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Review paper

Prostaglandins in the treatment of cancer

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Prostaglandins (PGs) with antiproliferative activity against tumor cells consist of the cyclopentenone PGs and the alkylidene cyclopentenone PGs. Such PGs are PGD_2, PGJ_2, Δ^{12}-PGJ_2, PGA_1, Δ^7-PGA_1 and PGA_2. Both PGJ_2 and Δ^{12}-PGJ_2 are ultimate metabolites of PGD_2 and have potent antiproliferative activity on tumor cells. Δ^{12}-PGJ_2 was identified in human urine, whereas Δ^7-PGA_1 has not been found in the human body. One important characteristic of both Δ^7-PGA_1 and Δ^{12}-PGJ_2 is that they have little cross resistance with cisplatin and adriamycin *in vitro* and *in vivo*. Δ^7-PGA_1 has 5-fold greater antitumor activity than Δ^{12}-PGJ_2. Methyl ester-$\Delta^7$$PGA_1$ (methyl-Δ^7-PGA_1) is stable chemically and can be easily synthesized in large amounts. All four isomers of methyl-Δ^7-PGA_1 showed the same antiproliferative activities on ovarian carcinoma cells. In addition, methyl-Δ^7-PGA_1 integrated in lipid microspheres (lipo-methyl-Δ^7-PGA_1) is more soluble in water than methyl-Δ^7-PGA_1 alone. Hence, lipo-methyl-Δ^7-PGA_1 was selected for extensive preclinical studies. Intravenous administration of lipo-methyl-Δ^7-PGA_1 could inhibit the growth of both HeLa S3 and Lovo colon cancer cells transplanted subcutaneously in nude mice. Lipo-methyl-Δ^7-PGA_1 by intraperitoneal administration could prolong the survival of scid mice bearing 2008C/13* cells resistant to cisplatin. The combined administration of cisplatin and lipo-methyl-Δ^7-PGA_1 prolonged the survival of nude mice bearing HRA cells compared with each single agent alone. Consequently, phase 1 clinical studies of lipo-methyl-Δ^7-PGA_1 in refractory ovarian cancer and colon cancer are planned. Δ^7-PGA_1 demonstrates irreversible binding to thiols, whereas PGA_1 shows reversible binding. Both Δ^7-PGA_1 and lipo-methyl-Δ^7-PGA_1 are metabolized to unknown products in human serum, whereas the latter is converted to Δ^7-PGA_1 in rat serum. The half-life of Δ^7-PGA_1 was 1.5 h in human serum, whereas that of methyl-Δ^7-PGA_1 was 13 min. The half-life of lipo-methyl-Δ^7-PGA_1 was almost the same as that of methyl-Δ^7-PGA_1. On the other hand, Δ^{12}-PGJ_2 was stable in human serum. Lipo-methyl-Δ^7-PGA_1 showed less toxicity than Δ^7-PGA_1 and Δ^{12}-PGJ_2. LD_{10} and LD_{50} for single administration were 17.4 and 33.5 mg/kg for male rats, and 38.1 and 45.9 mg/kg for female rats, respectively. All rats tolerated repeated administration of 10 mg/kg/day lipo-methyl-Δ^7-PGA_1 for 28 days. However, loss of weight was observed after 14 days of administration. Slight anemia was recognized with decrease of both red blood cell count and hemoglobin. However, the dose-limiting factors remain undetermined. The inhibition of DNA synthesis by antitumor PGs is independent of AMP. PGs were transferred into the nucleus, and Δ^{12}-PGJ_2 covalently bound to nuclear proteins and inhibited RNA synthesis. With respect to their antiproliferative activity, the primary effect of PGA_1, PGD_2, Δ^7-PGA_1 and Δ^{12}-PGJ_2 was to block cell progression from G_1 to S in the cell cycle. Both PGA_1 and PGJ_2 induced the synthesis of a 70 kDa protein (p70) which was identified as a heat shock protein related to the major 70 kDa heat shock protein group. The G_1 block is associated with both inhibition of *myc* gene family expression and induction of heat shock proteins. High dose administration of either PGA_2 or Δ^{12}-PGJ_2 resulted in significant G_2/M arrest and apoptosis. The PGs with a cyclopentenone ring have antiviral activity. PGJ_2 is a potent antiviral agent against Sendai virus and herpes simplex virus. In addition, PGA_1 and 16,16-dimethyl-PGA_2-methylester suppressed the proliferation of HIV-1-infected cord blood lymphocytes *in vitro*. The potency of antiviral activity was Δ^7-PGA_1 > PGA_1 > PGA_2. The cyclopentenone ring seems to have a universal action of both antitumor activity and antiviral DNA activity.

Key words: Antitumor activity, antiviral activity, mechanisms, prostaglandins.

Introduction

Prostaglandins (PGs) have been shown to be involved in the regulation of many physiological phenomena, including cell growth and differentiation[1–5] and immune function,[6–12] and to possess antiviral activity.[13–18] In several recent studies, prostaglandins have shown antitumor activity at the level of cell differentiation and proliferation, and enhance the host's immunity against tumors.[6–12] Most of the early studies on the antitumor effects of PGs examined the inhibition of animal tumors by PGA and PGE or their analogs. These PGs were shown to inhibit the growth *in vitro* and *in vivo* of several murine melanomas and Lewis lung carcinoma. Of the prostaglandins, PGD_2 has greater

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potency and few side effects.[5,19–21] Identification of PGJ_2 was epoch making in the development of antitumor PGs, since it was found to exhibit more potent tumor suppression than PGD_2 without other PGD_2 activity.[22] The conversion of PGD_2 to Δ^{12}-PGJ_2 in the presence of serum or albumin was demonstrated.[23] Δ^{12}-PGJ_2 has the most potent antitumor activity of the derivatives of PGD_2.[22,23]

Δ^{12}-PGJ_2 was found in human urine and was proven to be produced in the human body.[24] This suggests that Δ^{12}-PGJ_2 has some physiological roles, such as inhibition of tumors or viruses. PGA derivatives also have *in vivo* antitumor activity. Recently, both Δ^7-PGA_1 and PGJ_2 have been continued to be investigated for antitumor[25–30] and antiviral activities.[31–33] However, their precise mechanism of action is still unclear. Preclinical studies have continued to identify target tumors of both Δ^7-PGA_1 and Δ^{12}-PGJ_2, particularly in relation to drug-resistant tumors.[27,28] This supports the clinical application of antitumor PGs for drug-resistant tumors. At present, one such antitumor PG, lipo-methyl-Δ^7-PGA_1, has been selected for clinical studies, and both its antitumor activity *in vivo* and toxicity profile have been investigated. Hence, the present review focuses on the preclinical studies and potential of PGs as anticancer drugs.

Chemical structure of antitumor PGs

PGs that have antiproliferative effects on tumor cells are PGD_2, PGJ_2 Δ^{12}-PGJ_2, PGA1, Δ^7-PGA1 and PGA_2. Both PGJ_2 and Δ^{12}-PGJ_2 are ultimate metabolites of PGD_2,[23] and have potent antiproliferative activity on tumor cells. The chemical structures of the PGs responsible for their antiproliferative activity are cyclopentenone and alkylidene cyclopentenone.[34] Alkylidene cyclopentenone PGs such as Δ^{12}-PGJ_2 and Δ^7-PGA_1 have the most potent antiproliferative activity on tumor cells of the cyclopentenone PGs. Some octocorals produce Δ^7-PGA type alkylidene cyclopentenone eicosanoids such as clavulone and punaglandin which have similar antiproliferative activity to Δ^{12}-PGJ_2 and Δ^7-PGA_1. Such PGs contain 2-enone structures and are highly reactive with nucleophilic agents, thus forming Michael adducts.[34–36] This chemical reactivity may explain the biochemical activity of alkylidene cyclopentenone eicosanoid PGs. Modification of the Δ^7PGA_1 α- or β-side chains was performed to study the relation between the hydrophilicity/lipophilicity and the antiproliferative activity *in vitro*. Lipophilic derivatives of Δ^7-PGA_1 possess more potent antiproliferative activity on cultured cells *in vitro* than hydrophobic derivatives.[37] Δ^7-PGA_1 and the Δ^7-PGA_1 analog 5-[(*E*)-4,7-dihydroxy-(2*E*)-pentenylidene]-4-hydroxy-4-(4-phenoxybutyl)-2-cyclopentenone (TE10303) showed potent antiproliferative activity in ovarian cancer *in vitro* and *in vivo*.[38] Drug penetration of TE10303 into a multicellular spheroid was to a depth of about 150 μm from the outer layer. This suggests that hydrophobic PGs have poor tumor penetration (Sasaki and Terashima, unpublished observation). The optimal chemical structure of PGs for intraperitoneal or intravenous administration may be different from that of the potent antiproliferative PGs. The chemical stability of alkylidene cyclopentenone eicosanoids has been investigated in relation to their reaction with SH-containing compounds. Glutathione (GSH) reacts with Δ^7-PGA_1 methyl ester with a larger dissociation constant at neutral pH than with PGA_1 methyl ester.[36] Δ^7-PGA_1 demonstrates irreversible binding. Δ^{12}-PGJ_2 was identified in human urine, but Δ^7-PGA_1 has not been found in the human body. Methyl-Δ^7-PGA_1 is one of the synthesized PGs. Δ^7-PGA_1[38] and methyl-Δ^7-PGA^1 (Sasaki and Terashima, unpublished observation) have shown antiproliferative activity *in vivo*. Methyl-Δ^7-PGA_1 exhibits potent inhibition of cell growth *in vitro*;[26] however, it showed weak effects *in vivo*.[39] This suggests that methyl-Δ^7-PGA_1 may be metabolized in the human body. Furthermore, investigations are necessary on the metabolism of Δ^7-PGA_1 analogs in the human body. Four isomers of methyl-Δ^7-PGA_1 were synthesized and investigated for their antiproliferative activity on human ovarian carcinoma cells (2008 and A2780 cell lines). All isomers of methyl-Δ^7-PGA_1 showed the same antiproliferative activities on ovarian carcinoma cells.[40] This suggests that there may be no stereospecific receptors for the antiproliferative effects of methyl-Δ^7-PGA_1. Methyl-Δ^7-PGA_1 is chemically stable and can be easily synthesized in large amounts. In addition, methyl-Δ^7-PGA_1 integrated into lipid microspheres (lipo-methyl-Δ^7-PGA_1) is more soluble in water than methyl-Δ^7-PGA_1 alone. Hence, lipo-methyl-Δ^7-PGA_1 was selected for preclinical studies.[39]

Antitumor activity

Antiproliferative activity *in vitro*

The combination of PGE_2 and tumor necrosis factor (TNF) was effective in inhibiting proliferation of U-

937, ML-1 and HL-60 cells.[41] The effective concentration of PGE_2 was 100 ng/ml. This concentration of PGE_2 could induce the differentiation of U-937 cells. PGD_2, PGJ_2 and Δ^{12}-PGJ_2 either alone or in combination with TNF at a concentration of 100 ng/ml did not induce any differentiation in U-937 cells. However, the combination of TNF and Δ^{12}-PGJ_2 could inhibit the proliferation of three cultured cell lines (HeLa S3, HHUA and CAOV-3) derived from gynecologic malignancies.[42] The effective concentration of Δ^{12}-PGJ_2 was 1 μg/ml, which was less than that of PGE_2. Synergism between a series of PGs and interferon (IFN)-α has also been reported.[43] Idomethacin, an inhibitor of PGs, could modify tumor growth.[44,45] This could be derived from synergism between PGs and interleukin, IFNs or growth factors. The interaction between PGs and cytokines may play a role in the antitumor activity of PGs.

The antiproliferative effects of PGs alone on tumor cells continue to be studied for PGD_2 and the cyclopentenone PGs.[34,46] PGD_2 inhibited growth in over 20 different cancer cell lines.[47–49] The spectrum of antiproliferative activity of PGD_2 against tumor cells *in vitro* demonstrated that PGD_2 was active against human osteosarcoma, breast cancer, rhabdomyosarcoma, uterine cervical carcinoma, ovarian cancer and melanoma cells at a concentration of 5 μg/ml.[48] The antiproliferative activity of PGD_2 can be interpreted as being mediated by Δ^{12}-PGJ_2 which is the ultimate metabolite of PGD_2. PGD_2 is converted to Δ^{12}-PGJ_2 by human albumin and serum.[23,50] Δ^{12}-PGJ_2 has 5-fold greater antiproliferative activity on L1210 cells than PGD_2.[23] Kato *et al.* demonstrated antitumor activity of Δ^7-PGA_1 and Δ^{12}-PGJ_2 *in vitro* and *in vivo*.[51] Ikai *et al.* showed that Δ^{12}-PGJ_2 destroyed the cytoskeleton of transformed epidermal cells in culture on the basis of inhibition of protein synthesis.[52] The authors reported that Δ^{12}-PGJ_2 and an analog of PGA_1 could inhibit the growth of ovarian dysgerminoma cells *in vitro*.[38] Kikuchi showed that the cell proliferation of HRA cells was inhibited by Δ^{12}-PGJ_2 and an analog of PGA_1 *in vitro*.[29] Mitsuoka *et al.* reported an inhibitory effect of prostaglandin Δ^{12}-PGJ_2 on proliferation and α-fetoprotein expression in HuH-7 human hepatoma cells.[25] Methyl-Δ^7-PGA_1 and a Δ^7-PGA_1 analog (TE10303) based on modification of the side chains were synthesized and were shown to have potent inhibitory activity on cell proliferation *in vitro*.[37,38,50] Δ^7-PGA_1 has 5-fold greater antitumor activity than Δ^{12}-PGJ_2. One particularly important characteristic of both Δ^7-PGA_1 and Δ^{12}-PGJ_2 from the clinical aspect is their low cross resistance both to cisplatin and to adriamycin. The authors studied the antitumor activity of both Δ^7-PGA_1 and Δ^{12}-PGJ_2 on human ovarian cancer cell lines resistant to cisplatin, doxorubicin (ADR) and L-phenylalanine mustard (L-PAM). Drug resistance ratios based on IC_{50} were 62.5 for ADR in $A2780^{AD}$ cells[53] and 16.0 for cisplatin in 2008DDP cells,[54] whereas the ratios for Δ^7-PGA_1 and Δ^{12}-PGJ_2 were 1.5 and 1.8 in $A2780^{AD}$ cells, and 2.3 and 3.2 in 2008DDP cells, respectively.[28] $A2780^{AD}$ cells express the *mdr* gene, which is a major factor in their drug resistance.[55] DNA repair, a high level of intracellular glutathione and high activity of protein kinase C are thought to be factors related to drug resistance in 2008DDP cells.[54] It is an interesting phenomenon that both Δ^7-PGA_1 and Δ^{12}-PGJ_2 can overcome the factors of drug resistance.

The antiproliferative activity of PGs has been thought to be mediated by AMP.[4,55] However, Wiley *et al.* first reported that antitumor PG-induced inhibition of DNA synthesis is independent of AMP, because dideoxyadenosine, which is an inhibitor of adenylate cyclase, could not inhibit DNA synthesis.[56] In addition, Hughes-Fulford showed that PGJ_2 did not affect the intracellular level of AMP, while it inhibited DNA synthesis.[57] Narumiya and Fukushima measured the intracellular level of AMP of cancer cells treated with PGE_2, BW245C, PGD_2 and Δ^{12}-PGJ_2. They showed that Δ^{12}-PGJ_2 never increased the cellular cAMP level.[58] Analyses of the intracellular uptake and distribution of Δ^{12}-PGJ_2 and PGA_2 demonstrated that intracellular uptake occurred above 20°C and that both PGs were transferred into the nucleus at 37°C.[59] However, Δ^{12}-PGJ_2 differs from PGA_2 in relation to intracellular pharmacokinetics. Intracellular PGA_2 was washed out with PG-free medium and less than 15% of the initial amount remained in the nuclei, whereas 70% of the initial amount of Δ^{12}-PGJ_2 remained in the nuclei after intensive washing. Both hypotonic washing and 1% Triton X-100 solvent could not release Δ^{12}-PGJ_2 from the nuclei. Chromatographic analysis demonstrated that Δ^{12}-PGJ_2 was covalently bound to nuclear proteins.[60] These findings suggest that Δ^{12}-PGJ_2 binding in the nucleus was more stable than that of other PGs.

Bhuyan *et al.* reported that the primary effect of PGA_1, PGA_2 and PGD_2 was to block cell progression from G_1 to S in the cell cycle. At 2.5 μg/ml of PGD_2, blockade of cells in G_1 and normal progression through the other phases resulted in accumulation of 80–90% of the cells in G_1. At this concentration, there was no inhibition of DNA synthesis, and cells in S progressed apparently normally through S until

all cells were blocked in G_1.[61] Marui *et al.* demonstrated that the G_1 block was induced by both inhibition of N-*myc* gene expression and induction of heat shock proteins.[62] In addition, Holbrook *et al.* measured mRNA of the HSP70 gene family and showed that high-level induction is specific to HSP70 mRNA, resulting in an increase in the rate of transcription. Cycloheximide pretreatment, which inhibits the antiproliferative effects of PGA_2, prevents activation of the heat shock factor and induction of HSP70 mRNA by PGA_2.[63] In relation to the cell cycle modifying effects of PGs, G_2/M arrest has been reported. Treatment of L1210 cells with PGA_2 or Δ^{12}-PGJ_2 resulted in significant G_2/M arrest and subsequent DNA fragmentation at concentrations that are cytotoxic to the cells. When the morphology of cells was examined by electron microscopy, L1210 cells incubated with a cytotoxic dose of PGA_2 or Δ^{12}-PGJ_2 for 24 h showed characteristic morphological features of apoptosis such as chromatin condensation, nuclear fragmentation and formation of apoptotic bodies.[64] However, since this is only one report, further investigations are necessary regarding the induction of apoptosis by PGs.

Antiproliferative activity *in vivo*

Since the 1970s it has been well known that PGs of the E and A series inhibit the growth of some lines of tumor cells *in vivo*.[65,66] PGD_2 was also found to be a potent antitumor agent *in vivo*.[51,67] However, the discovery of PGJ_2 led to further studies of antitumor PGs including the PGA series, PGJ series and punaglandin. The antitumor activity of PGs *in vivo* is mainly classed as two types. One is derived from the immune effects of PGs. Combined administration of PGD_2 and cisplatin exhibited additive effects on human ovarian cancer cell growth in nude mice.[11,19,20,68,69] When antineoplastic PGs were administered to nude mice bearing human ovarian cancer cells, tumor growth in groups treated with PGJ_2 and Δ^{12}-PGJ_2 alone was significantly inhibited 63 days after tumor inoculation, compared to that in an untreated group.[70] Combination of Δ^7-PGA_1 and cisplatin could prolong survival of nude mice bearing human ovarian cancer cells.[29] Kita *et al.* demonstrated that Δ^{12}-PGJ_2 dose-dependently stimulated phytohemagglutinin (PHA)-induced blast formation of human lymphocytes in the concentration range of 10^{-8} to 10^{-6} M.[10,71] This may imply that the antitumor activity of antitumor PGs is based in part on their immune effects.

The other type of antitumor effects of PGs is derived from direct inhibition of tumor growth. The potency of antiproliferative activities of PGs on cultured cells originated from cancer is punaglandin > Δ^7-PGA_1 > Δ^{12}-PGJ_2.[70] However, punaglandin failed to produce significant prolongation of survival *in vivo*, while it induced significant weight loss.[70] Therefore, derivatives of Δ^7-PGA_1 were synthesized and their antitumor activities were tested in *in vivo* models. Although lipophilic analogs of Δ^7-PGA_1 have potent antitumor activity *in vitro*, hydrophobic analogs have greater activity than lipophilic analogs *in vivo*.[37] TE10303, which was obtained by modification of the side chains, showed some local effects of human ovarian dysgerminoma transplanted in nude mice.[38] However, TE10303 could not inhibit the growth of tumor transplanted subcutaneously in nude mice on intraperitoneal administration. Lipo-methyl-Δ^7-PGA_1 has been tested as a preclinical agent *in vivo*.[40] It has been shown to inhibit the growth of both HeLa S3 and Lovo colon cancer cells transplanted subcutaneously in nude mice when administered intravenously.[72] Lipo-methyl-Δ^7-PGA_1 by intraperitoneal administration could prolong the survival of scid mice bearing 2008C/13* cells resistant to cisplatin.[54] The combination of cisplatin and lipo-methyl-Δ^7-PGA_1 prolonged survival of nude mice bearing HRA cells compared to each single agent alone.[72] These findings suggest that lipo-methyl-Δ^7-PGA_1 could be a candidate for phase I and II clinical tudies to test its activity against refractory ovarian cancer and colon cancer.

Antiviral activity

It is well known that some viruses can cause cancer. Human papilloma virus and herpes simplex virus are thought to be causes of cancer of the uterine cervix.[74] HTLV-1 virus causes T cell leukemia.[73] Recently, AIDS associated cancers, including Kaposi sarcoma, lymphoma and cervical cancer, have been reported.[75]Antitumor prostaglandins that can inhibit viral replication may be useful for the treatment of virus-associated cancers.

PGs of the A and J series inhibit viral replication.[34,46] In 1980, Santoro *et al.* reported antiviral effects of PGA compounds[13] and furthermore demonstrated that PGJ_2 was a potent antiviral agent against Sendai virus.[18] D'Onofrio *et al.* demonstrated that PGA_1 and 16,16-dimethyl-PGA_2-methyl-ester (diM-PGA_2) blocked the proliferation of HIV-1-infected cord blood lymphocytes (CBL) *in*

vitro.[76] Yamamoto *et al.* investigated the relation between chemical structure and antiviral activity using herpes simplex virus. They showed that cyclopentenone PGs have potent antiviral activity. The potency of antiviral activity was Δ^7-PGA_1 > PGA_1 > PGA_2 and Δ^{12}-PGJ_2 > PGJ_2 > PGD_2.[77] The mechanisms of antiviral activities proposed are (i) inhibition of primary transcription of the viral genome,[77] (ii) alterations in the synthesis and/or maturation of specific viral proteins,[18,78] (iii) inhibition of mutant mRNA synthesis at 39°C[79] and (iv) inhibition of transcription of viral genomic RNA.[79] PGA and PGJ have been shown to affect RNA replication from DNA in the nucleus and, thus, inhibit viral proliferation. The cyclopentenone ring seems to have a universal action of both antitumor activity and antiviral DNA activity.

Toxicity

The antitumor PG lipo-methyl-Δ^7-PGA_1 is a candidate for clinical study.[39,46] Therefore, both the pharmacokinetics and toxicity of lipo-methyl-Δ^7-PGA_1 in rats have been investigated.[72] Both Δ^7-PGA_1 and lipo-methyl-Δ^7-PGA_1 are metabolized to unknown compounds in human serum; however, it is converted to Δ^7-PGA_1 in rat serum. The half-life of Δ^7-PGA_1 was 1.5 h in human serum and that of methyl-Δ^7-PGA_1 was 13 min. The half-life of lipo-methyl-Δ^7-PGA_1 was almost the same as that of methyl-Δ^7-PGA_1. On the other hand, Δ^{12}-PGJ_2 was stable in human serum.[72] This suggests that lipo-methyl-Δ^7-PGA_1 has lower toxicity than Δ^7-PGA_1 and Δ^{12}-PGJ_2. When lipo-methyl-Δ^7-PGA_1 was intravenously administered to rats once [Sprague-Dawley rats, crj:CD (SD), 4 weeks after birth], LD_{10} and LD_{50} were 17.4 and 33.5 mg/kg for male rats, and 38.1 and 45.9 mg/kg for female rats, respectively. All rats tolerated repeated administration of 10 mg/kg/day lipo-methyl-Δ^7-PGA_1 for 28 days. However, weight loss was observed after 14 days of administration. The dosage of 2.5 mg/kg/day did not induce loss of weight. Fifty percent of rats administered 10 mg/kg/day lipo-methyl-Δ^7-PGA_1 exhibited both reduced movement and increased respiratory rate from 3 min after administration, which showed recovery by 20 min after administration. Appetite loss continued throughout the administration period.[72]

In the urine examination, volume, color, SG, pH, protein, glucose, ketone bodies, bilirubin, urobilinogen, red blood cell count (RBC) and casts were within normal limits. Ocular examination was within normal limits. Hematologic tests revealed anemia with decrease in both RBC and hemoglobin. However, both leukocytes and platelets showed no differences compared to control. Serum albumin concentration increased and A/G ratio increased. Adrenal gland weight decreased with administration of 5 mg/kg of lipo-methyl-Δ^7-PGA_1 and higher doses. Furthermore, the relative weights of both lungs and heart to body weight increased with administration at 2.5 mg/kg/day and higher in rats. The weights of other organs did not change.[72] Histopathological examination is now on going. These results suggest that the dose-limiting toxicity is effects on the adrenal glands, lungs and heart.

Conclusions

Cyclopentenone PGs and alkylidene cyclopentenone PGs have antiproliferative activity. The alkylidene cyclopentenone PGs Δ^{12}-PGJ_2 and Δ^7-PGA_1 have the most potent antiproliferative activity on tumor cells, and both exhibit antiviral and antitumor activity. Δ^{12}-PGJ_2 was identified in human urine, whereas Δ^7-PGA_1 has not been found in the human body. Both Δ^7-PGA_1 and Δ^{12}-PGJ_2 have little cross resistance both to cisplatin and to ADR. Δ^7-PGA_1 has 5-fold greater antitumor activity than Δ^{12}-PGJ_2. Methyl ester-Δ^7-PGA_1 is stable chemically and can be easily synthesized in large amounts. In addition, methyl-Δ^7-PGA_1 integrated into microspheres (lipo-methyl-Δ^7-PGA_1) is more soluble in water than methyl-Δ^7-PGA_1 alone. Preclinical *in vivo* studies of lipo-methyl-Δ^7-PGA_1 have been conducted. Lipo-methyl-Δ^7-PGA_1 has been shown to inhibit growth of ovarian cancer cells resistant to cisplatin. In addition, the combination of cisplatin and lipo-methyl-Δ^7-PGA_1 showed additive antitumor effects on ovarian cancer cells *in vivo*. Lipo-methyl-Δ^7-PGA_1 showed low toxicity in rats. The toxic effects in rats consisted of decrease in RBC and hemoglobin, reduction of adrenal gland weight, and increase of relative weight of lungs and heart. This suggests that lipo-methyl-Δ^7-PGA_1 may be a candidate for phase I and II clinical studies to test its activity against refractory ovarian cancer and colon cancer.

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