 1 and 2, respectively. Finally, the animals were sacrificed to obtain samples of control and treated tumors for histological analysis, and samples of organs for biodistribution studies.

2.6. Dosimetric calculations

Radiation dose from ^{32}P silicone patch was estimated using the Monte Carlo MCNP5 code for simulation [17]. Cylinder was considered as the geometrical model (0.1 cm in height, 0.5 cm in diameter), with the density and chemical composition of silicon (1.2 g/cm^3 and with the chemical composition of SiC_2H_6O). Since ^{32}P was uniformly distributed in the patch, β-particle emission was considered isotropic. The region of the skin was simulated with water (density, 1 g/cm^3), and the other region was simulated with air. Electron histories (3×10^8) from the source were simulated with an uncertainty of <1%. The cutoff energy considered for electrons and photons was 10 keV. Taking into account the activity concentration of the patch (in MBq/cm^2) and the physical dose needed to administer within 7.5 mm, on average, of the maximum range of the β-particle of ^{32}P, exposition time was calculated.

The biologically effective dose (BED) of each scheme was calculated by taking into account the linear–quadratic model, as extensively explained elsewhere [18]:

$$\mathrm{BED} = D\left[1 + \frac{Dg}{(\alpha/\beta)}\right]$$

where D=dose rate×time, α/β=tissue radiosensibility and g=incomplete repair factor, with the following formula:

$$g = 2\left[\frac{\mu t - 1 + e^{-\mu t}}{(\mu t)^2}\right]$$

where t=time and μ=sublethal damage rate of repair, with the following formula:

$$\mu = \frac{\ln 2}{t_{1/2\mathrm{rep}}}$$

where $t_{1/2\ \mathrm{rep}}$=sublethal damage half repair time.

The α/β value is 10 Gy since it is the reference value for early-response tissue. μ was calculated using $t_{1/2\ \mathrm{rep}}$=1 h [19].

2.7. Histopathological studies

All samples were processed for conventional histological examination by formalin fixation and paraffin embedding. Sections were stained with hematoxylin–eosin. Photomicrographs of hematoxylin–eosin sections of all samples were taken at magnifications of ×10 and ×40 using a Canon PowerShot G5 camera (Canon, Japan).

2.8. Proliferating cell nuclear antigen staining

After deparaffinization, paraffin sections of control and treated tumors were placed in citrate buffer (10 mM, pH 6.0) and heated in a microwave oven twice for 2 min at boiling temperature for antigen retrieval. Endogenous peroxidase activity was blocked with 3% H_2O_2 in distilled water. Specimens were then incubated overnight in a humidified chamber at 4°C with primary mouse anti-proliferating cell nuclear antigen (PCNA) (1:100; DakoCytomation, Denmark) antibodies, as stated. Immunoreactivity was detected using horseradish-peroxidase-conjugated antimouse and visualized by 3,3-diaminobenzidine staining (Sigma Chemical Co.). Finally, the specimens were counterstained by immersion in hematoxylin. Light microscopy was performed on an Axiolab Karl Zeiss microscope (Karl Zeiss, Germany).

Immunostaining assessment was performed with the consensus of two observers. Overall examination of staining was carried out at a magnification of ×10, and a representative area of the specimen was then viewed at a magnification of ×100. Percentage scores based on the number of stained cells were as follows: 0 (*undetectable*), 1 (*1–20%*), 2 (*21–40%*), 3 (*41–60%*), 4 (*61–80%*) and 5 (*81–100%*). Determinations were made over at least 25 fields examined.

2.9. Statistical analysis

We tested the significance of the difference between two means as described by Evans [20] by fixing the level of significance at $P<.05$ in order to discriminate the radioactivity measurements of background and samples from the biodistribution and bioelimination studies.

Tumor growth at the end of the follow-up period, as well as PCNA staining of control and treated tumors, was compared using Student's t test, at a significance

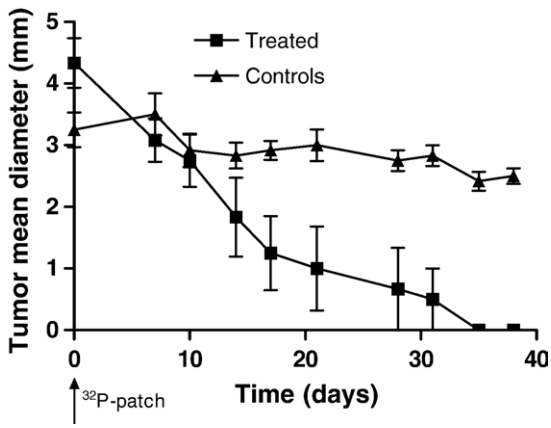

Fig. 1. Effect of a single dose of ^{32}P patch on tumor size in treated and control animals in Group 1. All tumors continued with TPA treatment after patch application.

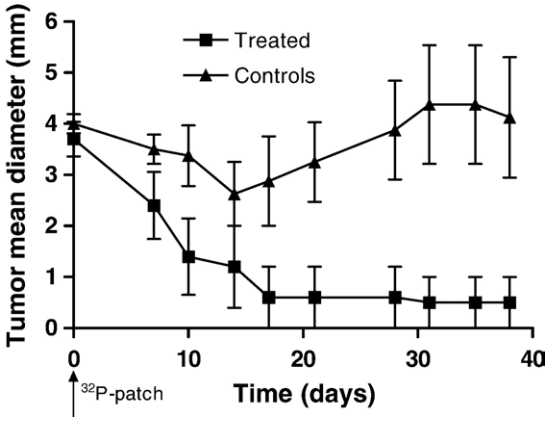

Fig. 2. Effect of a single dose of ^{32}P patch on tumor size in treated and control animals in Group 1. All tumors continued with acetone treatment after patch application.

level of $P<.05$ [21]. The results are presented as mean± standard deviation.

3. Results

Quality control of chromic phosphate ^{32}P and phosphoric acid ^{32}P used for patch production was performed in accordance with specifications (data not shown). Additionally, autoradiography performed on the patches showed that ^{32}P was uniformly distributed all over the surfaces (data not shown).

Bioelimination studies showed that neither the fecal samples nor the urine samples had ^{32}P activity when measured in the liquid scintillation counter. These results correlated with the results of the biodistribution studies since the organ samples of control or treated animals did not present ^{32}P activity. The activity values obtained from all these samples were not significantly different from those obtained from background activity.

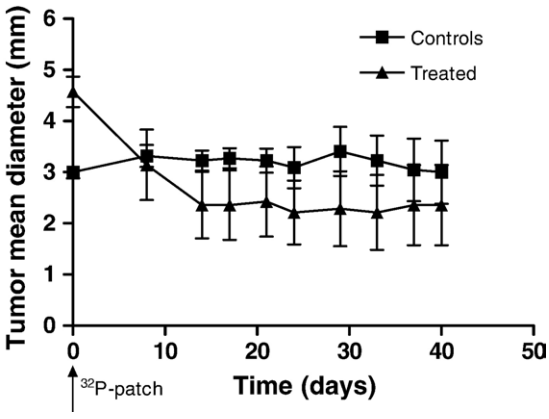

Fig. 3. Effect of a fractionated dose of ^{32}P patch on tumor size in treated and control animals in Group 1. All tumors continued with TPA treatment after patch application.

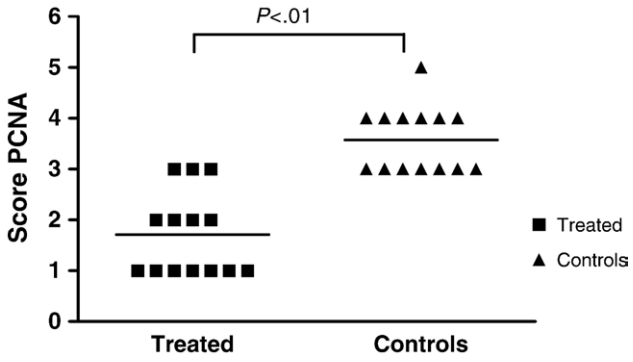

Fig. 4. PCNA staining after a single dose of ^{32}P patch in treated and control tumors in Group 1.

The two-stage carcinogenesis protocol produced both papillomas and keratoacanthomas, as described elsewhere [14–16], which were confirmed with histological analysis. After completion of therapy with the ^{32}P patch, dermatitis and skin ulceration developed in a few cases, but they gradually healed with tissue regeneration during the follow-up period. Therapeutic studies performed with the single-dose scheme (Group 1) showed a significant reduction in the diameter of treated tumors, in comparison with controls, independent of patch application received during the follow-up period: TPA ($P<.01$; Fig. 1) or acetone ($P<.05$; Fig. 2). Brachytherapy with the ^{32}P patch resulted in the macroscopic disappearance of 8 of 14 treated tumors, and the others significantly reduced their diameter independently of follow-up treatment (TPA or acetone). Histological analysis showed four total remissions and four partial remissions in the tumors. Animals in the fractionated dose scheme (Group 2) also presented a significant reduction in tumor diameter after patch application ($P<.01$), in comparison with controls (Fig. 3). In this case, brachytherapy treatment resulted in three complete remissions.

PCNA staining showed significant differences between treated and control tumors for both therapeutic schemes: single dose ($P<.01$; Fig. 4) and fractionated dose ($P<.05$; Fig. 5).

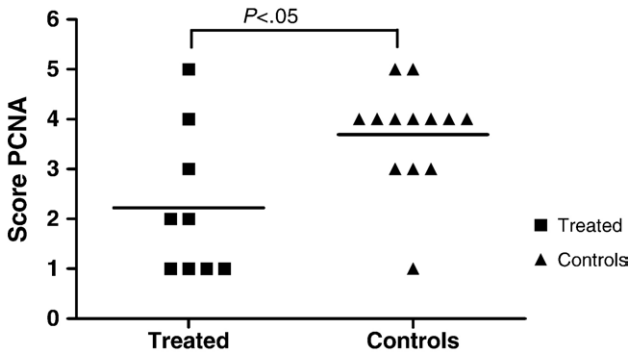

Fig. 5. PCNA staining after a fractionated dose of ^{32}P patch in treated and control tumors in Group 2.

Finally, the treatments of both therapeutic schemes were compared in terms of BED based on the linear–quadratic model. In this case, the BED for the single-dose scheme was 67.4 Gy, while that for the fractionated dose scheme was 51.1 Gy.

4. Discussion

Brachytherapy has been reported in previous years as a treatment modality for skin diseases such as skin cancer and keloid [19,22,23]. The development of patches, bandages and other devices containing β-emitters such as ^{166}Ho, ^{90}Y or ^{188}Re has demonstrated that these open sources, which behave like sealed ones and have been designed to deliver the dose uniformly, are effective in the treatment of different tumors [3–7]. The aim of our research work was to develop a patch containing ^{32}P, a β-emitter available in South America that possesses physical characteristics (E_{max}=1.7 MeV; maximum range in tissue, 7.5 mm) that justify its employment in contact brachytherapy.

Biodistribution and bioelimination studies were performed to evaluate possible mobilization of ^{32}P in the patch and later absorption through healthy or injured skin as a consequence of potential degradation processes occurring in the patch during brachytherapy treatment. Our results demonstrated that ^{32}P was not mobilized from the device, as a confirmation of stability studies performed in previous work [8].

Therapeutic effect was evaluated in an animal model of skin cancer by employing the Monte Carlo software for dosimetric calculations. Both the therapeutic schemes assayed (single and fractionated doses) resulted in tumor growth arrest. Nevertheless, the number of total and partial remissions, as well as tumor growth control, was higher for the single-dose scheme, and these results were confirmed by histopathology and PCNA staining. Taking into account that biological effects of radiation depend on dose in a linear–quadratic manner, these results correlated with the BED values obtained for the therapeutic schemes. This means that although a total physical dose of 40 Gy was selected for the studies, protracted treatment might allow the repopulation of tumors especially because of the overall time of treatment. Some authors suggest that for treatments longer than 7 days, BED diminishes by 1 Gy/day [18,19]; therefore, the BED value for fractionated dose scheme would be 50.1 Gy.

In conclusion, our results demonstrate that the ^{32}P patch fulfills the design and production specifications of a contact brachytherapy device. Bioevaluation of the radiopharmaceutical characteristics of the ^{32}P silicon patch demonstrates that this contact brachytherapy device is suitable for the treatment of skin diseases. Although the ^{32}P patch is an open source, it behaves like a sealed one, making it a safe device for the treatment of lesions in humans. Future perspectives must focus on assaying different therapeutic schemes including other radiation doses in order to improve cure rates and uses in various types of skin diseases.

References

[1] Behrens CF, King ER, Carpenter JWJ. Medicina nuclear. 1st ed. Barcelona, España: Editorial Salvat, 1972. p. 668–95, 762–78 [North American].

[2] Veall N, Vetter H. Técnicas con radioisotopos para la investigación y el diagnóstico en clínica. Protein metabolism and nutrition. Buenos Aires, Argentina: Editorial EUDEBA; 1964. p. 405–11.

[3] Lee JD, Park KK, Lee M, Kim E, Rhim KJ, Lee JT, et al. Radionuclide therapy of skin cancers and Bowen's disease using a specially designed skin patch. J Nucl Med 1997;38:697–702.

[4] Chung YL, Lee JD, Bang D, Lee JB, Park KB, Lee M. Treatment of Bowen's disease with a specially designed radioactive skin patch. Eur J Nucl Med 2000;27:842–6.

[5] Mukherjee A, Pandey U, Sarma HD, Pillai MRA, Venkatesh M. Preparation and evaluation of ^{90}Y skin patches for therapy of superficial tumors in mice. Nucl Med Commun 2002;23:243–7.

[6] Mukherjee A, Pandey U, Sarma HD, Gupta SK, Ingle AD, Pillai MRA, et al. Bioevaluation of radioactive bandages in a murine model of melanoma. Int J Radiat Biol 2003;79:839–46.

[7] Chung YL, Bang D, Lee JD, Sung YO, Park KB, Lee M. Treatment of multifocal Bowen's disease with a specially designed radioactive skin patch. Br J Dermatol 1998;139:938–9.

[8] Salgueiro MJ, Durán H, Palmieri M, Pirchio R, Nicolini J, Ughetti R, et al. Design and bioevaluation of a ^{32}P patch for brachytherapy of skin diseases. Applied Radiation and Isotopes [in press].

[9] United States Pharmacopeia-The National Formulary (USP-NF XXIX) Official monographs, NF 24, 2006, Rockville (versión español). Monograph phosphoric-^{32}P acid pp. 1113.

[10] Anghileri LJ. Second International Conference of Peaceful Uses of Atomic Energy, Génova, Italia; 1958. [15 p 1575].

[11] Anghileri LJ. ^{32}P en ratones. Influencia del tamaño de las partículas. Informe no. 158. Buenos Aires, Argentina: Comisión Nacional de Energía Atómica; 1967.

[12] Anghileri LJ, Marques R. New colloidal chromic radiophosphate (^{32}P) for local irradiation of the central nervous system. Int J Appl Radiat Isot 1967;18:97–100.

[13] United States Pharmacopeia-The National Formulary (USP-NF XXIX) Official monographs, NF 24, 2006, Rockville (versión español). Monograph phosphoric-^{32}P suspension pp. 1112.

[14] DiGiovanni J. Multistage carcinogenesis in mouse skin. Pharmac Ther 1992;54:63–128.

[15] DuBowski A, Johnston DA, Rupp T, Beltran L, Conti CJ, DiGiovanni J. Papillomas at high risk for malignant progression arising both early and late during two-stage carcinogenesis in SENCAR mice. Carcinogenesis 1998;19:1141–7.

[16] Aldaz CM, Conti CJ, Chen A, Bianchi A, Walter B, DiGiovanni J. Promoter independence as a feature of most skin papillomas in SENCAR mice. Cancer Res 1991;51:1045–50.

[17] X-5 Monte Carlo Team. MCNP — a general Monte Carlo *N*-particle transport code, Versión 5. Los Alamos (NM): Los Alamos National Laboratory; 2003.

[18] Steel G. Basic clinical radiobiology. 2nd ed. New York (USA): Oxford University Press; 1997.

[19] Kal HB, Veen RE. Biologically effective doses of postoperative radiotherapy in the prevention of keloids. Strahlenther Onkol 2005;181:717–23.

[20] Evans RD. The atomic nucleus. New York (USA): Ed McGraw-Hill Book Co.; 1955.

[21] Sokal RR, Rohlf FJ. Biometry. San Francisco, CA: Freeman and Co; 1981.

[22] Rudolph R, Zelac D. Squamous cell carcinoma of the skin. Plast Reconstr Surg 2004;114:82e–94e.

[23] Locke J, Karimpour S, Young G, Lockett MA, Perez CA. Radiotherapy for epithelial skin cancer. Int J Radiat Oncol Biol Phys 2001;51:748–55.