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REVIEW

Biochemistry of Prostaglandins A

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Abstract—This review considers modern concepts on the structural–functional properties and antiproliferative, antitumor, and antiviral effects of cyclopentenone prostaglandins A and mechanisms underlying their actions. Possible directions of pharmacological application of these compounds and their analogs are discussed.

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Key words: prostaglandins, arachidonic acid, heat shock proteins, cell cycle, prostanoid receptors, thermotolerance, antiviral activity

Prostaglandins (PG) are biologically active metabolites of arachidonic acid; they modulate various cell functions under physiological and pathological conditions [1-3]. According to chemical structure and molecular and cellular mechanisms responsible for their actions, PG are subdivided into two groups: cyclopentane (E, F, D) and cyclopentenone (A, B, C) PGs. The cyclopentenone PGs are characterized by the presence of a highly reactive α,β-unsaturated carbonyl ring [4]. In contrast to "classical" PGs acting at specific G-protein coupled plasma membrane receptors [5], the cyclopentenone PGs (15d-$\Delta^{12,14}PGJ_2$, $\Delta^{12}PGJ_2$, and PGA) lack their own receptors on the cell surface [4]. These PGs are actively transported into cells where they exhibit various biological effects including modulation of stress reaction, inhibition of the cell cycle, suppression of viral replication, and regulation of cell differentiation and development [4, 6-9]. Most effects of cyclopentenone PGs are associated with changes of gene expression (e.g., genes encoding heat shock proteins, γ-glutamyl-cysteine synthetase, collagen, and heme oxygenase [10-12]), induction of reactive oxygen species formation, and potentiation of inflammatory reaction caused by TNF-α [13, 14]. There is some evidence for the existence of specific receptor of pentenone PGs in the cell nucleus [15].

Here we review studies on the physiology and biochemistry of PGA and their possible pharmacological application.

Abbreviations: COX) cyclooxygenase; PC) prostacyclins; PG) prostaglandins; Tx) thromboxanes.

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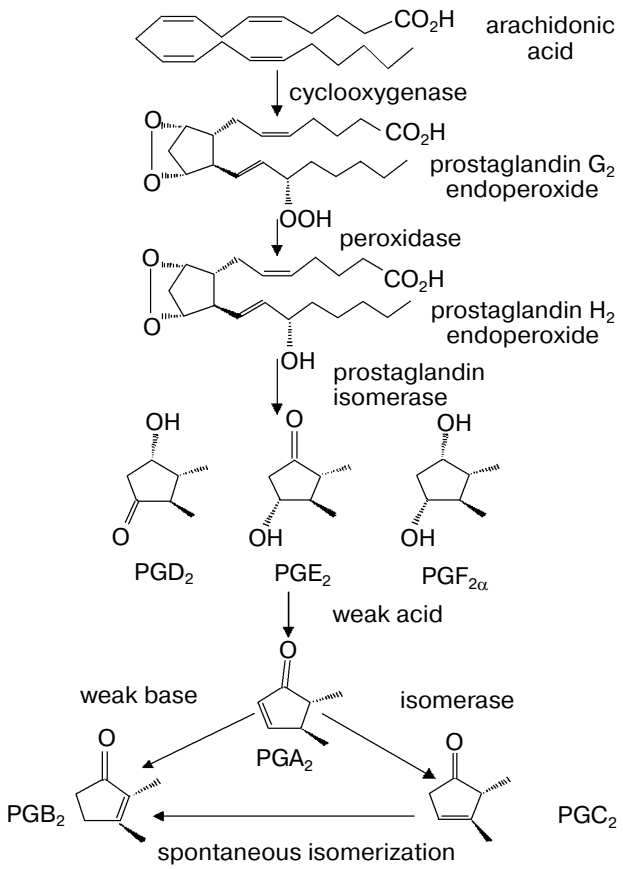
PGA AS BIOLOGICALLY ACTIVE METABOLITES OF PGE

Biosynthesis of PGA. The Scheme shows a common pathway of PGA biosynthesis. The first stage consists in the release of arachidonic acid from plasma membrane phospholipids catalyzed by activated phospholipase A_2 [1, 2, 16]. Arachidonic acid is then sequentially converted to PGH_2 by cyclooxygenase and peroxidase activities of PGH synthase, also known as cyclooxygenase (COX). There are two isoforms of cyclooxygenase: constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2); expression of the latter isoform is increased by pro-inflammatory cytokines [1]. This isoform (COX-2) is the main target for anti-inflammatory preparations such as aspirin and indomethacin [1, 2]. Formation of "classical" PGs involves specific synthases known as H-convertases; these are PGE_2 and PGD_2 isomerases and $PGF_{2\alpha}$ reductase [16]. PGH_2 may also be transformed into thromboxanes (Tx) or prostacyclins (PC) [16].

The main sources for PGA are PGE_1 and PGE_2; certain evidence exists that these PGs undergo non-enzymatic dehydrogenation yielding PGA_1 and PGA_2 [4, 16, 17] and mainly the latter appears in systemic blood circulation (Scheme) [17]. Interestingly, PGA_2 may be formed during storage of pharmaceutical preparations of PGE_2 [16].

Chemical properties of PGA. The chemical structure of PGA is characterized by the presence of an α,β-unsaturated carbonyl ring [16, 18], which has an electrophilic center. This center determines PGA reactivity for nucleophilic addition (Michael addition), including such nucleo-



Cyclooxygenase pathway of prostaglandin biosynthesis

Scheme

philes as free SH-group of cysteine residues of reduced glutathione or cell proteins [19, 20]. Adducts formed in the reaction of Michael addition with diene compounds (methyl ester of Δ^7-PGA_1) are less stable than the adducts formed by monoene components (methyl ester of PGA_1) [16]. Being formed, the glutathione conjugates are eliminated from the cell. This process involves specific proteins (MRP-1 and MRP-2) also known as ATP-dependent glutathione-S-conjugate exporting pump [16].

The specific biological activity of PGA requires a chemically active group of the cyclopentenone ring [16-18, 20]. Cyclopentane PGs (E, D, F) do not exhibit biological effects similar to those of PGA, and conjugation of the reaction center with glutathione eliminates the activity of cyclopentenone PGs [16]. Physiological effects of cyclopentenone PGs are eliminated by cyclopentenone (2-cyclopenten-1-one). Structurally related cyclopentane PGs (PGE_2, PGE_1, PGD, etc.) lacking biological activities typical for PGA illustrate the importance of the α,β-unsaturated cyclopentenone ring for manifestation of specific biological activity (of PGA) [16]. Chemical modification of the α- or ω-chain of these PGs by inserting heteroatoms and/or heterocycles may significantly influence their specific biological activity, as in the case of synthetic analogs of PGs from other groups [21-24].

CELLULAR AND MOLECULAR MECHANISMS OF PGA EFFECTS

PGA receptors. In 1967, it was found that the hypotensive substance "medullin" extracted from rabbit kidney medulla is identical to PGA_2 [2]. Medullin administration to rats caused a decrease in blood pressure due to arteriole dilation typical for the effects of PGE [25-30]. Later it was demonstrated that chemically and biologically unstable PGE readily underwent dehydrogenation followed by PGA formation [16]. Thus, some physiological and biochemical effects of PGE should be basically considered as a sum of PGE and PGA effects. However, effects of PGE and PGA are realized via different molecular mechanisms. For example, physiological effects of PGE, PGD, and PGF are realized by specific plasma membrane receptors coupled to G-proteins (EP, DP, and FP type receptors) and initiation of corresponding signal transducing system [16]. This problem has been exhaustively considered in several basic reviews [3, 5, 30]. However, involvement of these receptors (of "classical" PGs) in anti-inflammatory, antitumor, and antiviral effects of PGA was not demonstrated [16]. Only some "side" effects of PGA (hypotensive action *in vivo*) are mediated via low affinity binding to EP-, DP-, and FP-type receptors localized on plasma membranes [3, 16].

Molecular mechanisms of PGA effects mediated via plasma membrane receptors have not been determined yet. However studies have revealed the existence of specific receptor for PGA_2 on rat intestine plasma membranes [31]. This receptor has the following binding characteristics: dissociation constant (K_d) of 43.9 nM and B_{max} of 3.33 pmol/mg of protein. Binding of [^{3}H]PGA_2 was inhibited in competitive manner by sodium ions and noncompetitive manner by ATP [31].

Some authors consider PGA as a potential ligand for benzodiazepine receptor [32]. They found that PGA_1 and PGA_2 are competitive inhibitors of [^{3}H]diazepam binding with K_i values of 7.0 ± 0.1 and 15.0 ± 1.0 μM, respectively. Affinity of PGA to this type of receptors was two orders of magnitude higher than affinity of inosine, hypoxanthine, and nicotinamide and corresponded to affinity of endogenous benzodiazepine receptor ligand, 1-methyl-β-carboline [32]. PGA_1 concentration required for 50% inhibition of [^{3}H]diazepam binding corresponded to the PGA_1 concentration activating adenylate cyclase in neuroblastoma and inhibiting human myometrium contractility (0.3-3.0 μM) [32].

The discovery of specific nuclear receptors mediating the biological effect of cyclopentenone PGJ stimulated the search for nuclear receptors for structurally related

compounds of PGA [15]. Comparative analysis of [^{3}H]PGA_2 and [^{3}H]PGJ_2 accumulation in the nucleoplasm fraction and nuclear membrane and also in the chromatin fraction and nuclear matrix revealed accumulation of only 20% of [^{3}H]PGA_2 in the nuclear fractions. This suggests that in contrast to 12-PGJ_2 (which is in the nucleus mainly covalently bound to proteins of chromatin and nuclear matrix), nuclear PGA exists as the free molecule [33].

Non-receptor mechanisms of PGA effects. Analyzing experimental data obtained in different laboratories, Straus and Glass [16] proposed the existence of receptor-independent PGA regulation of expression of stress-inducible genes (Table 1). The list of genes responding to PGA by increased expression includes those of heat shock protein HSP70, transcription factors c-Fos, Gadd 153, and Egr-1, inhibitor of cyclin-dependent protein kinase (CDK) – $p21^{CIP1/WAF1}$, and heme oxygenase. The list of genes responding to PGA by decreased expression includes *c-Myc*, *N-Myc*, cyclin *D1*, and *Cdk4* required for cell transition through G1-phase of cell cycle, and also gene of insulin like growth factor 1 (IGF-1) acting as the autocrine factor in some tumor cells [16].

In spite of significant progress in understanding of signal transduction pathways and mechanisms involved in regulation of expression of some of the above mentioned genes, their molecular details have not been described in the literature. Activation of almost all genes listed in Table 1 requires the presence of the reactive center in the system of the cyclopentenone ring of PGA. For example, 2-cyclopenten-1-one induced expression of *HSP70* and *$p21^{CIP1/WAF1}$* and decreased expression of *IGF-1*, whereas related cyclopentanone and cyclopentene structures were ineffective [16].

Induction of *HSP70* increased DNA binding activity and transactivation potential of heat shock transcription factor (HSF). In the absence of stress stimuli, inactive HSF monomers are mainly localized in the cytoplasm in a complex with HSP70 chaperone molecules. Appearance of damaged cell proteins in the cytoplasm under stress conditions causes rapid dissociation of HSF–HSP70 complex and translocation of HSF into the nucleus, where HSF trimers bind to heat shock responsive elements of DNA molecules. Taking into consideration these molecular events, Santoro suggests that PGA may damage proteins and thus stimulate HSF translocation into the nucleus. However, the mechanism of possible damaging effect remains unknown [34].

PGA-induced Ca^{2+} mobilization may be one of major pathways responsible for gene activation [7, 16]. Rapid Ca^{2+} release from endoplasmic reticulum is associated with PGA_2-dependent induction of heme oxygenase, *HSP70*, *c-Fos*, *Egr-1*, and *Gadd 153* genes. Pretreatment of cells with the Ca^{2+} chelator BAPTA-AM prevented induction of these genes, supporting involvement of calcium ions in the regulation of gene expression [16].

Table 1. Receptor-independent regulation of gene expression by PGA [16]

Gene	Regulation direction	PG
HSP70	↑	A_1, A_2
Gadd 153	↑	A_2
$p21^{CIP1/WAF1}$	↑	A_1, A_2
c-Fos	↑	A_2
Egr-1	↑	A_2
Heme oxygenase gene	↑	A_1
γ-Glutamylcysteine synthetase gene	↑	A_2
c-Myc	↓	A_2
N-Myc	↓	A_2
Cyclin *D1*	↓	A_2
Cdk-4	↓	A_2
BiP	↑	A_1, A_2
IGF-1	↓	A_1, A_2

PGA may induce expression of a BiP encoding gene; this protein plays a crucial role in translocation of mature cell proteins through the endoplasmic reticulum membrane and their subsequent folding and assembly [15]. Induction of the *BiP* gene involving protein response element UPRE and induction of *HSP* genes involving heat shock responsive element may be important preconditions for cytoprotective regulation of protein folding under stress conditions, which makes cells more tolerant to stress.

Effects of PGA on expression of genes encoding heat shock proteins probably explain its neuroprotective action. PGA_1 significantly increased HSP70 and HSP72 levels in striatum of rats. Overexpression of genes encoding these proteins *in vivo* protected neurons of hippocampus and striatum against ischemic and kainic acid induced damage [35]. Thus, PGA mediated induction of HSP may be an important mechanism protecting neurons against exotoxin-induced apoptosis [35].

It should be noted that PGA can effectively regulate the activation of nuclear transcription factor κB (NF-κB); acting as a mediator of immune and inflammatory responses, it is involved in numerous pathological processes [36]. Inhibition of NF-κB activity by PGA involves inhibition of phosphorylation and prevention of degradation of NF-κB inhibitor, I-κB-α [6, 36, 37]. This

process does not require protein synthesis; it depends on the presence of the reactive cyclopentenone ring in PG [36]. Since NF-κB is involved in activation of immunoregulatory and viral genes, inhibition of its activity might represent the main mechanism underlying immunosuppressive and antiviral activity of PGA [16].

Antiproliferative activity of PGA. PGs may effectively inhibit proliferation of various cell types. PGA and PGJ are the most potent antiproliferative agents [12, 15]. In many cell types, PGA arrests the cell cycle at G1 phase. (There are basically no cell types insensitive to PGA inhibition.) The biochemical mechanism of this phenomenon remains unclear, but it certainly requires the presence of the α,β-unsaturated carbonyl ring [18, 38].

PGA_2 can form covalent bonds with glutathione [39, 40]. However, glutathione conjugate formation as well as stimulation of cAMP formation by these conjugates is not ultimate precondition for inhibition of cell proliferation [3, 41]. Taking into consideration covalent bonding of PGA with cell proteins [20], we suggest that interaction of PGs with one or more cell proteins is involved in inhibition of cell proliferation [3]. Indeed, concentrations of PGA_2 required for binding to proteins also cause inhibition of cell proliferation under identical conditions. Binding of PGA_2 to proteins occurs very quickly; this demonstrates that PGA_2 binding proteins exist in intact cells (untreated with this PG) [3]. PGA_2 can effectively interact with pig vascular endothelial cell proteins of 43, 50, and 56 kD. Binding of PGA_2 correlated with inhibition of proliferation. The biological functions of these proteins remain unknown; they are not HSP and glutathione-S-transferases. Moreover, they are not PG receptors [3]. The other mechanism of antiproliferative activity of PGA_1 in K562 cells involves induction of a 74 kD protein (p74) related to HPS70 proteins [12]. This protein is mainly localized in the cytoplasm of PG-treated cells, and its synthesis is ultimately related to manifestation of cytotoxicity. Doses of PGs causing cell replication stimulated p74 synthesis [12].

Antiproliferative activity of PGA may also be attributed to intracellular oxidative stress. Studies on human neuroblastoma SH-SY5Y cell line revealed the following PGA-induced signs of cytotoxicity [14]: changes in cell redox potential accompanied by exhaustion of antioxidant defense (glutathione and glutathione peroxidase); continuous decrease in mitochondrial membrane potential; formation of protein-bound products of lipid peroxidation (acrolein, 4-hydroxy-2-nonenal); accumulation of ubiquitinylated proteins. Interestingly, thiol-containing N-acetylcysteine significantly inhibited formation of reactive oxygen species induced by PGA and prevented cytotoxicity. This suggests that changes in redox potential are closely related to the prooxidant effect of PGA. Products of lipid peroxidation found in PG-treated cells stimulated formation of reactive oxygen species, which was also accompanied by accumulation of ubiquitinylated proteins. This suggests involvement of membrane lipid peroxidation products in augmentation of the cytotoxic effect of PGA by stimulating intracellular oxidative stress [14].

PGA transport. The main biological effects of PGA occur in receptor-independent manner, by changes in intracellular compartmentalization, regulation of gene expression, and binding to cell proteins. This implies the existence of transport system(s) responsible for translocation of this compound from extracellular space inside cells and further from cytosol into nucleus [15]. Several studies have demonstrated active and temperature-dependent transport of PGA_2 into mammalian cells [42-44]. Increase in temperature from 4 to 37°C significantly increased the initial rate of PGA transport into eukaryotic cells [43]. A major proportion of $[^3H]PGA_2$ accumulated at 4 and 20°C (90%) was detected in cytoplasm, whereas more than 50% of $[^3H]PGA_2$ transported into cells at 37°C was accumulated in the nucleus [43]. Intracellular level of glutathione plays an important role in PG transport. In glutathione depleted L-1210 cells, the total amount of PGA_2 was about two times less than in corresponding control cells. This was accompanied by a significant decrease in this PG in cytoplasm but not in the nucleus. The decrease in glutathione content caused inhibition of PG uptake and the decrease in cytosolic PGA_2 content; there were insignificant changes in its nuclear accumulation. PGA_2 caused similar inhibition of growth of control and glutathione depleted cells. This suggests that the glutathione level can significantly influence uptake of PGA_2 but not its accumulation and biological activity [41].

In cytosolic fraction, transported PGA_2 exists in a free form and also in glutathione- and protein-bound forms [41]. Reduction of glutathione content during cultivation of HeLa cells was accompanied by the increase in free PGA_2 without any changes in amount of protein-bound complexes. The increase in incubation temperature from 4 to 37°C was accompanied by gradual translocation of the PG–protein complexes into the nucleus [43]. Some authors described two cytosolic proteins of 100-150 and 25-35 kD transporting PGA from extracellular space inside cells and from cytoplasm into nucleus, respectively [43]. Employment of N-ethylmaleimide and *p*-chloromercuribenzoate revealed involvement of SH-groups of these proteins (interacting with the PG cyclopentenone ring) in binding and transport of PGA_2 [41, 43]. Binding and transport of PGA_2 into the nucleus were sensitive to inhibition by PGJ_2 and 4-hydroxycyclopentenone, whereas PG B_2, D_2, E_2, $F_{2\alpha}$, and also arachidonic and oleic acids were ineffective [41].

Biotransformation and neutralization of PGA. The major pathways for total biological inactivation of PGA include: oxidation of the allyl 15-hydroxy group catalyzed by 15-hydroxy-PG dehydrogenase; reduction of the *trans*-13,14-double bond by 13,14-PG reductase; two

sequential stages of β-oxidation of α-chain; ω-hydroxylation; oxidation of ω-alcohol to an acid; two sequential stages of β-oxidation of ω-chain. Besides these enzymatic reactions, there is non-enzymatic dehydration of the cyclopentenone ring followed by isomerization of the 10,11-double bond of PGA to position 8,12 yielding PGB [2].

Degradation of PGA mainly (90%) occurs in the liver, where PGA represents a natural substrate for the monooxygenase system. Liver monooxygenase catalyzes ω- and ω1-hydroxylation of PGA_1 [45, 46]. This enzymatic system is suggested to be involved in transformation of endogenous PG into hydrophilic metabolites excreted in the urine [47]. In the presence of NADPH, guinea pig liver microsomes catalyzed preferential hydroxylation of PGA_1 by more than 95% at ω1-position and less than 5% at ω-position; this resulted in the appearance of the 19- and 20-hydroxyderivatives of this PG [48]. Microsomal oxygenase inhibitors SKF525A, metirapon, nicotinamide, and CO inhibited hydroxylation of PGA_1 and PGF_1 [48]. A similar effect was observed in the case of cytochrome *c* and antibodies against NADPH-cytochrome *c* reductase; this indicates that PG hydroxylation involves the typical monooxygenase system [48].

Kidney is a very important organ involved into metabolism of PGA. Administration of PGA_1 into rabbit kidney artery was accompanied by quantitative conversion of this PG into less polar metabolites detected in the kidney venous system [17]. Studies employing mass spectroscopy, IR analysis, and NMR revealed that 15-keto,13,14-dihydro-PGA_1 and 13,14-dihydro-PGA_1 are the main (of five detected) metabolites [17]. Oxidation of the secondary OH-group at C15 of PGA_1 resulted in formation of 15-keto-PGA_1 causing 10-20-fold inhibition of depressor effects. Moreover, oxidation at C15 and reduction of 13-Δ-*trans*-double bond converts PGA_1 into 15-keto,13-14-dihydro-PGA_1, eliminating the hypotensive effect of PGA_1 [17]. Since PGA metabolites maintain high biological activity, it is suggested that at least part of physiological effects of PGA may be mediated by conversions of these PGs into active derivatives, as occurs with testosterone; the biological activity of this hormone is supplemented by its dihydroderivatives [17].

In all organs, PGA degradation by 15-hydroxy-PG dehydrogenase is lower compared with PGs of other groups. This probably explains higher metabolic resistance of PGA. For example, the half-time for PGA_2 elimination from systemic circulation is 1 min [2].

Antiviral properties. The first report on PGA inhibition of viral replication and prevention of development of persisting infections was published in 1980 [49]. Antiviral activity of natural PGA described in various *in vitro* and *in vivo* models was observed at concentrations nontoxic for the host cells (10^{-5} M) [4]. Effective antiviral cell protection was observed in the case of both DNA and RNA containing viruses including poxviruses, herpes viruses, paramyxoviruses, orthomyxoviruses, rabdoviruses, togaviruses, and retroviruses (Table 2).

Antiviral activity of PGA has a wider spectrum than that of other chemotherapeutic agents due to its ability to interact with both DNA and RNA viruses and to suppress viral replication even at relatively late stages of the viral cycle [50]. These properties make PGA an attractive class of easily available antiviral agents because PGA can be obtained by chemical synthesis and using natural sources [4].

The mechanism of antiviral activity of PGA was investigated using two RNA model viruses, rabdovirus (VSV) and Sendai paramyxovirus (SV). In both models, cyclopentenone PGs influenced more than one process during the viral cycle [51]. At late stages of VSV or SV infection, treatment of infected cells with PGA_1 caused sharp blockade of viral reproduction due to changes in maturation and intracellular translocation of glycoprotein G (VSV) or hemagglutinin-neuraminidase (SV). Moreover, PGA selectively blocked synthesis of VSV and SV proteins and protected host cells against virus-induced suppression of biosynthesis of host cell proteins. This blocking effect occurring at the translation level was due to HSP induction, especially HSP70; this effect was demonstrated in human and mammalian cell cultures, human peripheral blood lymphocytes, macrophages, and stem cells [56]. PGA-induced *HSP70* gene transcription was mediated via cycloheximide-sensitive activation of heat shock transcription factor, which binds to heat shock DNA responsive elements containing multiple reversed repeats of the pentamer nGAAn [56]. Synthesis of viral protein and HSP70 may compete for similar factors, limiting translation under these conditions [49, 56].

Besides induction of HSP gene expression, some authors have demonstrated PGA_1-dose dependent inhibition of viral RNA at the early stage of viral infection without any changes in stability of viral proteins. This effect was demonstrated using human cells infected with herpes-1 virus [61], murine cells infected with vesicular stomatitis virus [58, 59], and HeLa cells infected with poliovirus [51]. PGA_1 effectively suppressed HIV-1 replication during acute infection of CEM-SS lymphoblastoma cells [56]. A ten-fold decrease in production of p24-antigen and more than 1000-fold reduction in viral particle harvesting were observed at nontoxic PG concentrations, which did not inhibit nucleic acid biosynthesis in the non-infected cells. Thus, antiviral activity of PGA was comparable to that of azidothymidine [56].

These data together with better understanding of mechanisms underlying antiviral effect of PGA indicate that this class of PGs can be considered as a promising new group of therapeutic antiviral agents.

Antitumor activity. In 1970-1980 it was demonstrated that PGs stimulate proliferation of some cell types and inhibit proliferation of others [16]. It was also recognized that PGE stimulated proliferation of dog kidney MDCK

Table 2. Antiviral properties of PGA

Type of virus	Model	Effect	Reference
Rota virus SA-11	HeLa cells	inhibition of protein VP4 and VP7 synthesis, inhibition of glucosamine incorporation into the viral enterotoxin	[52]
Maiaro virus	Vero cells	inhibition of synthesis of viral capsid proteins	[53, 54]
Pig hyperthermia virus	PK-15 cells	inhibition of viral replication	[55]
Poliovirus P-1	HeLa cells	inhibition of viral protein synthesis	[51]
HIV-1	lymphoblastoma CEM-SS	inhibition of viral mRNA expression, maturation of glycoproteins and their intracellular translocation	[56, 57]
Vesicular stomatitis virus	mouse L fibroblasts	inhibition of viral glycoprotein G synthesis	[58, 59]
Sendai virus	37RC cells	inhibition of viral protein synthesis	[50, 60]
Herpes-1 virus	cultures of human stromal cells	inhibition of viral replication	[61]
Influenza virus A (Ulster 73)	LLC-MK2 cells	inhibition of viral mRNA expression, maturation of glycoproteins and their intracellular translocation	[8]
Encephalomyocarditis virus	mouse neuroblasts	inhibition of viral RNA synthesis	[62]

cell line, whereas PGF stimulated proliferation of Swiss 3T3 cell line, and this effect increased in the presence of PGE and PGF. Inhibitory effect of PGE on proliferation was observed using L929 cell line, normal human diploid fibroblasts, and aortal smooth muscle cells. PGE inhibited tumor growth *in vivo* and *in vitro* [16]. In 1984, Straus and Pang revealed that PGs of various classes inhibited DNA synthesis in transformed IMR-90 fibroblasts [63].

Antiproliferative activity of PGs reduced in the following order: $PGE_2 > E_1 > A_1 > A_2 > B_1 > B_2 >> F_{1\alpha} \approx F_{2\alpha} \approx TxB_2$ [63]. The antitumor effect was observed at relatively high (pharmacological) concentrations and effective concentrations of cyclopentenone PGs were one order of magnitude less than those of PGE. Moreover, in most tumor cells antiproliferative effects of PGE_1 and PGE_2 required preliminary dehydrogenation of the cyclopentane ring [63].

In 1986, Sujiura synthesized derivatives of PGE_1, PGA_1, PGD_2, and PGJ_2 [64]. Each compound was tested by inhibition of growth of leukemia L-1210 cells and Ehrlich ascites carcinoma cells. These studies revealed structural features of PGs associated with high antitumor activity. For example, in PGA series the highest activity was found in Δ^7-PGA_1, 12-epi-Δ^7-PGA_1, and 15-deoxy-Δ^7-PGA_1. In PGJ_2 series, 15-deoxy-$\Delta^{12,14}$-PGJ_2 was the most active analog; it was as active as Δ^7-PGA_1. These results provided convincing evidence that diene compounds possessing two electrophilic carbon atoms exhibited the highest activity; in the PGA_1 series the two stereoisomers at C_{12} exhibited equal biological activity; analogs possessing a hydroxyl group at C_{15} were the most active among compounds exhibiting this type of activity [64]. Testing of Δ^7-PGA_1 *in vivo* met a serious problem due to its instability in animal blood serum. The half-life of Δ^7-PGA_1 in rat and human serum was <1 and 13.9 min, respectively. This instability was due to rapid conversion of Δ^7-PGA_1 into Δ^7-PGC_1 catalyzed by PG isomerase followed by subsequent degradation of Δ^7-PGC_1 to a complex mixture of products [64].

This effect became a basis for the search for more stable analogs. The compound 13,14-dihydro-15-deoxy-Δ^7-PGA_1 was found to be one of them [65]. In contrast to Δ^7-PGA_1, this analog was stable in serum, but its antitumor activity was lower than that of Δ^7-PGA_1. The methyl ester of this compound (TE-19826) exhibited a unique antitumor profile in the COMPARE program using 38 tumor cell lines *in vitro*. TE-19826 encapsulated into lipid microspheres exhibited marked activity against human ovarian carcinoma cells in mice and some activity against tumor cells resistant to cisplatin [65].

For elucidation of structural features underlying instability of Δ^7-PGA_1 in serum, Suzuri et al. synthesized a series of analogs of this compound [66]. Inversion of the stereocenter at C_{12} from natural S-configuration to

unnatural R-configuration significantly increased PG stability in serum due to decreased recognition of such compounds by PG isomerase and therefore decreased catalytic conversion of them. Moreover, the R-isomer maintained high antitumor activity against tumor cells. It is suggested that due to increased stability of this isomer it may possess marked antitumor activity *in vivo* [63].

The methyl ester of Δ^7-PGA_1 attracts much attention of oncologists. This compound exhibits high chemical and biological stability and can be easily synthesized in significant quantities. All four isomers of methyl-Δ^7-PGA_1 possess similar antiproliferative properties tested using human ovarian carcinoma cells. Methyl-Δ^7-PGA_1 incapsulated into lipid microspheres (lipo-methyl-Δ^7-PGA_1) was characterized by better water solubility than methyl-Δ^7-PGA_1. Intravenous administration of lipo-methyl-Δ^7-PGA_1 to mice caused effective inhibition of malignant growth of HeLa S3 and Lovo cells [67]. Intraperitoneal administration of lipo-methyl-Δ^7-PGA_1 increased survival of mice bearing 2008C/13 cells resistant to cisplastin. This compound is now being intensively investigated in preclinical studies [67].

Another analog, 13,14-dihydro-15-deoxy-Δ^7-PGA_1 methyl ester (TE-19826), is also considered as a potential antitumor preparation [68]. Although this compound is easily hydrolyzed into the carboxyl form (TOK-4528), this form, TOK-4528, is as stable as methyl-Δ^7-PGA_1 in blood serum of man, mouse, and rat. The *in vitro* antitumor effect of TE-19826 with respect to Colon 26 cancer cells was more pronounced than that of Δ^7-PGA_1. Intravenous administration of TE-19826 four times a day provided marked suppression of malignant growth 3-4 days after the beginning of chemotherapy [68].

In the 1980s, Jaffe et al. described antitumor activity of 16,16-dimethyl-PGE_2; this effect correlated with its ability to stimulate cAMP formation in transformed cell (cited in [18]). Subsequent comparative *in vitro* study of the effects of various PGs on proliferation of tumors and cAMP production revealed that dimethyl-PGE_1 and dimethyl-PGE_2 inhibited proliferation of B16a cells by 80 and 75%, respectively, and increased cAMP production by 9000 and 1200%, respectively. PGA_1 and PGA_2 inhibited proliferation by 88 and 92% without any effect on cAMP production. This implies lack of correlation between antitumor activity of PGs and their effect on cAMP formation [18].

Further experimental evidence of independence of antitumor activity and cAMP generation was obtained during comparison of physiological effects of PGA_2 and 15-epi-PGA_2. Both compounds exhibited roughly the same antitumor activity. It should be noted that cAMP generation requires PG receptor binding and interaction of the PG molecule with receptor requires 15-OH in S-configuration, whereas 15-OH of 15-epi-PGA_2 has R-configuration. Inhibition of proliferation of B16a cells does not require stereospecificity of the 15-OH group in the PG molecule. Since antitumor activity of PGA is two times higher than that of PGE_1, this emphasizes the important role of active the α,β-unsaturated cyclopentenone ring in PGA-induced cell differentiation [18, 69].

Later studies demonstrated that in some tumor cell lines sub-toxic doses of PGA arrested the cell cycle at G1-phase. Cultivation of HeLa and MCF-7 cells for 48 h in the presence of PGA_2 (20 μg/ml) reduced the number of cells by 39 and 80%, respectively [9].

In some cases arrest of the cell cycle correlated with negative feedback of some proteins positively regulating transition of cell cycles (autocrine growth factor IGF-1 and cyclin D1) and positive feedback of cyclin-dependent protein kinase $p21^{CIP1/WAF1}$ (Cdk), suppressing cell cycle progression via inhibition of activity of cyclin/Cdk complex. In other tumor cells, PGA induced apoptosis before arrest of the cell cycle at G1 phase (HeLa and MCF-7 cells) [70]. In some transformed cells, non-apoptotic death is related to arrest at S-phase [16]. It is suggested that cells inducing $p21^{CIP1/WAF1}$ in response to PGA_2 are arrested at G1, whereas cells incapable of synthesizing this protein die [16, 70]. Some authors emphasize the importance of cytochrome *c* release and caspase-9 activation observed in the presence of PGA for triggering of apoptotic changes in tumor cells [71]. There was a correlation between antitumor activity of cyclopentenone PG and inhibition of nuclear topoisomerase II [6]. The precise mechanism of this phenomenon remains unknown.

OTHER PROTECTIVE MECHANISMS

Induction of thermotolerance. Thermotolerance, an increased short-term resistance to thermal treatments, is a universal (non-inherited) phenomenon induced by short-term exposure to increased temperatures [72]. PGA_1 provides increased resistance of eukaryotic cells to short (20 min) or prolonged (45 min) heat shock (45°C). The induced thermal resistance is maintained for 24-48 h after treatment with PG [72]. The mechanism responsible for this PG-induced phenomenon is now being actively investigated. Heat shock may cause some inhibition of protein biosynthesis; manifestation of this effect depends on temperature and type of cells. Thermotolerant cells exhibit normalization of protein synthesis reduced by thermal treatment; the presence of PGA_1 caused moderate induction of "translation tolerance" [72].

Studies of the contribution of HSP to the development of the phenomenon of thermotolerance revealed that cyclopentenone PGs can induce expression of some genes encoding stress proteins such as proteins of the HSP family [3, 12, 42, 73], heme oxygenase [11], and protein disulfide isomerase [16]. PGA_1 induced transcription of *HSP70* via activation of heat shock transcription factor HSF; in contrast to the effect of extreme temperatures, induction of HSF by PGA_1 required unaltered pro-

tein synthesis. The involvement of various mechanisms underlying induction of thermotolerance is also illustrated by the fact that pretreatment of cells with cycloheximide abolished the PGA_1 effect without decrease in thermally induced resistance [72].

As mentioned above, the presence of the α,β-unsaturated cyclopentenone ring in PGA structure determines the possibility of covalent bonding between these PGs and cysteine residues of cell proteins (via a thioester bond) [16, 18-20]. Results of experiments with cycloheximide also exclude the possibility of the development of PGA_1-induced resistance of cells due to direct binding of PGA_1 to temperature damaged proteins and confirm the role of induction of HSP70 expression in the development of thermotolerance [3, 12, 42].

Interestingly, only nontoxic concentrations of PGA increased cell survival *in vitro*; other PGs (PGJ_2, D_2, B_1, E_1, $F_{1\alpha}$) lacked this effect [15].

Cell immunity. PGs are potent local regulators of cell immunity [73, 74]. PGA_2 stimulated phagocytosis by mouse peritoneal macrophages without any effect on their proliferation [73]. Moreover, in contrast to PGE physiological concentrations of PGA_2 did not inhibit lymphocyte production of interleukin-2 required for proliferation of T-cells [74]. However, PGA_1 inhibited directed migration of polymorphonuclear leukocytes to chemoattractants of endotoxin-activated serum and their non-directed migration [75]. PGA_1 also inhibited migration of neutrophils to capillaries and significantly decreased activity of the hexoso monophosphate shunt. It is possible that release of PGA_1 during inflammation increases cell accumulation in lesion sites and amplifies the inflammatory process [75].

CLINICAL APPLICATION OF PGA

The unique biological properties of PGA described in this review suggest that these PGs represent a promising new group of compounds for development of therapeutic agents of certain spectrum of biological and pharmacological activity. Table 3 shows putative regions for application of PGA in clinical practice.

Results of long-termed studies have revealed that toxic signs are noted after chronic administration of PGA to mammals for nine months at a dose one order of magnitude higher than the therapeutic dose. Administration of such doses for 1, 3, and 6 months did not cause any side effects of this PG to cardiovascular, respiratory, and nervous systems [76].

Cyclopentenone PGA is a rather poorly studied class of natural PGs and so characterization of its physiological and biochemical effects definitely opens a new page in the history of prostaglandin research. Moreover, the existence

Table 3. Putative therapeutic applications of PGA

Disease	Pathogenesis	Physiological–biochemical basis of therapeutic effect	Reference
Acute renal failure	primary glomerular vasomotor dysfunction, crash syndrome	reduction of vascular resistance, increase of renal blood flow and rate of glomerular filtration	[77, 78]
Viral infection	viruses of various types	heat shock protein induction, inhibition of viral RNA synthesis	[49, 50, 58, 59, 79, 80]
Malignant tumors	multiple gene mutations	regulation of cell cycle, cytochrome *c* release, caspase-9 activation, inhibition of nuclear topoisomerase II	[6, 9, 18, 71, 81]
Hemodynamic impairments	various etiology	hypotensive, anti-arrhythmic effect	[25, 27]
Hepatitis	– " –	increase in hemostatic properties, decrease in hepatic fibrinolytic activity	[26, 82]
Gestosis	– " –	improvement of renal and cardiovascular hemodynamics, labor induction	[83]
Glaucoma	high intraocular pressure	increase of uveoscleral liquid outflow	[84-87]
Neurodegenerative diseases, Alzheimer's disease, stroke consequences	effects of toxins and viruses	protection of neurons against NMDA-mediated apoptosis; triggering up-regulation of heat shock proteins and inhibition of NF-κB activity	[35, 88, 89]

of protective effects in the spectrum of physiological actions of these compounds suggests possible employment of PGA analogs as antitumor and antiviral agents underlying the development of new therapeutic strategies.

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