



Recent advances in arsenic trioxide encapsulated nanoparticles as drug delivery agents to solid cancers

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Abstract

Since arsenic trioxide was first approved as the front line therapy for acute promyelocytic leukemia 25 years ago, its anti-cancer properties for various malignancies have been under intense investigation. However, the clinical successes of arsenic trioxide in treating hematological cancers have not been translated to solid cancers. This is due to arsenic's rapid clearance by the body's immune system before reaching the tumor site. Several attempts have henceforth been made to increase its bioavailability toward solid cancers without increasing its dosage albeit without much success. This review summarizes the past and current utilization of arsenic trioxide in the medical field with primary focus on the implementation of nanotechnology for arsenic trioxide delivery to solid cancer cells. Different approaches that have been employed to increase arsenic's efficacy, specificity and bioavailability to solid cancer cells were evaluated and compared. The potential of combining different approaches or tailoring delivery vehicles to target specific types of solid cancers according to individual cancer characteristics and arsenic chemistry is proposed and discussed.

Keywords: arsenic trioxide, solid cancer, nanotechnology, drug delivery, liposome

Introduction

Arsenic is a naturally occurring metalloid found as a trace element in the earth's crust^[1]. Instead of being present in its elemental forms; it exists as highly toxic arsenates of metals like calcium, sodium and potassium or as oxides and sulphides. Among its three inorganic forms, arsenic trioxide (ATO) manifests as white arsenic and is industrially produced as a by-product while roasting arsenic containing ores and purifying the smoke^[2]. The transformation of ATO from being the "king of poisons" to a "broad spectrum anti-cancer drug" is one of the most exciting stories in clinical oncology^[3].

Despite being the mainstay of *materia medica* since the past 2,400 years, ATO faced an escalating decline in its use as a therapeutic agent in the twentieth century due to an increasing awareness of its toxicity^[4]. Its resurgence transpired in the late twentieth century when a group of Chinese physicians recognized it as the main anti-leukemic ingredient in traditional Chinese medicine and decided to evaluate its therapeutic potency in treating patients with acute promyelocytic leukemia (APL), a subtype of acute myeloid leukemia^[5]. Clinical trials using ATO to treat APL patients as a single agent or in combination with conventional chemotherapy yielded highly encouraging results^[5]. ATO was consequently approved by the Food and Drug Administration

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(FDA) as frontline therapy for APL in 2000^[6]. Similar clinical successes were subsequently translated for other hematological malignancies, for instance, acute myeloid leukemia, chronic myelogenous leukemia, Hodgkins disease and multiple myeloma although not significantly for solid tumors^[7]. Rapid clearance of ATO and its products from the blood by the reticuloendothelial system (RES) limits the therapeutic dose reaching the tumor site. Increasing ATO dosage could not be a solution as it leads to systemic toxicities such as long-term memory damage, liver failure, cardiac failure and peripheral neuropathy^[8].

Since then, several attempts have henceforth been carried out to utilize arsenic's significant anti-cancer properties for treating solid tumors by increasing its bioavailability and specificity for carcinoma cells while reducing its administered dosage^[9]. These include sensitizing the cells prior to ATO treatment, employing it in combination therapy with other conventional chemotherapeutic agents to explore their synergistic actions or employing nanotechnology to increase bioavailability of ATO while reducing its systemic toxicity^[10-13].

This review aims to summarize the advances that have been made till date regarding therapeutic applications of ATO drug with an emphasis on the ongoing involvement of nanotechnology in the current ATO therapy in treating various malignancies.

Arsenic trioxide (ATO) history: rise, decline and resurgence

ATO presents a rich history in medicinal applications dating back to more than 2400 years^[14]. It was used in traditional Chinese medicine to treat syphilis, psoriasis and rheumatism^[2]. Other applications were also reported with ATO being effective as an anti-spasmodic, anti-pyretic, anti-periodic, caustic and sedative^[14]. However, the awareness of its toxicity began to rise when people realized that long-term chronic exposure to ATO could cause hyperpigmentation of the skin, cancers of the lung, kidney, bladder and prostate along with rare cases of neuropathy, encephalopathy, leucopenia and diabetes^[6]. Consequently, by the turn of the twentieth century, its medicinal use started to decline due to its acquired reputation of being a toxic substance and a carcinogen.

ATO resurged as a therapeutic compound in 1990s following an initial study at Harbin Medical University where its anti-leukemic properties were identified in treating APL^[14]. A group of physicians at Shanghai Second Medical University continued the study and

remarkable results were observed from the clinical trial with patients of relapsed and refractory APL after very low doses of intravenous ATO were administered^[15]. It was a major breakthrough in treating APL relapsed patients as the mortality rate with routine treatment i.e. all-trans retinoic acid (ATRA) and anthracycline based chemotherapy was 20-30% and bone marrow transplantation was the only option^[14]. The findings, which were later successfully replicated in United States, led to the approval of ATO (TrisenoxTM) by FDA as a frontline drug for relapsed and refractory APL in 2000^[6].

Although some successes of ATO therapy have also been observed in other hematological cancers, the therapeutic dosage administered is relatively low, and hence the commonly reported side effects are low grade and reversible after ceasing ATO therapy. However, the same kind of clinical success has not been translated for solid cancers in humans despite ongoing clinical trials to investigate arsenic's efficacy on a variety of solid cancers^[15] (<http://www.clinicaltrials.gov>). This is primarily due to the rapid clearance of ATO and its metabolites from the blood by RES. Compromised therapeutic dose of ATO reaches the tumor site, and, therefore, an increased dosage is required which might, in turn, lead to systemic toxicities and long-term memory damage and hormonal imbalance^[8,16]. Therefore, there is a crucial need for analyzing various possible approaches of increasing the bioavailability of ATO without raising its dosage.

Therapeutic strategies in using ATO alone or in combination

Anti-cancer activity of free ATO has been tested on a variety of solid tumor cell lines with positive experimental results but limited clinical success. Zhao and coworkers^[17] administered direct intra-tumoral injections of ATO to human esophageal carcinoma xenografts in mice and investigated ATO distribution and its efficacy within the tumor. Tumour growth inhibition of 13.56%, 62.37% and 76.92% was observed with administration of 1, 5 and 10 μg of ATO respectively without appreciable systemic side effects. These results provided encouraging evidence for potential clinical utility of direct intra-tumoral injections of ATO as one form of treatment for solid cancers. However, there is an escalating need to investigate other approaches where ATO can be delivered selectively to its site of action and to penetrate inside the tumor without being toxic to surrounding healthy tissue. Some of the approaches that have been tested or proposed are outlined below (**Fig. 1**).

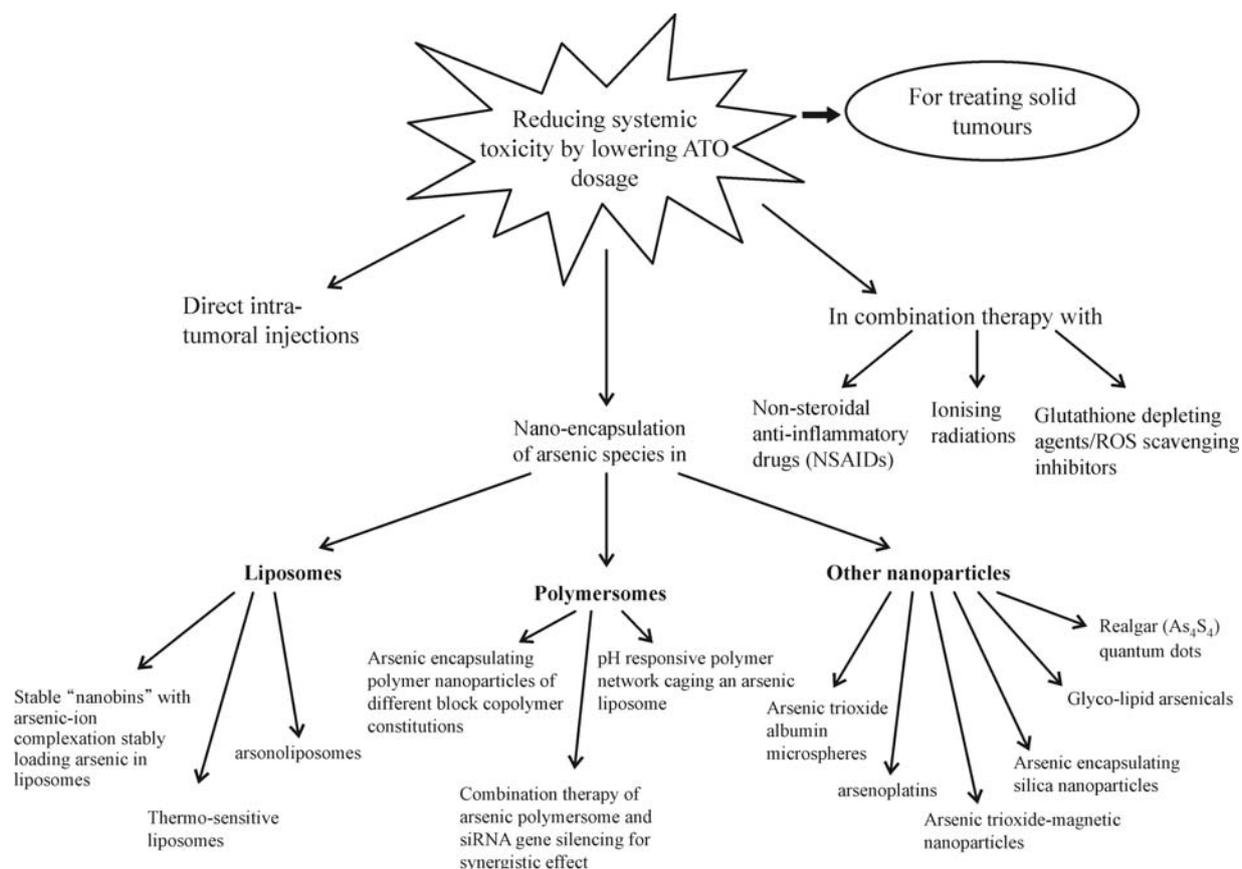


Fig. 1 Key therapeutic strategies employed in the attempt to increase the efficacy of ATO as a drug in treating solid tumors without escalating its dosage^[9,11-12,18-39]. ATO: arsenic trioxide; ROS: reactive oxygen species.

Glutathione depleting agents

One of the key targets of ATO is glutathione (GSH), a tripeptide molecule which protects the cells from oxidative stress. It is involved in a number of bio-reductive reactions and in the detoxification of xenobiotics^[11]. Glutathione titrates intracellular arsenic by forming a complex $\text{As}(\text{GS})_3$ ^[11]. This interaction is exothermic and spontaneous at pH 7.4 and temperature 37°C ($\Delta G = -8.8$ kcal/mol). The formation of this complex is preceded by its export out of the cell via multidrug resistance export proteins (MRP1-10) found in cells^[8]. Hence, substances which deplete glutathione can sensitize the cells to ATO anti-tumor activity by preventing its export out of the cells. To verify the importance of modulation of glutathione levels, in one experiment, glutathione content was increased by treating the cells with *N*-acetylcysteine. The anti-tumor activity of ATO was subsequently observed to be suppressed^[12]. This makes it evident that simultaneously depleting intracellular glutathione levels might be an effective strategy to intensify ATO action.

Gartenhaus *et al.*^[13] reported ascorbic acid to be one such compound which depletes intracellular glutathione

levels. Ascorbic acid further auto-oxidises to release H_2O_2 which might enhance pro-apoptotic effects of arsenic. *L*-Buthioninesulphoximine (BSO) is another drug which inhibits a critical step in glutathione synthesis by inhibiting the rate limiting enzyme γ -glutamyl cysteine synthetase along with generating reactive oxygen species (ROS). This sensitizes the cells to ATO treatment and was reported to enhance drug's efficacy in inducing apoptosis on a number of cell lines arising from the cervix, bladder, prostate, kidney, colon, pancreas and breast cancers^[18]. Further biochemical studies indicated that this combination treatment blocks the H_2O_2 scavenging system in the cells by interfering with glutathione along with catalase and glutathione oxidase enzymes. Therefore, this combination of an ROS producing drug and ROS removing inhibitor has the potential to offer selective and efficient anti-cancer treatment^[18].

Ionising radiation

When ATO is used in conjunction with ionising radiation, radiation response was reported to be intensified in tumor cells. ATO acts as a radiosensitizer

to tumor cells by increasing their susceptibility toward apoptosis. Combined with a low-dose irradiation, the dual treatment has been reported to hold a promise for effective cancer therapy. Kang and Lee^[9] observed synergistic effect when human cervical cancer cells were treated with ATO along with ionising radiation. The treatment caused regression in tumor growth by increasing ROS, destabilizing the mitochondrial membrane and activating caspases in cancer cells.

Non-steroidal anti-inflammatory drugs (NSAIDs)

These drugs are known to enhance the susceptibility of cancer cells toward various further anti-cancer treatments such as biologic treatment, chemotherapy and radiotherapy. One such drug sulindac has been reported to sensitize the cells due to its apoptosis inducing and anti-angiogenic properties^[40]. It increases the radiation sensitivity of cancer cells by interfering with tumor neovascularisation^[40]. Sulindac also exhibits synergistic augmentation of cytotoxicity when used alongside paclitaxel^[41]. Similarly, when sulindac was used alongside ATO on human lung cancer NCI-H157 cell line, it enhanced caspase activation *via* generation of ROS^[19]. This synergistic action allowed much lower concentration of ATO to be effective in carrying out its anti-cancer properties.

Another NSAID drug, indomethacin, is a known inhibitor of cyclooxygenase-2 (COX-2), whose expression is significantly increased in a variety of cancers^[19]. Hence, by acting as a COX-2 enzyme inhibitor, indomethacin exerts its cytotoxic effects on tumor cells. When it was used in combination with ATO, very low doses of both drugs achieve significant cytotoxicity^[42].

The synergism in their anti-cancer action was clearly evident as either drug was not as toxic when it was administered at the same concentration alone^[42].

Encapsulation of ATO as a single agent or co-encapsulated with another drug

The advent of nanotechnology has already changed the landscape of drug delivery. Improving the bioavailability of poorly water soluble drugs, selectively delivering the drug in a cell or tissue specific manner, co-delivering two or more drugs, transporting the drug to intracellular compartments/site of action and visualizing the site of delivery using imaging modalities are just few of the promises that nanotechnology holds for drug delivery^[43] (**Fig. 2**). ATO, along with few other organic arsenicals, has already been encapsulated in nanoparticles and their efficacy is summarized and evaluated in this review (**Table 1**).

Liposome encapsulation

Liposomes are lipid nanoparticles encapsulating a hydrophilic interior with a hydrophobic membrane. They are formed *via* self-assembly when certain phospholipids are hydrated in water^[44]. The hydrophobic membrane can entrap strongly lipophilic drugs whereas the hydrophilic interiors can encapsulate water soluble molecules like ATO. Zhao *et al.*^[17] prepared liposomes encapsulating ATO and intravenously injected them to investigate their therapeutic effect on C6 gliomas established in rat brains. Liposomal preparation led to a 5-fold increase in arsenic concentration in rat brains as compared to ATO solutions,

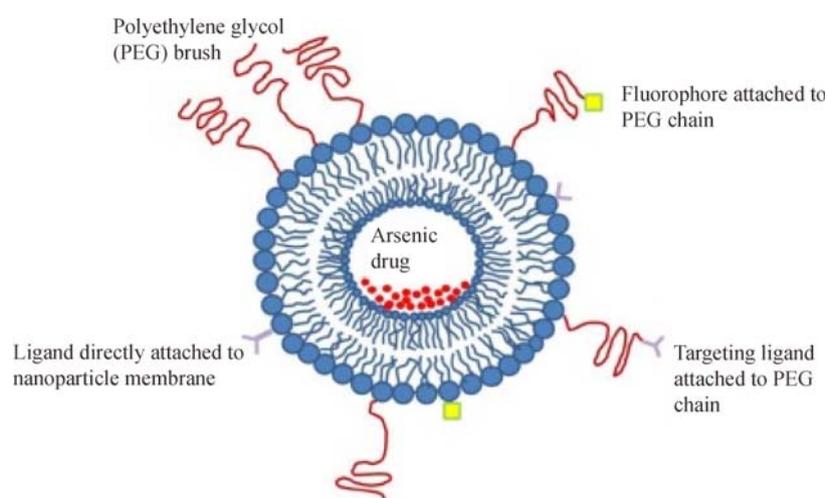


Fig. 2 Implementation of nanotechnology for ATO drug delivery. Amphiphilic phospholipids and block co-polymers self-assemble to form liposomes and polymersomes, respectively, which can effectively encapsulate ATO in their cores. Polyethylene glycol (PEG) layer acts as a brush to endow stealth, prevent immune recognition and recognition by serum proteins and increases their circulation half-life *in vivo*. Targeting ligands such as antibodies, peptides, proteins and other small molecules attached to the membrane bilayer or PEG facilitate the receptor-mediated endocytosis. Fluorophores attached promote the imaging of the nanoparticles and their fate *in vivo*.

Table 1 Applications of delivering ATO into cancer cells using nanotechnology.

Delivery vehicle	Co-encapsulated agent	Targeting ligand	Type of cancer tested	Treatment response	References
Liposomes	Manganese (Mn^{2+})	F(ab) ₂ fragments of PGN635, human monoclonal phosphotidyl serine (PS)-targeting antibody	Glioblastoma multiform (GBM)	Encapsulated As^{3+} - Mn^{2+} dissociates to release As^{3+} as active drug and Mn^{2+} as T ₁ contrast agent for MRI imaging.	[10]
Liposomes (Nanobins)	Transition metal ion (Ni, Cu, Zn, Co)	NA	Lymphoma; Breast cancer; SU-DHL-4 human lymphoma cell lines	Liposomal ATO is less harmful to the ovarian functions of female patients.	[20–22]
Liposomes	Transition metal ion (Ni, Cu, Zn, Co)	Urokinase plasminogen activator antibody	Ovarian cancer	Uptake of targeted liposomes by cancer cells is 4-fold higher than non-targeted ones.	[23]
Liposomes	NA	NA	NA	Liposomal ATO is more effective than free form in reducing the oncogene level (HPV-16) in HPV positive cervical cancers and inducing apoptosis	[24]
Liposomes (thermosensitive)	Transition metal ion (Ni, Cu, Zn, Co)	NA	NA	Drug release achieved by a small but rapid increase in temperature (< 4°C)	[25]
Liposomes	Transition metal ion (Ni, Cu, Zn, Co)	Folic acid	FR positive human nasopharyngeal and cervical cancer cell lines	Enhanced anti-cancer activity observed with the targeted liposomes	[26]
Liposomes (incorporating arsenolipid)	NA	NA	Three malignant cell lines (HL-60, C6, and GH3)	Induced morphological changes and inhibited survival of cancer cell lines while being non-toxic to control cells.	[27]
Polymersomes (PEGylated PLGA nanoparticles)	NA	NA	Murine peritoneal macrophages	Uptake by macrophages reduced indicating longer circulation half-life of nanoparticles	[28]
Polymersomes	NA	anti-CD44v6 single chain variable fragment	Pancreatic cancer cell line, PANC-1	Targeted nanoparticles display enhanced accumulation in tumor site and successfully induce apoptosis.	[29]
Polymersomes with thiol groups for loading of phenylarsine oxide (PAO) utilizing thiol-arsenic chemistry	NA	NA	Breast cancer, MDA-MB-435 cell line	The polymeric system displays similar toxicity as free PAO on the cell line tested.	[30]
Nanoparticles formed by linking sugar-lipoaminoacid to PAO	NA	NA	Breast cancer MCF-7 cell lines and HT-29 colon cancer cell lines	Inhibition of cell proliferation and apoptosis in MCF-7 but not in HT-29 cell lines showing high selectivity within these arsenicals.	[31]

Table 1 Applications of delivering ATO into cancer cells using nanotechnology. (continued)

Delivery vehicle	Co-encapsulated agent	Targeting ligand	Type of cancer tested	Treatment response	References
pH responsive polymer network surrounding ATO encapsulating liposome	Ni ²⁺ cation	NA	Human cervical HeLa cell lines	Simultaneous release of both Ni ²⁺ and As ³⁺ , with the former diminishing anti-oxidants levels aiding the apoptotic effect of As ³⁺ .	[32]
Liposomes (thermosensitive)	Mn _{0.5} Zn _{0.5} Fe ₂ O ₄ magnetic nanoparticles	NA	MDA-MB-231 breast cancer cell line	The magnetic nanoparticles serve as the source of hyperthermia causing the liposome to destabilize at its phase transition temperature and release As ³⁺ to induce apoptosis.	[33]
Nanoparticles (Arsenic platinum complex)	Pt ²⁺	NA	Colon HCT-116 and glioblastoma U-87 cell line	Combination of cisplatin and ATO proves more effective than either of the drug.	[34]
Magnetite doped mesoporous silica nanoparticles (MSN)	Drug camptothecin in the pores of MSN along with ATO on its surface using thiol binding	NA	Pancreatic cancer cells (BxPC-3)	Displays a synergistic effect and magnetite superparamagnetic crystals can be used as a contrast agent for MRI	[35]
Polymersome encapsulating arsenic	Co-treatment with si-RNA polyplex	NA	Human pancreatic cancer	Synergistic effect with si-RNA silencing KRAS oncogene and As ³⁺ inducing apoptosis	[36]
Fe ₃ O ₄ magnetic nanoparticles complexed with As ₂ O ₃	Iron oxide nanoparticles	NA	Human cervical cancer cell line (HeLa)	Inhibit cancer-related proteins expression when combined with hyperthermia	[37]
Liposomes	Platinum	Folic acid	Folic acid positive tumor cell line	Enhanced uptake and apoptosis induction of targeted liposomes	[26]
ATO nanoparticles	Coated with chitosan and 2,3-dimercaptosuccinic acid	NA	Human androgen dependent and independent prostate cancer cell lines	Coated nanoparticles are more stable and less toxic than bare As ₂ O ₃ nanoparticles.	[38]

inducing apoptosis and inhibiting tumor angiogenesis by interfering with the expression of vascular endothelial growth factor (VEGF) with fewer side effects^[17]. However, the liposomes formulated were stable for a maximum of three days, which limited their therapeutic use.

Nanobins

ATO forms arsenous acid, $\text{As}(\text{OH})_3$, when dissolved in water at physiologic pH^[8]. The latter is able to rapidly cross the lipid membranes; thereby, the liposomes formulated with ATO encapsulated are highly unstable with a stability life span of around 3 days. The issue of such "leaky liposomes" was solved when Chen *et al.*^[26] developed a novel method to stably encapsulate ATO in liposomes utilizing a metal-ion gradient loading mechanism. Arsenic complexes with transition metal ions such as Ni^{II} , Co^{II} , and Pt^{II} and forms a precipitate. Utilizing this property, liposomes formed are first loaded with acetate solution of a transition metal ion, followed by loading of ATO to the liposomes formulated^[22]. Efflux of protonated acetic acid drives ATO to get efficiently encapsulated within the liposomes where it stably precipitates with the transition metal ion in the form of a solid, crystalline substance^[8].

Loading of ATO *via* this mechanism was found to be very efficient and the lipid layer surrounding the drug attenuates its toxicity around 4 times compared with the free drug. These liposomes exhibit a pH dependency by releasing the arsenic payload on encountering lower pH in either acidic tumor milieu or the endosomes after being taken up by the cells. Ahn *et al.*^[21] tested the efficacy of these nanobins for orthotopic model of triple negative breast cancer. A visible shrinkage of tumor was observed in rats which were subjected to nanobin treatment. This could be attributed to the prolonged pharmacokinetics due to enhanced drug exposure to the tumor caused by better penetration of liposomes along with a reduced clearance of encapsulated arsenic within PEGylated liposomes. The shelf life of nanobins was greater than 6 months, probably due to stability provided by direct arsenic-metal bond^[8].

Most recently, in our laboratories, Wang and colleagues^[24] have also established that treatment with liposomal ATO is distinctively effective for HPV infected cervical cancer cells, by reducing the expression of HPV-18 E6 oncogene and inducing apoptosis more effectively than free ATO.

When acetate solution of aqua-cisplatin was employed for arsenic complexation, the liposomes formed had both arsenic and platinum species sequestered inside. Cisplatin (cis-diamine dichloroplatinum^{II})

is widely used to treat a variety of solid tumors such as bladder, ovarian, and testicular carcinomas^[44]. On being intracellularly hydrolysed, it yields aqua cisplatin, which induces apoptotic cell death in cancer cells by creating intra and inter-DNA strands crosslinks^[34]. This method hence provides an efficient co-encapsulation strategy to load both ATO and platinum drugs within the liposomal aqueous core. To further improve anti-cancer activity of the resulting nanobin, Chen *et al.*^[26] conducted a study using folic acid as a targeting ligand to introduce ATO into malignant epithelial cancer cell lines which overexpressed folic acid receptors (FR). The efficacy of such "targeted" liposomes was analyzed on FR positive KB (human nasopharyngeal) cell line and cellular uptake of ATO via FR mediated endocytosis was observed to be much more than FR negative MCF-7 (breast cancer) cell line. Moreover, the targeted liposomal uptake was 3-6 times higher than untargeted ATO liposomes^[26].

The targeting *via* nano-carriers opens a new horizon for selectively delivering the drug to specific cancer cells. Zhang *et al.*^[23] utilized the overexpression of urokinase system in ovarian epithelial cells and consequently conjugated urokinase plasminogen activator (uPA) antibodies to nanobins. The resulting targeted liposomes were reported to exhibit more than 4-fold increase in their uptake by the ovarian cancer cells than their non-targeted counterparts. They also inhibited tumor cell growth both *in vitro* and *in vivo* and induced apoptosis along with downregulating stem cell marker expression. Hence, utilizing specific cancer cell surface characteristics and identifying suitable ligands to be attached to ATO encapsulating liposomes are essential in enhancing the efficacy of ATO therapy.

Thermosensitive liposomes

Mild hyperthermia has frequently been employed as a stimulus to release the drug encapsulated either in the aqueous core or in the lipid bilayer of the liposome^[45]. Local mild heating can be applied directly within a narrow temperature range. Moreover, it renders the cells susceptible toward oxidative stress caused by ATO treatment. Needham *et al.*^[46] observed that inclusion of small fraction of lysolipids in double tail lipid liposomes causes the drug to be released quickly at phase transition temperature T_m . Winter *et al.*^[25] described a liposomal formulation for encapsulation of ATO which efficiently released the drug on being heated from 27°C to 35°C. This was achieved by incorporating lysolipid such as monopalmitoylphosphatidylcholine (MPPC) in liposomes composed of dipalmitoylphosphatidylcholine (DPPC). Ten mol % of MPPC enabled a very rapid

release of encapsulated arsenic within the first hour of temperature change. ATO loading was accomplished using the same principle as described in the nanobin above.

Arsonoliposomes

Arsonoliposomes are nanosized liposomes constituted from arsonolipids, which are analogs of phospholipids where arsenic replaces the phosphorus in the lipid polar head group^[47]. This type of nanoparticles is designed to combine the anti-cancer properties of arsenic with the enhanced efficacy and reduced toxicity of liposomes. Arsonolipids, when employed on their own, do not possess anti-cancer activity adequate enough to be of therapeutic value^[48]. However, on being incorporated in liposomes, they demonstrate increased toxicity to cancer cells while being less toxic to normal cells. Phosphatidyl choline (PC)-based arsonoliposomes suffered from rapid clearance from blood circulation after *in vivo* administration, along with their aggregating and fusing into larger particles. However, the PEGylation of arsonoliposomes was able to enhance their bioavailability and pharmacokinetic profile and was shown to be effective against brain glioma, pituitary and leukemia cell lines^[27].

Polymersome encapsulation

Polymersomes are nanoparticles constituted of block copolymers that are organized in a bilayer, similar to liposomes, with the hydrophobic polymer wall surrounding an aqueous core^[44]. PEGylated PLGA nanoparticles were synthesized as drug delivery vehicles for ATO. Loading the nanoparticles with ATO was achieved by slightly modified spontaneous emulsification solvent diffusion (SESD) method after transforming the amorphous ATO into cubic crystal form and thereby, increasing its solubility in organic solvents^[28].

Qian *et al.*^[29] synthesized PEG-PDLLA vesicles from amphiphilic diblock copolymer poly (*D, L*-lactide) and encapsulated arsenite ion in the nanoparticles' core. To further improve the specificity, they conjugated an antibody fragment, i.e. anti-CD44v6 single chain variable fragment onto the vesicles which demonstrated high specificity and affinity toward membrane antigen CD44v6 overexpressed on a number of epithelium derived cancer cells. The formulated nanoparticles were then tested against human pancreatic cancer cell line PANC-1 and were found to be very efficient in delivering the payload within the tumor site in comparison to the non-targeted ones.

A polymeric micelle system has been developed

recently by Zhang *et al.*^[30] utilizing a biodegradable block copolymer. Free thiol groups on the hydrophobic block of the polymer backbone were used to conjugate an even more toxic form of trivalent arsenic species compared to ATO, i.e. phenylarsine oxide (PAO). 65% of free thiol groups were reported to conjugate with PAO whose release from the formulated nanoparticles was triggered by the presence of glutathione, which perhaps acted as a competing agent for thiol group. PAO encapsulation further served to improve micellar stability and prevented micellar dissociation in water or in the presence of detergents like SDS^[17].

In addition to the above, a polymeric shell was also employed in caging ATO encapsulating liposome "nanobin" as described previously. The pH responsive polymeric network surrounding the nano-sized liposome allowed the simultaneous release of membrane permeable trivalent arsenic ions along with membrane impermeable Ni cations to which they form a complex. When the pH is reduced to the levels found in late endosomes or tumor acidic milieu, both ions were released simultaneously, unlike nanobins, where nickel ions remained inside the liposome while the arsenic diffused out^[49]. This is an attractive strategy because nickel ions have been known to deplete intracellular glutathione and ascorbate levels, thereby rendering the cells even more susceptible toward ATO toxicity^[26]. The cytotoxicity of these polymer caged nanobins was tested against HeLa cells and they were found to be more potent in inducing apoptosis than plain liposomes. The triggered release from pH change was due to the collapse of the cross-linked polymeric network which creates pressure points, hence pressing liposomes and consequently destabilizing their lipid bilayer membrane, making them release their payload^[49].

Nanoparticle mediated siRNA and arsenic co-therapy

Zeng *et al.*^[36] demonstrated the potential synergistic effect of silencing mutant *KRAS* gene and arsenic therapy on pancreatic tumor cell lines with excellent results. Mutant *KRAS* gene plays an important role in cell transformation and cancer development and is known to be activated in a number of pancreatic cancers. Therefore, downregulating it with si-RNA gene therapy becomes an attractive treatment which gave positive results such as inhibition of proliferative, migratory and invasive pancreatic tumor cells^[50]. When co-treated with ATO, the apoptotic effect of the latter was substantially improved. Arsenic was encapsulated in poly(ethylene glycol)-block-poly(*DL*-lactide) vesicles and siRNA-complexed polyplexes were

formed from poly(ethylene glycol)-block-poly(L-lysine) polymer. Both of the polymer nanoparticles were simultaneously administered to the human pancreatic cancer cell lines PANC-1 and BXP-3 and their cytotoxicity was analyzed and substantiated^[50].

Other nanoparticles

ATO magnetic nanoparticles

Encapsulating magnetic nanoparticles along with an anti-cancer drug has many advantages. These nanoparticles enable targeting under the influence of magnetic field and improve drug's pharmacokinetics profile in vivo. In addition, these ferromagnetic nanoparticles can also be utilized for inducing hyperthermia in the tumor tissue. Magnetic fluid (fluid comprised of superparamagnetic nanoparticles) absorbs much higher energy than usual materials and transfers all of it to the tumor tissue where temperature may rise to 45°C. This concept, magnetic fluid hyperthermia (MFH), where magnetic nanoparticles are heated by applying alternating magnetic field is gradually becoming an attractive anti-cancer treatment^[33]. By behaving as localized points of heat, they may prevent the damage to healthy tissues as is commonly induced in conventional hyperthermia treatment.

In a previous study, Fe₃O₄ magnetic nanoparticles were synthesized with ATO utilizing co-precipitation and impregnation techniques to form nanosized As₂O₃-Fe₃O₄ complexes. These nanoparticles were directly injected into the xenograft cancer tissue of human cervical cancer HeLa cells in nude mice and were found to be internalised by the cancer cells. When combined with MFH, these particles attained a steady rise in temperature ranging from 42° to 65°C which successfully ablated the tissue^[37]. In another study, As₂O₃-MgFe₂O₄ nanoparticles were synthesized via solvent displacement methods and characterized^[51].

When such a drug-magnetic nanoparticle complex was co-encapsulated in a thermosensitive liposome, the heat from these magnetic nanoparticles could lead to destabilizing of the liposomal membrane, and the drug could then be released at the specified location. Li *et al.*^[33] successfully used magnetic hyperthermia and thermosensitive liposomes in conjunction by co-encapsulating Mn-Zn ferrite magnetic nanoparticles with ATO in liposomes. Upon exposure to alternating magnetic field, the Mn-Zn ferrite nanoparticles served as the heating source, causing the lipids in liposomal membrane to reach their transition temperatures and thereby release ATO. Their efficacy was tested against human breast cancer MDA-MB 231 cell line and

a significant inhibition of cancer cell growth was observed^[33].

Song *et al.*^[52] prepared magnetic core-polymer shell drug delivery system encapsulating ATO. The magnetic core employed was a small magnetic nanoparticle like Fe₃O₄ and the shell was composed of a biocompatible and biodegradable polymer. This ATO drug delivering system has been utilizing double emulsion-solvent evaporation technique by coating the magnetite with the polymer poly lactic acid (PLA). These hybrid nanoparticles had high encapsulation efficiency and were effective in achieving anti-cancer response along with magnetic targeting.

ATO albumin microspheres

Bovine serum albumin microspheres containing ATO were prepared employing chemical crosslink and solidification method^[53]. The drug loading experiments exhibited 29% encapsulation efficiency and the release experiments in vitro suggested a slower release of ATO after an initial burst from the microspheres, with a cumulative percentage release close to 95%^[54]. When conjugated with cell penetrating peptides, the microspheres could display an increased cellular uptake and consequent intracellular delivery of ATO into the cells. For this purpose, trans-activating transcriptional activator (Tat) peptide was employed and covalently linked to the albumin microspheres for successfully delivering ATO into the bladder cells^[55].

ATO loaded silica nanoparticles

Magnetite doped mesoporous silica nanoparticles (MSN) have been recently fabricated where both the internal porous surface and external surface could be utilized for loading different carcinogenic drugs. Moreover, they possessed further attractive features including a large surface area, ease of surface functionalization, biocompatibility and stimuli responsive drug release. Muhammad *et al.*^[34] loaded camptothecin, a poorly hydrophobic drug, into the pores while conjugating ATO onto the surfaces of the designed silica nanoparticles utilizing the interaction between arsenic and sulphhydryl groups. Besides, superparamagnetic magnetite nanocrystals tethered onto their surface served two important purposes. They allowed the nanoparticles to deliver drug payload to the site of action and also enabled monitoring of therapeutic response via magnetic resonance imaging (MRI). This dual drug nanoformulation exhibited synergism with very low concentrations of nanoparticles showing cytotoxic effect to cancer cells.

Arsenoplatins

Arsenoplatins are small molecule complexes of aqueous form of ATO linked chemically to Pt(II). They are formed when cisplatin, an inorganic drug, is heated with ATO in an acetonitrile mixture for three days^[34]. The rationale behind their synthesis is that cisplatin, cis-diamminedichloroplatinum(II), widely used to treat ovarian, head, neck, bladder and testicular cancers, shows synergistic activity when used alongside with ATO. Both of them induce apoptosis in cancer cells albeit with different mechanisms, cisplatin by causing inter and intra-DNA crosslinking and arsenic by the mechanisms listed above. Miodragović *et al.*^[34] reported the formation of arsenoplatins, which are stable in solution, display superior anti-cancer activity and also overcome drug resistance which is a major limitation of platinum drugs. The As-Pt core is suitable for ligand substitution reactions and its efficacy has been demonstrated for a variety of cisplatin-resistant cancer cell lines, such as cisplatin resistant ovarian, colon and glioblastoma cell lines.

Others

Wimmer *et al.*^[31] developed a glycol lipid arsenical and reported its anti-proliferative effects on breast cancer cells MCF-7. This compound was formed by chemically attaching lipoamino acid to sugar. The former increased the lipophilicity of the compound and augmented arsenic's toxic effects. In addition, realgar (As₄S₄) quantum dots have been produced recently both as a colloidal dispersion and as a hydrogel; the latter is pH responsive and both forms have been reported to possess superior anti-tumor properties^[8]. Most recently, chitosan, a well-known surface modifier, has been reported to be used for coating ATO nanoparticles. The resulting nanoparticles were biocompatible and had an improved stability and toxicity profile against prostate cancer^[38]. Additionally, these nanoparticles induced cell cycle arrest by p21 WAF/CIP1 expression via epigenetic remodelling in androgen dependent and independent cell lines^[56].

Conclusion

Despite an increasing awareness of toxicity arising from arsenic compounds, ATO has established itself as an effective, broad spectrum anti-cancer drug. Following its successful establishment as the front line treatment for APL patients, researchers and clinicians have been working toward improving its effectiveness in treatment and reducing its side effects for solid cancers. Its poor bioavailability due to rapid clearance

from blood by RES limits the therapeutic dose of arsenic reaching the tumor site. Therefore, using nanotechnology to facilitate ATO delivery into solid tumors becomes the focus in this field. Up to date, immense progress has been made in delivering ATO compounds specifically to the cancer cells. ATO along with other arsenic compounds has successfully been encapsulated in liposomes, polymersomes and other nanoparticles. In addition, ligands unique to the receptors overexpressed on specific cancer cells have been conjugated to these nanoparticles. Hence, it is promising that delivering ATO using nanotechnology would be employed as an anti-cancer drug option in treating a variety of solid cancers with better efficacy and much less toxicity.

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