JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2023, 74, 2, 109-130
www.jpp.krakow.pl | DOI: 10.26402/jpp.2023.2.01

Review article

M. GONCIARZ[1], I. LOMBARD[1], L. KONECKI[1], P. GIETKA[1], R. PAJDO[2], T. BRZOZOWSKI[2]

POTENTIAL THERAPEUTIC ROLE OF MELATONIN IN HEPATOBILIARY DISEASES: CURRENT EVIDENCE AND CLINICAL OBSERVATIONS

[1]Department of Gastroenterology and Internal Medicine-National Research Institute, Military Institute of Medicine, Warsaw, Poland;
[2]Department of Physiology, Faculty of Medicine, Jagiellonian University Medical College, Cracow, Poland

Melatonin (MEL) is produced and secreted by the pineal gland as well as the small intestine, liver, retina, lymphocytes, and melanocytes in the skin in both experimental animals as well as in humans. While pineal and retinas MEL is closely related to the light/dark cycle, the production of MEL by other so called extrapineal tissues is independent of such circadian rhythm. Among the primary mechanisms of action of MEL in humans, the most important are interaction of MEL with specific receptors (M1, M2, M3) and the MEL 'scavenging' activity against the formation of free oxygen metabolites as a result of MEL's ability to transfer free electrons and stimulation of the expression of redox reaction enzymes. In addition, MEL binds to intracellular proteins such as calmodulin, thereby affecting the course of cell cycle, and it has been shown to activate of nuclear receptors belonging to the retinoid orphan receptors/retinoid Z receptors (ROR/RZR) subfamily. MEL exerts regulatory effects on the master clock regulating diurnal rhythms. This updated review presents current view on the synthesis and metabolism of MEL and the growing body of experimental evidence transferable to the practical medicine supporting a pleiotropic molecule beneficial effects on the health including protection against various organ abnormalities, including internal organs such as the liver. Although the beneficial effects of MEL in various types of liver damage have been well documented in experimental studies, there are relatively few studies on liver dysfunction in humans. Considering the worldwide obesity pandemic often associated with the occurrence of steatohepatitis and cirrhosis, the beneficial effects of MEL in liver pathology should be proven in randomized trials involving patients presenting with hepatic disorders.

Key words: *melatonin, liver diseases, biliary tract disorders, liver steatosis, steatohepatitis, liver cirrhosis, liver transplantation, free oxygen metabolites, fatty acids, proinflammatory cytokines*

INTRODUCTION

The modern history of melatonin (MEL) begins in the twentieth century with a study by McCord and Allen (1) from 1917 which showed that the pineal gland homogenate contained a substance that lightened the skin of tadpoles. In 1958 Lerner *et al.* (2), extracted MEL, N-acetyl-5-methoxytryptamine, an indoleamine from the bovine pineal gland. In fact, Lerner evaluated 50 g of lyophilized and powdered pineal gland from 200,000 of cattle. The earliest known report of the pineal gland was included in Galen's work, *De usu partium*, which described the location of the pineal gland immediately after what he called the 'central chamber' (now the third chamber). According to Galen, like all other glands (Greek: Ἀδήν) of the body, the pineal gland was supposed to be a support for the surrounding blood vessels. He gave the name to the pineal gland because of its shape resembling a cone. Galen also believed that the physiological role of the pineal gland is to fill the bifurcation of the great cerebral vein ('Galen's vein'), from which all choroidal plexuses of the anterior ventricles emerge (3). For a long time, MEL was considered as hormone occurring only in vertebrates, however, it turned out that MEL is a compound common in nature. It has been isolated in many different evolutionarily distant organisms such as bacteria (4), plants like Zea mays (5), invertebrates like *Dendrocoelum lacteum* or *Drosophila melanogaster* (6, 7). In mammals, there are three types of the pineal gland depending on its shape and location in relation to chamber III. Type A, with a conical shape, located in the epithalamus of the brain and connected to the rest of the epithalamus through the peduncle, occurs in humans. The intermediate type (AB) with an elongated shape occurs in, for example, a cat, and the medio-intermediate peripheral type (ABC), a strongly elongated and extending cerebellum, occurs in a guinea pig (8, 9). The mass of this gland depends on the species of mammal and is: 0.2 mg in mouse, 9 mg in dog (10), 100–200 mg in human (11, 12) and 1 g in a predatory marine mammal of the seal family called Weddel seal (13). In 1983 the pharmacological characteristics of the melatonin receptor in mammals were presented (14), and then the first human melatonin receptor was cloned (15). Noticeable progress in research on the importance of melatonin, expressed in the number of publications, has been observed from year to year, and the last quarter of a century was especially decisive. In humans MEL is produced and secreted by pineal gland as well

as small intestine, liver, retinas, lymphocytes and melanocytes in the skin, however only retinal MEL exhibits a circadian rhythm like that in the pineal gland. This kind of a distribution and prevalence of MEL suggest that it was a molecule that appeared at an early stage of the life and that is the reason that has an identical structure in all organisms in which it has been found. In contrast, the functions that MEL performs, is different in distinct species: regulation of the biological clock, protection against free radicals, regulation of reproduction and sexual maturation in mammals, sleep regulation, immunomodulation, and regulation of body weight and energy metabolism, and many others. The path the MEL has taken from its biochemical role in the metabolism of archaic bacteria, to an endocrine function, including the key regulatory place of diurnal behavior in humans is a fascinating story about the history of life on Earth.

MELATONIN - BIOSYNTHESIS, METABOLISM, AND PHARMACOKINETICS

Endogenous small molecule MEL is synthesized in pinealocytes and other tissues as the end product of the metabolic pathway in which tryptophan, after being transported into the cell, is further transformed by enzymes such as tryptophan hydroxylase (TPH), aromatic amino acid decarboxylase (AAAD), serotonin N-acetyltransferase (SNAT, aralkylamine N-acetyltransferase-AANAT) and N-acetylserotonin O-methyltransferase (ASMT) (16). The biosynthetic pathways of melatonin in animals and in plants are presented in *Fig. 1*. The MEL biosynthetic pathway in plants appears to be like that defined in animals, especially since its first two enzymes have been identified also in plant tissues. However, recent studies conducted on rice leaves, indicate that the first enzymatic step in the synthesis of MEL in plants is the decarboxylation of tryptophan (involving tryptophan decarboxylase (TDC) rather than its hydroxylation as in the vertebrates, and the product of this reaction is tryptamine rather than 5-hydroxytryptophan (17, 18). It is assumed that SNAT activity, which is under of the circadian rhythms control, plays a key role in regulating the rate of MEL synthesis (19, 20). It has been shown that in scotophase, the expression of the gene encoding SNAT in pinealocytes is significantly increased, which leads to raised concentrations of MEL in blood serum (21). This is important because no similar relationship has been found regarding the activity of other enzymes in the MEL biosynthetic pathways (22). At night and during darkness, the activity of post-ganglionic sympathetic fibers increases, which leads to the release of norepinephrine (NA) into the pineal gland, where it binds to pinealocyte β-AR receptors increasing intracellular cAMP, which

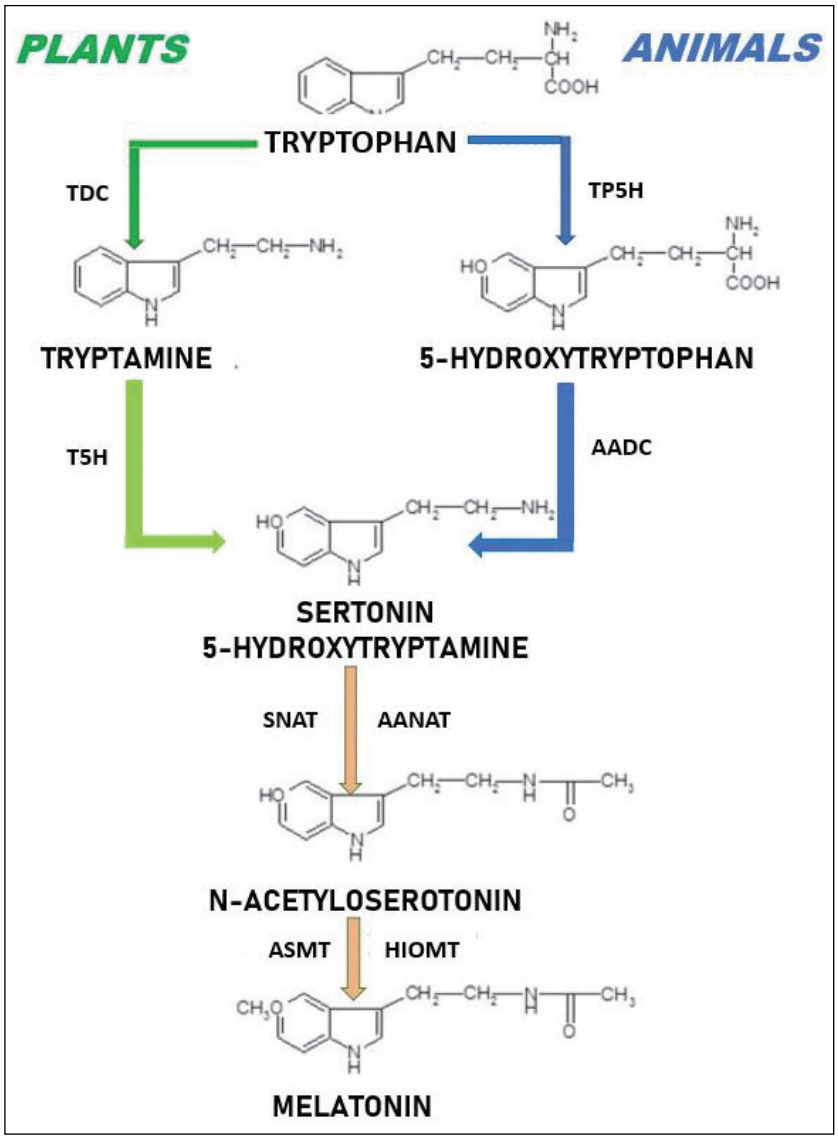

Fig. 1. Melatonin (MEL) biosynthesis in animals and plants.
AADC, aromatic-L-amino-acid decarboxylase; AANAT, arylalkylamine N-acetyltransferase; ASMT, N-acetylserotonin methyltransferase; HIOMT, hydroxyindole-O-methyltransferase; SNAT, serotonin-N-acetyltransferase; T5H, tryptamine 5-hydroxylase; TDC, tryptophan decarboxylase; TP5H, tryptophan 5-hydroxylase.

is responsible for the phosphorylation of CREB protein, a transcription factor initiating SNAT genes transcription (23, 24). Thus, after darkening, a >100-fold increase in the level of SNAT mRNA is observed, followed by SNAT protein expression and enzymatic activity (20). On the other hand, increased SNAT expression is also associated with post-translational modifications of the protein because of activating phosphorylation (25, 26). Some researchers question the major regulatory role of SNAT. Liu and Borjigin (27) showed that during the night hours, the pineal glands of the tested Sprague-Dawley and LEC rats contained SNAT in molar excess compared to MEL, and although SNAT expression increased steadily in the second half of the night, MEL concentration peaked much earlier. In vertebrate animals and humans, there is one SNAT homologue while other vertebrates show number more. For example, in *Solea senegalensis* fish, SNAT2 has been shown to be present in the pineal gland and homologs 1a and b in other tissues such as the retina (28). Related results were reported by Pomianowski *et al.* (29), who demonstrated the presence of two transcripts of genes encoding AANAT and two encoding ASMT in the eyeball at noon and midnight in sticklebacks. Day-night changes in MEL concentration did not correlate with changes in either gene expression or AANAT activity. At midday, high NAS synthesis and low concentrations were recorded in the eyeball of fish, and this suggests that NAS performs additional tasks beyond its function as a precursor of MEL biosynthesis. So, what mechanisms have resulted in the isolation of homologs in some species? Studies using gene sequencing have shown a common origin of SNAT homologs in vertebrate animals, and that the demonstrated differences are the result of mutations during evolution. It is thought that homologs such as 1a and b arose from genome duplication. Falcona *et al.* (30) showed that AANAT homologs (VT-AANAT, NV-AANAT) were already present in the evolutionary ancestors of *Agnatha* and *Gnathostomata*. Thus, different evolutionary pathways converged and hence the differences in SNAT (AANAT) expression and MEL concentrations. For example, some homologs may respond to photoperiodic changes, while others may be upregulated by oxidative stress and/or take part in detoxications of amines. This last function is extremely important because it prevents from binding arylalkilooamin to retinoaldehyd in photodetectors resulting in their inactivation. Additionally, cycle-related genes emerged during vertebrate evolution because of duplication of ancestral genes that were not related to vision (31). Moreover, other homologs of SNAT are involved in the detoxification processes. Samanta *et al.* (32) evaluated the effects of exogenous MEL on the regulation of endogenous plant growth regulators and their combined effects on metal-binding ligands in two indica rice cultivars: Khitish (arsenic-sensitive) and Muktashri (arsenic-tolerant) under arsenic stress conditions. MEL reduced the adverse effects of arsenic by stimulating endogenous biosynthesis of MEL and gibberellic acid in a mechanism to regulate the expression of key biosynthesis genes such as GA3ox, TDC, SNAT and ASMT. They also showed an increase in endogenous abscisic acid, which was in turn regulated by MEL in the mechanism of induction of the expression of the key anabolic gene NCED3 with simultaneous suppression of ABA8ox1 (32). The researchers also showed that MEL increased the accumulation of polyamines such as spermidine and spermine, thereby modulating arsenic-induced toxic stress conditions. Thus, MEL enhances tolerance to arsenic by inhibiting bioaccumulation of this element, by modulating the expression of selected arsenic transporters and controlling the homeostasis of endogenous phytohormones.

The metabolism of MEL, which takes place in the liver and partly in the kidneys, is extraordinarily complex and not fully understood. It has been shown that MEL can undergo enzymatic (33) and pseudo-enzymatic degradation, in which mitochondrial cytochrome C functions as an enzyme (34, 35) or can be eliminated by interaction with reactive oxygen species (ROS) or and others like reactive nitrogen species (RNS) or reactive chlorine species (RCS) (36, 37). MEL degradation products during detoxification of reactive species include such metabolites as cyclic 3-hydroxymelatonin (38) and N1-acetyl-N2-formyl-5-methoxycinnamate (AFMK) (33) characterized by their ability to further scavenge free radicals, which differs MEL from other 'scavengers'. After conjugation with sulfuric or glucuronic acid these metabolites are excreted in the urine. In the blood, MEL binds to albumin and in the liver, it is metabolized to 6-hydroxymelatonin by cytochrome P450 isoforms (mainly CYP1A2) and conjugated to 6-sulfatoxymelatonin, which is then excreted in the urine, and its concentration reflects the plasma concentration and can be used to assess pineal function. In the CNS, MEL is degraded to N-acetyl-N2-formyl-5-methoxycinnamate (AFMK), which is deformed to N-acetyl-5-methoxycinnamate (AMK) (34, 39).

MELATONIN - MECHANISMS OF ACTION AND PHYSIOLOGICAL EFFECTS

Most processes in living organisms are subject to cyclic variability in the form of self-sustaining oscillating physiological processes whose duration ranges from milliseconds to annual fluctuations. These reactions are called biological rhythms, and many of them have been with us since the dawn of life on earth undergoing evolutionary changes and participating not in passive assimilation but, above all, leading to the ability to modify our own vital functions in response to the external environment. The light-dark cycle is considered by most researchers to be the most essential, fundamental synchronizer of human endogenous rhythms (41). In mammals, the clock is a system of peripheral oscillators located in various organs and an overarching, so-called central clock, located in the suprachiasmatic nuclei (SCN) of the brain. It is a cluster of only 50.000 neurons in humans, which reach a size of only a few tenths of a cubic millimeter (42, 43). The SCN itself is subject to synchronization to the diurnal rhythm by light, which is why the rhythm generated by the central clock is a day/night rhythm receiving signals mainly from retinal cells containing melanopsin and directly responding to light (44). The neurotransmitter in this pathway is the glutamic acid. Biological rhythms are extremely conservative acting in analogous ways in evolutionarily distant organisms *e.g.*, humans and *Drosophila melanogaster*. The molecular mechanism of the clock is a feedback loop, mostly negative, in which the final protein product inhibits the expression of the gene encoding it. The primary mammalian clock genes belong to the Period - Per1, Per2, Per3, Cryptochrome - Cry1, Cry2 and Clock and Bmal1 families. A hallmark of central oscillator genes is expression at specific times of the day (45). For the discoveries of the clock in *Drosophila*, the 2017 Nobel Prize in Physiology or Medicine was awarded (46). In humans, MEL secretion is synchronized with the light/dark cycle carrying the primary information about the length of a given time of day (52). MEL biosynthesis increases rapidly during the dark period and its concentration peaks between 12 – 2 o'clock to then gradually decrease in the second half of the night. Blood concentrations of MEL range from 0–20 pg/ml during the day to 20–120 pg/ml at night (47, 48). Maximum concentrations are organism-specific and age-dependent. After 25 years of age according to some researchers, and after 40 according to others, MEL production in the pineal gland drops to 60% of young adult levels. From that point on, there is a steady decline to values as low as 20% of the young adult level in people over 90 years old, with average MEL values always higher in women (49-51).

In mammals, MEL exhibits four primary mechanisms of action in mammals:
1) interaction with MEL membrane receptors;
2) antioxidant action which is the resultant of MEL's ability to transfer free electrons and to stimulate redox reaction enzymes;
3) binding of intracellular proteins such as calmodulin, which affects cell cycle progression;
4) binding and activation of nuclear receptors belonging to the ROR/RZR subfamily (retinoid orphan receptors/retinoid Z receptors).

According to the IUPHAR nomenclature, the following types of MEL membrane receptors are distinguished:
1) ML1 - such as MT1 (Mel1a), MT2 (Mel1b) and Mel1c bound to G proteins. Their characteristic feature is their high affinity for MEL which determines their ability to bind the hormone at picomolar concentrations (52).
2) ML2 (MT3) - bound to quinone reductase and binds MEL at nanomolar concentrations of the hormone (52, 53).

MEL has demonstrated the ability to electron transfer and inactivate free radicals such as singlet oxygen, nitric oxide, hydroxyl radical hydroxide as well as a stimulating effect on antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. It has been shown that MEL's ability to inhibits lipid peroxidation *in vivo* is much more effective than other 'scavengers' like vitamin C or vitamin E (54-56).

Calcium-activated calmodulin participates in the initiation of the S and M phases of the cell cycle, in the expression of cell cycle-related genes, and in the return of cells from the resting G0 phase to G1 of the cell cycle. MEL binds to calmodulin, impeding the activation of the protein in question, its distribution, and the normal course of the cell cycle. It is thought that this mechanism may explain the antiproliferative effect of melatonin on dividing cells, including cancer cells (57-59).

MEL exhibits the ability to bind and activate nuclear receptors belonging to the ROR/RZR (retinoid orphan receptors/retinoid Z receptors) subfamily. Expression of diverse types of these receptors is demonstrated in various organs. Thus, RZRb were found in nerve cells bound to, for example, the limbic system, while RORa/RZRa are present in the pituitary gland and the liver, cartilage, or skin. The RZRg have primarily been localized to skeletal muscle (60, 61). These receptors participate in regulating immune processes, *e.g.*, maturation of T lymphocytes, and engage in the differentiation of the central nervous system. An example of the immunomodulatory effects of MEL resulting from RZRa stimulation is the inhibition of mRNA expression of 5-lipoxygenase, which participates in leukotriene biosynthesis in the arachidonic acid cascade (62).

MELATONIN - POTENTIAL MECHANISMS TARGETING LIVER AND BILIARY TRACT DISEASES

The potential mechanisms of action of melatonin in humans are shown in *Fig. 2*.

Alcoholism is a major health problem across the world and alcohol liver disease (ALD) remains the main cause of liver

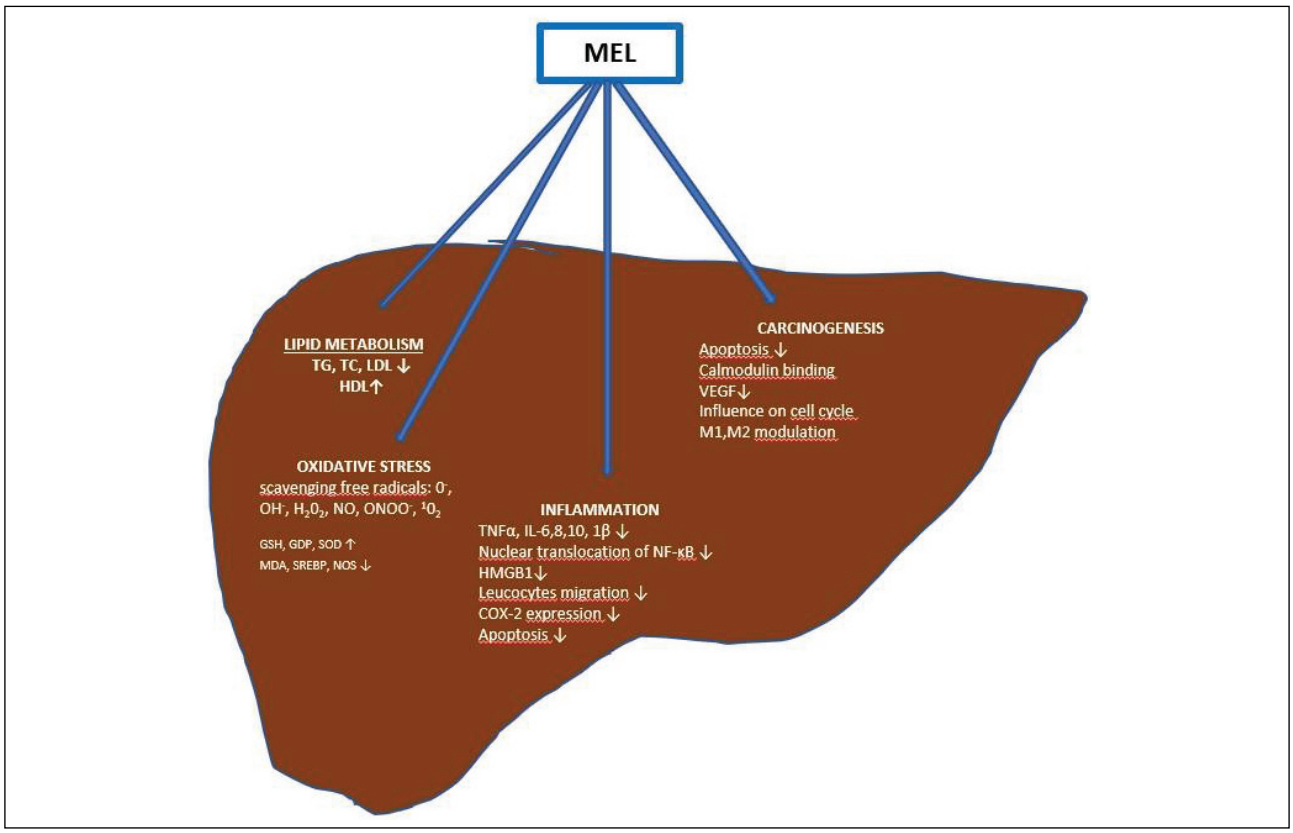

Fig. 2. Potential mechanisms of action of melatonin (MEL) in the liver.
$^{1}0_2$, singlet oxygen; COX-2, cyclooxygenase 2; GPD, glucose-6-phosphate dehydrogenase; GSH, glutathione peroxidase; H_20_2, hydrogen peroxide; HMBG1, high mobility group box 1; IL, interleukin; M1, M2, melatonin receptors; MDA, malondialdehyde; NF-κB, nuclear factor kappaB; NO, nitric oxide; NOS, nitric oxide synthase; O^{-}, superoxide; OH^{-}, hydroxyl group; $ONOO^{-}$, peroxynitrite; SOD, superoxide dismutase; SREBP, sterol regulatory element-binding protein; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

related mortality worldwide, the highest (41%) in the European region. The prevalence of ALD is approximately 2% in the US general population with an estimated mortality of 5.5 per 100,000 in 2010. In Europe, South-East Asia region including India and China percentage of drinkers continues to rise whereas in Africa it has decreased. The ALD encompasses a spectrum of disorders ranging from the simple fatty liver (hepatic steatosis) progressing at time with continued excessive alcohol ingestion to alcoholic steatohepatitis (histological evidence of hepatic inflammation or fibrosis), alcoholic cirrhosis and hepatocellular carcinoma. Among patients with simple fatty liver, approximately 35% progress to steatohepatitis and 10% develop cirrhosis. Multiple factors play a role in the pathogenesis of ALD, not all of which have been elucidated. Molecular mechanisms of ALD include direct hepatotoxicity, induction of ROS production by alcohol and its metabolites, activation of innate immunity and the production of proinflammatory cytokines. Most of the ethanol in the body is metabolized in the liver to acetaldehyde by the action of alcohol dehydrogenase, which, with the participation of aldehyde dehydrogenase, is converted to acetate, leading to increased production of NADH. However, in persons who consumed substantial amounts of alcohol the enzymes cytochrome P450 2E 1 and catalase have also a significant role in alcohol conversion to acetaldehyde and mediate liver impairment (63). Acetaldehyde is the first metabolite during ethanol detoxification, which can increase collagen I transcription directly and indirectly through transforming growth factor-β1 (TGF-β1). Excess NADH leads to the synthesis of glycerol phosphate and triglycerides deposited in the liver. Chronic alcohol consumption results in ROS formation, bacterial proliferation in the intestinal lumen, alteration of its permeability to macromolecules, bacterial translocation, and Toll-like receptor-dependent activation of Kupffer cells, which drives immune response and cytokine production, resulting in liver necrosis and fibrosis (64, 65). Studies in laboratory animals confirm the etiological role of cytokines in liver damage in ALD, with elevated levels of tumor necrosis factor alpha (TNF-α) and TNF-α-induced cytokines/chemokines such as interleukins (IL) - 6, 8 or 18, and that the levels of these cytokines correlate with markers of the acute phase response, liver function and the clinical picture of the disease (66). Another factor involved in the processes is nuclear hepatocyte factor-4, which acts as a major transcription factor for the regulation of certain genes involved in lipid metabolism and the oxidative process (67). A key molecule in the cascade of reactions leading to the abnormalities described above appears to be LPS derived from the gut microbiota (68, 69). Many studies in laboratory animals indicate that endotoxemia is a major risk factor for the development of ALD. Rats with ALD were characterized by high concentrations of endotoxins in the portal vein and a correlation between these concentrations and the severity of ALD (70). Similar observations were made by Bigatello *et al.* (71), showing that endotoxemia was present in 36 of 39 (92.3%) ALD patients at the time of cirrhosis. Circadian cycle genes have been shown to regulate the expression of genes encoding proteins of the apical complex in the intestine affecting its permeability and to regulate the expression of, for example, transporters for glucose. Balakrishnan *et al.* showed that the concentration of both SGLT1 and GLUT2 mRNA and/or protein in the intestine of rats and mice is dependent on diurnal rhythms (72). Equivalent results have been reported by other researchers (73, 74). Master clock and peripheral oscillator genes have also been shown to regulate lipid metabolism (75, 76), intestinal permeability (77) and other intestinal functions. Voigt *et al.* (78) found that disruption of diurnal rhythms (light/dark phase shift) disrupts the gut microbiota causing dysbiosis in mice. Thus, alcohol affecting the central clock can create favorable conditions for its toxic effects and the development of liver disease. This has been confirmed by studies in laboratory animals. Laboratory animals with ALD have high concentrations of alcohol and endotoxins in the portal vein, and this correlates with the degree of liver damage. Dysbiosis in alcohol-fed laboratory animals has been demonstrated previously. Mutlu *et al.* (79) found that male Sprague-Dawley rats developed dysbiosis and ALD at week ten of the alcohol rich diet, and that these phenomena were reversed by administration of a probiotic containing *Lactobacillus rhamnosus Gorbach-Goldin*. In 2011, Swanson *et al.* (80) published the results of a study in which they showed that alcohol, at a low concentration of 0.2%, induced a time-dependent increase in the permeability of a Caco-2 cell culture monolayer used as a research model of intestinal epithelial cells. This part of the experiment also showed an increase in the expression of CLOCK and PER2 proteins correlating with an increase in intestinal barrier permeability (80). Silencing of the Clock or Per2 genes with the siRNA technique led to a reversal of this phenomenon. The second part of the study analyzed intestinal barrier permeability in male Sprague-Dawley rats subjected to an alcohol-rich diet. Again, there was an increase in intestinal barrier permeability, as well as Per2 gene and PER2 protein expression, which were shown to be increased in duodenal and intestinal tissues (80). Currently, we have a small number of studies on the effects of MEL on alcohol-induced liver damage. Hu *et al.* (81) showed that MEL reduced aminotransferase activity, the degree of steatosis and inhibited inflammatory cell migration without affecting lipogenesis genes expression in hepatocytes in ethanol-fed mice. These authors also showed that MEL treatment led to a reduction in serum and tissue levels of pro-inflammatory cytokines, lipid peroxidation and neutrophil infiltration in tissues (81). MEL also inhibited apoptosis. Isolating Kupffer cells from ethanol-only-fed animals showed a prominent increase in the production of ROS and TNF-α, while Kupffer cells of MEL-treated mice had significantly less of them. Mishra *et al.* (82) showed that in ethanol-induced acute liver injury, MEL exhibited potent hepatoprotective effects by inhibiting oxidative stress accompanied by a decrease of alanine transaminase (ALT) activity in serum. The researchers showed that ethanol in a dose-dependent manner led to a significant increase in matrix metalloproteinase-9 (MMP-9) expression, which was significantly correlated with the expression of pro-inflammatory cytokines, such as TNF-α and ILS such as IL-1β and IL-6. In turn, the application of MEL had a hepatoprotective effect by downregulating the expression of MMP-9 and upregulating the expression of the tissue inhibitor of metalloproteases, TIMP-1 (82). It was shown that in the experimental animals, ethanol induced the translocation of the NF-κB playing a vital role in inducing the expression of inflammatory genes responsible for oxidative stress, and that the ethanol increased the degradation of the NF-κB inhibitor - IκBα. As the authors showed, MEL inhibited the process of nuclear translocation of NF-κB (82). Here it is worth noting that a similar mechanism of action is attributed to ginsenosides, natural steroids derived from Panax plants (especially Panax ginseng), widely used in traditional Chinese medicine (83, 84).

OTHER TOXIC LIVER DAMAGE

Acetaminophen (APAP) is an OTC drug often used for the symptomatic treatment of pain and fever. It has a particularly good safety profile; however, acute intoxication can result in liver damage (85), which is characterized by extensive oxidative stress (86). Matsura *et al.* (87) studied the preventive use of MEL (50 or 100 mg/kg) in mice in which toxic liver damage was induced by oral administration of APAP at a dose of 750 mg/kg. As shown, administration of MEL to mice 8 or 4 hours before toxicity inhibited the increase in aminotransferase activity in a dose- and time-dependent manner. In addition, MEL at a dose of 100 mg/kg

given 4 hours before APAP administration, significantly inhibited hepatic necrosis, reduced the intensity of inflammatory infiltration, and inhibited lipid peroxidation and myeloperoxidase activity in hepatocytes (87). MEL pretreatment inhibited also release of NOS and IL-6. However, MEL did not affect the content of reduced glutathione (GSH) in the liver, nor did it reduce GSH consumption as well as the expression of heat shock proteins HSP70 (87). Other studies showed also inhibitory effect of MEL on APAP-induced serine/threonine kinase and nuclear translocation of mitochondrial Bax and apoptosis-inducing factor (AIF), which prevented cell death (88). Interestingly, although APAP-induced liver damage was primarily due to its biotransformation to toxic metabolites by CYP4502E1, MEL had no effect on its expression. In another study, Kanno *et al.* (89) demonstrated a dose-dependent protective effect of MLT against hepatotoxicity induced by APAP. *In vitro*, at concentrations of 0.1, 1, 10 or 100 mM, MEL inhibited ROS production as well as lipid peroxidation. Similar *in vivo*, after subcutaneous administration at a dose of 10 mg/kg, MEL significantly reduced mortality and hepatotoxicity in experimental animals (89). In contrast to other reports, there was no synergistic effect of MEL on the antipyretic and analgesic effect of APAP (90). A different approach to the problem was presented by Karakus *et al.* (91), who evaluated the efficacy of agomelatine, a MEL receptor agonist, in the prevention of APAP-induced hepatotoxicity in rats. Agomelatine is a new antidepressant drug whose mechanism of action involves resynchronization of circadian rhythms and improvement of sleep architectonics. The authors found that administration of agomelatine in the test animals significantly corrected aspartate aminotransferase (AST) and ALT activity, TNF-α, IL-6 and 8-iso-prostane levels, and increased superoxide dismutase activity as well as glutathione concentration (91).

IMMUNOMODULATING AND IMMUNOSUPPRESSIVE DRUGS

Calcineurin inhibitors (CIn)

Cyclosporin A (CsA) and tacrolimus (TkL) hare used to treat autoimmune diseases and organ transplant patients. The action of the CIn is fraught with significant side effects such nephrotoxicity, cardiotoxicity and hepatotoxicity, caused by oxidative stress leading to a number of changes in the liver (92, 93).These changes include vacuolization of the hepatocytes cytoplasm, the appearance of numerous mitotic figures, intracellular changes in the concentration of GSH, MDA, expression of heat shock proteins (HSP) and apoptosis (92) and to increasing levels of TNF-α, IL-6 and/or NOS (93). Rezzani *et al.* (92) showed that MEL protected against cyclosporine-induced oxidative stress. Similar observations were made by Kurus *et al.* (94), who induced toxic liver damage in Sprague-Dawley rats using CsA at a dose of 10 mg/kg/28 days. They revealed that the group of animals treated with CsA and MEL together showed no significant histological changes compared to the control group. In turn, Akbulut *et al.* (95) found that the application of MEL in laboratory animals with liver damage after CsA, reduced the activity of histological lesions and increased hepatocellular glutathione, MDA, and increased the activity of superoxide dismutase and catalase.

NON-ALCOHOLIC CHRONIC LIVER INJURY

Nonalcoholic fatty liver disease (NAFLD) is regarded as the hepatic manifestation of metabolic syndrome, affecting at least a quarter of the global adult population and has become one of the most common reasons for liver transplantation, especially in Western countries. NAFLD encompasses a disease spectrum ranging from simple steatosis to steatohepatitis (defined histologically as hepatocyte injury due to inflammation and hepatocellular ballooning), liver cirrhosis and hepatocellular carcinoma. The most feared complication of nonalcoholic steatohepatitis (NASH) is the development of fibrosis, liver cirrhosis and hepatocellular carcinoma, respectively in 25–33%, 5–15% and 2–5% of people with NAFLD (96). Recently, the expert opinions issued by professional organizations have been proposed that the nomenclature of NAFLD should be updated to metabolic dysfunction-associated fatty liver disease (MAFLD). While the definition of NAFLD requires that there be evidence of fatty liver with no causes for secondary hepatic fat accumulation such as significant alcohol intake, chronic viral hepatitis, or use of steatogenic medication, MAFLD is diagnosed by detecting fatty liver together with metabolic risk factors without exclusion of other liver diseases (97, 98). No standard pharmacological treatment currently exists, however certain treatments including insulin sensitizing agents, lipid-lowering agents, antidiabetic agents, antioxidants and other classes of drugs have been studied in clinical trials. Lifestyle interventions and weight loss is indicated for all patients with NAFLD/MAFLD (99).

LIVER STEATOSIS

The first step on the path to liver damage is the accumulation of lipids in hepatocytes leading to steatosis and then, under the influence of factors that have not been fully clarified, to inflammation, fibrosis and eventually cirrhosis. The possible association of MEL with hepatic steatosis is based on historical observations that showed that pinealectomy increased serum glucose levels in laboratory animals, while administration of MEL corrected these abnormalities (100, 101). Moreover, glucose intolerance and decreased insulin sensitivity of adipocytes were observed after pinealectomy (102). Observations regarding the effect of MEL on these processes are unfortunately diverse and often contradictory. Thus, it was reported that adipocytes developed insulin resistance after pinealectomy, and that MEL administration led to hyperglycemia in laboratory rats. In birds, rabbits, on the other hand, showed both an increase, decrease and no effect of MEL on glycemia (103-105). Finally, Bojkova *et al.* (106) showed that 48 h fasting after prolonged MEL administration significantly altered changes in carbohydrate and lipid metabolism in young rats, and Markova *et al.* (107) revealed that MEL administration significantly decreased serum triacylglycerols and liver glycogen content in male rats, and increased liver phospholipid content in female rats. Since then, the number of experimental evidence has been increasing, and they point to a potential preventive role of MEL in the process of fat deposition in hepatocytes. In the end of 20th century, it was shown that MEL increased the concentration of total, free and esterified cholesterol and decreased the concentration of non-esterified fatty acids in the blood of rats. In turn, long-term use of MEL led to correction of hypercholesterolemia and hepatic steatosis on histological evaluation in animals fed a cholesterol-rich diet. MEL decreased leptin concentrations, which corrected the BMI of laboratory animals indirectly affecting the degree of hepatic steatosis (103, 107). By affecting the regulation of leptin concentrations, MEL also interferes with energy metabolism, reducing the body weight of laboratory animals (108, 109). In laboratory animals, a high-fat diet has been shown to induce oxidative stress leading to hepatic steatosis and steatohepatitis, and MEL inhibits these processes. Hoyos *et al.* (110) showed that the use of MEL in rats fed with a regular diet had no effect on

cholesterol and triglyceride concentrations, while in animals fed with a cholesterol-rich diet, MEL led to a significantly smaller increase in total cholesterol and low-density lipoprotein (LDL) concentrations compared to the fat-rich-fed control group (110). In the group of studied animals, MEL prevented a decrease in high-density lipoprotein (HDL) concentrations. In contrast, the authors showed no differences in very-low-density lipoprotein (VLDL) and triglyceride concentrations. In addition, they also showed that MEL slightly decreased urea and bilirubin concentrations, while it increased serum glucose concentrations (110).

An important observation made in this study was the demonstration of a protective effect of MEL on cell membrane damage (110). Comparable results were obtained in a study by Hatzis *et al.* (111), which showed that compared to the untreated group, rats treated with MEL at doses of 5 and 10 mg/kg had significantly lower mean liver weights and a significantly lower ($p<0.001$) ratio of liver weight to total body weight. Liver steatosis was also shown to be lower in the treated rat groups (111). Thus, III^0 steatosis was shown in 3.4% of animals using 10 mg MEL and in 11.1% 5 mg. Recently, the valuable studies have been published indicating the potential preventive effect of MEL in hepatic steatosis and the involvement of new mechanisms in this process. Wang *et al.* (112) evaluated the effects of MEL at a dose of 10 mg/kg/24h on fat metabolism and fat deposition in the liver and abdominal girdle in 20 Sprague-Dawley rats. After 60 days of feeding the rats with a high-fat diet, it was shown that liver weight and liver index (the ratio of liver weight to body weight) in the MEL group of animals decreased by 20.69% and 9.63%, respectively, similarly, abdominal fat weight decreased by 59.88% and 54.93%, respectively, and epididymal fat weight was lower by 45.34% ($p=0.049$) (112). Triglyceride, high-density lipoprotein, low-density lipoprotein, and total cholesterol concentrations were significantly lower in MEL-treated animals compared to controls. Genetic studies (Gene chip + qPCR) showed that MEL positively affected the expression of 289 genes and negatively affected the expression of 293, and that the mRNA expression of lipolysis-related genes increased, while the mRNA expression of lipogenesis-related enzymes decreased significantly ($p<0.05$) (112). In another study, Schneider *et al.* (113) evaluated the effects of MEL on the liver of zebrafish after feeding the test fishes with fructose for 8 weeks. In addition, the expression of genes related to appetite control (leptin, ghrelin and melanocortin 4 receptor - MC4R) was evaluated. As shown, the group of animals fed only with fructose developed hepatic steatosis, which did not occur in control animals and those additionally using MEL (113). Fructose-fed animals also had an increase in intestinal leptin expression compared to the MEL group. When considering the mechanisms by which MEL may exert the effects described above in humans, such as effects on gene expression, the following factors are considered: a) inhibition by various mechanisms of the electron transport chain, thereby reducing lipid peroxidation, b) effects on the regulation of sterol regulatory element binding protein (SREBP), which is activated by LPS and affects the expression of SREBP target genes (114, 115).

It has been known since years that insulin resistance is at the center of the pathogenetic events leading to hepatic steatosis. So, are there any scientific evidence about the impact of MEL on this phenomenon in humans? It seems that a research model of this problem could be type 2 diabetes (T2D) in the course of which steatosis and/or steatohepatitis are common. It is now known that membrane MEL receptors are present in cells of various organs including the gastrointestinal tract (116) with MT2 predominating (117). The presence of MT1 and MT2 receptors has also been confirmed in human pancreatic tissue and Langerhans' islets (118). The beneficial effects of MEL in T2D have been confirmed by studies in humans and/or in cell cultures of tissues obtained from humans. Thus, the use of MEL has been shown to increase glucose-stimulated insulin secretion in cell cultures obtained from people without diabetes (119), and that MEL in the same research model leads to both glucagon and insulin release (120). It has also been shown that the appearance of certain variants of the MEL receptor gene MTNR1B leading to inhibition of signaling is associated with an increased risk of T2D (121-124) although this issue has been a subject of controversy (125, 126). It is worth noting that views on the use of MEL in T2D are varied and some investigators raise harmful effects (127-129) therefore, the use of MEL in MAFLD and T2D patients must be subject to special supervision. We do not have well-documented studies on the prevention of simple hepatic steatosis in humans. All studies have been performed in steatohepatitis patients and will be discussed in the subsection dealing with this problem.

STEATOHEPATITIS

As mentioned earlier, the axis of disorders in MAFLD is insulin resistance manifested by hyperglycemia, hyperinsulinemia, increased gluconeogenesis, and *de novo* fatty acid synthesis, which leads to organ steatosis (130). In parallel, there is activation of lipolysis in adipose tissue and increased influx of fatty acids into the liver, increased production of adipokines and pro-inflammatory cytokines (131). All these processes lead to damage of the cell membrane system including mitochondria and the endoplasmic reticulum and increase in oxidative stress which expresses increased synthesis of ROS and other free radicals. The extent of the described disorders depends on aa number of factors including: a) the rate of fatty acid absorption, b) fatty acid biosynthesis in the liver, and c) the interaction of other factors such as the gut microbiome and/or genetic predisposition. The rate of fatty acid uptake by hepatocytes, depends on the amount and activity of fatty acid transport proteins (FATP, fatty acid transport protein; FAT, fatty acid translocase; CD36; FABP, fatty acid binding protein), the expression of which can be regulated in the short and long term. The first mechanism is mediated by intracellular insulin concentration, while the second is mediated by activation of peroxisome proliferator-activated receptor (PPAR) family transcription factors. The lipid metabolism in the liver involves biochemical pathways responsible for the body's energy balance, the most important of which are glycolysis, fatty acid biosynthesis, fatty acid desaturation and triglyceride synthesis. The key enzyme of fatty acid biosynthesis is fatty acid synthase (FAS), whose activity limits the rate of reaction. FAS expression is regulated by a group of transcription factors such as SREBP, liver X receptors (LXR) and carbohydrate response element binding protein (ChREBP) (132, 133).

The mechanisms by which MEL exhibits protective effects on hepatocytes in NASH and CIRR are not fully understood. Most studies indicate that the primary action of MEL is the direct scavenging of free radicals; as well as indirect by the stimulating of antioxidant enzymes and exhibit anti-inflammatory effects by inhibiting the synthesis of prostaglandins (134), adhesion molecules (135), leukocyte migration (136) and cyclooxygenase-2 (COX-2) expression (137, 138). The effect of MEL on the immune system and the profile of cytokines produced is the subject of intense research. MEL can directly affect the activity of immune cells, on the other hand, these cells produce MEL, and this situation is analogous to the functional immune synapse formed by immune-competent cells with the CNS. As shown, the effect of MEL on immune cells is mainly stimulatory but there are also opposing research available. This pro-inflammatory

effect is associated with increased production of pro-inflammatory cytokines such as IL-1, IL-2, IL-6, IL-12, TNF-α and interferon gamma (IFN-γ) in various cell types such as monocytes and T-helper type 1 (Th1) cells and IL-17 in T-helper type 17 (Th17) cells (139, 140). MEL has also been shown to cause increased production of cytokines involved in clonal differentiation and expansion. Thus, under the influence of MEL, increased concentrations of macrophage colony-stimulating factor (M-CSF) (141, 142), stems cell factor (SCF) (143, 144), transforming growth factor beta (TGF-β) (145, 146) and Tα and thymulin in thymocytes (147, 148).

The effect of MEL in autoimmune diseases is multidirectional. MEL may lead to improvement in others to worsening and the mechanisms responsible for this variation are unknown. MEL has found application in multiple sclerosis (149), rheumatoid arthritis (RA) (150, 151) in which improvement after its use has been described. The evaluation of the effect of MEL on the immune response in RA has its origins in the study by Chen and Wei (152), who showed that beneficial anti-inflammatory effect of MEL in a rat model of adjuvant-induced arthritis was related to reduced thymocyte proliferation and IL-2 secretion. In the other hand we have described a case of a patient suffering from ulcerative colitis accompanied by primary sclerosing cholangitis (PSC) with features of autoimmune hepatitis (AIH), who developed severe hepatitis manifested by multiple increases in aminotransferases activity after repeated MEL treatment (153). The histological picture of the liver was similar to that found in the original biopsy performed 2 years earlier resembled and included ballooning degeneration of hepatocytes, a mild inflammatory infiltrate in the portal zones composed mainly of lymphocytes with focal interface hepatitis and mild portal fibrosis (153). In addition, the presence of lymphocytes surrounding bile ducts was also noticed (153). The extended oxidative stress, activation of the immune response and apoptosis lead to increased inflammation and liver fibrosis. What is the current state of knowledge regarding the effects of MEL on these processes? One of the earliest reports on this problem came in 1999 from Ohta *et al.* (154), who assessed the efficacy of MEL at a dose of 50 or 100 mg/kg in rats by analyzing hepatic lipid peroxide levels and the decrease in reduced glutathione in the liver. The research model for liver fibrosis is carbon tetrachloride (CCl_4)-induced fibrosis (155). Noyan *et al.* (156) compared the efficacy of pentoxifylline (PTX) at a dose of 50 mg/kg/24 h and MEL at a dose of 10 mg/kg/24 h showing a reduction in MDA and LOOH levels in treated mice ($p<0.01$). Both MEL, PTX and MEL+PTX increased glutathione peroxidase (GSH-Px) and catalase (CAT) activities ($p<0.05$) (156). In the latter regard, MEL had greater efficacy than PTX and MEL+PTX ($p<0.05$). The use of MEL, PTH and MEL+PTH significantly ($p<0.01$) reduced apoptosis and bridging necrosis while only MEL had no effect on ballooning degeneration (156). In another study (157), the liver of rats was histologically evaluated after one month of preventive MEL treatment, showing essentially abolition of changes such as necrosis, fibrosis, mononuclear cell infiltration, hemorrhage, fatty degeneration and formation of regenerative nodules, apoptotic figures, and fine-droplet steatosis. Only mild ballooning degeneration of hepatocytes was observed in MEL-treated animals (157).

Similar observations have been made by other researchers (158-161). A remarkably interesting observation was made by Rafiq *et al.* (162) who published in 2022 the results of a study comparing the efficacy of MEL and mesenchymal stem cells (MSCs) obtained from the bone marrow of female BALB/c mice in preventing liver fibrosis after CCl_4 induction. It was shown that MSCs+MLT combination therapy had a significant beneficial effect on fibrosis and the parameters studied - a significant reduction in bilirubin concentration and ALT activity. The PCR method showed a decrease in Bax expression and an increase in Bcl-xl and albumin. Another mechanism for the preventive effect of MEL on liver fibrosis is related to its potential effects on cytokine production. In addition to assessing oxidative stress, Wang *et al.* (158) showed that MEL inhibited the expression of NF-κB in liver tissue and reduced the production of TNF-α and IL-1β. In another study, Crespo *et al.* (163) showed that MEL at a dose of 5 or 10 mg/kg/d administered intraperitoneally reduced aminotransferase activity, abrogated hepatic stellate cell activation, and significantly inhibited the expression of collagens I and III, TGF-β, platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), amphiregulin and Smad3 proteins, which are critical intracellular mediators of TGF-β. Choi *et al.* (164) evaluated the effects of MEL on protective necroptosis-associated mechanisms and damage-associated molecular patterns (DAMPs), which in fibrosis are mediated by activation of receptors associated with pattern recognition. In rats orally administered MEL at 2.5, 5 and 10 mg/kg/d, there was a reduction in hepatic hydroxyproline levels and hepatocyte damage, as well as TGF-β1 and α-smooth muscle actin expression (164). MEL also inhibited RIP1 receptor-interacting protein expression, RIP1/RIP3 necrosome complex formation, decreased serum concentrations of high-mobility group box 1 (HMGB1) and IL-1α, and inhibited HMGB1 interaction with receptors for advanced glycation end products (RAGE), Toll-like receptor 4 expression (TLR4), p38 phosphorylation and nuclear translocation of NF-κB factor (164).

Currently, only a few studies on the effects of MEL on steatohepatitis are available (165-172) involving humans and none regarding cirrhosis, not counting reports of MEL in cirrhotic patients used for sleep disturbances, circadian rhythms, and hepatic encephalopathy (173-178). Gonciarz *et al.* (165) published a study evaluating the use of MEL in NASH. At the time, it was the first such observation involving humans (165). Forty-two patients were enrolled in the study, whose diagnosis of NASH was based on, among other things, histologic findings obtained up to 6 months before starting MEL therapy at a dose of 2×5 mg. There was a significant reduction in baseline serum ALT activity in the MEL treatment group at week 4, 8 ($p<0.05$), reaching a nadir of activity at week 12 ($p<0.001$). Thirteen percent of patients showed normalization of ALT activity at week 12 (none in the control group) (165). Similarly, AST activity significantly decreases at week 4, 8 and 12 ($p<0.001$). In MEL-treated group also median gamma-glutamyltransferase (GGT) (IU/L) significantly decreased at week 4, 8 and 12 and among twenty-six patients with elevated GGT levels at baseline 13 (50%) showed GGT normalization: six cases at week four, five cases at week 8 and two cases at week 12 (165). Another observation involved the same group of patients in whom MEL was extended to 6 months (166). Significant difference in median ALT, AST and GGT activity between baseline and week 12 in both control and MEL treated group reported previously was also seen at week 18, 24 and at follow-up ($p<0.05$). In this study authors revealed that significant improvement in plasma ALT, AST and GGT activity were sustained throughout the next 12 weeks in which the patients were receiving treatment (166). Although AST level returned to the baseline value after discontinuation of MEL, the associated GGT decrease was not reversed (166). A major limitation of this studies was the lack of post-therapy histological follow-up. Continuing the study, the authors evaluated the effect of MEL, administered at a dose of 10 mg/day for 28 days to sixteen patients with histologically confirmed NASH on insulin resistance (HOMA-IR), plasma levels of adiponectin, leptin, ghrelin and resistin (167). In addition, aminotransferase activity, GGT and plasma MEL levels

were assessed. Median baseline values of HOMA-IR, leptin (ng/mL) and resistin (pg/mL) in patients with NASH were significantly higher compared to controls, while adiponectin (μg/mL) was significantly lower (167). There was no significant difference in ghrelin levels. After MEL treatment, median HOMA-IR values decreased by 60% compared with baseline values, while plasma concentrations of adiponectin, leptin and ghrelin increased significantly; the difference between pre- and post-treatment status in plasma resistin levels was not significant (167). Cichoz-Lach *et al.* (168) compared the efficacy of MEL, tryptophan and Essentiale Forte (EsF) in forty-five patients with histologically confirmed NASH, showing statistically significant reductions in GTP activity and triglyceride and pro-inflammatory cytokine concentrations in patients treated with MEL and L-tryptophan. Next Celinski *et al.* (169) compared the efficacy of MEL and EsF at a dose of 3×1 tablet/14 months in 70 NASH patients who were randomly assigned to one of the following groups: I) EsF + tryptophan 2×500 mg/d, II) EsF+MEL 2×5 mg/d, III) only EsF, showing a significant reduction in GGT activity and triglyceride and LDL-cholesterol levels in groups I and II (169). MEL concentration after therapy was significantly increased in groups I and II, with no change in group III. An important observation was the demonstration of statistically significantly lower levels of IL-1, IL-6 and TNF-α in patients receiving MEL and tryptophan, compared to group using EsF alone (169). In 2017, Pakravan *et al.* (170) reported the results of a randomized, double-blinded controlled trial that evaluated the efficacy of MEL in NAFLD patients compared to placebo. A total of one hundred patients were enrolled in the study, half of whom received oral MEL and the other half placebo for 3 months (170). Data analyzed included weight, waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), ALT and AST activity, high-sensitivity C-reactive protein (hsCRP) concentration, and degree of hepatic steatosis, which did not differ between groups before MEL treatment (170). In contrast, the group of patients treated with MEL showed a statistically greater reduction of DBP (p=0.0001), AST activity (p=0.005) and hsCRP (p=0.0001). Report lacks data on the MEL dose used (170). Another double-blind, randomized trial was the study by Bahrami *et al.* (171) published in 2020, which included fifty patients with NAFLD. Patients in the treatment group received 6 mg MEL/d/3 months (171). A significant improvement compared with the placebo group was observed in weight (p=0.043), waist circumference (p=0.027), abdominal circumference (p=0.043), SBP (p=0.039), DBP (p=0.015), leptin serum levels (p=0.032), hs-CRP (p=0.024), ALT (p=0.011), AST activity (p=0.034) and in the grade of fatty liver (p=0.020). Finally, in the same year, Mansoori *et al.* (172) published a systematic review and meta-analysis on the significance of MEL in NAFLD patients, which included only five studies (166, 168-171). The weighted mean difference (WMD) was computed with 95% confidence interval (CI) and I^2 statistic was used to determine heterogeneity (172). The significance level was defined as I^2 value >50 % or $p \leq 0.05$. The results showed that MEL had a significant effect on AST (WMD=2.29, [95%CI: 1.14, 3.43] IU/L, $p \leq 0.001$), ALP (WMD= –8.40, [95%CI: –11.33, –5.48] IU/L, $p<0.001$) and GGT (WMD= –33.37, [95%CI: –37.24, –29.49] IU/L, $p \leq 0.001$) activities, but had no effect on ALT (172). Based on the meta-analysis, the authors concluded that the use of MEL may improve some liver indices in NAFLD patients, but more randomized trials are needed. Recently, nanomolar concentrations of MEL were found to regulate insulin synthesis and secretion of this hormone in insulinoma cells of the INS-1E rat, suggesting the importance of this endocrine mechanism of diabetes, thus confirming the pleiotropic nature of this indoleamine (173). In addition, Mel supplementation counteracted psychosomatic symptoms in postmenopausal women, strongly recommending the usefulness of this MEL as an adjuvant in the treatment of mental disorders (174).

CIRRHOSIS HEPATIS

The cardinal pathologic features of cirrhosis reflect irreversible chronic injury of the hepatic parenchyma and include extensive fibrosis with the formation of regenerative nodules. The pathologic process represents the final common pathway of many types of advances liver injury, most often caused by excessive alcohol ingestion, obesity, and chronic viral infections. An inflammatory response is initiated in extra-parenchymal cells such as vascular endothelial, stellate and Kupffer cells, as well as cells of the immune-competent system (175). This process is made possible by the presence of PRRs (pattern recognition receptors), which exhibit the ability to recognize specific pathogen-associated molecular patterns known as PAMPs (pathogen-associated molecular patterns). Pathogen eradication can be achieved through the activation of complex signaling pathways that trigger an inflammatory response mediated by various cytokines and chemokines. To date, five classes of PRRs have been characterized, such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), C-type lectin-like receptors (CRLs) and AIM-2-like receptors (ARLs) (176, 177). Another mechanism mobilizes DAMPs, which are released by cellular stress and induce an inflammatory response (178). Activation of both PAMPs/DAMPs mechanisms triggers and amplifies the synthesis and release of inflammatory and pro-fibrogenic mediators that recruit immune-competent and matrix-producing cells: hepatic stellate cells (HSCs), myofibroblasts (MFBs) (179). More recently, the effects of MEL on DAMPs have become the focus of research. MEL was shown to inhibit the intracellular translocation of HMGB1 in intestinal epithelial cells (IECs), an effect that was partially abolished by the MEL antagonist luzindolem (180). HMGB1 is located in the cell nucleus and is integral to oxidative stress and signaling pathways regulating cell death and cell survival. However, HMGB1 released into the extracellular space induces inflammation by activating the NF-κB pathway through binding to TLR2, TLR4, TLR9 and RAGE (181). The effects of MEL on DAMPs have been demonstrated in few studies (182-184), and only a few of them focus on liver damage. In rats with streptozotocin (STZ)-induced diabetes, MEL showed hepatoprotective effects by preventing deterioration of hepatocyte morphology, DNA damage, and reducing the severity of necrosis. Serum ALP, ALT, and AST activity were also significantly lower than baseline ones ($p<0.05$). The improvement was due to a reduction in the total ROS load as a result of a decrease in the diabetes-induced increase in lipid peroxidation ($p<0.05$) accompanied by a decrease in acid polymerase 1 (PARP-1) cleavage and inhibition of cytoplasmic translocation and associated accumulation of serum HMGB1 protein (185). In rabbits treated with MEL for acute liver failure caused by rabbit hemorrhagic disease virus (RHDV), a significant reduction in the expression of TLR-4 receptor, HMGB1 and Decay-Accelerating Factor (DAF/CD55) were observed (186). Levels of IL-1β, IL-6, TNF-α and C-reactive protein were also lowered. The decreased expression of metalloproteinase-9, Janus kinase, as well as increased expression of hepatocyte growth factor (HGF), epidermal growth factor, PDGF-B, vascular endothelial growth factor and their receptors, mitogen-activated protein kinase (ERK) and signal transducer and activator of transcription 3 (STAT3) were also observed (186). In addition, Petrovic *et al.* (187) provided evidence of inhibition of HMGB1 and TLR4 fusion and apoptosis following MEL administration in diabetic rats (13).

The TLR4/NF-κB/NLRP3 signaling pathway has been shown to be crucial in the pathogenesis of hepatic ischemia/reperfusion (HIR) injury. Interestingly, MEL was found to act as a cardiac antiarrhythmic agent during experimental cardiac ischemia-reperfusion episodes, however, this effect was limited to the non-ischemic area of myocardial tissue (188). In addition, MEL attenuated protein expression of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, but was found to be ineffective in targeting recurrent arrhythmia triggers (188). Recently, attention has been drawn to the key role of NOD-like proteins not only in the formation of infections, cancer, autoimmune and neurodegenerative diseases, but also in the pathogenesis of metabolic diseases such as obesity, NAFLD/NASH, T2D. There is growing evidence from a number of laboratories that NOD-like receptors have a wide range of recognition, not only of microbial structures, but also of those, non-infectious factors, associated with tissue damage. They are involved in inflammatory processes and initiation of apoptosis. El- Sisi and colleagues (189) showed that octreotide (OCT) and MEL+OCT significantly inhibited oxidative stress, apoptosis and inflammation as expressed by decreased expression of HMGB1, TLR4, MyD88, TRAF-6, p-IκBα (S32), p-NF-κBp65 (S536), NLRP3, ASC, caspase-1(p20) and GSDMD-N. The similar observations were made by Mohamed *et al.* (190). Currently, we have no clinical studies evaluating the efficacy of MEL in human cirrhosis. However, most animal studies have established the protective potential of MEL against experimentally induced cirrhosis using thioacetamide (TAA). MEL has been shown to inverse TAA-induced phenomena such as activation of apoptosis and stellate cells, increases in lipoperoxide and reduced glutathione levels, catalase, and superoxide dismutase activities (191). These observations have also been confirmed in other studies. Czechowska *et al.* (192) revealed that in Wistar rats with cirrhosis induced by TAA, MEL at a dose of 10 mg /kg/b.w. for 4 weeks led to a significant improvement in liver enzyme activity ($p<0.001$). There was also a significant reduction in the levels of IL-1β ($p<0.05$), TNF-α, IL-6, TGF-β, IL-1β, PDGF-AB, GSH and oxidized glutathione GSSG ($p<0.001$). The improvement in liver histology was also observed. Lebda *et al.* (193) using the same experimental model showed that MEL decreases serum activity of aminotransferases and autotoxin (ATX) as well as serum concentration of bilirubin, hydroxyproline and urea. In fact, inhibiting ATX can have a promising therapeutic effect on many diseases, including NASH. The study also showed that MEL has led to an increase in the levels of glutathione, glutathione s-transferase, glutathione peroxidase and other antioxidant enzymes and decrease in malondialdehyde, protein carbonyl, nitric oxide (NO), and activation of DNA fragmentation. MEL also led to inhibition of the expression of pro-inflammatory cytokines and profibrogenic genes because of an increase in thioredoxin-1 expression (mRNA increase) (193). In rat model of biliary cirrhosis induced by bile duct ligation, MEL was shown to have an inhibitory effect on liver peroxidation and fibrosis and to increase the activity of antioxidant enzymes (194). Together with the aforementioned findings normalization of serum aminotransferases activity, reduction of the hepatosomatic and splenosomatic index (% of total organ weight relative to the total weight of the animal) were obtained (194).

As early as 1982, Iguchi *et al.* (195) showed that MEL concentrations were significantly elevated in cirrhotic patients compared to healthy volunteers. The study also showed correlations between daytime MEL concentrations and the retention rate of indocyanine green and serum total bilirubin levels, indicating reduced hepatic clearance, reduced of 6-beta-hydroxylase activity and competition with bilirubin in the intrahepatic transport system (195). Also, other studies show that cirrhotic patients are characterized by increased daytime MEL levels and delayed nocturnal peak, which generates in this group of patients a disturbance of the master oscillator manifested by a phase shift of the biological clock and thus the occurrence of abnormal circadian rhythms and excessive sleepiness of patients during the day, and insomnia at night (196-198) without association with hepatic encephalopathy (198). In the latter study, it was found that, patients with more severe liver failure (Child-Pugh score >5) had significantly ($p<0.04$) lower evening MEL levels compared to patients with less severe failure (Child-Pugh score <5) (199). Measurable MEL concentration was also found in ascites due to cirrhotic portal hypertension (200). However, the association of the increased MEL levels in cirrhotic patients with sleep disorders remains controversial because sleep disorders often occur in diseases that accompany liver pathology such as T2D (201) and obesity (202). In turn, sleep disorders can affect eating behavior, hyperinsulinemia, and obesity (203), with an increasingly raised correlation between the observations and the gut microbiota as a central pathogenetic point for the described phenomena in animals (204-206) and humans (207, 208). In patients with cirrhosis, differentiating sleep disorder from hepatic encephalopathy, especially its minimal form, is extremely difficult. It seems that within cases of encephalopathy, the primary sleep disorders may be subject to pharmacological correction, *e.g.*, with MEL treatment. Chojnacki *et al.* (196, 209) showed that in patients with alcoholic cirrhosis and encephalopathy, nocturnal serum MEL concentrations are statistically significantly increased ($p<0.01$), while urinary 6-HMS concentrations at the same time are decreased ($p<0.01$), and that serum serotonin concentrations are significantly decreased in patients with Child Pug B and C liver failure. In 2020, De Silva *et al.* (210) published the results of a study on the use of low-dose MEL (3 mg) in patients with early-stage (Child-Turcotte-Pugh class A or B) cirrhosis with sleep disturbances, without hepatic encephalopathy showing a statistically significant improvement in Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale (ESS) compared to both pretreatment ($p<0.001$) and post placebo scores ($p<0.001$). Esmaeili *et al.* (211) published the results of a randomized, double-blind, placebo-controlled study evaluating the antipruritic effects of MEL at dose 10 mg over night for 2 weeks using a Visual Analog Scale (VAS) and the 12-Point Pruritus Severity Score (12-PSS) in patients with chronic hepatic diseases of different origin. In the MEL group the VAS scale showed an alleviation of pruritus ($p<0.05$), and the 12PSS decreased by an average of 46.57% ($p<0.05$). Additionally, the study assessed Body Surface Area (BSA, adapted from the Severity Scoring of Atopic Dermatitis Index - SCORAD), which also improved by 51.71% ($p<0.05$).

Hepato- and cholangiocarcinoma

Cancer is one of the leading causes of death, a major public health problem and a barrier to increasing life expectancy. According to WHO analysis, cancer was the first or second cause of death before age 70 among 112 countries, and the second or third among another. Primary liver cancer (PLC) is the sixth most common cancer and the third cause of death globally (212). The highest incidence of this type of cancer traditionally has been seen in Asia, where the incidence is as high as 72.5% of the global incidence and the mortality rate is 73.3%. The most common diagnosis, 75%, is primary hepatocellular cancer (HCC). Trends observed in countries with traditionally exceedingly high incidence of HCC have improved between 2005 and 2015, with an estimated 3.9% annual decline in China's age-standardized incidence rate (ASIR). Unfortunately, during the same period, the incidence of primary biliary cancer

(ICC) was increasing, especially in the population of people ≥65 years old (213). The clinical course of HCC is insidious, at the onset of clinical symptoms patients are usually ineligible for effective therapy due to the advanced nature of the disease, and systemic chemotherapy has low efficacy, as demonstrated by several phase III trials using doxorubicin, sorafenib or the FOLFOX4 regimen (214). 70–80% of HCC cases develop in patients with cirrhosis, which is a complex, multi-stage process involving various initial factors such as steatosis and steatohepatitis (MAFLD), toxic damage and chronic infections with primary hepatotropic viruses, especially HBV whose DNA after insertion in the host genome can lead to deregulation of such gens like TERT, PDGFR, CCNE1, P53 which are involved in cell cycle, signaling and replication. In the remaining 30–20% of cases, the aforementioned factors are thought to trigger carcinogenesis bypassing the cirrhotic phase (215). A special subtype of HCC arising in normal livers of usually young individuals is fibrolamellar carcinoma, in which carcinogenesis is triggered by a characteristic somatic gene fusion, DNAJB1-PRKACA, resulting from a deletion in chromosome 19 and activating protein kinase A (216). The study of the relationship between impaired MEL secretion and cancer development has been conducted since the early 1970s. Many exploratory studies of tumor development in animals have been conducted using the possibility of chemical induction of cancer using the dye o-aminoazotoluene, 4-dimethylaminoazobenzene, 7,12-dimetylobenzanthracene or N-nitroso-N-methylurea. Also, a number of naturally occurring materials are hepatocarcinogens, *e.g.*, the aflatoxin B, a product of the Aspergillus family of molds, which may contaminate stored foodstuffs, especially grain and nuts. Administration of MEL to laboratory animals protected them from the carcinogenic effects of the inducer (217), while surgical pinealectomy had the opposite effect (218). Moreover, the use of MEL in the latter group of animals corrected this unfavorable trend (219). The effect of illumination on the development of carcinogen-induced tumors was also analyzed. Administration of MEL to pinealectomized animals and their exposure to constant 24-hour illumination led to a reduction in the number of adenocarcinomas compared to animals not using MEL. The timing of the illumination was also of similar importance; fewer cancers were observed in the group of animals exposed to light on a 10:14 h diurnal rhythm than in those after 24-hour exposure. A shorter latency time of chemically induced tumor was also observed in the group of animals undergoing pinealectomy and exposed to 24 hours of light exposure, in contrast to animals undergoing pinealectomy and exposed to 10 hours of light on a daily cycle (220). The epidemiological studies in humans indicate a link between night work and the risk of for example, breast cancer in women, while others do not confirm these observations. Despite the inconclusive results of epidemiological studies regarding the cancer risk of shift workers, considering some of them and the theoretical rationale behind them, the International Agency for Research on Cancer in 2007 identified shift as associated with circadian rhythm disturbances as a 'probable' carcinogen (group 2A). Using data from 2 prospective cohort studies, the Nurses' Health Study (1988–2012; n=78,516) and Nurses' Health Study II (1989–2013; n=114,559), Wegrzyn *et al.* (221) examined the associations between rotating night shift work and breast cancer risk. In both cohorts showed 9541 cases of breast cancer over 24 years of follow-up. In the Nurses' Health Study, women with 30 or more years of rotating shift work did not have a higher risk of breast cancer (HR=0.95, 95% confidence interval (95% CI): 0.77, 1.17; p for trend = 0.63) compared to those who had never worked a shift. In the second Nurses' Health Study II, the risk of breast cancer was significantly higher in younger women with 20 or more years of cumulative shift work, reflecting the exposure of younger individuals (HR=2.15, 95% CI: 1.23, 3.73; p for trend = 0.23), and was marginally significantly higher in women with 20 or more years of cumulative shift work when updated exposure information was used (HR=1.40, 95% CI: 1.00, 1.97; p for trend = 0.74). Thus, prolonged rotating night shift work was associated with a higher risk of breast cancer, especially among women who did shift work during young adulthood.

The mechanism of MEL's anti-neoplastic effects is diverse. In this regard, the effects of MEL on angiogenesis, oxidative stress, apoptosis, autophagy, and effects on various intracellular signaling pathways are considered. The effects of MEL on angiogenesis are multidirectional. In digestive gastric ulcers, MEL promotes angiogenesis (222, 223), which accelerates healing, while in tissue hypoxia, such as age-related or in cancer, it inhibits neovascularization through inhibitory effects on HIF-1, HIF-1α, VEGF and sphingosine kinase 1 (SPHK1) (224-227). The involvement of oxidative stress in the promotion of oncogenesis needs no justification, and MEL's action in this regard has been discussed previously. A distinctive feature of MEL is its effect on mitochondrial stress (MS), which makes its action unique. MEL has been shown to induce mitochondrial apoptosis by inhibiting PrPC prion protein expression in colon cancer cells (228, 229) and by activating the mitochondrial serine/threonine kinase PINK1 in rat hepatocytes (230). MEL has also been shown to alleviate MS stress-induced insulin resistance and that it sensitizes cells to apoptosis by inhibiting COX expression, increasing the Bax/Bcl-2 ratio and chemotherapy regimen (CHOP) (231). As for autophagy, the effect of MEL on this process is diverse and depends on the cell type. For example, Tran *et al.* (232) showed that MEL exhibited synergism with doxorubicin in activating apoptosis in breast cancer cells and enhances the therapeutic effect of doxorubicin by inducing autophagy. Other researchers have shown that autophagy may be a therapeutic target in colorectal cancer. Zhao *et al.* (233) found a synergistic anti-tumor effect of MEL and *Andrographis paniculata* (AnP) ($p<0.05$) in reducing the viability of colon cancer cells, as well as an inhibitory effect on colony formation and stimulation effect on apoptosis. The study also showed that the combination of MEL and AnP inhibited autophagy by affecting the expression of such autophagy-related genes as NR4A1, CTSL and Atg12 (223). Similar observations were made by Chok *et al.* (234), who analyzed autophagy pathways in HT-29, SW48 and Caco-2 cells and showed that MEL increased CRC death, oxidative stress and autophagic vacuole formation in a dose-dependent manner. All mechanisms of carcinogenesis described for other organs are considered in hepatic carcinogenesis. Kimball *et al.* (235) in cultured hepatic H4IIE cells evaluated the effects of MEL on hydrogen peroxide (H_2O_2)-induced activation of mitogen-activated protein kinase (MAPK) and the mTOR signaling pathway, demonstrating the inhibitory effects of MEL in this regard by inhibiting p38 phosphorylation and the ERK1/2 signaling pathway. This study also showed that MEL inhibited the phosphorylation of Akt and the final product of the mTOR signaling pathway, the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), which initiates the translation of key proteins for cell cycle progression. Remarkably interesting is a previously unpublished study by Lee *et al.* (236), who analyzed the effect of MEL on the behavior of mTORC1 activity and glycolysis-related gene expression in Hep3B and Huh7 HCC cell cultures. They showed that as early as 3 hours after MEL treatment, mTORC1 activity as measured by the expression of phosphorylated mTOR and ribosomal kinase S6 significantly decreased while the expression of c-Myc and glycolysis-related genes remained unchanged (236). If the test cultures were subjected to further prolonged MEL treatment, they showed a reduction in

glycolysis, cell proliferation and viability, and activation of apoptosis and in expression of c-Myc in a dose-dependent manner. It was also shown that in HepG2 liver cancer cell cultures, MEL inhibited the activity of metalloproteinase 9 (gelatinase) by suppressing the expression of the encoding gene and increasing the levels of the tissue inhibitor of metalloproteinases, TIMP-1, and reducing nuclear translocation of NF-κB (237).

In contrast, there were no significant changes in the activity of MMP-2 and its tissue inhibitor, TIMP-2. Yeh *et al.* (238) made similar observations, although the study was conducted on HSC3 and OECM-1 oral cancer cell cultures, showing that MEL decreased the activity of the MMP-9, as well as the mRNA and protein expression of MMP-9. In addition, MEL was shown to inhibit phosphorylation of the ERK1/2 signaling pathway, which inhibited MMP-9 gene transcription by affecting the expression of transcription coactivators such as CREB-binding protein (CREBBP) and E1A p300-binding protein (EP300), as well as reducing histone acetylation in the cultures studied (238). In contrast to the previous study, no effect on nuclear translocation of NF-κB was shown. The anti-angiogenic effects of MEL were demonstrated in HepG2 liver cancer cell cultures (239), in which MEL at a concentration of 1 mM was shown to reduce both cellular and secreted VEGF levels and prevent hypoxia-induced tube formation of HUVECs that was associated with decreased Hif1α protein expression, nuclear localization and transcriptional activity. While hypoxia increased STAT3, Hif1α phosphorylation and CBP/p300 recruitment as a transcriptional complex within the VEGF promoter, MEL reversed these processes (238). In another study, El-Magd (240) showed that preconditioning MSCs stem cells with MEL in female rats protected them from diethyl-nitrosamine-induced HCC by inducing apoptosis as indicated by increased expression of such proapoptotic genes as Bax and caspase-3 and decreased anti-apoptotic genes such as Bcl2 and survivin. Administration of MEL-conditioned cells simultaneously led to decreased inflammation and angiogenesis as indicated by decreased expression IL-1β, NF-κB, vascular endothelial growth factor, MMP-9, with increased metalloproteinase inhibitor gene 1. The same center also showed that in laboratory animals with DEN-induced HCC, liver tissue was characterized by low levels of apoptosis as indicated by a reduction relative to the control of DNA fragmentation and expression of genes such as p53, caspase 9 and 3, and an increase in IL-6 and TGF-β1. All adverse events were reversed by MEL and MSCs (241). Under experimental conditions, MEL prevented thioacetamide-induced liver fibrosis and hepatotoxicity in rats by modulating pro-inflammatory cytokines and attenuating thiobarbituric acid-reactive compounds (242).

Despite the theoretical rationale, studies regarding the use of MEL in human cancer patients are rare. Barni *et al.* (243) evaluated the efficacy of the combined use of IL-2 and MEL as second-line therapy in thirteen patients with CRC and liver metastases after initial therapy with 5-fluorouracil. IL-2 was administered at 3 million IU/day for 6 days/week for 4 weeks, and MEL was administered at 50 mg/day orally at 8.00 p.m. each day. As the study showed, there was no objective tumor regression, while disease stabilization was observed in 4/13 patients (median duration 5+ months), with the remaining 9 patients experiencing disease progression. The mean number of lymphocytes, eosinophilia, neopterin and TNF significantly increased during treatment. In a subsequent study, Lissoni *et al.* (244) evaluated the efficacy of the combined use of IL-2 and MEL in solid tumors other than renal cell carcinoma and melanoma, which are generally resistant to IL-2 alone. IL-2 was used similarly to the previous study while MEL was administered at a dose of 40 mg/day orally, starting 7 days before the first IL-2 administration. Eighty-two patients were analyzed, of whom seventy-two had distant organ metastases, and the histological types of tumors were as follows: non-small cell lung cancer - 19, HCC - 16, CRC - 15, gastric cancer - 11, pancreatic cancer - 11, breast cancer - 6; others - 4. Objective tumor regression was achieved in seventeen patients, disease stabilization was achieved in thirty patients, while the remaining thirty-five patients experienced progression. In one of the few human studies, Yan *et al.* (245) compared the efficacy of a trans-catheter arterial chemoembolization (TACE) with TACE in combination with MEL at a dose of 20 mg/d preceding 7 days before of TACE in the treatment of inoperable HCC. The efficacy of TACE vs. TACE+MEL was 16% and 28%, respectively ($p<0.05$). The survival rate at 6-month, 12-month and 2 years was 82%, 54% and 26% in the group of patients treated only with TACE, while in the TACE+MEL group it was 100%, 68% and 40% of all patients, respectively. Moreover, in patients treated with TACE+MEL, the percentage of patients who underwent two-stage resection was 14%, while in the group treated only with TACE it was 4% ($p<0.01$). Research on the use of MEL in patients with CCC are inconclusive. Reduction in the activity of enzymes such as N-acetyltransferase and serotonin O-methyltransferase in cell cultures and human biopaths and an increase in the expression of MEL MT1/MT2 receptors have been reported.

LIVER TRANSPLANTATION AND SURGERY

Liver transplantation is highly effective treatment for patients who suffer from a variety of irreversible progressive liver diseases for which there is no acceptable, alternative therapy. Unfortunately, the number of potential recipients significantly exceeds the number of available donors. Reducing this disproportion is very difficult, which is why it has become common to use organs with a risk of inferior function after transplantation, *e.g.* from older donors and/or those with brain death after prolonged hospitalizations and such risk factors for deterioration of liver function as systemic ischemia in the course of multi-organ trauma, hemorrhagic shock, heart failure and others, which within the liver lead to oxidative stress, inflammatory immune response, apoptosis, Kupffer cell activation and increased vascular cell adhesion molecule expression (245, 247). Thus, methods are still being sought that can improve the situation, and MEL, due to its pleiotropic properties, may play a vital role in this regard. The first step on the road to transplantation is to keep the donor organ in decent shape, and in this regard, preservation solutions play a significant role. Currently, four types of standard preservative solutions are in use: University of Wisconsin (UW), Institute Georges Lopez (IGL-1), Celsior solution (CE) and histidine-tryptophan-ketoglutarate (HTK) solution, whose effectiveness in sustaining transplantation has been evaluated in a study by Adam *et al.* (248) who showed that overall, 3-year graft survival was higher with UW, IGL-1 and CE (75%, 75% and 73%, respectively), compared to the HTK (69%) ($p<0.0001$) and the similar observation was made for partial grafts in which a 3-year graft survival was 89% for IGL-1, 67% for UW, 68% for CE and 64% for HTK ($p=0.009$). The use of MEL as a component of preservative solutions has been the subject of few studies before. For instance, Zaouali *et al.* (249) studied the use of MEL added to IGL-1 solution in an *ex vivo* perfusion model of isolated rat livers. The livers were stored at 4ºC/24 h in UW or IGL-1 solutions with or without MEL, and then reperfused at 37ºC. IGL-1+MEL-perfused organs showed lower aminotransaminase activity. Liver function was assessed by the degree of bile production and sulfobromophthaline clearance - both of which

were increased. In addition, IGL-1+MEL was also shown to reduce vascular resistance probably by a mechanism of e-NOS activation leading to increased of nitric oxide concentration, and to have anti-oxidative and anti-inflammatory effects associated with inhibition of the release of pro-inflammatory cytokines, especially TNF and adiponectin (249). In a further report, Zaouali *et al.* (250) published the results on the effects of MEL and trimethazine (TMZ) added to IGL-1. Analogous to the previous study, rat livers were preserved in UW or IGL-1 with or without MEL+TMZ and subjected to 24-hour reperfusion at 37ºC. As shown, liver preservation with IGL-1+MEL+TMZ caused a significant decrease in endoplasmic reticulum (ER) stress - a decrease in GRP78, PERK, and CHOP, activation of AMPK which in turn led to a decrease in ER stress and autophagy. Gnal *et al.* (251) evaluated the hepaprotective efficacy of a UW solution containing MEL at a concentration of 130 µmol/L in Wistar rats, showing that MEL exhibited significant anti-inflammatory and protective effects on Kupffer cells ($p<0.05$) and that the activity of enzymes such as LDH, AST and ACP were significantly lower compared to the control group. Another interesting observation in this study was the demonstration of an increase in the expression of heat shock proteins HSP70, which maintain normal cell homeostasis, restore normal cell function, and protect cells from damage by, among other things, reducing lipid peroxidation (251). Other researchers (252, 253) showed that the application of MDDP solution (pentoxifylline, glycine, deferoxamine, N-acetylcysteine, erythropoietin, MEL, and simvastatin) prior to liver reperfusion with HTK led to inhibition of malondialdehyde and IL-1 production, thereby completely abolishing the inflammatory response, reducing hepatocyte dysfunction and damage, and infiltration of the liver with inflammatory cells. Comparable results were reported in other studies by Vairetti *et al.* (254) and Freitas *et al.* (255). In the first one liver of Wistar rats were stored at UW and CE after perfusing with Krebs-Henseleit bicarbonate buffer (KHB) without or with MEL and a dose-dependent increase in bile production and tissue ATP levels were shown. The second group of investigators additionally showed that LDH and GSH activities in MEL-treated rats were similar to control values, with lower levels of ROS (255). Kireev *et al.* (256) studied the effects of MEL administered intraperitoneally and/or orally in Zucker rats, in which reperfusion of their own liver was performed after prior ligation of the portal vein and hepatic artery. As shown, MEL decreased ALT, AST activity and reduced the increase in oxidative stress exponents by both the mechanism of free radical scavenging and the increase in antioxidant enzyme expression (256). MEL also improved mitochondrial function and the ability of hepatocytes to produce ATP, and reduced the expression of pro-apoptotic genes and has also been shown to stimulate mitochondrial glutamate dehydrogenase activity, which reduces the release of cytochrome C and caspase-3 in rat hepatic IRI (257, 258). Other researchers have shown in animal models that the application of MEL inhibited the production of TNF-α (259, 260), iNOS and NO as well as exerted a hepatoprotective effect by inhibiting kinases such as IKK and the JNK pathway (261, 262). The last cited study does not involve liver IRI but acute cerebral ischemia, however, deserves attention because of its quality, and the conclusions are potentially transferable to all other tissues (262). As is well known, IRI leads to an immune inflammatory response during which there is an increase in the concentrations of many pro-inflammatory cytokines such as interleukins IL-1β, IL-6, TNF-α and IFN-γ which can lead to rapid rejection of the transplanted organ. In this regard, the use of MEL has been shown to reduce the concentrations of pro-inflammatory cytokines. In study by Kireev *et al.* (263) using the model described earlier, they evaluated the concentration of pro-inflammatory cytokines and the effect of age and intraperitoneal administration of MEL on the expression of multiple genes. Older animals showed greater liver damage, higher ALT and AST activity and expression of genes encoding such cytokines as IL-1β, MCP-1 and IFN-γ, as well as lower mRNA expression for IL-10 compared to young animals after IRI. The expression of proapoptotic genes (Bax, Bad and AIF) was significantly increased (263). MEL administration led to a reversal of these adverse phenomena. In experimental studies, MEL also influenced autophagy processes and phosphorylation of mTOR (mammalian target of rapamicin), which is a common, highly conserved serine/threonine kinase that binds to raptor or riptor and other proteins, forming mTORC1 and mTORC2 complexes in turn (264). The functions and signal transduction pathways of mTORC1, which affects key biological functions such as the cell cycle and gene expression, are fairly well understood (264). The complex has been shown to act by directly activating p70SK1 kinase and inhibiting 4E-BP1 binding protein (264), and that MEL inhibits mTOR-dependent autophagy (265). Similar observations were also made by Mohamed *et al.* (190), who compared the hepatoprotective effects of octreotide (OCT) and MEL in IRI by modulating autophagy, which was normalized by OCT by increasing the expression of Beclin-1, ATG7 and LC3, while decreasing the expression of p62 through induction of AMPK/S317-ULK1 and inhibition of PI3K/AKT/mTOR/S757-ULK1 signaling pathways. MEL affected autophagy by inhibiting AMPK/pS317-ULK1 but its effect was less pronounced than OCT. Kirimlioglu *et al.* (266) showed that the use of reservatrol (REZ) and MEL in Wistar rats after partial hepatectomy (70%) prevented lipid peroxidation and reduced hepatic GSH and NO levels ($p<0.05$) and Ki-67 expression, not a histone nuclear protein present in all phases of the cell cycle except G0. The treatment with REZ and MEL significantly enhanced apoptosis ($p<0.001$), with MEL more than REZ ($p<0.05$). One of the earliest clinical experiments on the use of MEL in human liver surgery was published as the PORTAL trial, which showed that a single preoperative dose of MEL was safe in patients with planned extensive liver resection (267). Subsequently, Nickkholgh *et al.* (268) showed that MEL in high dose (50 mg/kg b.w.) administered preoperatively through a nasogastric tube was remarkably high effectively absorbed from the gastrointestinal tract ($p<0.0001$) and resulted in a postoperative reduction in aminotransferase activity ($p=0.6$) with no serious adverse events. Improving the quality of organs intended for transplantation by mitigating I/R damage still remains a major challenge.

Recently an increased clinical use of MEL has been observed worldwide. In the United States, MEL is considered a dietary supplement and is available in health food stores. Sales of MEL in the United States increased by more than 500% between 2003 and 2014. In Japan, Australia and in the most countries of the European Union, MEL is available only with prescription (269). People commonly use MEL for insomnia and jetlag; however, MEL is also used for sleep-wake cycle disturbances in blind people and shift workers and in patients with neurodegenerative, liver, and cardiovascular diseases. Oral MEL is safe for adults in a dose up to 10 g daily, however, it can cause some side effects including nausea, headache, dizziness, less common others. However, special attention should be paid to patients with autoimmune diseases. Hong *et al.* (270) reported a patient in whom clinical, laboratory and histologic features of autoimmune hepatitis developed after beginning MEL therapy for the treatment of insomnia. Fourman *et al.* (271) described a case of autoimmune hepatitis that developed after starting ramelteon (agonist melatonin) for insomnia. As it was mentioned before we also described a case of a patient suffering from ulcerative colitis accompanied by overlap syndrome with PSC

and AIH, who developed a severe hepatitis manifested by multiple increases in aminotransferases activity after two separated courses of MEL treatment (153). Moshtagh-Sisan *et al.* (272) described a 78-year-old female in whom clinical and histological manifestations of autoimmune hepatitis developed during MEL therapy for insomnia. In all the above-mentioned cases MEL discontinuation led to normalization of liver enzymes and reduction of symptoms.

In the last years substantial progress has been accomplished in our understanding of the MEL pleiotropic mechanisms of action. The purpose of this review is to provide information regarding the potential benefits of MEL use in hepatobiliary diseases. Beside its well-known action as circadian regulator of physiological and neuroendocrine function, MEL and its metabolites possesses potent antioxidant properties by scavenging free oxygen metabolites and inducing the expression of antioxidant enzymes. Experimental studies demonstrated that MEL ameliorated hepatic injury caused by various etiopathology factors, however the translation of these observations to human hepatobiliary disorders was less well proven. The data from the literature indicate that MEL may be an important therapeutic tool for the management of a number of hepatic disorders, however, more extensive clinical studies with larger sample size are required.

Author's contribution: M. Gonciarz: designed the structure of this review and wrote the manuscript; I. Lombard participated in literature search and analysis of literature data; L. Konecki and P. Gietka participated in the literature search; R. Pajdo and T. Brzozowski critically evaluated the final version of MS.

Conflict of interests: None declared.

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Received: February 24, 2023
Accepted: April 30, 2023

Author's address: Prof. Maciej Gonciarz, Clinic of Gastroenterology and Internal Medicine, Military Institute of Medicine, 128 Szaserow Street, 04-141 Warszawa, Poland.
E-mail: mgonciarz@wim.mil.pl