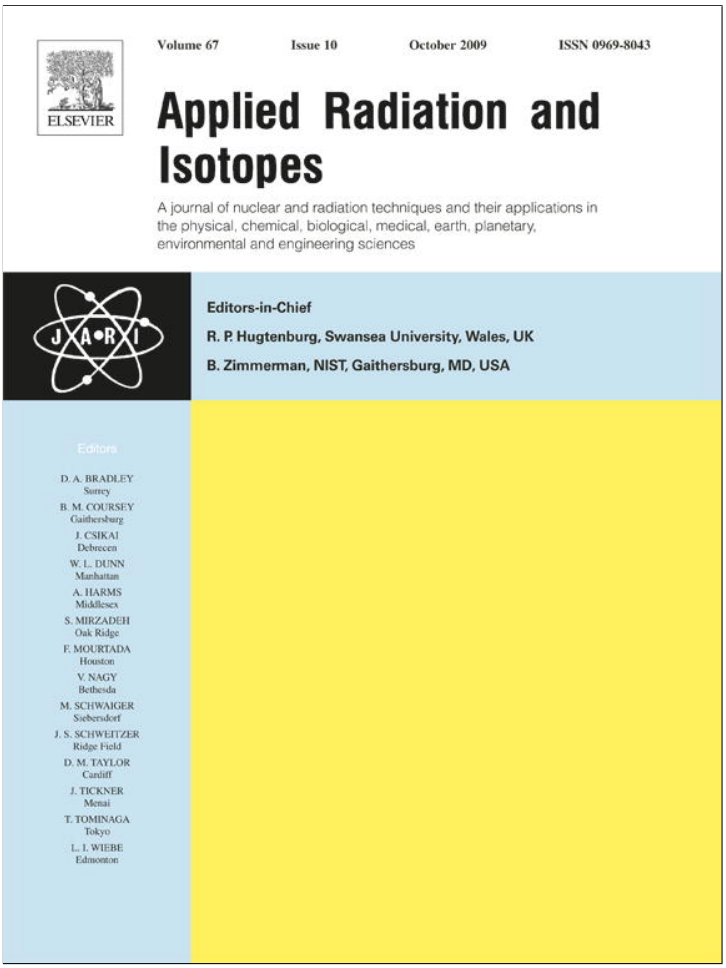

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# Biological effects of brachytherapy using a $^{32}$P-patch on the skin of Sencar mice ☆

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ABSTRACT

In recent years, specially designed patches containing beta emitters have been developed for contact brachytherapy of skin lesions. The aim of the present work was to evaluate the biological effects of the $^{32}$P-patch on the skin of Sencar mice as a result of a brachytherapy treatment. For this purpose, a $^{32}$P-patch was prepared with Chromic $^{32}$P-phosphate and silicone and the classical model of two-stage skin carcinogenesis was reproduced in Sencar mice. Animals were divided in six groups. Four groups received the contact brachytherapy treatments using a scheme of a single session of 40 and 60 Gy (SD40 and SD60) and a scheme of two sessions of 40 and 60 Gy each (FD40 and FD60). The other two groups were used as controls of the single (CSD) and the fractionated (CFD) treatments. Radiation doses were estimated with equations derived from the MIRD DOSE scheme, and biologically effective doses (BED) were calculated according to equations derived from the linear-quadratic model. The endpoint to evaluate the treatments effects was tumor size after a follow-up period of 44 days. Finally, animals were sacrificed in order to get samples of all tumors for histological analysis and PCNA staining. Erythema, dermatitis and skin ulceration developed in almost all treated animals, but they gradually healed with regeneration of tissue during the follow-up period. Radiation effects on the skin of SD40, SD60, FD40 and FD60 showed a significant reduction of the tumor size with regard to controls, independently of the scheme and the radiation dose considered. PCNA staining scores of control groups were higher than for treated groups, independently of the scheme and the radiation dose considered. This radioactive $^{32}$P-silicone-patch which is easy to prepare and use in the treatment of skin diseases, seems promising as a radioactive device for clinical use.



## 1. Introduction

Treatment modalities for skin diseases such as melanoma, non-melanoma skin cancer and keloids are surgical excision, radiotherapy and chemotherapy (Diepgen and Mahler, 2002; Kal and Veen, 2005; Locke et al., 2001). Each therapeutic mode has its own advantages and disadvantages, but brachytherapy has been reported as a promissory effective alternative. In recent years, specially designed patches containing beta emitters such as $^{90}$Y, $^{188}$Re and $^{166}$Ho have been developed for contact brachytherapy of skin lesions (Lee et al., 1997; Chung et al., 1998, 2000; Mukherjee et al., 2002, 2003; Pandey et al., 2008). The experience in treating ophthalmologic diseases was the basis for the design of such brachytherapy treatments, employing both gamma and beta emitters. These kinds of devices fulfil the advantages of localized brachytherapy treatments and were designed according to the shape of the lesions with variable activity of the radionuclide with regard to the different types of treatment schemes applied. Since the availability of these beta emitters, their cost and their physical characteristics are of importance when considering the possibility for their employment for treatment, we designed a $^{32}$P-patch, as this radioisotope is commercially available in Southamerica. In previous works we evaluated the $^{32}$P-patch with regard to its radiopharmaceutical characteristics demonstrating the absence of radioactivity leakage in vitro and in vivo as well as the

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**Table 1**
Dosimetric calculations results.

| Treatment scheme (Gy) | Sessions (#) | Interval between sessions | | *As* $^{32}$P-patch | | $t$ (h) | $g$ | BED per session (Gy) | Total BED per treatment (Gy) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | ($\mu$Ci/cm$^2$) | (kBq/cm$^2$) | | | | |
| Single dose | | | | | | | | | |
| $\bar{D} = 40$ | 1 | – | | 263 | 7.1 | 15.5 | 0.169 | 67.0 | 67.0 |
| $\bar{D} = 60$ | 1 | – | | 263 | 7.1 | 23.0 | 0.118 | 102.5 | 102.5 |
| Fractionated dose | | | | | | | | | |
| $\bar{D} = 40$ | 2 | 1 week | #1 | 341 | 9.2 | 12.0 | 0.212 | 73.9 | 141.7 |
| | | | #2 | 274 | 7.4 | 15.0 | 0.174 | 67.8 | |
| $\bar{D} = 60$ | 2 | 1 week | #3 | 341 | 9.2 | 18.0 | 0.147 | 112.9 | 216.1 |
| | | | #4 | 274 | 7.4 | 22.5 | 0.120 | 103.2 | |

homogeneous distribution of the radionuclide in the patch (Salgueiro et al., 2008a, b). The aim of the present work is to evaluate the biological effects on the skin of Sencar mice as a result of a brachytherapy treatment.

## 2. Materials and methods

### 2.1. $^{32}$P-patch production and control

The patches were designed taking into account the size and the shape of the tumors and were not shielded.

For the production of patches, 10 mCi of $^{32}$P were purchased as Phosphoric-$^{32}$P-acid (CNEA, Buenos Aires, Argentina). The patches were prepared as previously described (Salgueiro et al., 2008a, b) using Chromic $^{32}$P-phosphate (30–70 nm) and silicone (Silastic® J-White 80, Dow Corning, The Dow Chemical Company and Corning, Inc., Michigan, USA). The Chromic $^{32}$P-phosphate was washed with isopropilic alcohol (Anhedra, Buenos Aires, Argentina), centrifuged to 2000 rpm during 10 min and finally dried at 80 °C in order to obtain a fine powder. Afterwards, the Chromic $^{32}$P-phosphate powder was mixed with the silicone and dried at room temperature for 4 h.

The activity concentration of patches was measured as previously described (Salgueiro et al., 2008a, b). Briefly, a sample of the patches was dissolved with hyamine hydroxide (MP Biomedicals, LLC, Ohio, USA) at room temperature overnight. An aliquot of 3 mL of the obtained solution was added to a vial containing a complete phase combining system for liquid scintillation counting (PCS® Amersham Biosciences, Piscataway, NJ, USA). The activity measurement was performed in a liquid scintillation counter (Wallac 1410 Liquid Scintillation Counter, Pharmacia Wallac OY, Turku, Finland) according to the $^{32}$P protocol, with a relative error $<1\%$. Results were expressed as kBq/cm$^2$ (or $\mu$Ci/cm$^2$) taking into account the weight of the sample and the density of the patches.

### 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". The animals were fed *ad libitum* with balanced standard food and maintained in stainless steel cages with cycles of 12/12 h light/darkness and controlled room temperature.

The classical model of mouse two-stage skin carcinogenesis (DiGiovanni, 1992; DuBowski et al., 1998; Aldaz et al., 1991) was reproduced in female mice of the Sencar strain (CNEA, Buenos Aires, Argentina) of 7–9 weeks old. Briefly, the backs of the mice were shaven with surgical clippers at least three days prior to treatment and only mice in the resting phase of the hair cycle were used. Tumors were initiated in mice by a single topical application of 20 nmol of DMBA (7,12-dimethylbenz[a]anthracene, Sigma Chemical Company, St. Louis, MO, USA) in 0.2 ml of acetone (Anhedra, Buenos Aires, Argentina). Afterwards, they were topically treated twice a week with 12-O-tetradecanoyl-phorbol-13-acetate (TPA, Sigma Chemical Company, St. Louis, MO,USA) 3.25 nmol in 0.2 ml of acetone, starting 10 days after initiation and over a period of 20 weeks. Control animals were submitted to the same treatment described but acetone was used instead of DMBA and TPA.

### 2.3. Radiation treatments

Contact brachytherapy treatments were performed employing a single and a fractionated-like dose schemes. For the single dose schemes unique sessions of 40 and 60 Gy were used. For the fractionated dose schemes two sessions of 40 and 60 Gy each, were administered with an interval of a week between them (for clarification, see Table 1).

### 2.4. Biological effects of radiation on skin

We used 65 animals that were randomized in two main groups: controls without carcinogenic treatment (C, $n = 10$); and treated with carcinogen ($n = 55$). Animals treated with carcinogen were afterwards divided in the following groups: controls without brachytherapy treatment (CWB, $n = 7$); treated group with $^{32}$P-patch in a single dose scheme of 40 Gy (SD40, $n = 10$); treated group with $^{32}$P-patch in a single dose scheme of 60 Gy (SD60, $n = 9$); controls treated with a decayed $^{32}$P-patch mimicking the single dose scheme (CSD, $n = 6$); treated group with $^{32}$P-patch in a fractionated-like dose scheme of two sessions of 40 Gy each (FD40, $n = 9$); treated group with $^{32}$P-patch in a fractionated-like dose scheme of two sessions of 60 Gy each (FD60, $n = 10$); and controls treated with a decayed $^{32}$P-patch mimicking the fractionated-like dose scheme (CFD, $n = 4$). Initial tumor sizes were recorded for all animals. Afterwards, each patch was placed directly in contact with the lesions or on the normal skin selected to be treated, and firmly affixed with a hypoallergenic adhesive tape to prevent displacement. The time of exposition for each radiation dose was determined according to dosimetric calculations described below. After 10 days of the end of the treatments, TPA was again administrated to all animals with the same frequency during the follow-up period except for C group which received acetone. The follow-up period was of 44 days for all groups during which tumor size was recorded as the endpoint. Finally, animals were sacrificed in order to get samples of all tumors for histological analysis and PCNA staining.

### 2.5. Dosimetric calculations

Eq. (1) describes the estimation of the average absorbed dose in a tumor of thickness equivalent to the maximum range of $^{32}P$ in the absorber considered. This equation derives from the MIRD DOSE scheme (MIRD Committee, 1988). Although there is no activity accumulated *in vivo* during this external treatment, we assumed that the effect of the radiation dose is equivalent to the effect of the internally accumulated activity in order to use this equation.

$$\bar{D} = 2.13 \frac{\tilde{A}}{m} \sum_i ni\bar{E}\Phi \,(\mathrm{cGy}) \tag{1}$$

where:

(a) 2.13 is a constant $k$ to convent units to express the result in cGy.

$$k = 1.602 \times 10^{-6} \frac{\mathrm{erg}}{\mathrm{MeV}} 3.7 \times 10^{4} \frac{dis}{\mathrm{s\,\mu Ci}} 10^{-2} \frac{\mathrm{cGy}\,g}{\mathrm{erg}} 3600 \frac{\mathrm{h}}{\mathrm{s}}$$

(b) $\tilde{A}/m$ represents the accumulated activity per unit mass (μCi h/g), which can be calculated as described in

$$\frac{\tilde{A}}{m} = \frac{As(1 - e^{\ln 2t/t_{1/2}})}{\ln 2/t_{1/2} R_\beta \delta} \tag{2}$$

where $As$ is the activity per unit of surface expressed in (μCi/cm$^2$), $R_\beta$ the maximum range in tissue expressed in cm (for $^{32}P$ is 0.75 cm), $\delta$ the density of tissue simulated as water 1 g/cm$^3$, $t$ the time of exposition expressed in hours (h), and $t_{1/2}$ the half life of the radionuclide (for $^{32}P$ is 14.3 days or 343.2 h).

(c) $\sum_i ni\bar{E}i$ represents the probability of the transition and its energy associated according to the disintegration mechanism of the radionuclide. In the case of $^{32}P$, $\bar{E}i$ is 0.7 MeV.

(d) $\Phi$ represents the fraction absorted which is 0.5 since the source is plane and only one face is in contact with skin.

The biologically effective dose (BED) of each scheme was calculated taking into account the linear-quadratic model (Eq. (3)) as extensively explained elsewhere (Steel, 1997).

$$BED = D[1 + Dg/(\alpha/\beta)] \tag{3}$$

where $D$ is the dose rate × time, $\alpha/\beta$ the tissue radiosensibility considered as 10 Gy (2), and $g$ the incomplete repair factor calculated as

$$g = 2\left[\frac{\mu t - 1 + e^{-\mu t}}{(\mu t)^2}\right] \tag{4}$$

where $t$ is the time and $\mu$ the sublethal damage rate of repair calculated as

$$\mu = \frac{\ln}{t_{1/2rep}} \tag{5}$$

where $t_{1/2rep}$ is the sublethal damage half repair time considered as 1 h (Kal and Veen, 2005).

### 2.6. Histological studies

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

### 2.7. PCNA staining

Paraffin sections of control and treated tumors after deparaffinization 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-PCNA (1: 100, DakoCytomation, Glostrup, Denmark) antibodies, as stated. Immunoreactivity was detected by using horseradish peroxidase-conjugated anti-mouse and visualized by 3,3-diaminobenzidine staining (Sigma Chemical Co., St. Louis, MO, USA). Finally, the specimens were counter-stained by immersion in haematoxylin. Light microscopy was performed on an Axiolab Karl Zeiss microscope (Gottingen, Germany).

The immunostaining assessment was performed by consensus of two independent observers. An overall examination of staining was carried out at 10 × magnification, and representative area of the specimen was then view at 100 × magnification. A percentage score based on the number of stained cells assigned as: 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. Photomicrographs of PCNA staining sections of all samples were taken at 10 × and 40 × magnification using a Canon PowerShot G5 camera (Cannon, Tokyo, Japan).

### 2.8. Statistical analysis

ANOVA test or the Kruskall–Wallis test followed by the Student–Newmans–Keuls test or by the Dunn test, respectively, were used to compare tumor growth and PCNA staining scores. The level of significance was $p < 0.05$ in all cases (Sokal and Rohlf, 1981). The results are shown as mean ± standard deviation.

## 3. Results

The results of dosimetric calculations as well as a brief summary of the treatment protocols is shown in Table 1. In order to determine the time of exposition of the $^{32}P$-patches to each group according to the physical dose ($\bar{D}$) selected in the protocol, the accumulated activity per unit mass was calculated from Eq. (1). Then, the activity per surface unit of the $^{32}P$-patches

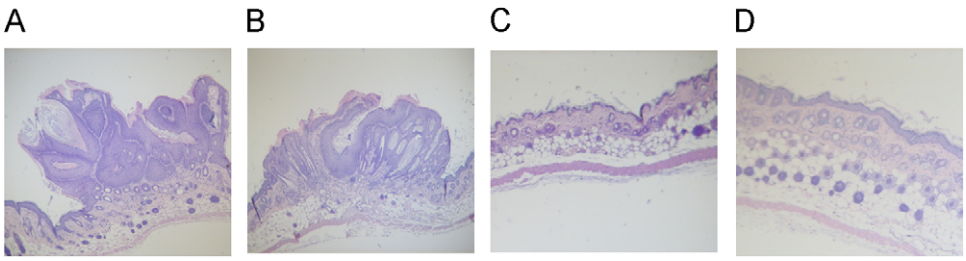


**Fig. 1.** Hematoxilin–eosin staining photographs. Panel A: control papilloma. Panel B: control keratoacanthoma. Panel C: normal skin irradiated with $^{32}P$-patch. The photograph shows the hypotrophy of the skin as well as the lost of hair follicles as a consequence of radiation, with no alteration of epidermis and dermis. Panel D: normal skin of Sencar mice.

measured by liquid scintillation counting was applied in Eq. (2) to finally obtain the time of exposition. Additionally, Table 1 also shows the calculated $g$ factor as described in Eq. (4) and the BED calculated according to the linear-quadratic model as described in Eq. (3).

The two-stage carcinogenesis protocol produced both papillomas and keratoacanthomas as describe elsewhere (DiGiovanni, 1992), which were confirmed with histological analysis (Fig. 1, panels A and B). After completion of therapy with the $^{32}$P-patch, erythema, dermatitis and skin ulceration developed where the patches were applied, but they gradually healed with regeneration of tissue during the follow-up period. Histological findings of the skin of animals in group C, showed the hypotrophy of the skin as a consequence of radiation, with no alteration of epidermis and dermis (Fig. 1, panel C). The lost of hair follicles shows the limits of the irradiated zone of the skin (for comparative purposes a sample of normal skin of Sencar mice was included in Fig. 1, panel D).

Radiation effects on the skin of the other groups showed a significant reduction of the tumor size, expressed as diameter in millimeters, of treated tumors with regard to controls, independently of the scheme and the radiation dose considered. Fig. 2 (panel A) shows the results for tumor size of groups in the single dose scheme, with significant tumor size reduction ($p<0.05$) in SD40 and SD60 with regard to control groups CWB, CSD. No significant differences in tumor size were found between SD40 and SD60 or between control groups. Brachytherapy with the $^{32}$P-patch resulted in the macroscopic disappearance of 3 of the 10 treated tumors with 40 Gy in a single dose and of 5 of the 9 treated tumors with 60 Gy. Histological analysis showed 5 total remissions (1 from SD40 and 4 from SD60) and 3 partial remissions (2 from the SD40 and 1 from the SD60) of the disappeared tumors. Animals in the fractionated dose scheme groups also presented a significant reduction in tumor diameter after patch application in comparison with controls. Fig. 2 (panel B) shows that tumor size of FD40 and FD60 significantly differed from control groups ($p<0.05$) but no differences were found within treated groups and neither within controls. In this case, brachytherapy treatment resulted in the macroscopic disappearance of 7 of the 9 treated tumors with a total dose of 40 Gy in fractionated doses and of 8 of the 10 treated tumors with a total dose of 60 Gy. Histological analysis showed 9 total remissions (4 from the FD40 and 5 from the FD60) and 6 partial remissions (3 from the FD40 and 3 from the FD60) of the disappeared tumors. Fig. 3 shows an example of a partial (panel A) and a complete regression (panel B) of treated tumors, where recovery of some hair follicles can be observed as well as skin characteristics.

PCNA staining scores of groups in the single dose scheme were higher for control than for treated tumors, showing significant differences for SD40 and SD60 with regard to CSD and CWB, ($p<0.01$, Fig. 4, panel A). The same pattern was observed for groups of the fractionated dose scheme, with significant differences for FD40 and FD60 with regard to CFD and CWB ($p<0.01$, Fig. 4, panel B). On the other hand, we did not found differences in PCNA scores between control groups (CSD, CWB, CFD and CWB) neither between treated groups (SD40, SD60, FD40

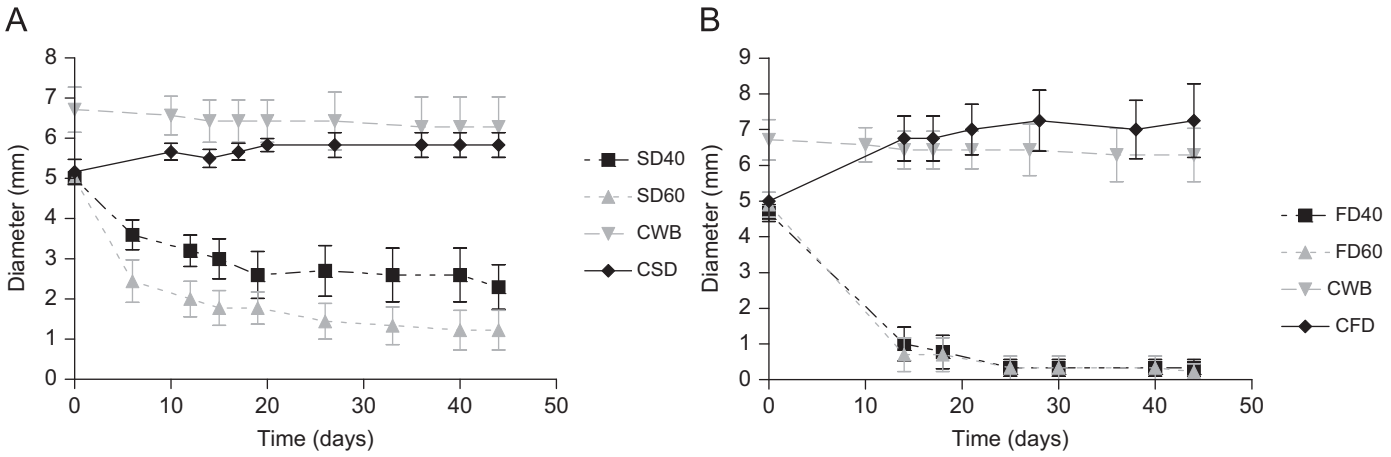


**Fig. 2.** Tumor size (diameter in mm) evolution after $^{32}$P-patch application. Panel A shows the results for groups in the single dose scheme. Panel B shows the results for groups in the fractionated dose scheme.

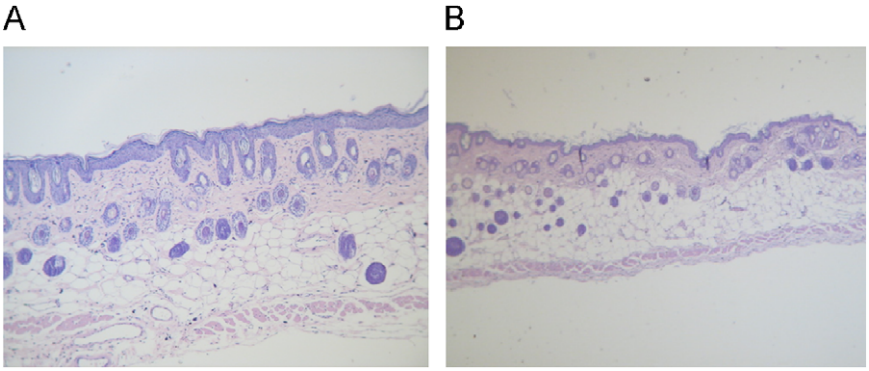


**Fig. 3.** Hematoxilin–eosin staining photographs. Panel A: partial regression of a treated tumor showing the recovery of the hair follicles after brachytherapy treatment. Panel B: complete regression of a treated tumor showing the recovery of the skin with no alterations in dermis and epidermis.

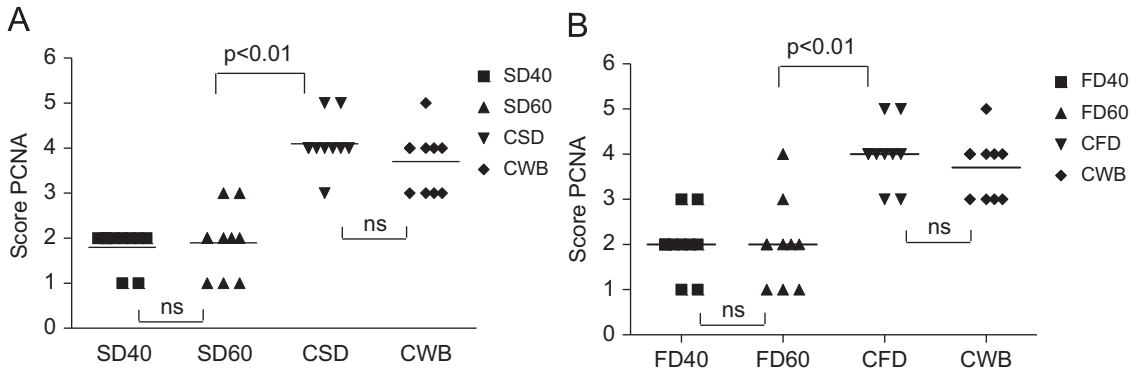


**Fig. 4.** PCNA scores of tumors. Panel A shows the results for groups in the single dose scheme. Panel B shows the results for groups in the fractionated dose scheme.

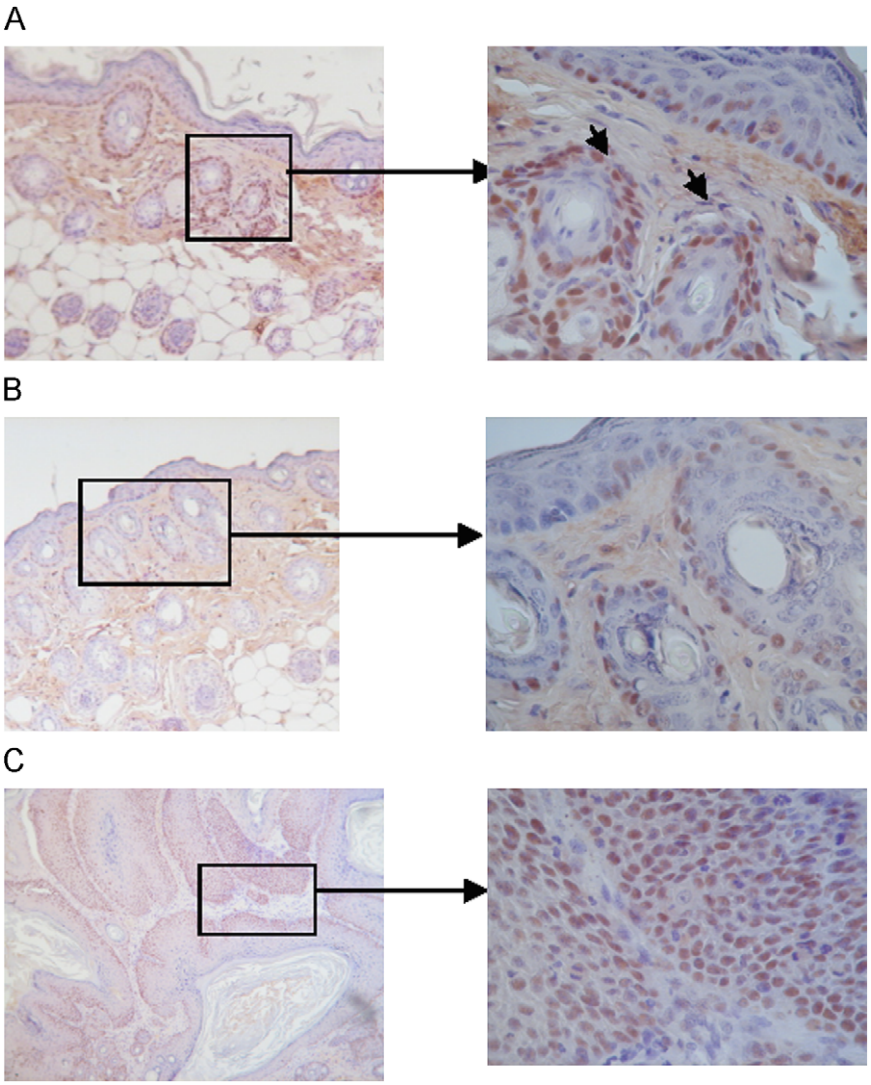


**Fig. 5.** PCNA staining photographs. Panel A: normal histological appearance of the skin in a treated, completely regressive papilloma. In detail normal proliferative activity restricted to basal layer. Arrows indicate pillar follicles entirely regenerated. Panel B: normal Sencar skin appearance, with normal epithelial layers and pillar follicles showing moderate PCNA expression. Panel C: control tumor. Acanthosis, papilomatosis and nuclear anomalies in induced papilloma. Intense positivity of PCNA all skin layers.

and FD60). Fig. 5 shows examples of PCNA staining results of a complete regression (panel A), a normal Sencar skin for comparative purposes (panel B) and a control papilloma (panel C) at 10× and 40× magnification.

## 4. Discussion

Brachytherapy has been reported as a valid alternative treatment modality for these diseases, in the last years (Kal and

Veen, 2005; Locke et al., 2001; Lee et al., 1997; Chung et al., 1998, 2000; Mukherjee et al., 2002, 2003; Rudolph and Zelac, 2004). In this work, we used $^{32}$P which emits $\beta^-$ radiation ($E_{max} = 1.7$ MeV) and it is produced in nuclear reactor. $^{32}$P has a half life of 14.3 days and a maximum range in tissue of 7.5 mm. This beta emitter, extensively available in our country and South America, has similar characteristics to those used by other groups with the additional advantages of its long half life, low cost and the absence of gamma radiation. Altogether, these characteristics made the $^{32}$P-patch a valid option as a radiopharmaceutical to be dispensed from a centralized radiopharmacy for this kind of treatment with minimal risk of irradiation for physicians.

In order to evaluate the biological effects of the $^{32}$P-patch application on the skin of Sencar mice, we selected two therapeutic schemes employing physical doses of 40 and 60 Gy given in a single dose (single dose scheme) or two equal doses of 40 or 60 Gy with an interval between them of a week (fractionated dose scheme). Doses were calculated with a formula derived from MIRD DOSE scheme where the physical calculated dose is the average absorbed dose in a tumor of thickness equivalent to the maximum range of $^{32}$P in water and taking into account that the radiation source is plane and it is in contact with the skin. All treatments were administered at the lower limit of a medium dose rate (MDR) treatment, which for comparative purposes were expressed in terms of BED, calculated according to the linear-quadratic model. In this way, it is interesting to note that BED values are higher than radiation dose values estimated using the MIRD DOSE equations for both schemes as it is shown in Table 1. All these differences may be explained by differences in the $g$ factor of the BED formula, which takes into account tissue reparation as a consequence of the time elapsed during the treatments. In this case, repopulation was a factor not taken into account since the overall time of all treatments was less than a week or equivalent to a week (Kal and Veen, 2005; Steel, 1997). According to these calculations, radiation dose from $^{32}$P-patch application was higher for both fractionated scheme groups than for single dose groups and biological effects on skin related to these doses were observed as explained below.

Sencar mice that were not submitted to the carcinogenic protocol but received radiation from the $^{32}$P-patch application on skin, developed erythema, dermatitis and ulceration of skin which healed during the follow-up period. These observations correlated with the hypotrophy of the skin and the lost of hair follicles that appeared at the histological level. On the other hand, mice that developed papillomas and keratoacanthomas as a consequence of the carcinogenic protocol and treated with the $^{32}$P-patch showed tumor growth arrest as the principal biological effect independently of the therapeutic scheme assayed. Nevertheless, the higher the total dose, the higher the number of remissions observed. Indeed, the number of total and partial remissions as well as tumor growth control in terms of tumor size at the end of the follow-up period, was higher for the fractionated dose scheme and these results were confirmed by the histopathology and PCNA staining.

## 5. Conclusion

Our results show that the $^{32}$P-patch designed as a contact brachytherapy device is easy to prepare and use in the treatment of skin diseases. Biological effects of radiation from $^{32}$P-patch in Sencar mice skin showed promissory results in order to consider it as a valid therapeutic modality for clinical use in the treatment of skin diseases. Future perspectives will be focused in assaying different therapeutic schemes including other radiation doses in order to improve cure rates, as well as its use in other types of skin diseases.

## References


Aldaz, C.M., Conti, C.J., Chen, A., Bianchi, A., Walter, B., DiGiovanni, J., 1991. Promoter independence as a feature of most skin papillomas in SENCAR mice. Cancer Res. 51, 1045–1050.

Chung, Y.L., Bang, D., Lee, J.D., Sung, Y.O., Park, K.B., Lee, M., 1998. Treatment of multifocal Bowen's disease with a specially designed radioactive skin patch. Br. J. Dermatol. 139, 938–939.

Chung, Y.L., Lee, J.D., Bang, D., Lee, J.B., Park, K.B., Lee, M., 2000. Treatment of Bowen's disease with a specially designed radioactive skin patch. Eur. J. Nucl. Med. 27, 842–846.

Diepgen, T.L., Mahler, V., 2002. The epidemiology of skin cancer. Br. J. Dermatol. 146, 61–66.

DiGiovanni, J., 1992. Multistage carcinogenesis in mouse skin. Pharm. Ther. 54, 63–128.

DuBowski, A., Johnston, D.A., Rupp, T., Beltran, L., Conti, C.J., DiGiovanni, J., 1998. Papillomas at high risk for malignant progression arising both early and late during two-stage carcinogenesis in SENCAR mice. Carcinogenesis 19, 1141–1147.

Kal, H.B., Veen, R.E., 2005. Biologically effective doses of postoperative radiotherapy in the prevention of keloids. Strahlenther. Onkol. 181, 717–723.

Lee, J.D., Park, K.K., Lee, M., Kim, E., Rhim, K.J., Lee, J.T., Yoo, H.S., Kim, Y.M., Park, K.B., Kim, J.R., 1997. Radionuclide therapy of skin cancers and Bowen's disease using a specially designed skin patch. J. Nucl. Med. 38, 697–702.

Locke, J., Karimpour, S., Young, G., Lockett, M.A., Perez, C.A., 2001. Radiotherapy for epithelial skin cancer. Int. J. Radiat. Oncol. Biol. Phys. 51, 748–755.

MIRD Committee, 1988. MIRD PRIMER for absorbed dose calculations. The Society of Nuclear Medicine, Inc., NY, USA.

Mukherjee, A., Pandey, U., Sarma, H.D., Pillai, M.R.A., Venkatesh, M., 2002. Preparation and evaluation of $^{90}$Y skin patches for therapy of superficial tumors in mice. Nucl. Med. Commun. 23, 243–247.

Mukherjee, A., Pandey, U., Sarma, H.D., Gupta, S.K., Ingle, A.D., Pillai, M.R.A., Venkatesh, M., 2003. Bioevaluation of radioactive bandages in a murine model of melanoma. Int. J. Radiat. Biol. 79, 839–846.

Pandey, U., Saxena, S.K., Sarma, H.D., Tandon, P., Ramu Ram, P.T., Samuel, G., Dash, A., Venkatesh, M., 2008. Bioevaluation studies of 32P incorporated mould brachytherapy sources for potential application in treatment of superficial tumors. Nucl. Med. Commun. 29, 717–723.

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

Salgueiro, J., Durán, H., Palmieri, M., Pirchio, R., Nicolini, J., Ughetti, R., Papparella, M.L., Casale, G., Zubillaga, M., 2008a. Design and bioevaluation of a 32P-patch for brachytherapy of skin diseases. Appl. Radiat. Isot. 66, 303–309.

Salgueiro, J., Durán, H., Palmieri, M., Pirchio, R., Medina, V., Ughetti, R., Croci, M., Nicolini, J., Zubillaga, M., 2008b. Bioevaluation of a 32P-patch, designed for the treatment of skin diseases. Nucl. Med. Biol. 35, 233–237.

Sokal, R.R., Rohlf, F.J., 1981. Biometry. Freeman & Company.

Steel, G., 1997. Basic Clinical Radiobiology, second ed. Oxford University Press, NY, USA.