**Brachytherapy on restenosis.**

**32 P radioisotope in animal model.**

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**INTRODUCTION**

Despite a notorious decline in age-adjusted death rates for cardiovascular disease, coronary artery illness still remains as the major cause of mortality after the 40th in men and the 60th in women. More than 25% of death in persons over the age of 35 are due to coronary disease. In about 50% of men and 30% of women the first manifestation of the disease will be an acute myocardial infarction and 10% sudden cardiac death. At present, it is estimated that in Argentina about 100,000-115,000 people per year suffer a myocardial acute infarct as first manifestation of coronary illness.

Angioplasty has an important and well established site in the treatment of the coronary illness. All over the world a million of interventions per year are performed, and throughout the last ten years an increase of about 20% per year in the number of interventions for re-vascularization was registered.

Restenosis (loss of lumen cross-sectional area) represents the principal complication of angioplasty for myocardial re-vascularization. About a 35-40% of arteries treated with angioplasty present restenosis within the first sixs month after the intervention (1, 2, 3), with the concomittal need of re-interventions, re-hospitalizations, need of by-pass surgery, work discontinuity and the significant cost for the public health system. Following a coronary intervention such angioplasty, the artery may remain open, may re-close during the early phase due to thrombosis or dissection or a combination of both, or else may close in the late phase due to a process of wound healing called restenosis. The understanding to the relative contribution of these processes is important in planning methods to control and prevention. With availability of modern techniques, the acute closure of arteries following angioplasty is relatively uncommon, and restenosis remains the major limitation of the technique. Although the size of the lumen decreases following almost all interventions, restenosis is defined as a compromise of the lumen which is felt to approach an obstruction that could result in hemodynamic alteration. The components of restenosis are: 1) the elastic recoil that occurs promptly after the overstretch of the artery; this has been quantified as approximately 50% of the cross-sectional area. 2) the intimal proliferation resulting in new tissue growth occupying the cracks and tears in the vessel wall; sometimes this growing produces a severe re-obstruction of the artery; this process begins a few days after angioplasty and continues for weeks or months; 3) the entire artery may become contracted so that the external elastic lamina occupies a smaller circumference. Damage to the artery wall and its surrounding tissue results in transformation of cells in the media and in the adventitia, so that they are signaled to proliferate, migrate, and elaborate extracelular matrix. These same cells may then undergo phenotype transformation and become cells capable of causing constrictiveness.

In order to control the process of restenosis, a number of drugs were tested as anti-restenostic: anticoagulants, aspirin, antispasmodics and lipid lowering agents (4, 5, 6). Despite results from these various trials, no drug has yet generally used for restenosis prevention. Recently, experimental researches were focused on the delivery of drugs locally administered, principally the transfer of antisense oligonucleotides directly into the vessels wall to signal the cells to stop replicating and block neointimal formation after a vascular intevention (7).

Different animal models have been utilized to restenosis study. One is the pig coronary artery balloon overstretched injury model (8). In this model, juvenile female pigs weighing 25-30 kg receive coronary artery angioplasty with oversized balloon catheters under general anesthesia using standard cardiac catheterization techniques from a femoral artery approach (8, 9). The acute response to balloon overstretch in the pig coronary arteries is dissection of the media with dehiscence of the medial ends resulting in a crater-like defect of the arterial wall. A treatment which decreases the intima lesion size but increases luminal and/or vessel wall size would be favored for potential anti-restenotic effects. Such effects have been studied in this model with systematically administered compounds as antioxidants, growth inhibitors, somatostatin analogs as well as endovascular brachytherapy using beta or gamma emitters (9, 10). Some limitations of this model ser due to repair process. In coronary injury model using normal coronaries in young and growing animals this process is more rapid and different from the response of mature and aged humans with atherosclerotic plates.

Other experimental model is the induced atherosclerosis in iliac or femoral rabbits vessels (11, 12).
Pharmacological and radiation effects were evaluated using this model. Another experimental designs were performed using rats carotids atherosclerotic model (13, 14).

Other method used to study the restenosis is the stent implantation. Neointimal lesions are formed after implantation of metallic stent protheses and for this reason this procedure is used by some researchers as a restenosis model in various animal species and arteries (15).

Finally, different doses of ionizing radiation were utilized for therapy of some non-neoplastic diseases as restenosis on the basis of the inhibitory effect on cellular proliferation that this radiation exert (16, 17, 18, 19). The potential role of radiation in preventing restenosis following angioplasty or stent placement was first discussed by Dawson (20). In experimental studies in which intravascular irradiation to prevent restenosis was utilized, changes in different layers of arteries were observed and attributed to differences in source types utilized, energies, radiation doses and doses rate. However, there is no consensus on the nature of intravascular brachytherapy. The better alternative seems to be a high dose rate from removable beta or gamma sources (32P, 90Y, 90Sr / 90Y, 192Ir), or a low dose rate from a radioactive stent (32P) (21, 22). No available radioisotope has shown to be ideal. Each technique and isotope present its own difficulties. Nevertheless, endoluminal brachytherapy seems to be a promising technique for the prevention of restenosis after angioplasty (23, 24).

Despite the number on the experimental studies and trials, there is no yet consensus in many aspects of the restenosis prevention.

The first step in this work was to induce the experimental model in rabbits. Afterwards, by means of the balloon methodology brachytherapy experiments were carried out to evaluate the biological effect on different layers of arteries, with different doses using a beta emitter particle radioisotope (32P).

MATERIAL AND METHODS

Animals: A total of 24 male New Zeland rabbits of 3-4 months were kept single in cages with water ad-libitum, at 16-20 °C and a minimum floor area of 4000 sq cm per animal and a height of 45 cm (conditions recommended by Institute of Animal Technology, UK).

Atherosclerosis induction: the experimental atherosclerosis lesions were induced in femoral arteries of 21 rabbits by mean of surgery intervention and administration of a hypercholesterolemic diet during 30 days. Under general anesthesia with ketamine (35 mg/Kg BW) and xylazine (5 mg/Kg BW), the femoral arteries were surgically exposed in each animal; the induced lesion mimic the sclerotic plates that illness produce in humans. Three rabbits were healthy control (six arteries).

Angioplasties were performed on femoral arteries when rabbits attained 3,0-3,5 Kg. Body weight with balloon methodology and the interventions were controled by fluoroscopy to localize the atherosclerotic lesions.

Radiation system: Studies were done with two different doses with a source of beta ionizing radiation (32P: half life, 14.3 days; maximal energy, 1.71Mev/dis) administered with ballon dilatation catheter. The inflate balloon dimentions were 20 mm in length and 2.5 mm in diameter. Exposed femoral arteries received brachytherapy with 32P during four minutes. The operations were performed with the adequate radioprotection criteria. The total of experiments included: six normal control arteries without intervention (group A); the atherosclerotic arteries randomly received: 12, 10 Gy on the internal wall (group C); 12, 20 Gy on the internal wall (group D); 10, non received brachytherapy (Group B). The delivery catheter without the radioactive source was placed in the control injured arteries (Group B) in the same manner as for the treated goups (C and D). All radioactive waste generated during the practices were disposed in accord with the guides of Nuclear Regulatory Autority of Argentina. Regarding safety mesures in the staff, excersices of stent implantation with non-radioactive sources were practiced in order to achieve non-risk technique for the methodology.

Analyzed parameters: Animals were sacrificed by overdose of phenobarbitone at 4 days post-brachytherapy; vasodilatation, edema, hemorrhages, mononuclear infiltrate in the periadventitial tissue and other biological changes were observed and compared with the control non irradiated arteries.

Dosimetric calculus: Each dose was estimated using the appropriate methods employing different equations (25, 26, 27).

Histopathological studies: Samples of all arteries were sectioned at different six levels each and were harvested for histological examination. Specimens were fixed in 10% formaldehde and embedded in paraffin. Haematoxylin-eosin (HE) stained sections were microscopically examined. Figure 1 ilustrate the six samples analized of each artery.

Reagents: Radiactive source of 32P (specific concentration 50 mCi/ml = 1.85 x 10^9 Bq/ml.) as ortophosphoric acid was purchased from National Atomic Energy Comission of Argentine. The contrast medium utilized was Omnipaque 240.
Fig. 1- Scheme illustrating the levels of arteries from where samples were taken of.

RESULTS

Atherosclerotic lesions were successfully induced in 100% of surgically intervened arteries. Eight arteries (19%) have been totally occluded and brachytherapy was not possible at the moment of fluoroscopy.

Total dose and dosis rate were evaluated by different methods (25, 26, 27). All calculus indicate that internal wall of the arteries of group C received an average dose of 10 Gy and group D an average dose of 20 Gy. The intraluminal irradiation in arterial wall was done by the balloon dilatation cateter with a liquid radioactive source of $^{32}$P. The employed balloon dilated technique have the advantage that a dose delivered to the arterial wall is more uniform than other procedures (32).

The histological results indicate important differences between intraluminal irradiated arteries and control ones. The atherosclerotic arteries (Group B) present always neointima proliferation with fibrosis and lipid deposits on histiocytes, with partial occlusion of the arterial lumina. Generally, also hemorrhagic areas and inflammation of adventitia were shown in these arteries. Figure 1 illustrates a representative atherosclerotic lesion with important intimal proliferation and occlusion of lumina. The first picture of the Figure 1 shows the intimal area with proliferative appearance (H.E. 20X); second and 3rd. ones show the hemorrhagic area and histiocytes with lipid accumulation (H.E. 20X and 100X, respectively); the 4th picture shows the adventitial vessels with normal characteristics (H.E. 100X).
Figure 1. Characteristic atherosclerotic lesions obtained in the rabbit atherosclerotic model.

The intraluminal irradiated arteries showed distinct degree of differences with respect to controls non irradiated. Arteries irradiated with 10 Gy (Group C) showed moderate intimal proliferation with scarce compromise of arterial lumina and normal permeability. Figure 2 illustrates a representative artery irradiated with 10 Gy; the first, 2nd and 3rd pictures show the arterial lumina with characteristics like normal, with little hyperproliferation into the lumen (H.E. 20X, 100X and 100X, respectively). In this case, the adventitial vessels does not suffer changes with respect to control ones, as the 4th picture of Figure 2 shows.

Figure 2. Characteristics atherosclerotic lesions on the rabbit atherosclerotic model irradiated with 10 Gy.
Regarding to the atherosclerotic lesions intraluminal irradiated with 20 Gy, interesting results were obtained. In general, a disorganized intimal proliferation was observed. Figure 3 shows a representative irradiated artery of Group D rabbits. In the first picture (H.E. 20X) it can be seen the important partial luminal occlusion with intraluminal protrusion of the intima and presence of histiocytes. The majority of the samples showed an important alteration on *vasa vasorum*, probably due to the high dose received; the 2nd picture shows the *vasa vasorum* lesions (H.E. 400X).

![Figure 3](image1.png)  ![Figure 3](image2.png)

Figure 3. Characteristic atherosclerotic lesions on the rabbit atherosclerotic model irradiated with 20 Gy.

**CONCLUSIONS**

The potential role of ionizing radiation in preventing restenosis following angioplasty or stent placement was firstly discussed by Dawson (20) and carried out by Schwartz et al (28) in the porcine stent model, based in the biological effects of ionizing radiation (18, 19, 29). In our work we demonstrated that ionizing radiation produces inhibitory effect on restenosis in New Zealand rabbits.

It has been proved that, to develop an effective strategy for prevention of restenosis, an appropriate animal disease model is essential. In our case induction and treatment of lesions shown a success of 81% (34/42), taking into account that at the moment of brachytherapy the relatively few arteries (8/42) were occluded enough as for not allow to perform this therapy. In that sense, the selected model seems to be satisfactory.

With respect to histologic observations, we can conclude that atherosclerotic non-irradiated arteries showed in all cases typical lesions, with thrombosis, histiocytic aculus, intimal proliferation, lipid deposits and presence of abundant eosinophiles. The intimal proliferation was seen over the entire artery wall, with important and sometime complete occlusion.

Irradiated arteries with high-energy beta particles ($E_\beta = 0.71$ MeV/dis) showed different biologic effects according to received dose. In this way, those irradiated with doses near 10 Gy (Group C) did not show intimal proliferation but in some slices that condition was seen in moderate quantities and with scarce histiocytes.
Arteries were seen dilated in a normal way, with intraluminal section preserved and vascular permeability. Arteries irradiated with higher dose, near the 20 Gy (Group D) in numerous occasions presented intimal proliferation in almost the entire arterial wall and with presence of eosinophiles; in some opportunities it was seen the rupture of the intima and media with loosening at internal elastic layer. It was also observed infiltration of granulocites, eosinophiles and repair histiocytic tissue. It is probable that doses so high make the quadratic component predominant in the cellular survival curve. It is known that at doses of interest here, dominant cellular response is the cell death. This model of cell death leads to the dose-response relationship where the fraction of surviving cells, S, is a linear quadratic function of dose, D

\[ S = e^{-\alpha \cdot D - \beta D^2} \]  

(18)

At high doses, the quadratic term begun to dominate and the cell-surviving fraction is very low.

In reference to the adjacent striated muscle, in all the cases it is shown the typical normal characteristics with absence of alterations. Even in spite that studies on radiosensitivity done with human muscular or endothelial cell lines shown that there is no significant differences between them with respect to the radiosonsobility (30), the obtained results are in accord with the type of the employed radioactive source; as the $^{32}$P is a beta emitter with a range of 7.5 mm in the biological tissue (31), it is known that cells at greater distance will not receive irradiation.

With respect to the adjacent connective tissue and the vascular-nervous bundle they also mantained their normal structures.

When compared the biological effects obtained through both doses, the higher dose employed (20Gy) causes non-wished histologic changes, whilst the lower dose (10 Gy) seems more adequate for therapeutic uses. However, 12 and 15 Gy intermediate doses will be tested in further investigations in order to obtain a more data. In this sense, it is very important to consider the ALARA principle (32) and determine the dose with the low cost and the high benefit; a beta particles emitter source has considerable advantages over other emitters in terms of reduced dose delivery to normal tissues and radiation safety of the attending researchers and/or medical staff. However, recent reports indicate that the balloon dilatation catheter technique with a radiactive solution has advantage over other proposed intraluminal irradiation procedures (33), more studies are necesary to complete this aspect of experiments.

Another problem to resolve was the non-uniform dose distribution in the artery wall. In the calculus, based on dosimetry reported by Wildermann (34) it is possible to assume a linear radial dose loss of 10% across the arterial wall. However, this point needs more research.

By the other hand, and in spite that dosimetric calculus give coincidental results, a better adjustment is needed with the use of the Monte Carlo method (35). Also, the isodose curves in tissue adjacent to arteries are necessary to complete the dosimetric estimation and to determine the possible late biological effects.

Finally, many discrepancies have been reported between different researchers which found benefit or disadvantages at the same doses. We think that, in this sense, more practical guidance are necesary.

At present, different trials are undertaken (36, 37, 38). Results obtained in our work demonstrated that intraluminal irradiation of arteries in conjunction with angioplasty reduces the neointimal proliferation in the experimental model induced in rabbits and may open the posibility to apply this technique in human patients in Argentina.

REFERENCES
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