R&D of Novel Medicinal Materials for Curing Cancer: Sugar Modified Gd-DTPA MRI Contrast Agents and Phospha Sugar Anti-cancer Agents

Junko Yamashita, Mitsuji Yamashita, Michio Fujie, Kazuhide Asai Takuya Suyama, Satoru Ito, Valluru Krishna Reddy, Manabu Yamada, Keisuke Ogawa, Nobuhisa Ozaki, Satoki Nakamura, Takashi Aoki, Gang Yu, Kengo Aoshima, Tatsunori Kato, Nao Kamikage, Keita Kiyofuji, Yasuo Takehara, Harumi Sakahara, Hisao Takayanagi, Tatsuo Oshikawa, Sophie Laurent, Carmen Burtea, L. Vander Elst, Robert N. Muller

Abstract:

Novel Sugar Dendritic Gd-DTPA Complexes for MRI Contrast Agents were prepared and evaluated by in vitro and in vivo methods. The sugar dendritic MRI contrast agents have a good blood vesse pool character, and draw blood vessels and liver cancer remarkably clearer than the clinically using Gd-DTPA. Phospha sugar derivatives or phosphorus heterocyclic derivatives provided by functional groups such as epoxide, bromide, etc., were prepared and evaluated by MTT in vitro method. These phospha sugar derivatives showed excellent activities against leukemia cells as well as solid cancer cells in fashions of (i) higher activity, (ii) wider spectra, (iii) higher selectivity and specificity distingushing healthy and cancer cells, etc., compared with the molecular targeting chemotheraputic anti-cancer agent, Gleevec.

Keywords: *MRI contrast agent, sugar-ball-dendrimer, Gd (III)-DTPA complex, phospha sugars, phospholanes, anticancer agent, tumor.*

1. Introduction

Cancer is one of the most serious diseases, and the disease is expected to be more and more serious if the innovation in cancer therapy will not be realized hereafter. To innovate in cancer therapy it is very important that medicinal materials or technologies to find tumors safely at the very early stage (early diagnosis) and to cure tumors by improving the quality of life (QOL) of the patients. To develop and realize such medicinal materials, highly functionalized MRI contrast agents [1], [2], which provide clearer images of very small cancers, are required. The currently quite often used MRI contrast agent is Gd-DTPA (Magnevist) which is safe and potential MRI contrast agent, however, the MRI contrast agent has poor characters for imaging blood vessels and cancers. To improve the poor characters of Gd-DTPA to draw cancers as well as blood vessels (Magnetic Resonance Angiography; MRA), Gd-DTPA was chemically modified by sugars. The results are described in the first part of this paper.

Molecular targeting chemotherapeutic agents play one of the most important role in curing cancers. Among chemotherapeutic agents Gleevec (Imatinib) is one of the most commonly used medicines. Gleevec has potential activity against cancers, especially leukemia cells, nevertheless, Gleevec has lower activity towards some of leukemia cells. Gleevec is also used for solid tumors, however, to cure larger tumor tissues by Gleevec some times faces lower efficiency for the complete cure. Therefore, new researches to develop alternative anticancer agents to Gleevec are steadily demanded.

Phospha sugar is one of sugar analogs which have a phosphorus atom in place of the ring oxygen atom of normal sugars and is assigned to a category of *pseudo* sugars. *Phospha* sugars are not found in nature yet, and then all of them reported until now are chemically synthesized. On the other hand, the alternative pseudo sugars, such as *aza-*, *carba-*, and *thia-sugars* [3], [4], having a nitrogen, carbon, and sulfur atom, respectively, in the hemiacetal ring of sugars, are widely known in nature and are also chemically synthesized and modified extensively. Many of them are known to have important biological activities.

Sugar starting materials, which have an oxygen atom in the hemiacetal ring, are basically and usually used to prepare *pseudo* sugars. To prepare phospha sugars from sugar staring materials is rather difficult compared with the other pseudo sugars, therefore, less kinds of *phospha* sugars are prepared and a little is known about the character of phospha sugars compared with *aza-*, *thia-* sugars, etc.

As an alternative preparative method, we have developed *phospha* sugar chemistry starting from phosphorus heterocyclic compounds, e.g., 2-phospholene derivatives, by chemical modification at their reactive sites [3], [4]. Addition of bromine to the unsaturated C=C double bond of the starting 2-phospholene derivatives produced 2-bromo- or 2,3-dibromophospholane derivatives. Substitution reaction of 2-bromophospholane derivatives, which correspond to 2-bromo-2-deoxyphospha sugar derivatives, with amine nucleophiles gave *N*-glycosides of *phospha* sugar derivatives. Further, nucleic acid bases such as uracil were introduced into 2-phospholene 1-oxide derivatives by the cyclization reaction of acrylamide derivatives to prepare phospha sugar nucleosides [3], [4].

We are continuously searching biological activity for these *phospha* sugars or phosphorus heterocycles by *in vitro* and *in vivo* bio-assays. In the second part of this paper, we will deal with the successful preparation of many kinds of *phospha* sugars or phospholane derivatives from 1-phenyl-3-methyl-2-phospholene 1-oxide and the related derivative. The biological activities of the prepared *phospha* sugars or phospholane derivatives were evaluated by MTT *in vitro* method for leukemia cell. These deta will be reported in this paper.

2. Results and discussion

2.1. Sugar-Ball-Dendritic MRI contrast agents

2.1.1 Preparation of Sugar-Ball-Denderimers of Gd-DTPA-Dn-Sugar structure

Currently, one of the most often used clinical MRI contrast agent is Gd-DTPA complex (Gadolinium Diethylenetriamine pentaacetic acid: Magnevist) (Fig. 1), whose molecular size is small and then the contrast agent penetrates the blood vessel. To make the MRI contrast agent remain in the blood vessels so as to draw clearer blood vessels (Magnetic Resonance Angiography (MRA)) and tumors, sugar ball dendritic structure of Gd-DTPA complexes were designated and synthesized. These dendritic Gd-DTPA complexes are generally represented in this paper as Gd-DTPA-Dn-Sugar. The reaction to prepare Gd-DTPA-D1-Glc(OAc) (four peracetylated glucose derivative) is exemplyfied in Scheme 1 [1], [2]. The product of the alikaline hydrized Gd-DTPA-D1-Glc(OH) is represented here as DEN-OH.

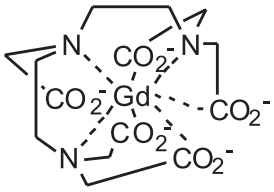
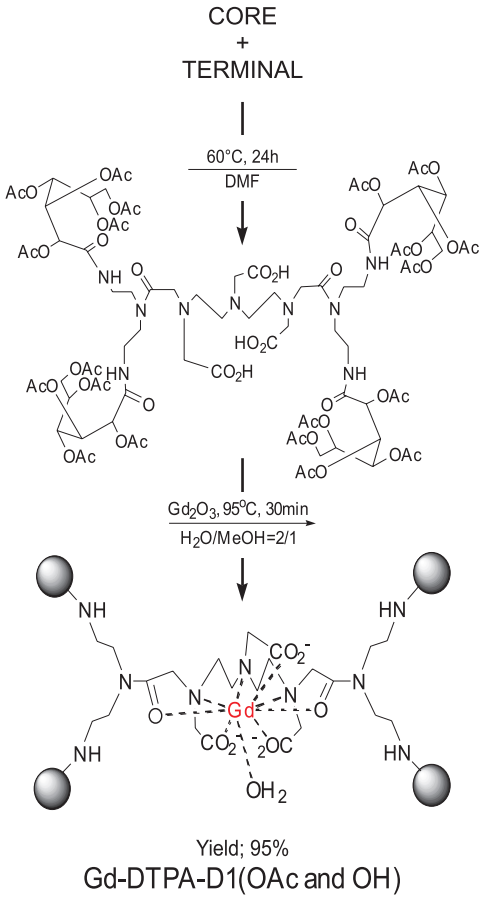


Fig. 1. Gd-DTPA (Magnevist).



Scheme 1. Preparation of Gd-DTPA-D1-Glc (OAc). The hydrized product of Gd-DTPA-D1-Glc(OAc) is simpely represented here as DEN-OH.

2.1.2 In vivo evaluation of DEN-OH as the MRI contrast agents to draw MRA and tumour

The Gd-DTPA-D1-Glc(OAc) and DEN-OH were subjected to *in vivo* evaluation by using rats. The MRA by DEN-OH drew blood vessel clearly as shown in Fig. 2 and tumors on the liver of rats were also drawn quite clear images as shown in Fig. 3.

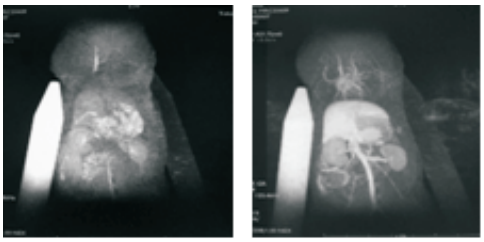


Fig. 2. MRA of rat at 30 min after injection (Left: by Gd-DTPA; Right: by DEN-OH).

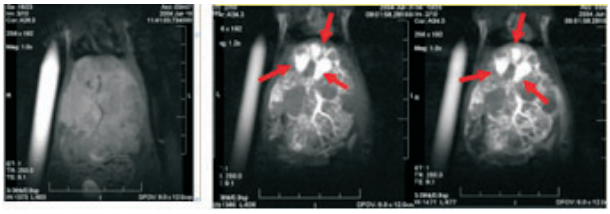


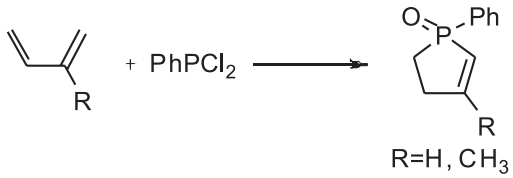
Fig. 3. MRI of liver cancer of rat (Left: by Gd-DTPA at 3 min after injection; Right two: by DEN-OH at 3 min and 30 min after injection).

Fig. 2 shows that DEN-OH draws boold vessels remarkably clearer than Gd-DTPA. And Fig. 3 shows that DEN-OH draws the liver cancer quite clearly. These results strongly indicate that Gd-DTPA-Dn-Sugar must be novel good MRI contrast agent for early stage tumor drawing.

2.2. Phospha sugar anti-cancer agents

2.2.1 Preparation of phospholenes, phosphorus heterocyclic compounds

The McCormack reaction of 1,3-dienes with phosphorus chlorides, e.g., phosphorus tichloride, phenylphosphonus dichloride, afforded 2-phospholene derivatives (Scheme 2), which were used as the starting materials of *phospha* sugar derivatives or phospholanes.

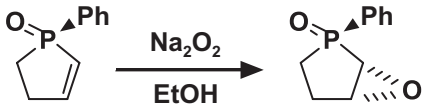


Scheme 2. Synthesis of 2-phospholenes (McCormack Reaction).

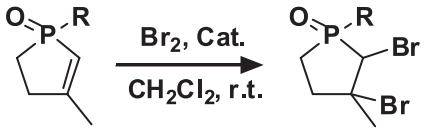
2.2.2 Preparation of *phospha* sugars or phospholanes

1,2-Anhydro-*phospha* sugars or 2,3-epoxy-1-phenylphospholane 1-oxides were prepared by an epoxidation of 2-phospholenes with sodium peroxide as shown in Scheme 3. The epoxidation reaction was stereospecific and stereoselective and gave essentially *threo* epoxide. The *threo* epoxide was defined by the two oxygen atoms

of epoxide and phosphoryl locate on the same side of the sugar ring skeleton. The 1,2-dibromo-1,2-dideoxy-*phospha* sugars or 2,3-dibromo-1-phenylphospholane derivatives were prepared by an addition reaction of bromine to the double bond of 2-phospholenes as shown in Scheme 4 [3,4].



Scheme 3. Epoxidation of 2-phospholenes with sodium peroxide.



Scheme 4. Preparation of 1,2-dibromo-1,2-didoxy-phospha segars.

The substitution, addition, homologation reactions, etc., were carried out to prepare new phospha sugars or phospholane derivatives.

2.2.3 Evaluation of phospha sugars or phospholanes by *in vitro* MTT method

MTT method of *phospha* sugars against leukemia cell lines, K562 and U937, were carried out for *in vitro* evaluation as the anti-tumor agents [5]. The results are shown in Fig. 4 and Fig. 5. Fig. 4 shows that some of phospha sugars, e.g., bromohydrin, epoxide, dibromo derivatives, were active and many of the other derivatives were inactive.

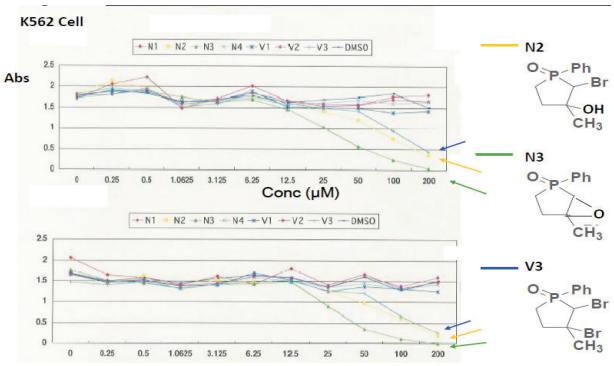


Fig. 4. MTT evaluation of phospha sugars as anti-tumor agents against K562 cells.

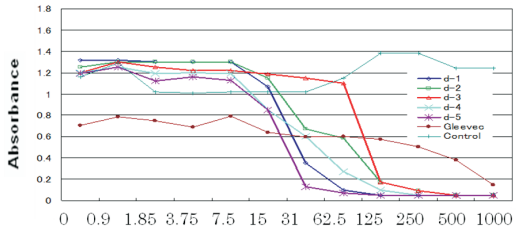


Fig. 5. MTT evaluation of phospha sugars (dibromophospha sugars) as anti-tumor agents (comparison with Gleevec) against U937 cells.

Fig. 5 shows that the dibromo derivative was also active against U937 cell lines. The diastereomers of the dibromide showed different activities, and they are much more active against the U937 cell lines than Gleevec. These findings strongly indicate that the *phospha sugars* must be quite active and wide spectral anti-tumour agents.

The dibromide showed that the *phospha* sugar were active not only against solution cancer (leukemia cells) but also against solid cancer (stomach cancer). Flow cytomertry for the preliminary mechanistic study indicated that these *phospha* sugars induced apoptosis for leukemia cells. Further studies on the optimization of the structure-activity of *phospha sugar* derivatives against cancer and the mechanistic studies are under progress.

3. Conclusion

The novel Gd-DTPA-Dn-Sugar structured MRI contrast agents could image quite small sized tumors, and then could be used for MRI contrast agents for MRA and initial stage tumor drawing. The novel *phospha* sugars could kill the leukemia cells in (i) high activity, (ii) selective and specific manner, (iii) wide spectra, by induction of apoptosis of cancer cells. Together with these novel medicinal materials, early stage findings and early stage chemotherapeutic treatment to cure cancers should be realized in the near future.

4. Experiment

4.1. Synthesis of 2,3-dibromo-3-methyl-1-phenylphospholane 1-oxide

To CH_2Cl_2 (10ml) solution of 3-methyl-1-phenyl-2-phosholene 1-oxide (0.27 g, 1.4 mmol) and Mn(IV) dioxide (0.24 g, 2.8 mmol; 2.0 eq.) was added drop wise CH_2Cl_2 (10 ml) solution of bromine (0.40 ml, 7.8 mmol; 5.6 eq.) and the reaction mixture was stirred for 8 h at room temperature. The reaction was quenched by addition of saturated sodium sulfite aqueous solution. The aqueous mixture was extracted with chloroform (10 ml x 3). The organic layer was neutralized with saturated $NaHCO_3$ aqueous solution, washed with saturated NaCl solution and dried over with anhydrous sodium sulfate. The solvent of the filtrate was evaporated under a reduced pressure to give an oily mixture of product. The mixture was purified by column chromatography on silica gel by using chloroform and methanol (30 : 1) as the eluent to give 2,3-dibromo-3-methyl-1-phenylphosholane 1-oxide (0.37 g) in 75% yield; m.p. (Shimadu Simultaneous DTA-TG Apparatus (DTG-60A50AH)) 189.20 °C; b.p. 280.24 °C; TLC (Silica gel: Wako Chromato Sheet and/or Merk Kieselgel 60; Eluent: $CHCl_3$: MeOH = 20 : 1), Rf = 0.42; MS (MALDI-TOF-MS: GL Science (Voyager-DE Porimerix); Matrix: α-Cyano-4-hydroxy-cinnamic acid (m/z)), 349.29 (M - H^+ (Molecular peak - 1); isotope peaks: 349.29, 351.29, and 353.29) and 351.29 (M + H^+ (Molecular peak + 1); isotope peaks: 351.29, 353.29, and 355.29); IR (JASCO FT/IR 410 (KBr)): 1126 cm^{-1} (P=O), 748 cm^{-1}, 1396 cm^{-1} (C-Br); ^{1}H-NMR (JEOL JNM-AL300 (300 MHz) and Hitach R90H (90 MHz); Solvent: $CDCl_3$, δ(ppm)); 1.67 (s, 3H, CH_3), 2.36-2.46 (m, 2H, H-4), 2.97-3.02 (m, 2H, H-5) 4.28-4.31 (m, 1H, C-2), 7.51-7.70 (m, 5H, Ph-H). HPLC (Apparatus:

JASCO HPLC Set (JASCO 860-CO, 880-PU, 875-UV, RI-930, and 807-IT; Column: Silica gel (Analysis: Wakopac, Wakosil Φ 4.6 mm × 250 mm, Eluent: $CHCl_3$: MeOH = 30 : 1, Flow rate: 0.5 ml/min), RT (retention time: min) values of diastereo isomers were 8.1, 9.1, 9.9, and 11.5.

AUTHORS
Junko Yamashita, Mitsuji Yamashita*, Michio Fujie, Kazuhide Asai, Takuya Suyama, Satoru Ito, Valluru Krishna Reddy, Manabu Yamada, Keisuke Ogawa, Nobuhisa Ozaki, Takashi Aoki, Gang Yu, Kengo Aoshima, Tatsunori Kato, Nao Kamikage, Keita Kiyofuji - Graduate School of Science and Technology, Shizuoka University, Hamamatsu 432-8561, Japan.
E-mail: tcmyama@ipc.shizuoka.ac.jp.
Yasuo Takehara, Michio Fujie, Harumi Sakahara, Satoki Nakamura - Faculty of Medicine, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan.
Hisao Takayanagi - Pharmaco-chemical Reserach Center, Tanabe Mitsubishi Pharmacy, Yokohama 227-0033, Japan.
Tatsuo Oshikawa - Department of Materials Chemistry, Numazu College of Technology, Numazu 410-8501, Japan.
Sophie Laurent, Carmen Burtea, L. Vander Elst, Robert N. Muller - NMR Laboratory, Department of Organic Chemistry, University of Mons-Hainaut, B-7000 Mons, Belgium.
* Corresponding author

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