Original Article

Conjugation of Antibodies with Radiogold Nanoparticles, as an Effector Targeting Agents in Radiobioconjugate Cancer Therapy: Optimized Labeling and Biodistribution Results

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Abstract

Purpose of the Study: Drug accessibility to the tumor cells is an important area of concern with an anticipation of increasing the efficacy of the drug to be delivered to a specific site. The biogenesis of gold nanoparticles using plant-mediated phytochemical extracts and their possible linkage to cancer antibodies with an aim at delivering the conjugate specifically to the tumor-associated antigen is the basic objective of the research. **Materials and Methodology:** Radiolabeling of antibodies with gold nanoparticles was carried out by a protocol, and the labeling extent of antibodies was compared with that of a radiogold solution to ordinary particulate size (AuNO-Ab). The amount of radiolabeling was estimated by subjecting the reaction mixtures to thin layer chromatography (ITLC-Silica-gel) in different solvent mediums, both by visual inspection of images of the Siemens Orbitor Gamma Camera ZLC-7500 and also by *in vitro* counting of the radioactive counts in different quarters of the chromatographic strips. Biodistribution relating to the deposition of injected dose in nontargeting sites (reticuloendothelial system [RES]-localization) was studied and efforts were made for reducing the same. **Results:** Much improved gold incorporation was confirmed at various molar ratios of gold to immunoglobulin (antibody) using nanogold solution (>85%). The RES uptake in the liver, spleen etc., was observed as a problem and the prior administration of unlabeled nonspecific gammaglobulin (before the actual radiolabeled product) was identified as the suitable blocking agent for this purpose. **Conclusion:** The study signifies the potential for PEGylated gold nanoparticles of a precise size range, suitable to use as a delivery vehicle for targeting small biomolecules (antibody etc.) to the tumor site. The stability of this labeled immunoconjugate and other toxicity effects under physiological conditions needs further evaluation. If successful, this could be a role model for attaining high tumor/nontumor ratio.

Keywords: *Monoclonal antibody, radioimmunotherapy, radiolabeled gold nanoparticles, reticuloendothelial localization*

Introduction

Cancer is one of the foremost problems affecting human health. Its impact globally is significant in all the strata of society and there are several projections emphasizing the increasing magnitude of the problem with both the developed and developing nation.[1,2]

Radiobioconjugate targeting in cancer relates to the specific and selective targeting of cancer cells by the delivery of a localized radiation, using an appropriate radionuclide war head coupled to a biological carrier molecule (antibody), which has a relative specificity for tumor tissue. Radiobioconjugate targeting using monoclonal antibodies (MoAbs) (radioimmunotargeting) linked to a high energy radionuclides is a promising approach for treating metastatic cancer. MoAbs with favorable characteristics should produce high tumor uptake accompanied with low background activity i.e., high target/nontarget ratio, thus representing the measure of its efficiency.[3-7]

Gold has been advocated as a promising radionuclide for radioimmunotherapy of cancer cells. Gold (Au-199), because of having an effective beta emissions of the range 0.30 MeV (70%), 0.2530 MeV (24%), 0.46 MeV (6%), along with useful gamma radiation of 0.158 MeV range, considered as an ideal radionuclide both for therapy and scintigraphic (imaging) purposes. It has a favorable half-life of 3.15 days, which is compatible with the time course of accumulation of antibody

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in a tumor making it more attractive. This also gives time to carry out the radiochemical linkage.[8,9] Hazra *et al*. reported that gold Au-199 is suitable to use for internal radiotherapy under Indian conditions as it can be produced in a carrier free form by Pt-198 (n, γ) reaction (^{198}Pt (n, γ) ^{199}Pt → ^{199}Au). Pt-198 being stable permits the time-lag during transportation over large distances from reactors to users. The radio-gold (Au-199), can be obtained from (Pt-198) by an irradiation method. However, the labeling chemistry of gold with antibody in terms of high labeling yield is still unexplored and a question mark.[10-14]

The advent of nanotechnology has revolutionized our ability to obtain nanoparticle of precise size. Due to this, substantial work has been done and going on in developing noninvasive and targeted tumor treatment for nanoscale metallic particles.[15,16] Gold nanoparticles because of their unique size dependent physicochemical and optical properties, biocompatibility, easy adaptability, and subcellular sizes, offers a two-fold means of transporting small and macro biomolecules, both as a targeting agent to the diseased cells, as well as a therapeutic agent, thus creating a force multiplier effect.[17]

Aim

The present study was carried out with an aim at the direct labeling of antibody (both polyclonal nonspecific antibody and specific MoAbs with radiogold nanoparticles, and the comparison of the labeling extent of the radiolabeled product was then studied with that of the gold solution to ordinary particulate size. Biodistribution studies in animals relating to the deposition of the radiolabeled injected dose in the nontargeting sites (reticuloendothelial system [RES]-localization) were also investigated and efforts were made for reducing the same.

Materials and Methodology

Materials

Radioisotopes

Radionuclide gold (Au-199) was available from Board of Radiation and Isotope Technology, Mumbai, India, and produced in carrier-free form by Pt-198 (n, γ) reaction.

Antibodies

The antibodies whose labeling was studied include the following:

Human immunoglobulin

Polyclonal nonspecific mixture marketed as Bharglob (Bharat Serums and Vaccines Limited, Thane, Mumbai).

The composition of Bharglob is as follows:
- Protein content: 16.5 mg/ml
- Stabilizer - glycine: IP 0.3 M
- Preservatives - thiomersal: IP 0.01% w/v.

M3-Monoclonal antibody

Monoclonal antibody used to direct against the tissue polypeptide specific antigen, which is a Pancarcinoma proliferation antigen and belongs to Cyto-Keratin 8-18 family of IgG1 class. The antibody was kindly made available by Professor Bjorklund, President of Swedish Cancer Council, Stockholm, Sweden.

The initial *in vitro* labeling study was carried out with a nonspecific gammaglobulin (Bharglob). However, the *in vivo* biodistribution studies were performed both with the nonspecific antibody Bharglob and a specific MoAb M3 available with us.

Chelating agents and other salts

Chelates and other salts used for linking the antibody with radiogold were obtained from "M/s Sigma-Chemicals," while other general laboratory chemicals used were obtained from the central research laboratory of Bio-Chemical Sciences Department, of the institute.

Equipment

Imaging of experimental animals was performed using Orbiter-ZLC 7500 SPECT Gamma Camera (SIEMENS, Germany). Sacrificing of mice with periodical counting of radioactivity in different organs in a nontumor-bearing mice was performed using "Auto Gamma Counter" (PACKARD, Germany). Radioactivity before injecting into the animals was measured using "Deluxe Isotope Calibrator" (VICTOREEN, USA).

Experimental animal models

Swiss albino mice were used for experimental studies on animal models.

Methodology

The methods employed for labeling a nonspecific antibody (Bharglob) with gold nanoparticles includes the following steps:

Purification of nonspecific gammaglobulin (Bharglob)

To concentrate the gammaglobulin and render it free of preservatives for radiolabeling, the commercial Bharglob preparation has to be dialyzed. For this, Bharglob was taken in a dialyzing membrane bag dipped in normal saline for an overnight at room temperature to remove preservatives and then concentrated by putting the dialyzing bag in a polyethylene glycol (PEG; Mw = 6000) solution.

Reduction of nonspecific gammaglobulin (Bharglob)

The sulfide (-S-S-) bond present in the gammaglobulin (nonspecific antibody) was reduced to -SH-group using 2-mercaptoethanol (2ME). For that, the antibody was first concentrated by ultrafiltration to an approximate concentration of 10 mg/ml, and a sufficient amount of 2ME was added to its stirred solution, to provide a molar ratio

(1000:1; 2ME:antibody). The reaction mixture was then incubated at room temperature for 30 min with continuous rotation. The reduced antibody was further purified by gel filtration on a Sephadex G-50 column using a phosphate-buffered saline as the mobile phase. The antibody fractions were collected and divided into 0.5 ml aliquots, frozen immediately at −20°C, and stored ready for use.

Biosynthesis of gold nanoparticles

Ecofriendly approaches using flavonoid containing plant extract (polyphenolic components) were adopted for the biosynthesis of stabilized gold nanoparticles.[18-21] We followed the Amendraiz *et al*., 2004 reported method for green synthesis of nanoparticles using either the plant extract from lemon grass or using Avena sativa extract suggested by Shankar *et al*.[22,23] As per the reported methods, 10 mg of the plant extract dissolved in equal volumes of dimethyl sulfoxide and the reaction mixture was stirred continuously, at least for 15 min at 25°C. A volume of 10 ml of Auric chloride solution ($HAuCl_4.3H_2O$), with concentration (1 mM) was added to the above solution. The color of the reaction mixture changes from pale yellow to brownish red, indicating the formation of gold nanoparticles. The solution was then stirred continuously for 45 min at 25°C for its stabilization and preventing it from agglomeration. At a low PH range (2–3) and mild temperature conditions, gold nanoparticles of variable sizes of desired range (25–85 nm) were obtained. The nanoparticles so obtained were characterized using X-ray diffraction and ultraviolet spectroscopy. The particles obtained were further conjugated with PEG and folic acid. The average molecular weight of PEG used in coating the Au-nanoparticles is ~ (5000 Mw). Coating of PEG with gold nanoparticles (AuNPs) increases stability, both *in vivo* and *in vitro*. The influence of surface/capping density of PEG on the aggregation behavior of AuNPs in different solvent media can be related with the change in size of functionalized gold nanoparticles. In our research, we prefer to use PEG of ~5000 Mw, as the AuNPs functionalized with PEG of ~5000 Mw has an overall average diameter in the size range of 50–90 nm, which is desirable. Folic acid serves as a ligand for generating tumor specificity on these particles, while PEG provides a sheath coating on the particles, making them long circulatory by reducing their nonspecific RES uptake. Finally, to prevent aggregation of synthesized nanoparticles, a stabilizing agent sodium citrate was added during the synthesis process.[24,25]

Labeling of nonspecific gammaglobulin (antibody) with gold nanoparticles

The labeling of radiogold nanoparticles with antibody was attempted by a protocol. In the method studied at our center for direct radiolabeling of antibody, the nanogold solution so prepared was adjusted to pH = 4.5, using 0.5 M NaOH added drop by drop. 500 µl of the gammaglobulin (antibody) at a concentration of 5.2 mg/ml was added to the 1000 µl (~1.5 mCi activity) of radiogold nanoparticle (AuNP) solution (pH = 4.5) and incubated for a period of 4 h. The reaction mixture was then purified on a Sephadex PD-10 column and collected in a glass vial (1). For comparing the labeling extent of the radiolabeled product, the same reaction mixture as prepared for was taken in a separate vial (2), only with a difference that the radioactive nanogold solution in this was replaced with the gold solution of ordinary particulate size (AuOP).

Experimental observations

Study of the Radiolabeled product (chromatography to assess labeling)

The labeling of gammaglobulin (antibody) with a radiogold solution was tested and observed both with the vial (1) and (2) reaction mixtures. The reaction mixtures of the two vials were subjected to thin layer chromatography (TLC). The TLC spots were developed in three different solvent mediums – normal saline, acetone and triple mixture solvent (ethyl alcohol:water:ammonia – 2:5:1). For comparison, the free Gold radio metal was also run with these. All the strips were imaged with our Siemens Orbitor Gamma Camera ZLC-7500 and the pictures acquired on the computer [Figure 1]. The amount of radiolabeling was

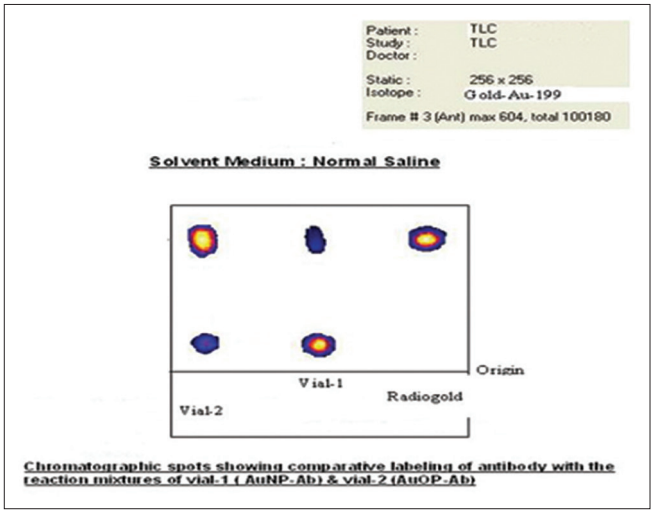

Figure 1: Chromatographic spots imaged with Siemens Orbitor Gamma Camera ZLC-7500 showing comparative labeling for both vial (1) and vial (2) reaction mixtures. The resultant reaction mixtures of the two vials (1) and (2) were spotted on the instant thin layer chromatography paper sheet and were subjected to thin layer chromatography in three different systems – normal saline, acetone, and triple solvent mixture (normal saline-as a solvent medium shown in a figure). For comparison, free (control) radio gold was also run with these. All the strips were imaged with our Siemens Orbitor Gamma Camera ZLC-7500 and the pictures acquired on the computer. The amount of radiolabeling was estimated by both visual inspections of these images as well as by *in vitro* counting of the quarters of the chromatograph strips. It was observed that in all the three systems free pertechnetate moved with the solvent front, with nothing or very little remaining at the origin. Labeling was observed with both the reaction mixtures, with a considerable amount remaining at the origin. However, the amount that remains at the origin is significantly higher for the reaction mixture of vial (1) (gold nanoparticle labeled immunoglobulin) in comparison to that for a vial (2) (gold ordinary particulate labeled immunoglobulin), representing better labeling of an antibody with radioactive nanogold. Labeling efficiency was reported to be optimized, up to >85%–90% using nanogold particles of precise size and shape

estimated by both visual inspections of the images as well as by *in vitro* counting of the quarters of the chromatograph strips. It was observed that in all the three systems free radiometal moved with the solvent front, with nothing or very little remaining at the origin, while the gold labeled gammaglobulin and a considerable amount of hydrolyzed radiometal stays at the origin. The relative quantities of both these products (Gold labeled gammaglobulin and hydrolyzed gold) were estimated further by evaluating them in a triple solvent mixture of ethanol:water:ammonium hydroxide – 2:5:1), where the hydrolyzed radiometal finally stays at the origin and the radiolabeled product like the free radiometal moved with the solvent front [Figure 2].

Results

Analyses of the results obtained from *in vitro* experimental studies

Quantitative analyses of radiochromatograms showing comparative labeling were carried out with both the vial (1) – (reaction mixture containing gammaglobulin labeled with nanosize gold particles and vial (2) (reaction mixture containing gammaglobulin labeled with ordinary particulate size gold) in three different solvent mediums [Table 1 (a, b, c)]. From the experimental data onto Table 1(a), the radioactivity in the first quarter of the chromatographic strip representing both the radiolabeled product (radiogold labeled gammaglobulin) and the hydrolyzed gold together is 90.20% for the vial (1) and 65.22% for the vial (2). From the experimental data onto Table 1(b), the radioactivity in the first quarter of strip representing both the radiolabeled and hydrolyzed product together is 87.96% for the vial (1) and 64.84% for the vial (2). From the experimental data onto Table 1(c), the amount of hydrolyzed radiometal can be separately estimated out at a triple solvent mixture and is reported to be 4.78% for the vial (1) and 7.85% for the vial (2).

Considering the analyses onto Table 1, the net percentage yield of labeling of antibody with nanogold (AuNP) is 90.70%–4.78% = 85.92% while the labeling of antibody with ordinary particulate size gold (AuOP) is 65.22%–7.85% = 57.37%. Similarly, gold labeling with antibody can also be computed by Acetone and Triple Solvent Mixture analyses – Table 1. From the analyses of 1(b) and 1(c), the net percentage of labeling of antibody (Bharglob) with radio nanogold is 88.95%–4.78% = 84.17% while with ordinary particulate size radiogold, the net percentage of radiolabeling of antibody is 64.84%–-7.85 = 56.99%. These are comparable to the figures obtained considering the analyses of 1a and 1c, i.e., normal saline and triple mixture solvent [Figure 3a and b].

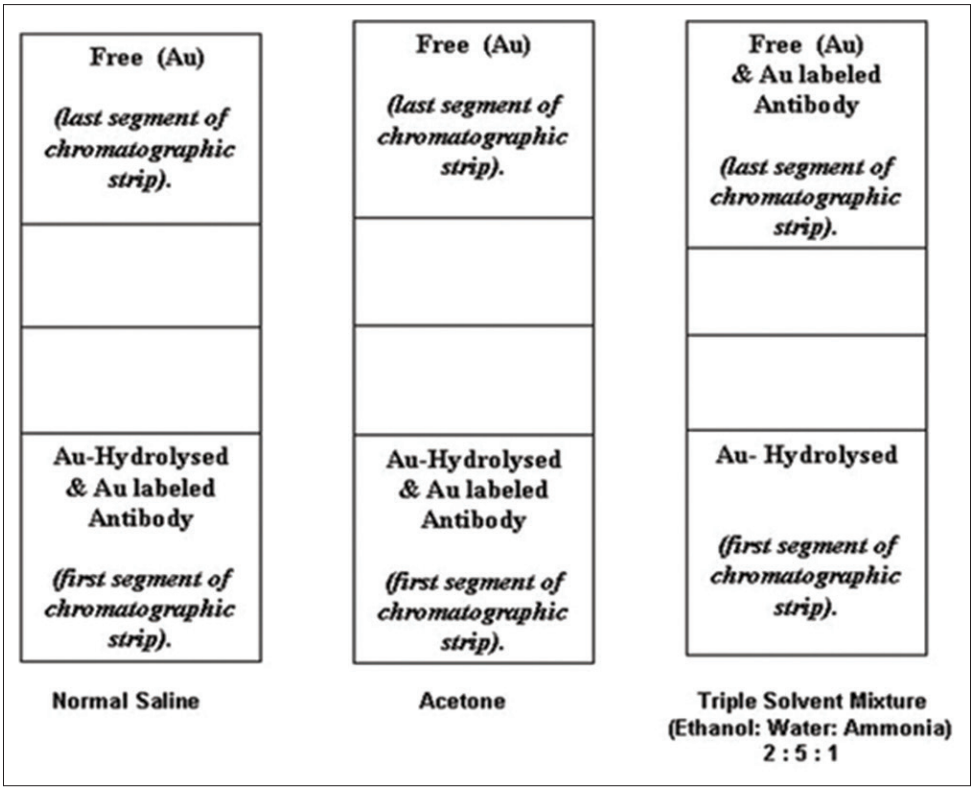

Figure 2: Radiochromatograms showing the behavior of labeled gold, hydrolyzed (colloidal) gold and free (control) gold in different solvent mediums: The thin layer chromatography spots when developed in solvent mediums such as normal saline and acetone, then the radiolabeled ligand, as well as the hydrolyzed (colloidal) radiometal, remain at the point of spotting, while the free radiometal moves with the solvent front. However, using the system of "triple mixture solvent" (ethanol:water:ammonia – 2:5:1) the hydrolyzed radiometal stays at the origin and the radiolabeled compound like the free radiometal moves with the solvent front

Table 1: Quantitative analysis of the radiochromatograms showing comparative labeling of nonspecific antibody with radio nanogold and ordinary particulate radiogold (n=3) experiments

Labeling preparation	Radioactivity first quarter of strip (counts/s) ($a_1 + a_2$)	Radioactivity last quarter of strip (counts/s) (b)	Ratio first/ last (counts/s)	Total counts of strip (counts/s) (c)
(a) Solvent medium: Normal saline (NaCl)				
AuNP-labeled gamma globulin (vial 1) (%)	76,990±396 (90.70%)	5,860±102 (6.90)	13.14	84,880±476
AuOP-labeled gamma globulin (vial 2) (%)	54,085±318 (65.22%)	26,070±174 (31.43)	2.07	82,925±418
Control (radiogold)	1812±54	78,728±412	0.02	86,310±436
(b) Solvent medium: Acetone (CH_3COCH_3)				
AuNP-labeled gamma globulin (vial 1) (%)	79,220±412 (88.95%)	7,516±126 (8.43)	10.54	89,060±482
AuOP-labeled gamma globulin (vial 2) (%)	56,730±316 (64.84%)	28,610±186 (32.70)	1.98	87,482±412
Control (radio-gold)	2716±86	82,605±398	0.033	89,320±438
(c) Solvent medium: (Triple solvent mixture [alcohol:water:ammonium hydroxide] 2:5:1)				
AuNP-labeled gamma globulin (vial 1) (%)	4280±96 (4.78%)	80,890±424 (90.48)	-	89,402±516
AuOP-labeled gamma globulin (vial 2) (%)	7,095±116 (7.85%)	76,500±398 (84.74)	-	90,270±492
Control (radio-gold)	1210±86	80,360±408	-	88,410±478

AuNP: Gold nanoparticle, AuOP: Gold ordinary particulate

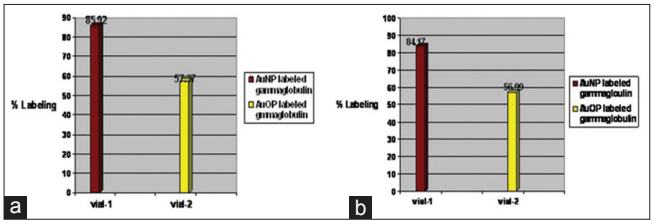

Figure 3: Charts showing comparative labeling of a nonspecific antibody (Bharglob) with radio nanogold and ordinary particulate radio gold in different solvent mediums. Complete statistical analysis of the Tables 1 was reported, representing the percentage of labeled, unlabeled, and hydrolyzed product The net percentage labeling of antibody with radioactive gold was calculated comparatively with the reaction mixtures of two vials (1) and (2) and was shown in the panels (a and b)

From the results so obtained, it can be reported that labeling of antibody with gold was observed both in the vial (1) and (2), but the fraction of the radiolabeled product was much greater, using radioactive nanogold solution as compared to the ordinary particulate size radiogold solution, (approximately 85%–90% labeling was reported in vial (1) as against about 55%–60% reported to the vial (2).

It was also observed from the results that the TLC of the two reaction mixtures of the vial (1) and (2) when carried out in a triple mixture solvent medium, marked a reduction in the hydrolyzed species was observed using radiogold nanoparticles as compared to ordinary particulate size gold solution. With best conditions, labeling efficiency was reported to be optimized, up to >85%–90% using nanogold particles of precise size and shape.

Statistical analyses

All data were evaluated by comparison tests and the values are expressed as the mean ± standard deviation. Each experiment was done in three separate experiments (n = 3).

In vivo biodistribution studies and the results obtained

Gold labeled nonspecific antibody (Bharglob) biodistribution study was performed for 120 h. It was observed that that the nanogold labeled antibody showed considerable activity in the stomach, liver, spleen, lungs, kidneys etc.The biodistribution study in a nontumor-bearing control mouse with 199-AuNP-labeled nonspecific gammaglobulin (Bharglob) was shown in Table 2. It was observed from the experimental data, that a significant amount of injected dose (activity) gets deposited in liver/spleen, and other organs which could represent RES localization. The reason for high activity in the stomach upto 24 h is not certainly explored. However, in case of Au-immuno conjugates, it may be because of unlabeled (free) gold deposited in the linings of stomach or may due to anti-inflammatory effect of unlabeled gold causing peptic ulceration of stomach or duodenum showing more frequent activity in this region.[26] In all the studies, it was observed that RES system is the major site of accumulation of radiolabeled compound using both specific and nonspecific labeled antibodies, thereby limiting the attainment of high tumor/nontumor ratios [Figure 4]. The toxicity study related to the administration of PEGylated gold nanoparticles reports no appreciable change, either in the weight loss or behavioral attitude of animal even after *in vivo* permeation, indicating no sign of elementary toxicity.

Studies relating to reticuloendothelial system blocking

Several approaches have been compared to improve the target/nontarget ratio. Some of the attempts include the use of Fab and $F(ab)_2$ constructs of antibody devoid of Fc component, but it was expensive in terms of using a tumor-specific immunoglobulin, generally, constructs by chemical methods. The use of cytokines for enhancing permeability was also suggested, but it also may have pharmacological adverse reactions.

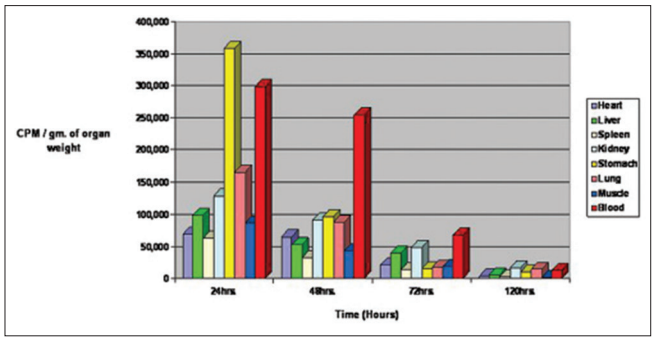

Figure 4: Biodistribution data of control mouse with (gold nanoparticle) labeled nonspecific Gammaglobulin. Nanogold-labeled nonspecific antibody (Bharglob) biodistribution was performed for 120 h. It was observed that the gold-labeled antibody showed considerable activity in the stomach, liver, spleen, and other organs which could represent reticuloendothelial system localization. In all the studies, it was observed that reticuloendothelial system is the major site of accumulation of labeled compound using both specific- and nonspecific-labeled antibodies, thereby limiting the attainment of high tumor/nontumor ratios

Table 2: Biodistribution study in control mouse with nanogold (^{199}AuNP)-labeled nonspecific gamma globulin (Bharglob)

Organs	CPM/gram of organ weight Time (h)			
	24 h	48 h	72 h	120 h
Heart	68,846	65,218	21,275	4016
Liver	98,708	52,610	39,554	5288
Spleen	62,357	32,405	12,920	2178
Kidney	128,515	90,572	48,338	16,488
Stomach	358,715	95,980	15,705	10,040
Lung	165,180	87,165	17,808	14,020
Muscle	86,264	42,510	19,216	1098
Blood	298,726	254,989	68,208	12,216

CPM: Counts per minute

Table 3: Mean percentage reduction in reticuloendothelial system uptake with different blocking agents injected 4-24 h prior to the actual radiolabeled products (n=3 mice each group)

Blocking agents	Time-gap between blocking and radiolabeled agent (h)	Radiolabeled products	
		AuNP-IgG (vial 1)	AuOP-IgG (vial 2)
Indian Ink	4	1.8±8.2	1.1±10.4
	24	1.2±7.4	1.3±8.6
Gamma-globulin	4	28.7±5.1	22.1±5.8
	24	49.2±6.2	38.0±4.4
Dextran	4	18.1±7.7	18.6±8.3
	24	32±7.6	28.9±9.3
Polygeline	4	13.6±5.3	Not done
	24	29.8±9.3	23.8±4.7
Hydroxy ethyl starch	4	14.6±4.8	Not done
	24	28.7±9.8	Not done

AuNP-IgG: Gold nanoparticle labeled immunoglobulin, AuOP-IgG: Gold ordinary particulate labeled immunoglobulin

An attempt to study whether liver/spleen uptake could be reduced by the blocking RES was made in experimental animals. The use of different blocking agents like (a) Indian-ink (b) dextran (c) nonspecific gammaglobulin (d) polygeline (e) hydroxy ethyl starch to reduce RES uptake was studied. These blocking agents were administered in quantities of 1 mg. Each through tail vein of an experimental animal either 4 or 24 h prior to the administration of the actual radiolabeled product (gold labeled antibody). From the study, it was confirmed that the blocking agent most effective, was the unlabeled nonspecific gammaglobulin, which could reduce RES localization effectively (as reported from the experimental data of [Table 3 and Figure 5].

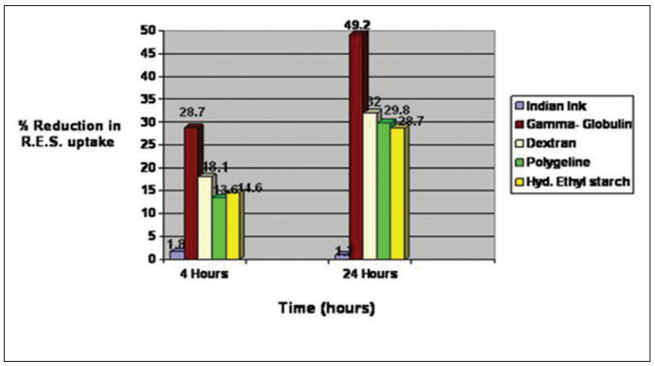

Figure 5: Comparative reduction in reticuloendothelial system uptake with different blocking agents injected 4–24 h before radiolabeled agent (gold nanoparticle labeled immunoglobulin). The use of different blocking agents such as (a) Indian-ink ((b.) Non-specific gammaglobulin (c.) Dextran (d) polygeline (e) hydroxy ethyl Starch to reduce reticuloendothelial system uptake was studied. From the study, it was confirmed that the blocking agent most effective was the unlabeled nonspecific gammaglobulin, which could reduce reticuloendothelial system localization effectively when administered in prior, before the administration of the actual radiolabelled product (gold labeled antibody)

The biodistribution results provides a combined effect of permeability, distribution, metabolism, and excretion and can give an idea of certain sets of pharmacokinetic parameters and toxicology end points, which are helpful in expediting the future plans of research. The biodistribution studies were performed, to compare the values of mean percentage reduction in the RES uptake with different blocking agents using two different radiolabeled products (vial (1) radiolabeled nanogold immunoconjugate, vial (2) ordinary particulate size radiolabeled-gold immunoconjugate).

Discussion

Gold nanoparticles were synthesized using an ecofriendly method, to eliminate the formation of chemical derivatives and its effect on the nature. The synthesis of gold nanoparticles is highly dependent on the pH maintained, which in turn depends on its interaction to the binding site. A low pH range of (2–3) was adopted for the synthesis of spherical shaped nanoparticles of precise size range (25–85 nm). At this low pH value, the biomass may carry more positive functional groups that allow the Au (III) ions to get more closely to the binding site. A mild temperature reaction condition aids the formation of a stable and

increased quantity of production, with a well-defined dimensional distribution reported.[27,28]

The optimized antibody gold nanoparticle labeling results (>85%) achieved, using folic acid, and PEG showed the potential of folic acid coupled PEGylated nanoparticles as a useful addition. Folic acid served as a ligand for generating tumor specificity on these particles while the inclusion of PEG provides sheath coating onto the particles, make them longed circulatory by reducing their RES uptake.[29,30] The PEG-coated gold nanoparticles do not cause an obvious decrease in body weight or any appreciable change in the behavior of animal noticed by us even their breakdown *in vivo*. However, in some research reports, it is pointed out that the physical dimensions and surface chemistry of gold nanoparticles play an important role in generating toxicity. Smaller particles accumulate themselves more rapidly in the liver and spleen and can show an adverse effect on the immune system. More biochemical investigations relating to the change in blood counts (Hb. TLC, SGOT, SGPT etc), toxicity related to liver & kidney dys-function, estimated by change in bilirubin and creatinine level, needs further evaluation.

Chanda *et al*.[31] in their research study reported the use of thioctic acid for conjugation of gold nanoparticles with bombesin peptides (AuNP–BBN conjugates). However, our preference was much for the use of folic acid, on account of its biocompatible nature. Further to prevent aggregation of these biosynthesized nanoparticles, a stabilizing agent sodium citrate was added during the synthesis process. Sodium citrate plays a dual role: (a) as the reducing agent and subsequently, (b) as a stabilizer, as it gets absorbed onto the surface.[32,33]

Biodistribution *(in vivo)* studies of the radiolabeled product (nanogold-labeled antibody) was carried out in a nontumor-bearing control mouse. The animal model used in experimental trials is a Swiss Albino mouse (*Mus musculs*). Sacrificing of mouse with periodical counting of radioactivity in different organs takes place in a nontumor-bearing animal. The nontumor-bearing animal does not show any histological signs of viral induced toxicity. A considerable amount of the injected radiolabeled activity was observed in the stomach, liver/spleen etc., indicative of the RES uptake. To reduce these alternate approaches have been tested, which includes the degalactosylation of antibodies, use of antibody constructs Fab and $(Fab)_2$ devoid of Fc component, but the tumor dwell time of Fab and $(Fab)_2$ was relatively short and therefore inadequate for delivering an adequate therapy dose. Moreover, it was also expensive in terms of using a specific antibody constructs by chemical methods. The use of cytokines for enhancing permeability was also reported, but it may also have a widespread pharmacological adverse reaction. The use of hyperthermia and ultrasonic beams, but this is only limited to superficial tumors.

Finally, it was decided to test the use of different large molecular weight blocking agents to block the RES uptake and it was again confirmed by us that the prior administration of unlabeled nonspecific gammaglobulin was found to be the most effective agent among all in reducing the RES uptake up to 30%–50%.[34-36]

Although it appears that the ultimate clinical solution may involve the combination of all the above approaches, but for reducing RES localization using different blocking agents, the use of unlabeled, non-specific gammaglobulin, appears to be one of the most attractive and technically easy options.[37-40]

Conclusion

The work has been both challenging and interesting. Labeling antibody (both specific and nonspecific) with radioactive nanogold reaction mixture was successfully achieved in terms of high labeling yield >85%. The study signifies the potential of folic acid coupled PEGgylated gold nanoparticles of precise size range suitable to use as a delivery vehicle for targeting small biomolecules (antibody) to the tumor site.

The RES uptake in the liver and spleen was a problem and the nonspecific gammaglobulin was identified as the best blocking agent for this purpose compared to other alternate approaches. However, the stability of the radiolabeled product under physiological conditions and its biodistribution needs further evaluation.

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Conflicts of interest

There are no conflicts of interest.

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