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Effects of gamma-irradiation on PLGA microspheres loaded with thienorphine

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Ionizing radiation can be used as a drug sterilization technique, provided that the drug itself is not modified and that no toxic products are produced; moreover, if the irradiated product is a drug delivery system, its drug release characteristics must not be significantly altered by radiation. The aim of this work was to study the effects of sterilization by ionizing radiation on PLGA microspheres, containing thienorphine. Thienorphine PLGA microspheres were prepared by the O/W solvent evaporation method and characterized by HPLC, SEM and laser particle size analysis. Our experimental results showed that gamma-rays did not alter the drug content, and did not modify the kinetics of drug release from microspheres. Moreover, no significant changes in the shape and in the size distribution of microspheres were found after irradiation. In conclusion, the sterilization method is adequate because microspheres not underwent any change after exposition to gamma-irradiation.

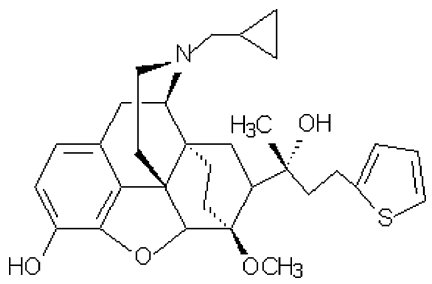
1. Introduction

Opioid abuse and dependence remains a serious worldwide health problem. Relapse of addiction is often caused by poor compliance and lack of retention in programs (Hulse et al. 2005). Therefore, it is of great importance to be able to reduce the level of involvement of the subject with medicinal treatments, particularly those treatments involving a specific regimen.

Many experts have adopted sustained-release methods to reduce the involvement of subjects in compliance. Biodegradable injectable microspheres have been studied widely in the last 30 years. They can prolong the duration of a drug effect significantly and improve patient compliance. The total dose and some adverse reactions may be reduced because a steady plasma concentration can be sustained. There is also no need for them to be implanted by surgical operation and removed after the drug is completely released. Among various biodegradable polymers, poly (lactic-co-glycolic acid) (PLGA) is the most widely studied and used (Fu et al. 2005).

Thienorphine[*N*-cyclopropylmethyl-7 (-[(R) -1-hydroxy-1-methyl-3-(thien-2-yl)-propyl]-6, 14-endo-ethano-tetrahydrono roripavine] is a compound, synthesized in our institute. The pharmacology studies showed that thienorphine is a potent, long-acting partial opioid agonist and may have a possible application in treating addiction (Gong et al. 2001). Inspired by the result, we prepared thienorphine loaded PLGA microspheres. Our objective was to develop a controlled release system that is effective for a period of 1 month

Microspheres intended for subcutaneous administration, as with other injectable delivery systems, have to meet the pharmacopoeia requirements of sterility. It is well known that most sterilization techniques, such as sterilization by steam and dry heat cannot be used for biodegradable aliphatic polyesters, such as PLGA, since they alter the physical chemistry of the polymer. Chemical sterilization with ethylene oxide causes serious toxicological problems due to residues of the sterilizing agent (Athanasiou et al. 1996). Moreover, the stability of a drug incorporated into the polymer matrix can also be altered (Bittner et al. 1999).



Thienorphine

In recently years, ionizing radiation has gained much interest in sanitization/sterilization processes for food, drugs, pharmaceutical systems and medical supplies as an alternative method to heat, gas exposure and other sterilization techniques. Ionizing radiation, such as gamma-irradiation generated from a ^{60}Co source, is able to kill the pathogens that could contaminate therapeutic products. The advantages of sterilization by irradiation include high penetrating power, low chemical reactivity, low measurable residues, small temperature rise and fewer variables to control.

Gamma-irradiation has been successfully employed for the sterilization of biodegradable microspheres intended for parenteral use. However, ionization events activate in the microspheres numerous chemical reactions with different effects on drug release, depending on the active ingredient used.

Accordingly, in this study, the effects of gamma-irradiation at various doses on microspheres made of PLGA and loaded with thienorphine, which was prepared by a O/W emulsifica-

Table 1: Characteristics of thienorphine loaded PLGA microspheres

Batch	Actual drug loading (%)	Encapsulation efficifency (%)	Particle size(μm)
1	4.42	88.40	55.38 ± 0.17
2	7.16	71.60	37.84 ± 1.32
3	10.10	67.33	49.79 ± 0.07
4	6.45	64.50	42.69 ± 0.15
5	8.31	83.10	55.61 ± 0.27
6	7.54	75.40	52.37 ± 0.63
7	5.19	51.90	30.19 ± 1.17
8	7.24	72.40	53.74 ± 0.50
9	4.64	46.40	38.45 ± 0.11
10	6.83	68.30	47.19 ± 0.14
11	7.08	70.80	59.15 ± 0.67

tion/solvent evaporation method, were evaluated in order to continue with *in vivo* studies (Yang et al. 2010). The influence of irradiation on the selected formulation was investigated. Besides the evaluation of physicochemical characteristics, thienorphine release profiles of sterilized and non-sterilized microspheres were also compared.

2. Investigations, results and discussion

2.1. Effects of formulation parameters

It was reported that the encapsulation efficiency of microspheres was mainly dependent on drug partition coefficients in the internal and external phases (Bodmeier et al. 1987). The acceleration of microsphere solidification may reduce drug partitioning into the external aqueous phase and increase the encapsulation percentage (Zhu et al. 2001). The extracted rate of organic solvent from the oil phase and its evaporation rate from the aqueous phase were proved to be important factors.

As shown in Table 1, higher concentration of PLGA in oil phase increases drug encapsulation of PLGA microspheres, because an increase in the viscosity of the oil phase prevents thienorphine from diffusion. However, an increase in the concentration of PVA in the external phase leads to a decrease in the encapsulation efficiency. This might be explained by the fact that the solubility of thienorphine is increased in aqueous PVA solution.

Encapsulation efficiency of microspheres was also affected by the theoretical drug loading. As the amount of polymer decreased, encapsulation efficiency decreased; this is due to the fact that less amount of polymer would produce small size droplets with increased surface area, such that diffusion of drug from such microspheres will be fast, resulting in the loss of drug with a consequent lowering in encapsulation efficiency.

Other preparation parameters which have significant influence on encapsulation efficiency and particle size are shown in Table 1.

Our objective was to develop a controlled release system that is high encapsulation efficiency and small particle size. Thus, batch 2 was selected for gamma radiation studies.

2.2. Effects of gamma-irradiation

2.2.1. Morphology and particle size

Figure 1 shows the scanning photomicrographs of non-irradiated microspheres and after irradiation at 10, 15 and 20 kGy: the microspheres irradiated were of good morphological characteristics, spherical shape and smooth surface, as the non-irradiated ones; this result shows that the irradiation does not cause any morphological alteration.

Mean diameters of irradiated at 10 kGy (33.46 ± 10.18 μm), 15 kGy (35.13 ± 9.87 μm) and 20 kGy (35.87 ± 8.75 μm), and non-irradiated (34.52 ± 4.56 μm) microspheres were not significantly different. The histograms in Fig. 2 show the dimensional distribution of non-irradiated microspheres, and those irradiated at 15 kGy; in both cases, about 85% of microspheres were in the same size range between 25 and 45 μm. Moreover, the t-test did not show any significant difference for each interval, between percentage content of irradiated and non-irradiated microspheres, even in the interval 30–35 μm ($P < 0.01$), where the highest difference was observed (41 and 30%, respectively). Similar results were obtained after irradiation at 20 k Gy. Since no effects due to gamma-irradiation were observed at 15 and 20 kGy, experiments were not repeated at lower doses. These results showed that no morphological change due to sterilization, e.g. particle fusion, took place after gamma-irradiation treatment.

2.2.2. Encapsulation efficiency and drug loading

Thienorphine mean loading efficiency was not affected by the irradiation process at 15 kGy ($P < 0.05$), with values 4.51 ± 0.12% and 4.57 ± 0.04%, for irradiated and non-irradiated microspheres, respectively. However, the drug loading (4.41 ± 0.08%) was little affected by irradiated at 20 kGy. Although data obtained by HPLC before and after irradiation did not show significant differences ($P < 0.05$), further studies would be necessary to confirm the absence of bond scission of the drug molecule in the microspheres after gamma-irradiation exposure.

2.2.3. In vitro drug release

Figure 3 shows the *in vitro* release profiles of thienorphine loaded microspheres before and after sterilization. In the initial 7 days, release was nearly linear with 10% released per day. Thereafter a gradual slow release was observed and about 90% drug released by day 21. Similarity factor (f_2) was calculated to compare release profiles of thienorphine from microspheres before and after sterilization. The f_2 value was greater than 50 between non-irradiated and 15 kGy, so both curves can be considered similar; analogous results were observed for the other irradiated samples.

The *in vitro* release data were analyzed with Higuchi ($Q = 25.365\ t^{1/2} - 22.433$ ($R^2 = 0.9704$)), first-order ($\ln(100\text{-}Ft) = -0.1697\ t + 5.0483$ ($R^2 = 0.8759$)) or zero-order ($Ft = 4.3915t + 7.3355$ ($R^2 = 0.9972$)) equation, respectively. As a result, the method of least squares showed that zero-order was the most appropriate for describing the kinetics of drug release: for non-irradiated microspheres the correlation coefficient was 0.9972; analogous results were observed for irradiated samples.

The drug release rate from irradiated microspheres was negligibly faster than from the non-irradiated ones. For instance, at the first day after the beginning of the experiment, the percentage of thienorphine released from non-irradiated microspheres, and microspheres irradiated at 10 kGy, 15 kGy and 20 kGy, was 10.82, 10.57, 10.81 and 13.23%, respectively.

These results demonstrate that not only the microspheres irradiated at 15 kGy, but also those irradiated at 20 kGy show the same kinetic behavior of drug release as the non-irradiated microspheres. It is therefore possible to conclude that gamma-rays, at 10 kGy, 15 kGy and 20 kGy for sterilization, do not determine crosslinking or modification reactions in the matrix components, and that the delivery system under test is stable and the safe use of irradiated microspheres is not endangered. In conclusion,

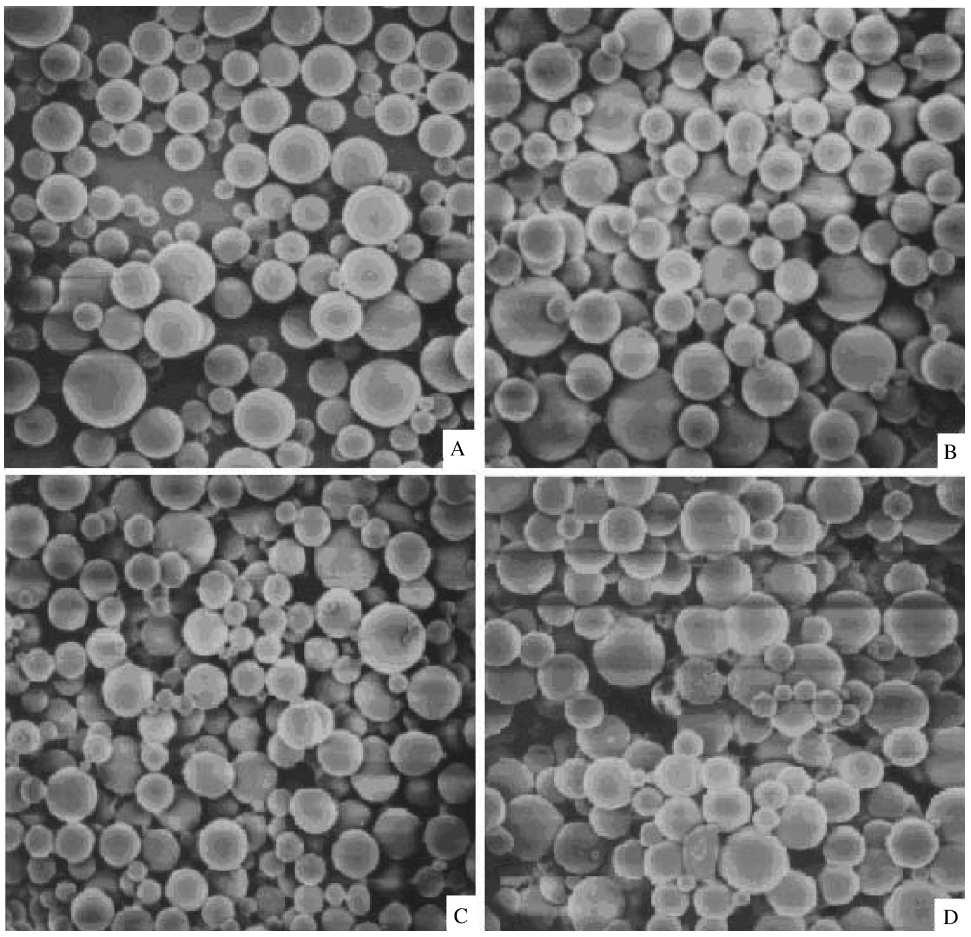


Fig. 1: Photomicrographs of microspheres containing thienorphine: (A) before irradiation; (B) after irradiation at 10 kGy; (C) after irradiation at 15 kGy; (D) after irradiation at 25 kGy (magnification 50 times)

gamma-sterilization at the recommended dose of 15 kGy did not affect the release rate of the drug.

3. Experimental

3.1. Materials

PLGA (Wh15000, lactide/glycolide ratio, 75/25) was generously donated by Prof. Shao-bing Zhou of Southwest Jiaotong University. Thienorphine (99% purity) was supplied by Beijing Institute of Pharmacology and Toxicology. Polyvinyl alcohol (PVA-124), dichloromethane (DCM), ethanol were obtained from Beijing Chemical Reagents Company. All other materials or solvents were of reagent or analytical grade.

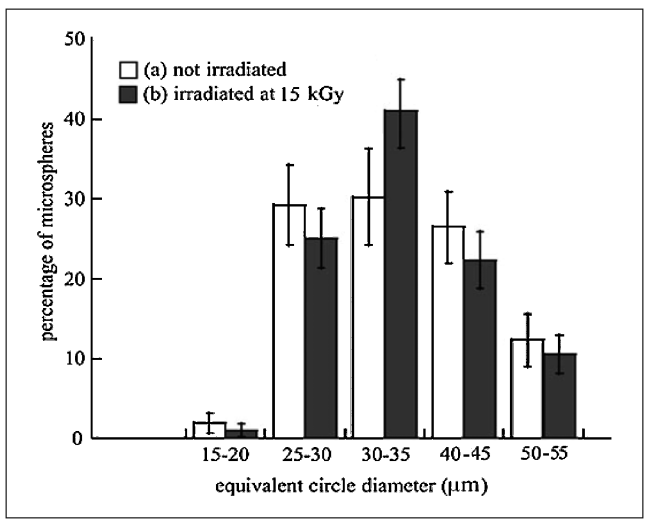


Fig. 2: Dimensional distribution of microspheres before irradiation (a), and after irradiation at 15 kGy (b)

3.2. Microspheres preparation

The formulation conditions of thienorphine loaded PLGA microspheres are shown in Table 2. The thienorphine loaded PLGA microspheres were prepared using o/w solvent evaporation method which was used widely in the preparation of microspheres. Briefly, an amount of PLGA and thienorphine were added to 1 ml of a mixture of DCM. After being completely dissolved, it was poured into PVA-124 aqueous solution and then the mixture was emulsified by a propeller stirrer (SXJQ-1, Zhengzhou, China) at various rates for 10 min at room temperature (T). Then the thienorphine PLGA microspheres were solidified while DCM being extracted for 8 h under stirring at 300 rpm. The resulting microspheres were washed three times with distilled water and dried under vacuum.

3.3. Gamma-irradiation of microspheres

The thienorphine loaded microspheres were weighed (100 mg) and transferred to 5-ml glass aluminum sealed cap vials. Vials were labeled and

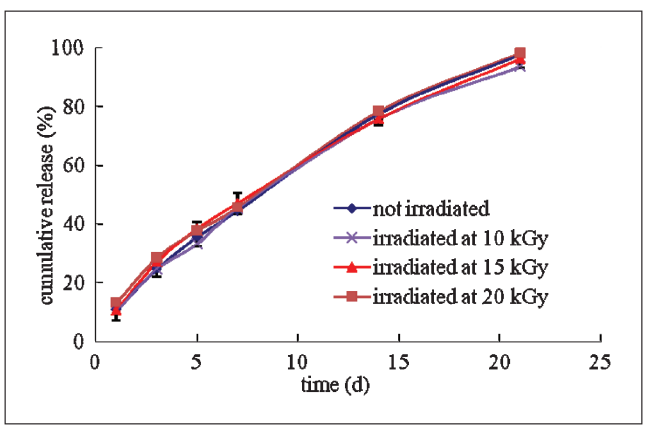


Fig. 3: Percentage of thienorphine released from microspheres before, and after irradiation at 10 kGy, 15 kGy, and 20 kGy

Table 2: Formulation conditions of thienorphine loaded PLGA microspheres

Batch	Theoretical drug loading	PLGA concentration (mg·ml^{-1})	PVA concentration	Homogenization speed (rpm)	DCM (ml)
1	5	200	4%	800	10
2	10	200	4%	800	10
3	15	200	4%	800	10
4	10	100	4%	800	10
5	10	300	4%	800	10
6	10	200	2%	800	10
7	10	200	6%	800	10
8	10	200	4%	600	10
9	10	200	4%	1000	10
10	10	200	4%	800	5
11	10	200	4%	800	20

packed surrounded with dry ice into a polyurethane container, assuring a low temperature during the irradiation process. Although gamma irradiation causes only a minimal temperature rise, keeping low temperature avoids a possible acceleration of PLGA hydrolytic degradation. The samples were carried out at room temperature with a ^{60}Co chamber GC-900 (Beijing Institute of Pharmacology and Toxicology) at dose values of 10, 15 and 20 kGy. The dose of 15 kGy was chosen since it is the recommended dose for sterilization treatment of drugs. The 20 kGy irradiation was performed to study the effects caused by doses higher than recommended. The dose rate (measured with the Fricke dosimeter) was 1.3 kGy/h. Uncertainty in dose value was less than 2%. Non-irradiated samples were kept as reference.

3.4. Determination of thienorphine content of microspheres

Thienorphine was extracted from the microspheres with 25 mL of a mixture of DCM and ethanol (1:1). The solution was directly measured by using HPLC (LC-10AT VP, Shimadzu, Japan). HPLC conditions were as follows: C_{18} column (250 mm × 4.6 mm, 5 μm), a mixture of acetonitrile-methanol-0.02 mol·L^{-1} phosphate (40:15:45) buffer containing 0.2% triethylamine (pH = 3) as eluant, detection at 220 nm. The polymers did not interfere with absorbance of the drug at the specified wavelength. The encapsulation efficiency is expressed as the ratio of the amounts of drug detected and added. Each measurement was performed in triplicate.

3.5. Particle size analysis

A light-scattering particle size analyzer (BT-9300, BETTER, China) with a circulation disperser (BT-600, BETTER, China) was used to determine the size distribution of the prepared microspheres. The lyophilized particles were suspended in a large volume of distilled water and analyzed under continuous stirring. Particle size was expressed as volume mean diameter in micrometers (SEM, n = 3) of three batches.

3.6. Microscopic observations

Microsphere morphology was determined by optical microscopy (OLYMPUS BX-50, Japan) and scanning electron microscopy (Hitachi S-450, Japan). In the latter case, a layer of gold was deposited by evaporation on the sample.

3.7. In vitro release assays

About 25 mg of microspheres were weighed and added to a dialysis bag with molecular weight cut off 1 kDa, 1 ml phosphate buffered saline (PBS, 0.1 M, pH 7.4) was then added. The dialysis bag containing the microsphere suspension was placed in a flask filled with 30 ml of 0.01 M phosphate buffered saline (PBS) (pH 7.4, prepared according to the ChP monograph) containing 0.02% sodium azide. Incubation was conducted at 37 °C by shaking at a rate of 72 rpm. At predetermined intervals, 1 ml of medium was drawn out and replenished with the same volume of fresh medium. Thienorphine concentration was determined at 220 nm by HPLC. Each measurement was performed in triplicate.

The similarity factor (f_2) was calculated to compare release profiles of thienorphine from microspheres. The similarity factor is a measurement of the similarity in the percent release between the two curves and is defined as the "logarithmic reciprocal square root transformation of the sum of the squared error" in the FDA guidance document (FDA Guidance for Industry, 1997).

$$f_2 = 50 \times \log_{10}\left\{\left[1 + \left(\frac{1}{n}\right)\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-\frac{1}{2}} \times 100\right\}$$

where n is the number of time points, R_t is the experimental data at time t, and T_t is the predicted *in vitro* percent cumulative release at time t.

It is noted in the FDA guidance document that generally, f_2 values greater than 50 suggest equivalence of the two profiles.

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