TOPICAL REVIEW

Telomerase inhibitors in anticancer therapy: Gossypol as a potential telomerase inhibitor

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Abstract

Telomerase is a reverse transcriptase which helps to stabilize the length of telomeres by adding TTAGGG repeats onto the telomeres that enables unlimited division of the cell. Absence of telomerase activity causes replication senescence and cell death.
Activity of telomerase was detected in embryonal, gonadal and cancer cells, but was not detected in adult somatic tissues with the exception of tissues containing stem cells.
Telomerase inhibitors are being considered as potential anticancer drugs with the hope that the lack of telomerase expression in normal somatic cells would result in a higher specific therapy with fewer side effects than conventional chemotherapy.
Because of high activity of telomerase in gonadal cells, we hypothesized that one of the drugs which selectively interfere with spermatogenesis may act like a telomerase inhibitor.
Gossypol is a male contraceptive derived from cotton seeds. It causes suppression of spermatogenesis by an unknown mechanism. It has a non-endocrine inhibiting effect on spermatogenesis, gradual onset of action, and irreversible suppression of spermatogenesis with increasing length of use. It also has an antiproliferative effect on cell lines derived from tumors, mild antineoplastic action in vivo with few side effects. It has mild inhibiting influence on the replication of HIV via possible inhibition of reverse transcriptase HIV. This shows its possible telomerase inhibiting activity.
It appears that gossypol is a potential new drug for testicular cancer therapy, because of its spermatotoxic and cytotoxic activity on cell lines derivided from testicular tumors.
We posit that the use of gossypol in combination with other anticancer chemotherapeutics can lead to a more effective therapy of human tumors. ***(Ref. 47.)***
Key words: telomerase, gossypol, inhibition, anticancer chemotherapy.

Unicellular organisms are immortal. In an optimal enviroment they divide unlimitedly. Cells of multicellular organisms with evolutional advancement have lost ability, and they are not able to divide autonomously in the organism. These cells are even not able to divide unlimitedly under optimal conditions in vitro (Hayflick et al, 1961). Permanent stimulation of one of the signal transduction pathways is also not sufficient for unlimited cell division (Lodish et al, 1995).

We can make an assumption that in the process of evolution the cellular competence related to division was encoded by certain group of genes for the benefit of a multicellular organism. These genes are partialy or completely repressed and are not required for cell division until a certain number of divisions when the cell is not able to divide without their function. This regulation prevents unlimited dividing of the cell to the detriment of organism; on the other hand it facilitates natural processes of regeneration and reparation without requiring activation of the above mentioned genes (risking the desintegration of organism). In cancer cells and immortalised cell lines there is probable derepression of these genes, by means of which cells aquire their autonomous character. We can say that this group of genes is inevitable for the survival of the unicellular organism as species. If it had a limited number of divisions, it would soon become extinct.

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Cells are not able to divide boundlessly in a multicellular organism, because they have repressed the group of genes providing this process. We can then ask what kind of mechanism secures the survival of species, i.e. its reproduction. Reproduction in a multicellular organism is supplied by gametes. That is why their production must be on a sufficient level. We suppose that there is some similarity between division of the unicellular organism and production of gametes from the viewpoint of reproduction of the species.

A multicellular organism has groups of cells that specialize in certain functions while in a unicellular organism one cell performs all operations. We suppose that there is an analogy between one function of a unicellular organism – reproduction – and the function of the specialized cells of a multicellular organism – gametes. Because in a unicellular organism there is a group of genes necessary for its reproduction which facilitates its unlimited dividing, we assume the required presence of these genes in those cells of a multicellular organism that provide its reproduction — the cells of testis and ovary. A cancer cell is like a unicellular organism, concerning this behaviour; that is why we can assume that it will require activation of these genes, too.

If we take into consideration that ontogenesis imitates phylogenesis, we can assume activity of this group of genes in embryonal human cells and their gradual repression during ontogenesis, except gonadal cells. This assumption partialy correlates with telomerase activity in human cells.

Telomerase is a cellular reverse transcriptase which helps to stabilize the length of telomeres by adding TTAGGG repeats onto the telomeres which enable unlimited division of the cell (Shay et al, 2001; Holt et al, 1996). The absence of telomerase activity leads to gradual telomere shortening at each replicative cycle and causes replication senescence, a non-dividing state of the cell, and cell death (Broccoli et al, 1995).

Telomerase activity is present in cells of embryonal tissues, (Counter et al, 1994) in germ cells of the testis and ovary, cancer cells, and immortal and tumor-derived cell lines. It has not been detected in normal adult somatic tissues or cultured human diploid cells, with the exception of renewal tissues that contain stem cells (Aragona et al, 2000; Hiyama et al, 1995; Kim et al, 1994; Piatyszek et al, 1995; Tahara et al, 1995; Wright et al, 1996; Holt et al, 1996). Telomerase activity is high in cells of embryonal tissues, but its activity decreases during ontogenesis in connection with differentiation of tissues (Counter et al, 1994).

In adult tissues, high expression is present in germ cells, moderate expression in lymphoid follicles, and weak expression is present in proliferating cells of renewal tissues (Holt et al, 1997; Yashima et al, 1998). Telomerase activity in activated lymphocytes and hematopoietic stem cells is not able to prevent the loss of telomeres (Engelhardt et al, 1997; Norrback et al, 1997; Pan et al, 1997).

Telomerase activity is present in 85–90 % of human malignancies, but not in benign tumors, so the difference in telomerase activity cannot be explained simply as a difference in cellular proliferation (Kim et al, 1994; Piatyszek et al, 1995; Holt et al, 1996).

Approximately 5 % of tumors and 25 % of immortalised cell lines have no detectable telomerase activity. It is believed that these cells have an alternative mechanism for the lengthening of telomeres (ALT), but the nature of ALT is not yet known (Bryan et al, 1997; Bryan et al, 1997; Finkel et al, 1998).

Telomerase activity partialy correlates with risk coefficient after whole body irradiation (Aghová et al, 1993). Telomerase probably represents the susceptibility factor of tissue to malignant transformation, although the activation of telomerase does not lead to transformation but only to immortalization of cells (Broccoli et al, 1995; Morales et al, 1999).

Telomerase inhibitors are being considered as potential antitumor agents, in the hope that the lack of telomerase expression in normal somatic cells would result in a highly specific treatment with fewer side effects than conventional chemotherapy (Finkel, 1998; Holt et al, 1996).

Telomerase is an enzyme important for immortalization, i.e. for unlimited cell dividing ability, and for reproductive ability. Gonads, respectively germ cells with the highest telomerase activity are specialized for reproduction in multicellular organisms. Because of the high activity of telomerase in gonadal cells, we think that the drugs that selectively interfere with spermatogenesis like male contraceptives, and drugs with side effects of oligo-azoospermia, may act like telomerase inhibitors.

Gossypol is the only male contraceptive used to a large extent. It is a polyphenolic compound derived from cotton seeds.

Subjects received gossypol as a contraceptive at daily doses being 20 mg per day for 2 months and as maintenance doses 60 mg per week (Nieschlag et al, 1981; Porat et al, 1990), respectively 15 mg per day from 3 to 4 months and maintenance doses 7.5–10 mg per day for 40 weeks (Coutinho et al, 2000). In both dosing schedules, suppression of spermatogenesis was achieved in 99 % (Porat et al, 1990) respectively 100 % (Coutinho et al, 2000) of probands. The suppression was related to decrease of testicular volume without influence of endocrine function of testis (Coutinho et al, 2000; Porat et al, 1990). Inhibition of spermatogenesis occurs independently of concentration, whereas spermatogenesis recovery appears to be concentration-dependent (Coutinho et al, 2000). With the increasing duration of gossypol use the risk of irreversible changes in spermatogenesis increased (Porat et al, 1990). The most common side effects of gossypol were hypokalemia, irreversible azoospermia, and hepatotoxicity (Coutinho et al, 2000; Deoras et al, 1997; Porat et al, 1990; Yu et al, 1998).

Gossypol also has antiproliferative and antineoplastic effects on tumor cell lines derived from testis, lung, breast, cervix, melanoma, colorectal carcinoma, and others (Tanphaichitr et al, 1984; Balci et al, 1999; Blackstaffe et al, 1997; Hu et al, 1994; Shelley et al, 1999; Wang et al, 2000). In some cases its effect was significantly higher than the effect of clinically used cytotoxic drugs (Blackstaffe et al, 1997; Shelley et al, 1999; Wang et al, 2000).

In an experimental study of adrenal tumors in mice, gossypol inhibited tumor growth and achieved a better survival.

In the phase I of clinical trials in metastatic adrenocortical cancer a 20 % partial response rate was found (Flack et al, 1993; Wu et al, 1989). Gossypol was also used in the treatment of adult glial tumors that had recurred after radiation therapy (Bushunow et al, 1999). It was tested in 27 pacients at daily doses 10 mg per day, i.e. a lower dose than used for contraception. Treatment was continued until the disease progressed. Two patients (10 %) had partial response, lasting for 8 or 78 weeks. Toxicity was mild and did not differ from toxicity of contraception (Bushunow et al, 1999). Gossypol was also studied in phase I/II clinical trial in the treatment of patients with anthracycline and taxane refractory metastatic breast cancer (Van Poznak et al, 2001). Twenty women received gossypol at daily doses between 30 to 50 mg per day. One patient had a minor response and two patients achieved stabilization of the disease with more than a 50 % decline of the serum tumor markers. The most common toxicities were nausea, fatigue, emesis, and diarrhoea. Dermatologic toxicity was limited to the daily dose of 50 mg.

Understanding the mechanisms of action of gossypol is essential for including this drug in clinical use (Wang et al, 2000). This mechanism is unknown but is considered to be related to the inhibitor of proteinkinase C (Balci et al, 1999), dehydrogenase (Bushunow et al, 1999), lipoxygenase (Yang et al, 1998), DNA polymerase alpha (Rosenberg et al, 1986), and topoisomerase II (Adlakha et al, 1989). Today gossypol may be considered as a leading compound for a new class of antineoplastic agents (Van Poznak et al, 2001).

We think that the possible contraceptive and antineoplastic mechanism of action of gossypol might cause inhibition of telomerase activity in cancer and in gonadal cells.

The inhibition might be direct or indirect according to upregulation of tumor growth factor 1 (TGF-1) (Shidaifat et al, 1997; Zhang et al, 1998) which leads to downregulation of telomerase activity (Engelhardt et al, 1997; Katakura et al, 1999; Zhu et al, 1996). Its non-endocrine inhibiting effect on spermatogenesis, gradual onset of action, irreversible suppression of spermatogenesis with increasing length of use, antiproliferous effect on cell lines derived from tumors, slight antineoplastic action in vivo, and few side effects (which may be related to weak respectively absent telomerase activity in adult human tissues) show its possible telomerase inhibiting activity. This assumption is partialy supported by the fact that gossypol has a slight inhibiting influence on replication of HIV via a possible inhibition of reverse transcriptase HIV, because telomerase is also a reverse transcriptase (Vlietinck et al, 1998).

Because of its direct, non-endocrine inhibitory effect on spermatogenesis, and cytotoxic effect on tumor cell lines derived from testis, it might be interesting to use gossypol in the treatment of testicular cancer especially the platine refractory cases.

Other drugs with possible antitelomerase activity are cimetidine and phenytoin, which also cause hypospermia (Fody et al, 1985). In cimetidine, beside an antiandrogenic effect, other biochemical factors necessary for normal spermatogenesis could be involved in the testicular alternations (Sasso-Cerri et al, 2001). Some studies refer to the possible anticancer effect of cimetidine, use of which might cause tumor regression (Klener, 1996). In a phase II study in the treatment of metastatic malignant melanoma with interleukin-2 and interferon-alpha plus cisplatin, there was no difference in response or survival in the patients treated with or without cimetidine seen (Schmidt et al, 2000).

The anticancer use of gossypol and other potential telomerase inhibitors may affect the function of telomerase in tumors. Telomerase inhibitors may prove most effective when used in combination with traditional chemotherapeutic drugs that damage DNA, and together accelerate the accumulation of an intolerable disarray in the genome of a cancer cell (Douglas, 2000).

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