Doping with growth hormone/IGF-1, anabolic steroids or erythropoietin: is there a cancer risk?

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Abstract

Anabolic steroid and peptide hormones or growth factors are utilized to increase the performance of athletes of professional or amateur sports. Despite their well-documented adverse effects, the use of some of these agents has significantly grown and has been extended also to non-athletes with the aim to improve appearance or to counteract ageing. Pre-clinical studies and epidemiological observations in patients with an excess of hormone production or in patients chronically treated with hormones/growth factors for various pathologies have warned about the potential risk of cancer development and progression which may be also associated to the use of certain doping agents. Anabolic steroids have been described to provoke liver tumours; growth hormone or high levels of its mediator insulin-like growth factor-1 (IGF-1) have been associated with colon, breast, and prostate cancers. Actually, IGF-1 promotes cell cycle progression and inhibits apoptosis either by triggering other growth factors or by interacting with pathways which have an established role in carcinogenesis and cancer promotion. More recently, the finding that erythropoietin (Epo) may promote angiogenesis and inhibit apoptosis or modulate chemo- or radiosensitivity in cancer cells expressing the Epo receptor, raised the concern that the use of recombinant Epo to increase tissue oxygenation might favour tumour survival and aggressiveness.

Cancer risk associated to doping might be higher than that of patients using hormones/growth factors as replacement therapy, since enormous doses are taken by the athletes often for a long period of time. Moreover, these substances are often used in combination with other licit or illicit drugs and this renders almost unpredictable all the possible adverse effects including cancer. Anyway, athletes should be made aware that long-term treatment with doping agents might increase the risk of developing cancer.

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Keywords: Growth hormone; Insulin-like growth factor-1; Erythropoietin; Anabolic steroids; Cancer

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Several substances such as stimulants, narcotics, anabolic agents and peptide hormones are improperly utilized to increase performance of athletes. Some of these agents such as growth hormone (GH) or anabolic steroids (AS) are taken or administered to enhance the muscular mass and strength. Recently, their use has significantly extended also to non-athletes with the aim to counteract ageing, combat obesity and improve appearance or libido [1–3].

Media and economic interests contribute to encourage to excel in the sport or in the life at all costs. However, success is sometimes achieved with the aid of illicit drugs or with the improper use of legal substances. A lot of factors push both top-level and amateur athletes to use ergogenic drugs, such as the prospective of huge earning, the desire of becoming famous and of raising individual’s social status [2]. The “win at all costs” mentality prevails mainly in teenagers and young athletes despite the never enough publicized deaths due to the adverse effects of performance-enhancing drugs. Addiction or psychological dependency on these substances [4], regardless of their pharmacological class, develop rapidly and doping market is becoming as lucrative as the narcotic market [5]. For all these reasons too little attention is paid to the long-term adverse effects of the use of doping substances. Actually, it is very difficult to know and identify all the consequences of long-term treatment with these compounds at the extremely high dosages required to enhance athletic performance. It should also be noted that these agents are often used in combination and that it is difficult to be aware of all the possible drug interactions [6]. Moreover, different individual or environmental risk factors may contribute to exacerbate toxicity. Therefore, it is very hard to establish a direct correlation between the drugs taken by doped athletes and the adverse effects on their health.

Clinical reports indicate a link between doping with AS and cancer [7–21], while basically no clinical data are presently available on the risk of cancer development and progression which may be associated to the use of GH/IGF-1 or erythropoietin (Epo) as performance enhancing substances. In fact, whether these drugs could directly cause or favour cancer development still remains an unresolved problem. A number of evidences in support to this fear come from pre-clinical studies and from circumstantial epidemiological observations in patients with an excess of hormone production or in patients chronically treated with hormones or growth factors for various pathologies [22–29]. Post-marketing pharmacosurveillance for recombinant peptide hormones is still too short and it is too early to draw any definitive conclusion. However, it should be pointed out that the cancer risk associated to doping might be different, since the doses taken by the athletes are higher than those used in the clinics [6,21,30,31].

This review is focused on the experimental evidence and clinical data indicating a link between hormones, growth factors and peptides used as doping agents and cancer.

1. GH/IGF-1

1.1. Physiological effects

GH is a polypeptide hormone produced in the pituitary gland under the control of hypothalamic factors such as growth-hormone-releasing hormone (GHRH) and somatostatin. GH exerts either direct or indirect effects on hepatic or extra hepatic target tissues. The indirect effects of GH are mediated by stimulating the release of insulin-like growth factor-1 (IGF-1) from the liver and hepatic synthesis of IGF-1 is regulated by a number of hormonal and nutritional factors. The principal metabolic effects of GH include increase of glucose uptake and protein synthesis (especially in the liver and muscles) and inhibition of lipolysis in adipose tissue. Similar effects are induced by IGF-1.

The most striking physiological effect of GH is the stimulation of the longitudinal growth of bones. Other effects of GH include the stimulation of myoblast differentiation, the increase of muscle mass and of glomerular filtration rate.

1.2. Clinical indications and use as doping agents

In clinics, recombinant GH is used to treat severe GH deficiency both in children and adults, short stature despite adequate GH production or idiopathic short stature, chronic renal insufficiency, children born small for gestational age, AIDS-associated wasting and for malabsorption associated with the short bowel syndrome [32].
As performance-enhancing drug GH has gained popularity especially in the last two decades due to the availability of the recombinant form and is now largely used in the sport world almost replacing the supremacy as doping agent of AS [6,33–35]. Nowadays, athletes such as cyclists, swimmers, power lifters and body builders take recombinant GH or IGF-1 for their anabolic effects assuming that they will improve their performance, strength and look [6,33–35]. It should be noted that the doses of GH used as doping agent are hard to evaluate and that the few controlled studies on efficacy of GH as performance enhancer have been done with doses lower than that claimed to be effective by body-builders [6].

1.3. Adverse effects

Severe side effects have been described both in GH treated patients or athletes: intracranial hypertension, visual changes, headache, nausea, vomiting, peripheral edema, carpal tunnel syndrome, arthralgia, myalgia, acromegalic features such as nose and jaw enlargement, hypertension, cardiomegaly, increased cardiovascular risk, arthralgias, insulin resistance and diabetes [31].

1.4. GH/IGF-1 and cancer development

1.4.1. Molecular mechanisms linking GH/IGF-1 to cancer

The ability of GH, via its mediator peptide IGF-1, to influence cellular growth has been the focus of much interest in recent years. Since its identification, there have been unresolved concerns about the potential cancer-enhancing properties of treatment with GH. In fact, IGF-1 signalling is highly involved in cancer development and progression [36] exerting powerful effects on each of the key stages of cancer formation: cellular proliferation, apoptosis, angiogenesis, metastasis and resistance to chemotherapeutic agents (Fig. 1).

The intracellular signalling pathways activated following IGF-1 binding to its tyrosine kinase receptor (IGF-1R) involves [22] initial phosphorylation of insulin-receptor substrate-1, which in turn activates phosphatidylinositol-3-kinase (PI3K)/Akt/target of rapamycin TOR and Ras/Raf/mitogen activated protein kinase (MAPK) pathways. The IGF-1R is crucial in transformation and survival of tumour cells while it is only partially required for normal cell growth. This receptor is ubiquitously expressed in normal tissues and in a wide range of haematologic neoplasias or solid tumours, such as prostate, breast and colon cancer.

Activation of the IGF-1R pathway induces post-transcriptional and post-translational modifications that influence the function of a number of molecules, such as proteins involved in cell cycle regulation (e.g., the tumour suppressors retinoblastoma and p53), in DNA synthesis and repair. Moreover, IGF-1/IGF-1R signalling inhibits apoptosis by up-regulation of caspase inhibitors or anti-apoptotic regulators of the Bcl-2 family, activates transcriptional regulators such as nuclear factor-kB, which is implicated in cell survival and chemoresistance, and activates telomerase, a ribonuclear protein involved in telomere elongation and in the unlimited proliferative potential of cancer cells [37]. The anti-apoptotic effects of IGF-1 could cause a regulatory imbalance of cell proliferation that is considered the starting point of cancer development [22]. The imbalance of proliferation/cell death could potentially favour survival of stem cells that may be exposed to genetic hits over a longer period of time accelerating the process of carcinogenesis. Moreover, signalling through IGF-1R increases the expression of vascular endothelial growth factor (VEGF), the main proangiogenesis factor responsible for neo-vascularisation of many tumours and this effect is mediated by the activation of the hypoxia-inducible factor-1α (HIF-1α) [38,39]. Indeed, administration of IGF-1 to animals is associated with an increase of VEGF expression and metastases [40].

GH/IGF-1 axis may also participate to deregulate the signalling pathway activated by Wnt, a large family of highly conserved growth factors that plays a crucial role in cell fate, survival and proliferation in a variety of tissues [41]. Aberrant activation and up-regulation of the Wnt pathway is a key feature of many types of cancer [42]. For instance, deregulation of this pathway, is the first, virtually obligatory step, on the pathway leading to colorectal neoplasia and IGF-1 signalling affects this pathway favouring the adenoma–carcinoma progression sequence [43].
Considerable evidence suggests a complex cross-talk between oestrogen receptor signalling and GH/IGF-1 axis [43]. In fact, the majority of breast cancers harbour IGF-1R and IGF-1 has been found to be mitogenic for breast cancer cells in vitro and, interestingly, high levels of IGF-1 predict subsequent risk of breast cancer in pre-menopausal patients [44,45].

1.4.2. Animal studies

In vivo studies in pre-clinical models suggested that GH may affect cancer risk [46]. High doses of GH can induce tumour formation in rats and transgenic mice overexpressing GH or IGF-1 in mammary gland have an increased incidence of breast cancer [47,48]. Recently, it has been shown that the behaviour of experimental IGF-1R-positive cancers is influenced by variations in the circulating levels of IGF-1, indicating that these neoplasms can be stimulated in their growth by IGF-1 produced in tissues distant from the tumour [49]. Moreover, IGF-1R signalling potentiates the transcriptional activity of the androgen receptor (AR), favouring the progression of androgen-independent prostate cancers [50].

More compelling, even though indirect, proof of the mechanistically relationship between GH/IGF-1 and cancer derives from animal studies that involve manipulation of the GH/IGF-1 axis. Homozygosity for the Lir mutation, which inactivates the GHRH receptor, results in small mice with subnormal levels of circulating GH and IGF-1 and less permissive for neoplastic proliferation than controls [51]. Another indirect evidence comes from the development of experimental strategies for cancer treatment targeting the GH/IGF-1 signalling. These strategies include the use of blocking antibodies directed against the IGF-1R or molecules which inhibit signal transduction at different levels of the GH/IGF-1 pathway (e.g., the growth hormone antagonist pegvisomant) [52–56].

1.4.3. Clinical studies

A number of epidemiological studies examined the possible link between cancer and increased levels of GH/IGF-1. A role of GH/IGF-1 in cancer development is suggested by the finding of an increased frequency of colon carcinoma among patients affected by acromegaly with high IGF-1 levels (reviewed in Ref. [23]). Moreover, it was observed that women with breast cancer had elevated serum GH levels [24] and patients with breast and prostate cancer had increased circulating IGF-1 levels [25]. High circulating levels of IGF-1 have been associated with increased risk for several common cancers, including those of the breast, prostate, lung and colorectum [26–29]. Moreover, birth weight and size or levels of cord blood IGF-1 have been positively correlated with risk of breast, prostate, colorectal and childhood cancers [22].

Nutritional status and dietary energy intake are critical regulators of IGF-1 levels, which decrease in association with dietary restriction, while they raise in response to increased energy and protein intake [22]. Recent evidence suggests that dietary and related factors such as physical activity and body size may influence cancer risk through their effects on the serum concentration of IGF-1 and its binding proteins. Therefore, it cannot be excluded that diet composition might amplify the potential cancer risk associated to treatment with GH/IGF-1 as doping agents.

However, it is important to point out that other studies did not detect any association between IGF-1 levels and risk of cancer [57]. Discrepancies may be due to the complexity of the relation between circulating IGF-1 levels and IGF-1R signalling. In fact, bioavailability of IGFs can be influenced by concentrations of specific IGF-binding proteins (IGFBPs). These proteins are present in plasma as well as in extra-vascular fluids and IGFBP3 provides most of the serum binding capacity greatly extending IGF-1 circulating half-life and increasing IGF-1 signalling. On the other hand, IGFBPs can also decrease IGF-1 signalling competing with IGF-1R for IGF-1 binding.

The long-term risk of developing cancer has been studied in patients treated with human pituitary GH in childhood and early adulthood. The results of this work indicate that the incidence of colorectal cancer and Hodgkin lymphoma was increased in people who received GH for growth deficiency at young age [58]. Actually, a case of Hodgkin’s lymphoma was also reported in a cyclist treated with GH [59]. Other studies observed a raised risk of colorectal cancer in patients treated with GH as replacement therapy [60,61]. Several cohort studies have found enhanced leukaemia risk for GH treated patients, but this observation was not confirmed by other studies in which prior leukaemia risk factors were excluded [46]. The current international surveillance data do not support the increase of malignancy after recombinant GH treatment but the duration of follow-up is still 4 years and longer period of observation is needed [57].

1.5. Conclusive remarks

In conclusion, strong experimental in vitro evidences or in vivo pre-clinical studies support the potential role of GH/IGF in cancer from a mechanistic point of view. Epidemiological studies performed in patients taking GH as therapeutic agent show the association between the IGF-1 levels and cancer incidence, but do not substantiate the existing concern that GH/IGF therapy could enhance the risk of developing a cancer (Table 1).

2. Anabolic steroids

Another category of substances frequently used to increase performance are AS, which are related to the male sex hormones.

2.1. Physiological effects

Male hormones are partially responsible for the developmental changes that occur during puberty/adolescence and for the changes in primary and secondary sexual characteristics. The anabolic effects of androgens include accelerated growth of muscle and bone, enhanced production of red blood cells and increased neural conduction. The main male sexual hormone testosterone is essentially produced by Leydig cells in testes where it takes origin from cholesterol. Testosterone may also derive from the direct precursors, dehydroepiandrosterone (DHEA) and androstenedione that are released from gonads and adrenal cortex and converted to testosterone in the liver.
Table 1
Medical literature on the association between AS, GH/IGF-1 or Epo and cancer

A. Cancer cases attributed to doping

<table>
<thead>
<tr>
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<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GH/IGF-1 doping</td>
<td></td>
<td></td>
<td>Hodgkin’s lymphoma [59]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epo doping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No cases reported to date</td>
</tr>
</tbody>
</table>

B. Clinical studies on AS, GH/IGF-1, Epo and risk of cancer

<table>
<thead>
<tr>
<th>Long-term AS therapy, high serum androgen levels</th>
<th>Liver [18,31,75]</th>
<th>Breast [81]</th>
</tr>
</thead>
<tbody>
<tr>
<td>High plasma levels of GH/IGF-1</td>
<td>Breast [24-26]</td>
<td>Prostate [27]</td>
</tr>
<tr>
<td>rGH replacement therapy</td>
<td>Colorectal [46,58,60,61]</td>
<td>Hodgkin’s lymphoma [58]</td>
</tr>
<tr>
<td>rEpo to prevent or treat malignancy-associated anaemia</td>
<td>Dubious impact on progression-free survival time [107–113]</td>
<td></td>
</tr>
</tbody>
</table>

In women, testosterone is synthesized both in the corpus luteum and adrenal cortex by similar pathways. Testosterone can also give rise to oestrogens after conversion to estradiol by the enzyme aromatase.

Testosterone acts as an androgen either directly, by binding to the AR, or indirectly through conversion by the enzyme 5α-reductase to dihydrotestosterone (DHT), which also binds to the androgen receptor even more avidly than testosterone and activates gene expression more efficiently. As a result, testosterone, via DHT, is able to exert effects in a variety of tissues expressing 5α-reductase that it could not have if it were present only as testosterone. Upon binding of testosterone or DHT to the receptor, the ligand–AR complex undergoes a conformational change and translocates to the nucleus, where it behaves as transcription factor.

2.2. Clinical indications and use as doping agents

In the clinics AS are primarily used to treat hypogonadism, a condition in which testosterone is not sufficiently produced by the testes causing growth defects, delayed puberty or development and sexual dysfunction. These compounds are also administered to treat body wasting caused by HIV infection or malignancies and anaemia [18].

The use of AS is very diffused in sport and can be prolonged for the whole course of athlete career. In addition, a wide range of AS is self-administered at high doses by bodybuilders to achieve a rapid increase in muscle mass [20]. AS administration can be done by different modalities: stacking, which describes the simultaneous use of different steroids in combination; cycling, a regimen in which AS administration is alternated to a period of rest; pyramiding, when the doses are increased in a first period and then decreased [21].

In sport, the AS employed as ergogenic substances include testosterone, DHT, DHEA and derivatives such as testosterone esters, alkylated androgens (e.g., nandrolone and stanozolol) and other compounds, such as tetrahydrogestrinone [18]. Synthetic modifications of AS have been performed with the aim of modifying some pharmacokinetics properties such as solubility, half-life, sensitivity to enzymatic destruction and of enhancing the anabolic properties, minimizing the androgenic properties [18].

Steroid hormones stimulate receptor molecules in muscle cells, which activate specific genes to produce proteins. AS are considered to prevent tissue from breaking down following an intense workout and are used to speed recovery after muscle trauma. In order to exert any beneficial effect on physical performance AS must be associated with intense training, which increases the number of unbound AS receptor sites. These drugs prevent muscle catabolism that often accompanies intense exercise, blocking the effects of cortisol which is involved in tissue protein catabolism during and after training. In fact, cortisol is increased during exercise to feed muscles and to suppress inflammation that accompanies tissue injury. When the athlete stops taking AS the catabolic effects of cortisol are enhanced and strength and muscle size decrease rapidly. This rebound effect mediated by cortisol can give origin to AS addiction, which induces athletes to take the drugs for long periods and long-term administration increases the chance of serious side-effects. Moreover, the rapid loss of strength and muscle size may provoke depression which in turn may lead to the abuse of psychostimulants such as amphetamines and cocaine [19,62]. Euphoric and aggressive behavioural effects or the decrease of the athlete’s sense of fatigue during training may also contribute to AS dependence.

2.3. Adverse effects

High and multi-doses of AS used to enhance athletic performance can induce serious and irreversible organ damage. Among the most common adverse effects of these drugs are reduced fertility and gynecomastia in males and masculinization
in women or children [18]. Of particular concern is premature physical closure in any child/adolescent, which results in a decrease in adult height. Other adverse effects include hypertension and atherosclerosis, blood clotting, jaundice and liver dysfunction, increased rate of muscle strains/ruptures, tendon damage, increase of aggressiveness, psychiatric and behavioural disorders. Orally administered AS may exert profound adverse effects on the liver, whereas parenterally administered AS seem to have less serious hepatic untoward effects [18].

2.4. AS and cancer development

2.4.1. Molecular mechanisms linking AS to cancer

Even though AS are synthetic derivatives of testosterone modified to enhance the anabolic properties minimizing the androgenic actions of the hormone, steroids completely devoid of androgenic effects are still lacking [63]. Androgens are critical in prostate cancer cell growth and survival interacting with androgen response elements and stimulating the expression of genes implicated in cell proliferation or apoptosis/survival (Fig. 1). Transcriptional activation of target genes relies on co-activators, some of which possess intrinsic histone acetyltransferase activity required for modulation of gene expression [64]. In addition, AR can also act as cytoplasmic signalling molecules activating the Src/Raf/extracellular signal-related kinase (ERK) pathway [65]. Among the pathways that could play a role in androgen-dependent prostate cancer growth the IGF-1 signalling has been suggested to modulate AR function. IGF-1 mediated activation of PI3K/Akt or Ras/MAPK, would lead to AR phosphorylation and sensitization to low androgen concentrations [66]. Moreover, androgens induce the expression of Mdm2 that in turn decreases the level of p53, a key regulator of cell cycle and apoptosis [67]. Androgens protect cells from apoptosis also by inhibiting Fas signalling and the expression of caspase-8, which play a critical role in initiation and execution of programmed cell death [68]. Recently, it has been shown that androgens stimulate angiogenesis in prostate cancer increasing the expression of VEGF through activation of the transcriptional factor HIF-1 [69].

2.4.2. Animal studies on AS and cancer development

Initially, DHEA gained interest in cancer research because of its anti-carcinogenic effects, which has been proven in various organs (including the liver) of laboratory rats [70]. Despite the anti-carcinogenic effects, in rats DHEA itself may also act as non-genotoxic hepatocarcinogen when it is given at high doses for more than one year [71]. This effect proved to be dependent on the treatment scheme, mainly determined by dosage and treatment time.

Testosterone alone or in combination with carcinogens, can induce prostate carcinomas after long-term administration or high dose [72]. Moreover, testosterone in combination with 17-β estradiol is capable of inducing breast cancer in rat experimental model [71]. Treatment with a combination of four AS at the doses used by athletes and body builders reduced the life span of experimental animals due to the development of tumours in the liver or kidney, lymphosarcomas or heart damage [73]. Molecular studies of testosterone related carcinomas have revealed that the Ki-ras gene, Cyclin D and TNFα may play a mechanistic role in carcinogenesis [72–74].

2.4.3. Clinical studies

The results of clinical studies indicate that AS can contribute to the initiation and development of benign and malignant tumours and, in particular, hepatic carcinoma (reviewed in Ref. [75]). The majority of human studies on AS and tumour formation involves hospitalized patients who are treated for prolonged periods for various diseases. The association between AS and liver tumours was first noted in patients with Fanconi’s anaemia, which is characterized by genomic instability, and in patients affected by other types of refractory anaemia [75]. Moreover, patients treated for endocrine, gynecological or other pathologies are also at risk of liver tumours (reviewed in Ref. [75]). In the case of athletes or bodybuilders a number of case reports on liver tumours or cancers of different tissue origin have been reported, such as prostate, renal, testicular cancers and non-Hodgkin’s lymphoma [7–21]. Most information on AS related tumours are anecdotal and does not take into account the concomitant presence of other liver associated risk factors for hepatocarcinoma such as heavy alcohol consumption, low vegetable diet, contraceptives, smoke, viral exposure to HBV, HCV or carrier status. There are strong indications that tumours of the liver are caused when the AS contain a 17-alpha-alkyl group, such as danazol, methyltestosterone, nandrolone, oxymetholone and stanozolol [75]. Although most of the reported tumours are benign, early detection is important in order to avoid the associated risks of life threatening hemorrhages and malignant degeneration.

Evaluating the potential cancer risk associated to AS abuse is very difficult since these drugs are often used at very high doses and in combination with other licit or illicit drugs [2,21,76]. Another alarming factor is that exposure to AS starts more and more prematurely since it has been shown a continuing and significant increase of their use among adolescent athletes and non-athletes [2,77,78].

AS hormones and their metabolites have been postulated to be involved in the aetiology of prostate cancer. Prostate cancer is initially partially androgen-sensitive, which is the basis for the initial treatment of metastatic prostate cancer by androgen deprivation but then becomes unresponsive to continued deprivation even though AR signalling remains active. Although it has been established that sex steroid hormones, particularly androgens, are essential to the growth, development, and maintenance of healthy prostate epithelium, and to the progression of prostate cancer, a review of 10 prospective epidemiologic studies failed to support the hypothesis that circulating androgens are positively associated with prostate cancer risk [79]. Moreover, it should be noted that polymorphisms in enzymes involved in AS metabolism might also influence the individual prostate cancer risk [80].

2.5. Conclusive remarks

Overall, several clinical reports attributed to doping with AS the development of liver and renal tumours (Table 1). Moreover,
since AS may favour tumour progression of an early androgen-dependent tumour the possibility of a higher risk of prostate cancer in athletes or body builders subjected to long-term and high-dose intake of steroids should be considered.

The role of androgens in women’s health has been generally neglected. Prospectively conducted epidemiologic studies have found that high levels of serum testosterone are associated with an increase in post-menopausal breast cancer risk [81]. Although in vitro studies report both proliferative and anti-proliferative effects of testosterone on the growth of various breast cancer cell lines, it is not yet clear how testosterone might exert these effects in vivo. Also in this case, it would be highly recommended for female athletes who used AS for doping purpose to monitor themselves for breast cancer risk.

### 3. Erythropoietin

#### 3.1. Physiological effects

Epo is the principal haematopoietic growth factor, regulating cellular proliferation and differentiation along the erythroid lineage with the aim to maintain the oxygen-carrying capacity of peripheral blood (Fig. 1). This factor is produced primarily in the adult kidney under the control of an oxygen-sensing mechanism. In fact, at low oxygen tension Epo transcription is markedly enhanced through the activation of HIF [82]. Normally HIF-1α is degraded by two intracellular enzymes (i.e., asparaginyl hydroxylase and prolyl hydroxylase), whereas in low oxygen conditions the activity of these enzymes is inhibited and, consequently, HIF-1α can join to HIF-1β forming a heterodimer which translocates to the nucleus to activate gene transcription. Besides hypoxia, there are several other factors that modulate Epo production, such as hypoglycemia, increased intracellular calcium, insulin release, oestrogens, AS, and various cytokines [83]. The biological effects of Epo in haematopoietic cells are mediated through its binding to the specific cell surface Epo receptor (EpoR).

EpoR expression and signalling in haematopoietic tissues is essential for normal mammalian erythropoiesis during development. Functional EpoR expression has been documented also in many nonhaematopoietic cell types, including vascular endothelial cells, smooth muscle cells, skeletal myoblasts, cardiac myocytes, neurons, retinal photoreceptors, liver stromal cells, placenta, kidney and macrophages.

Epo possesses pleiotropic effects, distinct from its essential role in the regulation of red blood cell production and exogenous administration of recombinant Epo (rEpo) has been associated with diverse effects in nonhaematopoietic tissues. For instance, expression of EpoR in muscle cells, is associated with the ability of rEpo to induce cellular proliferation [84]; in the central nervous system, Epo is produced by astrocytes and plays an important role in the response of the brain to neuronal injury [85].

#### 3.2. Clinical indications and use as doping agents

In the clinics rEpo is used for the treatment of a number of anaemias, such as those associated with a poor erythropoietic response, chronic kidney disease, surgery, AIDS, cancer chemotherapy, prematurity, and certain chronic inflammatory conditions [86].

Endurance athletes such as cyclists, long-distance runners, cross-country skiers are often seeking new ways to increase tissue oxygenation as a means to improve performance. The currently known methods include rEpo, hypoxia chamber, nitrogen tents, and autologous blood transfusions. In addition, haemoglobin based substitutes such as perfluorochemicals and the synthetic allosteric modifier of haemoglobin efaproxiral (RSR13) have been used by athletes to enhance oxygen delivery to tissues particularly in endurance sports [87].

Since rEpo became available as an erythropoiesis-stimulating drug, it has been widely used by athletes in aerobic sports and the performance enhancing effect of this application is well documented [88–91].

#### 3.3. Adverse effects

The most common adverse effects of rEpo are related to the increase in red blood cell mass and include migratory thrombophlebitis, microvascular thrombosis, pulmonary embolism, high blood pressure, hypertensive encephalopathy. It should be noted that the risk of blood clotting and heart failure is particularly high in athletes, since dehydration that occurs during endurance training and competition, further increases blood viscosity. Rarely, pure red cell aplasia has been observed in patients treated with rEpo. The pathogenesis of this anaemia is mainly related to certain formulations and to improper storage that may lead to increased immunogenicity of the recombinant product [92,93].

### 3.4. Epo and cancer development

#### 3.4.1. Molecular mechanisms linking Epo to cancer

Epo-EpoR signalling is associated with activation of a cytoplasmic, nonreceptor protein Janus tyrosine kinase 2 (JAK2) and the downstream molecule signal transducer and activator of transcription 5 (STAT5), a cytoplasmic transcription factor that plays an important role in the regulation of in vivo erythropoiesis [94,95]. Moreover, Epo-EpoR signalling activates PI3K-Akt pathway that has been associated with primary erythroid survival signalling and the MAPK pathway, required for synergistic expansion of erythroid progenitor and precursor cells in response to Epo [96,97]. Abnormal regulation of Epo-EpoR signalling in haematopoietic cells has been associated with proliferative disorders of the bone marrow, such as polycythaemia, a disease characterized by erythrocytosis. Mutation of EpoR or constitutive activation of JAK2, as a consequence of a missense mutation in its pseudokinase domain, has been shown to underlie the Epo-independent erythropoiesis that is characteristic of polycythaemia vera [98,99].

Recent studies have reported the expression of EpoR in tumour cell lines as well in primary cancers, suggesting the potential existence of an autocrine or paracrine growth-stimulatory Epo-EpoR loop in tumours [100]. Finally, activation of Epo-EpoR signalling in cancer cells may be associated...
with modulation of cellular proliferation, apoptosis, metastatic behaviour and sensitivity to radio-chemotherapy [100,101] (Fig. 1). Moreover, the expression of EpoR in vascular endothelium of tumours has suggested an influence of Epo on the tumour microenvironment, such as the stimulation of angiogenesis. Indeed, Epo has proved to be a pro-angiogenic cytokine in a variety of experimental systems, stimulating the proliferation and migration of endothelial cells [102–106].

3.4.2. Clinical studies

rEpo has been widely used in the clinics to prevent or treat malignancy-associated anaemia and for reducing transfusion requirements in cancer patients. However, in randomized trials patients in the rEpo arm fared worse than their placebo-treated counterparts in terms of progression-free survival, suggesting a role for Epo in tumour growth or in tumour resistance to radio-chemotherapy [107–109]. Nevertheless, doubts on the negative impact of erythropoietin on tumour progression have been recently raised. Phase II and III studies in head and neck or breast cancer patients did not observe poorer survival in the rEpo arms, and studies in pelvic and cervical cancer patients indicated improved tumour control with rEpo treatment [110–113]. Some reports questioned the validity of an increasing number of studies showing the expression of functional EpoR in many non-haematopoietic tissues and solid cancers, invoking methodological problems and limited specificity of commercially available antibodies against the EpoR [114].

The concern that chronic administration of pharmacologic doses of rEpo could have several types of deleterious consequences, such as thrombo-embolic events and enhancement of cancer growth, has been evaluated by a systematic review on the effects of rEpo or of the long-acting analogue of erythropoietin Darbepoeitin alfa [115]. Uncertainties remain as to whether and how these molecules affect overall survival but caution is advised when they are administered in combination with thrombogenic chemotherapeutic agents or in cancer patients who are at high risk for thrombo-embolic events [115].

3.5. Conclusive remarks

The role of EpoR in tumour growth is still controversial and ongoing trials are currently underway to clarify the actual risk of increasing tumour aggressiveness by rEpo treatment. If these studies will confirm the detrimental effects of rEpos observed in cancer patients, it cannot be excluded that the improper use of Epo as performance enhancing substance in healthy subjects might favour the cancerogenesis processes (Fig. 1).

4. Conclusions

Appropriate information about the potential carcinogenic risk associated with the use of performance-enhancing substances is highly recommended, in view of the fact that there has been a substantial increase in the use of doping agents by athletes (despite the anti-doping controls introduced in the 1960s).

Presently, medical literature documents clinical reports on cancers developed especially in athletes taking AS to increase performance (Table 1A). As far as GH/IGF-1 or rEpo are concerned, beside one case of Hodgkin’s lymphoma in a cyclist treated with GH [59], no clinical reports are available on tumour formation associated with doping. It should be noted that biological studies and epidemiological observations in patients with an excess of hormone production or in patients chronically treated with hormones/growth factors for various pathologies (Table 1B) indicate the ability of these peptides to stimulate tumour growth or to promote tumour formation.

The paucity of tumour case reports regarding doping which have been published so far should not reassure the athletes who used these substances about the lack of cancer-related risk. Difficulties in the detection of several performance enhancers and the small number of self-admissions by doped athletes have contributed to the lack of retrospective studies.

Therefore, given the total absence of controlled scientific reports dealing with athletes treated with AS, GH/IGF or rEpo, the potential risk of developing cancer in doped athletes should not be underestimated and should be more deeply evaluated.

A new threat has emerged from the promising therapeutic specialty of gene therapy: gene doping. A super mouse has been created using adenoviral vector to insert directly into muscle the IGF-1 gene [116]. These animals have 20–30% greater muscle mass and strength, live longer and recover more quickly from injuries with respect to controls. However, untoward effects associated to gene therapy are not easily predictable from experimental studies performed in animal models, since they may change moving from an animal species to another or from animals to humans. In fact, several unexpected effects have been described such as liver damage, autoimmune disease and tumour development [117,118]. A scenario in which some athletes with injuries could use IGF-1 to speed healing and repair of the damaged muscles or to strengthen their muscle mass or in which athletes would inject themselves with the gene that produces Epo, allowing the body to naturally produce more red blood cells, must take into account that the complication of gene doping may arise years after treatment.

Already two decades ago it was noted that medicine, which has long been concerned with the health and welfare of people, in sport shows more interest in finding new ways of enhancing the performance of athletes than in caring about their physical well being [119]. It is now time to pay more attention especially to long-term effects of performance enhancing agents taking into account that teenagers are exposed more and more prematurely to these drugs and for a long period. In fact, younger can have many decades of uncertainty during which the unpredictable complications of these kinds of drugs might appear.

References


