

Application of superparamagnetic nanoparticles for magnetically guided release of anti-cancer drug: An Ivvivo study on Rats

Jyoti Choubey *

Department of Chemistry,
RDVV, Jabalpur(M.P.) - 482 001, India.
email: jyoti_choubey67@rediffmail.com
Phone:0761-2426681

&

Madhu Swami

Department of Veterinary Pathology,
College of Veterinary Science & AH
Jabalpur(M.P.) 482 001, India.
Email: vetpath@rediffmail.com

ABSTRACT

The specific delivery of chemotherapeutic agents to their desired targets with a minimum of systemic side effects is an important, ongoing challenge of chemotherapy. Magnetically targeted iron oxide incorporated gelatin nanoparticles (IOIGNP) containing doxorubicin were selectively targeted to tumors in rats by utilizing an external magnetic field, it also reduces the toxic side effects from generalized systematic distribution by use of a much smaller dose. Tumor were inoculated subcutaneously in the tail of rats by using tumor inducers. When nanoparticles containing drug (0.5 mg/kg) were infused with the magnet adjacent to the tumor, there was a significant (83%) decrease in tumor size.

Keywords: Gelatin, iron oxide, nanoparticles, doxorubicin, drug targating.

INTRODUCTION

The difference between the success or failure of chemotherapy depends not only on the drug itself but also on how it is delivered to its target. Because of the relatively nonspecific action of chemotherapeutic agents, there is almost always some toxicity to normal tissue even under optimal conditions.[1] Therefore, it is of great importance to be able to selectively target the antineoplastic agent to its tumor target as precisely as possible, to reduce the resulting systemic toxic side effects from generalized

systemic distribution and to be able to use a much smaller dose, which would further lead to a reduction of toxicity. In the past, chemotherapy targeted by magnetic fields using magnetic albumin microspheres has shown encouraging results [2,3]

The important properties of magnetic particles for medical applications are nontoxicity, biocompatibility, injectability, and high level accumulation in the target tissue or organ; the most important property among those mentioned above is nontoxicity. Magnetic nanoparticles offer attractive and versatile applications in the field of biotechnology, such as DNA and RNA separation, cell separation, drug delivery system (DDS), magnetic resonance imaging (MRI), and hyperthermia [4-9] For these applications, magnetic iron oxides such as Fe₃O₄ or gamma-Fe₂O₃ are employed as a magnetic phase because they are stable and harmless to the living bodies [10].

For achieving high target-area concentrations of drug carrier, we designed small (10-100nm average diameter) magnetically responsive gelatin nanoparticles capable of responding to external magnetic fields (11). Virtually water-soluble drug can be entrapped in these nanoparticles. By varying magnetic parameters, we have shown in vitro experiments that the nanoparticles could be selectively retained at capillary-level flow rates (11). By using appropriate magnetic parameters, 50-80% of the injected nanoparticles could be targeted, thus eliminating systemic distribution

and subsequent toxicity of the entrapped agent. In this report we present data on the use of magnetic nanoparticles bearing doxorubicin in the treatment of subcutaneous tumors in rats.

PLACE OF WORK

The study was conducted at Department of Veterinary Pathology, College of Veterinary Science & AH Jabalpur. The required permission for animal experimentation was obtained from the IAEC (institutional animal ethics committee) of the college. All the protocols followed were in accordance to the guidelines given by Committee for the purpose of Control and Supervision of Experiments on Animals(CPCSEA).

MATERIALS:

(i) Animals: Adult Wistar albino rats of either sex, 2 months old and weighing between 80 to 150 gm were used in the experiment.

(ii) Carcinogens: Rat skin tumors were induced by chemical carcinogens Dimethylbenzanthracene (DMBA) as initiator and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) as promoter.

(iii) Magnets Rare earth magnets were used for the experiment.

(iv) Chemicals: Acid processed gelatin (Type A, isoelectric point 7.6) in yellowish granular form, was supplied by Loba Chemie, Mumbai, India and used without any pretreatment. Type B gelatin (Bloom No. 240, isoelectric point 4.8) extracted from human bone was a kind gift from Shaw Wallace Gelatins Ltd., Jabalpur, India. Genipin was employed as a crosslinker of gelatin and obtained from Wako Chemicals Japan. Paraffin oil was used as oil phase. Other chemicals and solvents were of analytical reagent grade. Doxorubicin (DOX) was used as a model drug and obtained from Dabber Pharma LTD.

(v) Iron Oxide Incorporated Nanoparticles:

The IOIGNP used in the experiments were prepared by the method described in our previous publication (16) and consisted of a colloidal particles formed by solvent evaporation method. The particles were surrounded by magnetite(Fe_3O_4). For loading of drug on to the nanoparticles, known volume of drug injections were taken and diluted with appropriate amount of phosphate buffer saline and shaken vigorously for mixing of drug injection and distilled water.

The loading of DOX was performed by allowing the nanoparticles to swell in the freshly prepared drug solution till equilibrium, and then drying to obtain the release device. The percent loading of drug was calculated by the following eq.

$$\% \text{ Loading} = \frac{W_d - W_o}{W_o} \times 100 \text{---- (1)}$$

where W_d and W_o are the weights of loaded and unloaded nanoparticles, respectively.

METHODOLOGY

The study was conducted in two phases:

In the **first phase** cutaneous tumour were induced in rats by using chemical carcinogens.

In the **second phase** of the study the effect of nanoparticles (with and without magnetic field) on the chemically induced tumourous growth was observed.

OBSERVATION

After completion of the **first phase** Gross tumor appearing features were observed, counted and measured weekly.

RESULT

A well formed elevated papule was observed after twelve weeks of start of the experiment which grew in size and by twenty two weeks circumscribes about 2 to 3mm in diameter tumorous growth.

Effect of nanoparticles and design of experiment given in table:1.

Group	Treatment	No of Animal	Sacrifice of animals	Studies Undertaken
A	Control	6	Two animals each were sacrificed after 24 , 48 and 72 hours	The gross and microscopic appearance of the tumorous growths were studied
B	Nanoparticles without MF	10	Three animals each were sacrificed after 24 and 48 and four after 72 hours	---do---
C	Nanoparticles with MF	10	Three animals each were sacrificed after 24 and 48 and four after 72 hours	---DO---

After completion of the **second phase** of the study Grossly, no change in the size of tumorous growths was observed in animals of the control group. However in group B animals, treated with drug only, tumor size regression was visibly observed and a dried scab like lesion was visible although the mean size of tumorous growth remained unchanged. In group C animals given drug along with application of magnetic field the effect was visible. A shriveled up dried scab was observed at the site of tumorous growth.

The final evaluation of the treatment on the tumorous growth was done after histopathological observations .

HISTOPATHOLOGICAL OBSERVATION

Control Group A

Well formed elevated papules were observed .

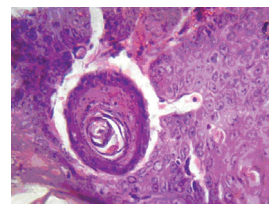


FIG 1 Histopathological observation of control group

Group B Nano particles without Magnetic field

Areas of necrosis were prominently visible.

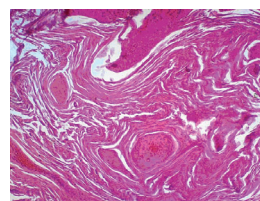


FIG 2

Histopathological observation of Group B Nano particles without Magnetic field

Group C Nano particles with Magnetic field

The tumor regressive features like increased necrosis and apoptosis were more prominent in this group at all stages.

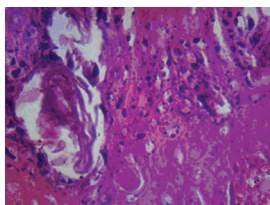


FIG 3

Histopathological observation of Group C Nano particles with Magnetic field

CONCLUSION

By using appropriate magnetic parameters, 50-80% of the injected nanoparticles could be targeted, thus eliminating systemic distribution and subsequent toxicity of the entrapped agent.

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