Anabolic Androgenic Steroids Abuse and Liver Toxicity

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Abstract: In the athletes the wide use of Anabolic Androgenic Steroids (AAS) cause series damage in various organs, in particular, analyzing the liver, elevation on the levels of liver enzymes, cholestatic jaundice, liver tumors, both benign and malignant, and peliosis hepatis are described. A prolonged AAS administration provokes an increase in the activities of liver lysosomal hydrolases and a decrease in some components of the microsomal drug-metabolizing system and in the activity of the mitochondrial respiratory chain complexes without modifying classical serum indicators of hepatic function. Liver is a key organ actively involved in numerous metabolic and detoxifying functions. As a consequence, it is continuously exposed to high levels of endogenous and exogenous oxidants that are by-products of many biochemical pathways and, in fact, it has been demonstrated that intracellular oxidant production is more active in liver than in tissues, like the increase of inflammatory cytokines, apoptosis and the inhibitors of apoptosis NF-κB and Heat Shock Proteins.

Keywords: Anabolic androgenic steroids, apoptosis, liver, inhibitors of apoptosis, oxidative stress, peliosis hepatis.

INTRODUCTION

Anabolic-Androgenic Steroids (AAS) are synthetic hormone derivatives of testosterone chemically modified to maximize anabolic effects, promoting growth of skeletal muscle and enhancing athletic performances [1], and to minimize androgenic ones, consisting in development of male sexual characteristics [2].

Testosterone derivatives have been categorized in three classes [3]:

1. Class A: 17-β-hydroxy esters requiring intramuscular injection
2. Class B: alkylation at the 17-α-hydroxy position allows compounds that can be given orally
3. Class C: alkylation in the A, B, or C rings of the steroid backbone results in orally available AAS resisting hepatic metabolism.

Most commonly used Anabolic-Androgenic Steroids include nandrolone, oxandrolone, stanozolol, and oxymetholone (Fig. **1**).

Pharmacologic doses of AAS and/or testosterone have been successfully used for the treatment of patients with retarded growth, hypogonadism, impotence, infertility, eunuchoidism, cryptorchidism, sarcopenia [4], muscle wasting or debilitation [5], decline of cognitive functions [6], paroxysmal nocturnal hemoglobinuria, depression, HIV-related cachexia [7], anemia [8,9], hepatic regeneration following an injury or a transplantation [10], endometriosis, and many nonendocrine disease (thrombocytopaenia, panmyelopathy, hereditary angio-oedema, breast cancer and osteoporosis) [11].

The beneficial potential of AAS accompanies undesirable side effects. Short and long term side effects have been demonstrated in various organs and the liver alterations are related with androgenic anabolic steroids use [12-15].

Steroids abuse cause both reversible (spontaneous regression of lesions on AAS cessation) [16] and irreversible changes, whereas side effects develop virtually only during long-term use at high dose [17].

The restart of medical complications after recidivistic steroid abuse needs to be stressed [18], as well as the fact that ill effects of steroids misuse can also manifest many years after cessation of AAS abuse.

Although the use of anabolic steroids is associated with a number of side effects, the prevalence of toxic effects following AAS administration is difficult to ascertain because of underreporting. Nevertheless, it is becoming increasingly clear that the abuse of AAS is associated with serious adverse effects [19], such as the suppression of endogenous testicular function, gynecomastia, erythrocytosis, psychological disorders, sudden death resulting from cardiac disease, induction of atherogenic lipoprotein pattern, activation of hemostatic system, virilization, hepatotoxicity, premature epiphyseal fusion, stunting of growth, infections, and musculoskeletal damages [20].

In men, the multi-organ damage has been explained also by the hypercalcemia [21] that could occur as a result of AAS modulation of steroid hydroxylase activity [22].

Among these side effects, hepatotoxicity is the most common and prominent of them. Hepatotoxicity has never

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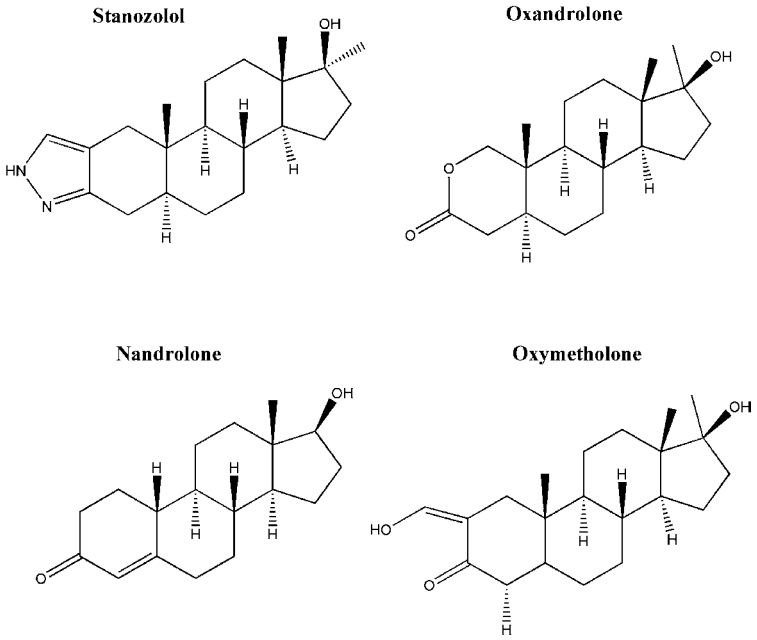

Fig. (1). Chemical structures of most commonly used Anabolic-Androgenic Steroids.

been described with the parenteral use of testosterone, while class B and C are highly and potentially life-threatening hepatotoxic leading to: elevation of liver enzymes (aspartate and alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, and gammaglutamyltransferase), high levels of amylase, lipase and creatine protein kinase [21]; jaundice; cholestasis [15]; peliosis hepatis [23-25]; hepatic adenomas [26] and haemorrhages up to spontaneous hepatic rupture [18,27-28]; liver cysts; hepatocellular necrosis and hepatitis [19,29]; hepatocellular carcinoma [30,31]; decreased liver capacity for metabolizing xenobiotics [19]. AAS also increase hepatic triglyceride lipase activity and low-density lipoprotein, and simultaneously reduce level of high-density lipoprotein cholesterol, mainly in cirrhotic malnourished patients [32,33]. However it is not clear whether these findings are the result of a direct effect of AAS-induced liver toxicity [13]. Schwingel *et al.* demonstrated that AAS abuse is also correlated to a toxicant-associated fatty liver disease (TAFLD), whose spectrum is inclusive of steatosis, non-alcoholic steatohepatitis, cirrhosis and hepatocellular carcinoma [34]. Moreover, it is well known that most AAS, when used exogenously, inhibit the normal process of steroid biosynthesis [35]. This means that the first step in steroidal hormone production is hindered and that cholesterol is not converted into pregnenolone, resulting in cholesterol storage and so creating the proper environment for fatty liver accumulation.

In the athletes who use steroids elevation on the levels of liver enzymes (aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase) is described [30]. Cholestatic jaundice occurs occasionally with steroid use and typically resolves within 3 months of discontinuing the drugs. Increase of triglycerides and cholesterol serum levels is also reported [15]. Liver tumors, both benign and malignant, have been linked to the administration of steroids [30], in particular hepatocellular carcinomas were more often associated with oxymetholone and methyl testosterone. There is also an increased risk of peliosis hepatis with steroid use.

LIVER PELIOSIS

Peliosis is a pathological entity characterized by multiple blood-filled cavities, mostly involving the liver (peliosis hepatis). Peliosis hepatis is uncommon; however it is one of the most serious complications associated with the use of androgen-steroids [25,36,37]. It has been reported to be associated with long-term treatment with oral contraceptives, tamoxifen, estrogens, intravenous drug abuse and chronic alcoholism too [38]. Peliosis hepatis is characterized, at macroscopic examination, by multiple blood-filled pools of various sizes without lobular systematization, while histologically, by the presence of scattered, small, blood-filled cystic spaces throughout the liver parenchyma. Some of the cysts may be lined by sinusoidal cells whereas others are not. Secondary changes, including fibrosis, may occur secondarily. Often blood-filled spaces are near to areas of hepatocellular necrosis [39]. The correlation between AAS and peliosis was first noted in 1952 [40], but the pathogenesis of peliosis hepatis is still unclear. It was discussed a congenital [41] and an infectious mechanism. Paradinas *et al.* hypothesized that hyperplasia of the hepatocytes could partly be responsible for the formation of cystis through mechanical obstruction of hepatic veins and for the formation of nodules and tumors [42]. Other authors discussed about the pathogenesis of peliosis hepatis postulating that an acquired malformation of the sinusoidal system may be triggered by altered local intravascular pressure conditions, toxic substances, or active proliferation of endothelial cells. Proliferation of endothelial cells in turn can be triggered by angiogenetic factors. *Bartonella* is

thought to be relatively unique among bacteria in inducing such factors [43-45].

LIVER TUMORS

Sportsmen, especially bodybuilders, taking anabolic androgenic steroids over a long period should be considered a group at risk of developing hepatic sex hormone related tumors. In particular hepatocellular adenoma (HA) is a rare benign liver neoplasm that is strongly associated with oral contraceptive use and androgen steroid therapy [46]. So in recent times the use of anabolic androgenic steroids have been proved to be involved in the development of HA [26]. Although more than 750 cases of oral contraceptive induced HA have been reported, apparently androgen induced HA are relatively rare. However, the possibility that oral AAS such as stanozolol can induce liver cell proliferation must be taken into account [20]. The risk of androgen-associated liver tumors appears to correlate with the cumulative androgen dose and the potency of the steroid used [46].

Boada *et al.* examined the hepatic effects of high doses of the anabolic steroid stanozolol in rats. Steroid treatment decreased the levels of cytochrome P450 and cytochrome b5, indicating reduced ability to metabolize xenobiotics. The livers exhibited inflammatory and degenerative lesions in centrilobular hepatocytes and there was also an increase in the percentage of S-phase cells among those cells, indicating an increased risk for liver cancer [47].

CHOLESTASIS

Several deaths from cholestatic jaundice have been attributed to steroids, but they occurred in elderly, debilitated patients, and the evidence of causality is far from convincing. Based on animal studies, the mechanism for bile accumulation appears to involve a disruption of the microfilaments within the hepatocytes that reduces the ability of the cells to transport bile. Histologically, steroid-associated cholestasis is characterized by the occurrence of bile accumulates in the canaliculi but without evidence of inflammation or necrosis [39].

Ultrastructural changes AAS-induced including alterations in the canaliculi and degenerative changes in mitochondria and lysosomes have been also demonstrated [47,48].

LIVER TOXICITY AND OXIDATIVE STRESS

Despite the widespread physiological, biochemical, pathological and stereological [49] investigation of the effects of androgenic anabolic steroids on the liver, the cause of hepatotoxicity still remains speculative. An intrinsic direct hepatoxicity of AAS, correlated to individual susceptibility, particularly on genetic factors, was also hypothesized in men [50] due to the fact that androgen receptors (ARs), specifically activated by testosterone, are present in normal liver tissue [51]. In rat hepatocytes a direct toxic AAS-induced effect has been showed with inflammatory and degenerative lesions in centrilobular hepatocytes [47,52], with a dose-dependent increase in plasma levels of enzymatic markers of liver toxicity, as well as hepatocytic hyperplasia [10]. The determination of transaminases in the serum is generally considered to be of great value to detect toxic effects on the liver, and various authors have previously described the increase in levels of serum transaminases exerted by AAS, in particular stanozolol [53]. Despite previous observations, this increase was not found in animal model, which showed no change in the levels of these hepatic enzymes [47].

Inflammation is an inseparable component of both acute and chronic liver injury, in view of eliminating cell debris and invading microorganisms as well as promoting tissue healing. With its large population of Kupffer cells (tissue resident macrophages), dendritic cells, natural killer (NK) cells, and NK T cells, the liver acts as an “immune organ” and it has the unique milieu of close interaction between these immune cells and the non-immune cells of the liver. The resident inflammatory cells will become activated during any apoptotic or necrotic insult. Additionally, there may be an infiltration of circulating inflammatory cells. Among other functions, the cells of the innate immune system are the sources of fibrogenic mediators and inflammatory cytokines, especially tumor necrosis factor alpha (TNF-α) in diseased livers [54]. TNF-α plays a key role in a wide variety of physiological processes, including inflammation, proliferation and programmed cell death, as well as the activation of the anti-apoptotic and pro-inflammatory transcription factor NF-κB [55]. Cytokine production by resident cell populations in the liver contributes significantly not only to local cytokine appearance and organ homeostasis, but also to their emergence into the systemic circulation. In experimental study the efflux of TNF-α and IL-6 from the splanchinc bed after intravenous endotoxin administration was shown. Almost 40% of the TNF-α that appeared in the systemic circulation was derived from the splanchinc bed [56]. Not only Kupffer cells are a potential source of proinflammatory cytokines in the liver, but also biliary epithelial cells and venous endothelial cells can make significant quantities of cytokines, especially TNF-α, during liver regeneration [57]. TNF-α first gained notoriety for its ability to cause necrosis of solid tumors and its propensity to produce endothelial injury, hypotension, and shock. TNF-α also seems to be a prominent ligand for the activation of cellular apoptosis. TNF-α-induced apoptosis in hepatocytes is not completely explained. Although hepatocytes are particularly sensitive to TNF-α-mediated apoptosis, the cells first must be exposed to transcriptional inhibition. This requirement for trascriptional inhibition is likely due to the observation that TNF-α simultaneously invokes signaling pathways (Fig. **2**) [58,59]. Induction of the proinflammatory properties of TNF-α, induced in part through activation of the transcription factor NF-κB seems to inhibit apoptosis directly (Fig. **3**).

The transcription factor NF-κB is involved in the activation of immediate early response genes in response to injurious and inflammatory stimuli, namely by TNF-α [60]. NF-κB is a ubiquitous trascription factor that plays a crucial role in the cellular response to signal trasduction by TNF-α and IL-6 (Fig. **4**).

Hepatic stellate cells (HSC) are perisinusoidal cells residing in the space of Disse, which, during injury in response to inflammatory and other stimuli, like AAS, adopt a myofibroblast-like phenotype and represent the cornerstone

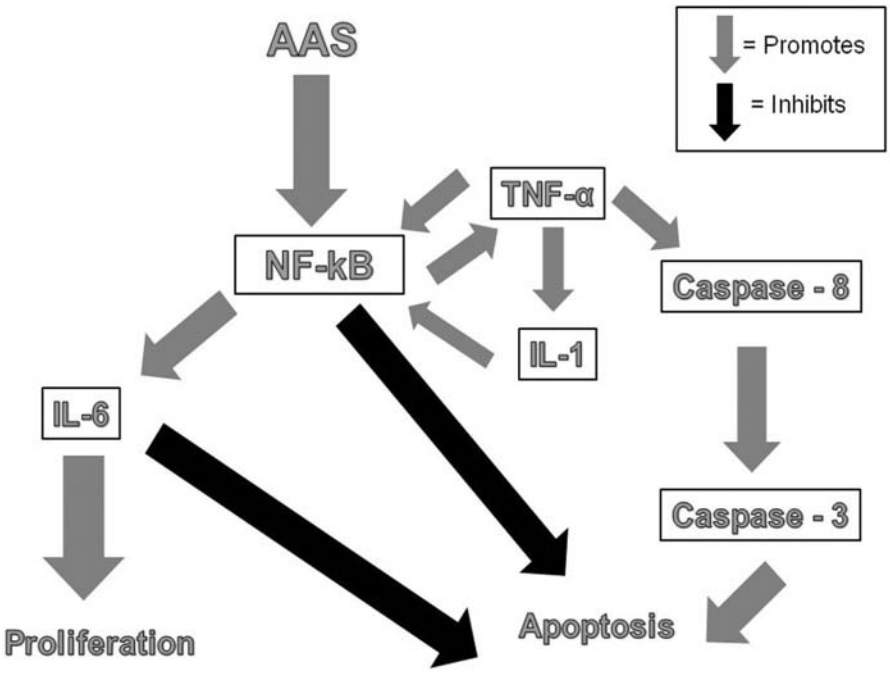

Fig. (2). Hepatocyte fate is determined by the balance between TNF-α-mediated NF-κB activation and apoptosis. TNF-α signaling simultaneously activates pathways that induce and antagonize caspase-dependent apoptosis. Drugs or agents that inhibit NF-κB activation promote apoptosis, whereas cytokines that induce NF-κB protect against TNF-α-mediated apoptosis.

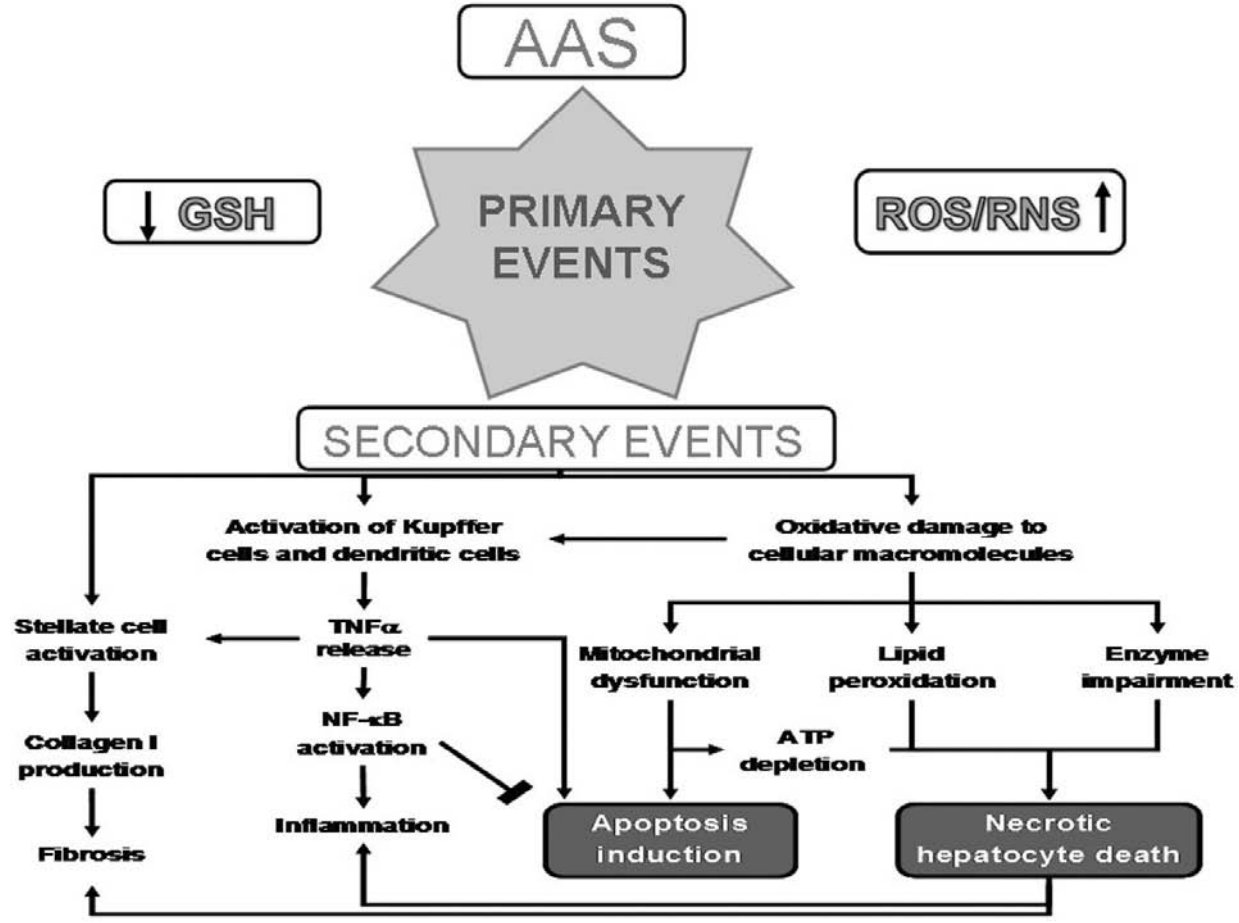

Fig. (3). Schematic illustration of putative mechanisms involved in AAS-mediated hepatotoxicity.

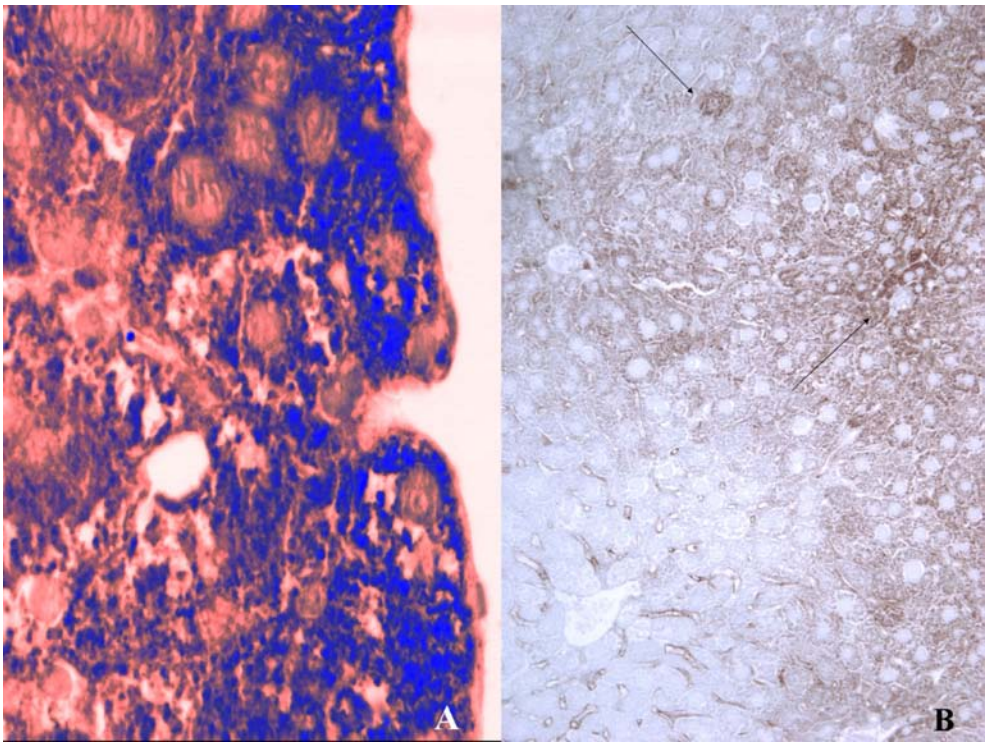

Fig. (4). Mouse liver treated with nandrolone decanoate (**A**) Confocal laser scanning microscope. Increase of NF-κB with intense positive reaction (blue). (**B**) Immuhistochemistry revealed TNF-α over-expression in centrilobular zone (arrows) with a reddish positive reaction.

of the fibrotic response in the liver [61]. AAS presents a dual and dose-dependent effect on a cell line of HSC, inducing collagen production at low doses and cell death by apoptosis at higher concentrations through an oxidative stress-dependent manner [52,62]. NF-κB is also activated by steroids in an oxidative stress independent fashion and plays a protective role in the steroids pro-apoptotic effect, indeed, in an experimental study on cells of rat liver was demonstrated that steroids induced DNA fragmentation, DNA repair and apoptosis in primary rat hepatocytes [63].

Polymorphonuclear leukocytes (neutrophils) are part of the innate immune response to infection and tissue trauma. Because of the high mobility of these leukocytes and the capability to either release or generate potent cytotoxic mediators, the main function of neutrophil recruitment to sites of inflammation is to rapidly eliminate invading microorganisms and/or remove dead or dying cells. These cells accumulate in the liver vasculature in response to the exposure to inflammatory mediators [64]. Though neutrophil-generated cytotoxic mediators are important for the inflammatory healing effect, their overproduction may result to detrimental effects to the affected organ. AAS-induced hepatotoxicity, in humans, was shown to be associated with an increase in the infiltration of lymphocytes, neutrophils and eosinophils in liver tissue, following repeated exposure [65]. Furthermore, the hepatotoxic recurrence has been reported in various studies about patients on re-challenge to other drugs, like MDMA, along with the infiltration of inflammatory cells, reinforces the postulated immunologically mediated mechanisms [66]. This data clearly indicates immunomodulatory responses after exposition to steroids, with some consumers evidencing a clear neutrophil activation effect.

Oxidative stress could represent another mechanism of liver toxicity [67] resulting *e.g*. in the impairment of the canalicular bile salt export [68]. A previous study conducted by Welder *et al.* underlined an oxidative stress-induced damage showing the GSH depletion in cultured hepatocytes of rats treated with 17-α-alkylated AAS [52].

An experimental study demonstrated that the prolonged AAS administration provokes dysfunction of mitochondrial respiratory chain complexes and mono-oxygenase systems [69] leading to an increased Reactive Oxygen Species (ROS) generation. Afterwards, when ROS production exceeds the high levels of enzymatic and non-enzymatic antioxidant defences and liver repair capacity, the oxidative stress-induced liver damage appears [67].

Vieira *et al*. recently confirmed this hypothesis by demonstrating that subchronic administration of nandrolone decanoate leads to an increase in collagen deposition in liver parenchyma, portal space, and centrilobular vein, probably correlated to the increase in the number of Kupffer cells. When activated, Kupffer cells produce many harmful by products such as TGF-β1, reactive oxygen species, NF-κB, TNF-α, and IL-1β, which are compounds that act directly by stimulating the liver fibrosis process [13]. In another recent study the results show that prolonged stanozolol treatment can cause an oxidative stress situation in rat liver as indicated by enhanced lipid peroxidation extent. Interestingly, ROS overproduction and lipid peroxidation have been implicated in the pathogenesis of many types of

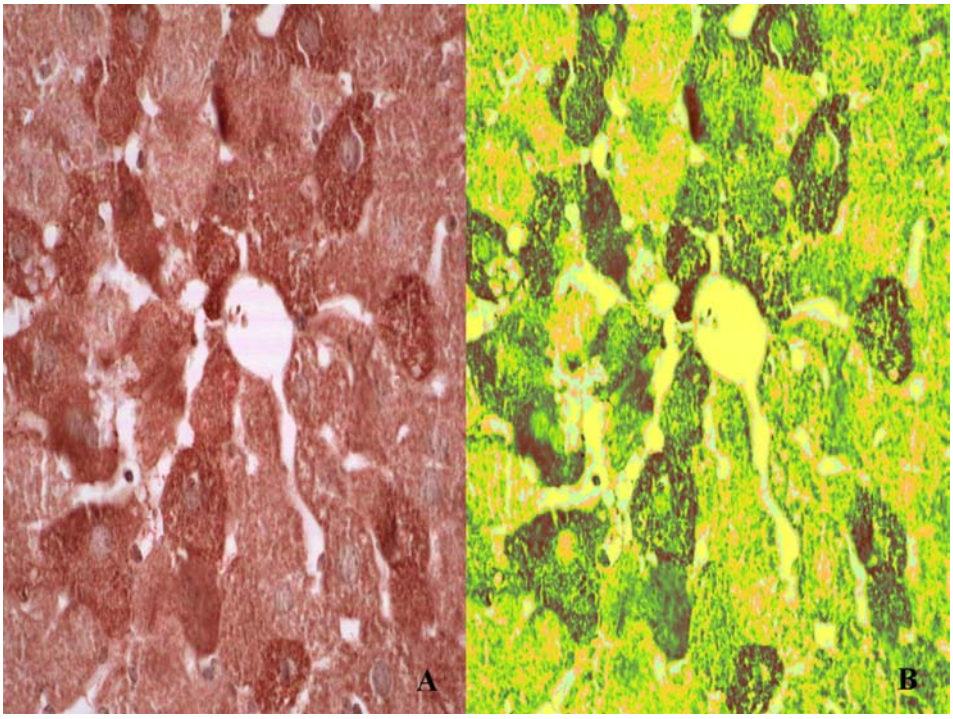

Fig. (5). (**A**) Numerous and wide positive reactions of HSP70 (in reddish) were observed in a rat liver treated with stanozolol. (**B**) The same field seen with confocal laser scanning microscope shows a more selective expression in hepatocytes cytoplasm.

liver injury and especially in the hepatic damage induced by several toxic drugs showing a marked accumulation of HSP72 conferring to liver cells protection was detected [65] (Fig. **5**).

CONCLUSIONS

AAS use is associated with various adverse effects that are generally dose related; therefore, illicit use of the high doses taken by sportsmen carries substantial risks for health [70-72]. A major side effect of AAS therapy is hepatotoxicity, including elevated levels of liver enzymes, cholestatic jaundice, peliosis hepatis, and various neoplastic lesions. When hepatic function is monitored in healthy athletes abusing AAS, liver serum parameters show no change or slight elevation, which revert to normal levels after discontinuing the drug. However, conventional biochemical liver tests do not always reflect liver abnormalities particularly at the initial stages. Stanozolol, and other orally active AAS, has been shown to cause inflammatory or degenerative lesions in centrilobular hepatocytes, ultrastructural alterations in the canaliculi and degenerative changes in mitochondria and lysosomes [47,48]. Furthermore a prolonged AAS administration provokes an increase in the activities of liver lysosomal hydrolases and a decrease in some components of the microsomal drug-metabolizing system and in the activity of the mitochondrial respiratory chain complexes without modifying classical serum indicators of hepatic function. Liver is a key organ actively involved in numerous metabolic and detoxifying functions. As a consequence, it is continuously exposed to high levels of endogenous and exogenous oxidants that are by-products of many biochemical pathways and, in fact, it has been demonstrated that intracellular oxidant production is more active in liver than in tissues, like the increase of inflammatory cytokines, apoptosis and the inhibitors of apoptosis NF-κB and Heat Shock Proteins [65].

REFERENCES

[1] Shahidi, N.T. A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. *Clin. Ther*., **2001**, *23*(9), 1355-1390.

[2] van Amsterdam, J.; Opperhuizen, A.; Hartgens, F. Adverse health effects of anabolic-androgenic steroids. *Regul. Toxicol. Pharmacol.*, **2010**, *57*, 117-123.

[3] Hall, R.C.; Hall, R.C. Abuse of supraphysiologic doses of anabolic steroids. *South Med. J.*, **2005**, *98*(5), 550-555.

[4] Fontana, K.; Aldrovani, M.; de Paoli, F.; Oliveira, H.C.F.; de Campos Vidal, B.; da Cruz-Hofling, M.A. Hepatocyte nuclear phenotype: the cross-talk between anabolic androgenic steroids and exercise in transgenic mice. *Histol. Histopathol.*, **2008**, *23*, 1367-1377.

[5] Vermeulen, A. Androgen replacement therapy in the aging male: a critical evaluation. *J. Clin. Endocrinol. Metab*., **2001**, *86*(6), 2380-2390.

[6] Resnick, S.M.; Maki, P.M. Effects of hormone replacement therapy on cognitive and brain aging. *Ann. N. Y. Acad. Sci.*, **2001**, *949*, 203-214.

[7] Hengge, U.R.; Baumann, M.; Maleba, R.; Brockmeyer, N.H.; Goos, M. Oxymetholone promotes weight gain in patients with advanced human immunodeficiency virus (HIV-1) infection. *Br. J. Nutr*., **1996**, *75*(1), 129-138.

[8] Velazquez, I.; Alter, B.P. Androgens and liver tumors: Fanconi's anemia and non-Fanconi's conditions. *Am. J. Hematol.*, **2004**, *77*(3), 257-267.

[9] Deicher, R.; Horl, W.H. Hormonal adjuvants for the treatment of renal anaemia. *Eur. J. Clin. Invest.*, **2005**, *35*(Suppl. 3), 75-84.

[10] Sileri, P.; Palmieri, G.; Gentileschi, P.; Perfetti, A.; Sica, G.; Venza, M.; Benavoli, D.; Gaspari, A.L. Anabolic steroid nandrolone augments hepatic regenerative response in rats. *Transplant. Proc*., **2005**, *37*(10), 4563-4566.

[11] Evans, N.A. Current concepts in anabolic-androgenic steroids. *Am. J. Sports Med*., **2004**, *32*(2), 534-542.
[12] Gerez, J.R.; Frei, F.; Camargo, I.C. Histological assessment of ovaries and uterus of rats subjected to nandrolone decanoate treatment. *Contraception*, **2005**, *72*(1), 77-80.
[13] Vieira, R.P.; França, R.F.; Damaceno-Rodrigues, N.R.; Dolhnikoff, M.; Caldini, E.G.; Carvalho, C.R.; Ribeiro, W. Dose-dependent hepatic response to subchronic administration of nandrolone decanoate. *Med. Sci. Sports Exerc.*, **2008**, *40*(5), 842-847.
[14] Sánchez-Osorio, M.; Duarte-Rojo, A.; Martínez-Benítez, B.; Torre, A.; Uribe, M. Anabolic-androgenic steroids and liver injury. *Liver Int.*, **2008**, *28*(2), 278-282.
[15] Kafrouni, M.I.; Anders, R.A.; Verma, S. Hepatotoxicity associated with dietary supplements containing anabolic steroids. *Clin. Gastroenterol. Hepatol*., **2007**, *5*(7), 809-812.
[16] Modlinski, R.; Fields, K.B. The effect of anabolic steroids on the gastrointestinal system, kidneys, and adrenal glands. *Curr. Sports Med. Rep*., **2006**, *5*(2), 104-109.
[17] Thiblin, I.; Petersson, A. Pharmacoepidemiology of anabolic androgenic steroids: a review. *Fundam. Clin. Pharmacol*., **2005**, *19*(1), 27-44.
[18] Martin, N.M.; Abu Dayyeh, B.K.; Chung, R.T. Anabolic steroid abuse causing recurrent hepatic adenomas and hemorrhage. *World J. Gastroenterol.*, **2008**, *14*(28), 4573-4575.
[19] Stimac, D.; Milic, S.; Dintinjana, R.D.; Kovac, D.; Ristic, S. Androgenic/anabolic steroid-induced toxic hepatitis. *J. Clin. Gastroenterol.*, **2002**, *35*(4), 350-352.
[20] Socas, L.; Zumbado, M.; Perez-Luzardo, O.; Ramos, A.; Perez, C.; Hernandez, J.R.; Boada, L.D. Hepatocellular adenomas associated with anabolic androgenic steroid abuse in bodybuilders: a report of two cases and a review of the literature. *Br. J. Sports Med.*, **2005**, *39*, e27.
[21] Samaha, A.A.; Nasser-Eddine, W.; Shatila, E.; Haddad, J.J.; Wazne, J.; Eid, A.H. Multi-organ damage induced by anabolic steroid supplements: a case report and literature review. *J. Med. Case Reports*, **2008**, *2*, 340.
[22] Bhasin, S.; Woodhouse, L.; Casaburi, R.; Singh, A.B.; Bhasin, D.; Berman, N.; Chen, X.; Yarasheski, K.E.; Magliano, L.; Dzekov, C.; Dzekov, J.; Bross, R.; Phillips, J.; Sinha-Hikim, I.; Shen, R.; Storer, T.W. Testosterone dose-response relationships in healthy young men. *Am. J. Physiol. Endocrinol. Metab*., **2001**, *281*(6), E1172-E1181.
[23] Walter, E.; Möckel, J. Images in clinical medicine. Peliosis hepatis. *N. Engl. J. Med*., **1997**, *337*(22), 1603.
[24] Choi, S.K.; Jin, J.S.; Cho, S.G.; Choi, S.J.; Kim, C.S.; Choe, Y.M.; Lee, K.Y. Spontaneous liver rupture in a patient with peliosis hepatis: a case report. *World J. Gastroenterol*., **2009**, *15*(43), 5493-5497.
[25] Tsirigotis, P.; Sella, T.; Shapira, M.Y.; Bitan, M.; Bloom, A.; Kiselgoff, D.; Levin, M.; Libster, D.; Abdul Hai, A.; Gesundheit, B.; Or, R.; Slavin, S.; Resnick, I. Peliosis hepatis following treatment with androgen-steroids in patients with bone marrow failure syndromes. *Haematologica*, **2007**, *92*(11), e106-e110.
[26] Nakao, A.; Sakagami, K.; Nakata, Y.; Komazawa, K.; Amimoto, T.; Nakashima, K.; Isozaki, H.; Takakura, N.; Tanaka, N. Multiple hepatic adenomas caused by long-term administration of androgenic steroids for aplastic anemia in association with familial adenomatous polyposis. *J. Gastroenterol.*, **2000**, *35*(7), 557-562.
[27] Bagia, S.; Hewitt, P.M.; Morris, D.L. Anabolic steroid-induced hepatic adenomas with spontaneous haemorrhage in a bodybuilder. *Aust. N.Z. J. Surg*., **2000**, *70*(9), 686-687.
[28] Patil, J.J.; O'Donohoe, B.; Loyden, C.F.; Shanahan, D. Near-fatal spontaneous hepatic rupture associated with anabolic androgenic steroid use: a case report. *Br. J. Sports Med*., **2007**, *41*(7), 462-463.
[29] Masumori, N.; Ikeda, H.; Endo, T. Acute hepatitis induced by replacement oral testosterone product in a female-to-male patient with gender identity disorder. *Int. J. Urol*., **2009**, *16*(5), 530-531.
[30] Maravelias, C.; Dona, A.; Stefanidou, M.; Spiliopoulou, C. Adverse effects of anabolic steroids in athletes. A constant threat. *Toxicol. Lett*., **2005**, *158*(3), 167-175.
[31] Gorayski, P.; Thompson, C.H.; Subhash, H.S.; Thomas, A.C. Hepatocellular carcinoma associated with recreational anabolic steroid use. *Br. J. Sports Med*., **2008**, *42*(1), 74-75 discussion 75.
[32] Thompson, P.D.; Cullinane, E.M.; Sady, S.P.; Chenevert, C.; Saritelli, A.L.; Sady, M.A.; Herbert, P.N. Contrasting effects of testosterone and stanozolol on serum lipoprotein levels. *JAMA*, **1989**, *261*(8), 1165-1168.
[33] Bagatell, C.J.; Bremner, W.J. Androgens in men – uses and abuses. *N. Engl. J. Med*., **1996**, *334*(11), 707-714.
[34] Schwingel, P.A.; Cotrim, H.P.; Salles, B.R.; Almeida, C.E.; Dos Santos, C.R. Jr.; Nachef, B.; Andrade, A.R.; Zoppi, C.C. Anabolic-androgenic steroids: a possible new risk factor of toxicant-associated fatty liver disease. *Liver Int.* **2010**, doi: 10.1111/j.1478-3231.2010.02346.x. [Epub ahead of print].
[35] Rone, M.B.; Fan, J.; Papadopoulos, V. Cholesterol transport in steroid biosynthesis: role of protein-protein interactions and implications in disease states. *Biochim. Biophys. Acta*, **2009**, *1791*(7), 646-658.
[36] Arnold, G.L.; Kaplan, M.M. Peliosis hepatis due to oxymetholone – a clinically benign disorder. *Am. J. Gastroenterol.*, **1979**, *71*(2), 213-216.
[37] Bagheri, S.; Boyer, J. Peliosis hepatis associated with anabolic-androgenic steroid therapy. A severe form of hepatic injury. *Ann. Intern. Med.*, **1974**, *81*(5), 610-618.
[38] Nadell, J.; Kosek, J. Peliosis hepatis. Twelve cases associated with oral androgen therapy. *Arch. Pathol. Lab. Med*., **1977**, *101*(8), 405-410.
[39] Hausmann, R. Long-Term Effects of Anabolic-androgenic-steroid abuse. Morphological findings associated with fatal outcome. In: *Forensic Pathology Reviews*; Tsokos, M. Ed.; Humana Press Inc.: Totowa, NJ, **2005**; Vol *2*, pp. 273-289.
[40] Burger, R.A.; Marcuse, P.M. Peliosis hepatis: report of a case. *Am. J. Clin. Pathol.*, **1952**, *22*(6), 569-573.
[41] Zak, F.G. Peliosis hepatis. *Am. J. Pathol*., **1950**, *26*(1), 1-15.
[42] Paradinas, F.J.; Bull, T.B.; Westaby, D.; Murray-Lyon, I.M. Hyperplasia and prolapse of hepatocytes into hepatic veins during longterm methyltestosterone therapy: possible relationships of these changes to the developement of peliosis hepatis and liver tumours. *Histopathology*, **1977**, *1*(4), 225-246.
[43] Schulte, B.; Linke, D.; Klumpp, S.; Schaller, M.; Riess, T.; Autenrieth, I.B.; Kempf, V.A. Bartonella quintana variably expressed outer membrane proteins mediate vascular endothelial growth factor secretion but not host cell adherence. *Infect. Immun*., **2006**, *74*(9), 5003-5013.
[44] Edwards, R.; Colombo, T.; Greaves, P. "Have you seen this?" peliosis hepatis. *Toxicol. Pathol*., **2002**, *30*(4), 521-523.
[45] Wong, A.K.; Alfert, M.; Castrillon, D.H.; Shen, Q.; Holash, J.; Yancopoulos, G.D.; Chin, L. Excessive tumor-elaborated VEGF and its neutralization define a lethal paraneoplastic syndrome. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*(13),7481-7486.
[46] Gelfand, M.M.; Wiita, B. Androgen and estrogen-androgen hormone replacement therapy: a review of the safety literature, 1941 to 1996. *Clin. Ther*., **1997**, *19*(3), 383-404.
[47] Boada, L.D.; Zumbado, M.; Torres, S.; López, A.; Díaz-Chico, B.N.; Cabrera, J.J.; Luzardo, O.P. Evaluation of acute and chronic hepatotoxic effects exerted by anabolic-androgenic steroid stanozolol in adult male rats. *Arch. Toxicol*., **1999**, *73*(8-9), 465-472.
[48] Gragera, R.; Saborido, A.; Molano, F.; Jiménez, L.; Muñiz, E.; Megías, A. Ultrastructural changes induced by anabolic steroids in liver of trained rats. *Histol. Histopathol*., **1993**, *8*(3), 449-455.
[49] Karbalay-Doust, S.; Noorafshan, A. Stereological study of the effects of nandrolone decanoate on the mouse liver. *Micron*., **2009**, *40*(4), 471-475.
[50] Ishak, K.G.; Zimmerman, H.J. Hepatotoxic effects of the anabolic/androgen steroids. *Semin. Liver Dis*., **1987**, *7*(3), 230-236.
[51] Giannitrapani, L.; Soresi, M.; La Spada, E.; Cervello, M.; D'Alessandro, N.; Montalto, G. Sex hormones and risk of liver tumor. *Ann. N. Y. Acad. Sci.*, **2006**, *1089*, 228-236.
[52] Welder, A.A.; Robertson, J.W.; Melchert, R.B. Toxic effects of anabolic-androgenic steroids in primary rat hepatic cell cultures. *J. Pharmacol. Toxicol. Methods*, **1995**, *33*(4), 187-195.
[53] Camerino, B.; Sciaky, R. Structure and effects of anabolic steroids. *Pharmacol. Ther. B.*, **1975**, *1*(2), 233-275.
[54] Malhi, H.; Gores, G.J. Cellular and molecular mechanisms of liver injury. *Gastroenterology*, **2008**, *134*(6), 1641-1654.
[55] Schwabe, R.F.; Brenner, D.A. Mechanisms of liver injury. I. TNF-alpha-induced liver injury: role of IKK, JNK, and ROS pathways. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **2006**, *290*(4), G583-G589.

[56] Fong, Y.M.; Marano, M.A.; Moldawer, L.L.; Wei, H.; Calvano, S.E.; Kenney, J.S.; Allison, A.C.; Cerami, A.; Shires, G.T.; Lowry, S.F. The acute splanchnic and peripheral tissue metabolic response to endotoxin in humans. *J. Clin. Invest.*, **1990**, *85*(6), 1896-1904.

[57] Bradham, C.A.; Plümpe, J.; Manns, M.P.; Brenner, D.A.; Trautwein, C. Mechanisms of hepatic toxicity. I. TNF-induced liver injury. *Am. J. Physiol.*, **1998**, *275*(3 Pt 1), G387-G392.

[58] Beg, A.A.; Baltimore, D. An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. *Science*, **1996**, *274*(5288), 782-784.

[59] Van Antwerp, D.J.; Verma, I.M. Signal-induced degradation of I(kappa)B(alpha): association with NF-kappaB and the PEST sequence in I(kappa)B(alpha) are not required. *Mol. Cell. Biol.*, **1996**, *16*(11), 6037-6045.

[60] Chen, F.; Shi, X. Signaling from toxic metals to NF-kappaB and beyond: not just a matter of reactive oxygen species. *Environ. Health Perspect.*, **2002**, *110*(Suppl 5), 807-811.

[61] Iredale, J. Defining therapeutic targets for liver fibrosis: exploiting the biology of inflammation and repair. *Pharmacol. Res.*, **2008**, *58*(2), 129-136.

[62] Montiel-Duarte, C.; Ansorena, E.; López-Zabalza, M.J.; Cenarruzabeitia, E.; Iraburu, M.J. Role of reactive oxygen species, glutathione and NF-kappaB in apoptosis induced by 3,4-methylenedioxymethamphetamine ("Ecstasy") on hepatic stellate cells. *Biochem. Pharmacol.*, **2004**, *67*(6), 1025-1033.

[63] Mattioli, F.; Garbero, C.; Gosmar, M.; Manfredi, V.; Carrozzino, R.; Martelli, A.; Brambilla, G. DNA fragmentation, DNA repair and apoptosis induced in primary rat hepatocytes by dienogest, dydrogesterone and 1,4,6-androstatriene-17beta-ol-3-one acetate. *Mutat. Res.*, **2004**, *564*(1), 21-29.

[64] Jaeschke, H. Mechanisms of Liver Injury. II. Mechanisms of neutrophil-induced liver cell injury during hepatic ischemia-reperfusion and other acute inflammatory conditions. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **2006**, *290* (6), G1083-G1088.

[65] Pey, A.; Saborido, A.; Blázquez, I.; Delgado, J.; Megías, A. Effects of prolonged stanozolol treatment on antioxidant enzyme activities, oxidative stress markers, and heat shock protein HSP72 levels in rat liver. *J. Steroid. Biochem. Mol. Biol*., **2003**, *87*(4-5), 269-277.

[66] Yeh, M.M.; Brunt, E.M. Pathology of non-alcoholic fatty liver disease. *Am. J. Clin. Pathol.*, **2007**, *128*(5), 837-847.

[67] Carvalho, M.; Pontes, H.; Remião, F.; Bastos, M.L.; Carvalho, F. Mechanisms underlying the hepatotoxic effects of ecstasy. *Curr. Pharm. Biotechnol.*, **2010**, *11*(5), 476-495.

[68] Pérez, L.M.; Milkiewicz, P.; Elias, E.; Coleman, R.; Sánchez Pozzi, E.J.; Roma, M.G. Oxidative stress induces internalization of the bile salt export pump, Bsep, and bile salt secretory failure in isolated rat hepatocyte couplets: a role for protein kinase C and prevention by protein kinase A. *Toxicol. Sci*., **2006**, *91*(1), 150-158.

[69] Molano, F.; Saborido, A.; Delgado, J.; Morán, M.; Megías, A. Rat liver lysosomal and mitochondrial activities are modified by anabolic-androgenic steroids. *Med. Sci. Sports Exerc*., **1999**, *31*(2), 243-250.

[70] Fineschi, V.; Baroldi, G.; Monciotti, F.; Paglicci Reattelli, L.; Turillazzi, E. Anabolic steroid abuse and cardiac sudden death: a pathologic study. *Arch. Pathol. Lab. Med*., **2001**, *125*(2), 253-255.

[71] Fineschi, V.; Riezzo, I.; Centini, F.; Silingardi, E.; Licata, M.; Beduschi, G.; Karch, S.B. Sudden cardiac death during anabolic steroid abuse: morphologic and toxicologic findings in two fatal cases of bodybuilders. *Int. J. Legal Med.*, **2007**, *121*(1), 48-53.

[72] Riezzo, I.; Di Paolo, M.; Neri, M.; Bello, S.; Cantatore, S.; D'Errico, S.; Dinucci, D.; Parente, R.; Pomara, C.; Rabozzi, R.; Turillazzi, E.; Fineschi, V. Anabolic Steroid - and Exercise-Induced Cardio-Depressant Cytokines and Myocardial 1 Receptor Expression in CD1 Mice. *Curr. Pharm. Biotechnol*., **2011**, *12*(2), 275-84.

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