Antiproliferative Effects of Molecular Iodine in Cancers

Pompilio Elio Torremante*[,1] and Harald Rösner[2]

[1]*Praxis für Gynäkologie und Geburtshilfe, Ochsenhausen, and* [2]*Institut für Zoologie, Universität Hohenheim-Stuttgart, Stuttgart, Germany*

Abstract: Iodine is a scarce element in soil that is essential for human beings. It constitutes the basis of thyroid hormones, which are important for mammalian metabolism and development and are indispensable for fetal brain development. Iodine deficiency causes multiple disorders and is still the major cause of endemic cretinism. Comparison of different national cancer statistics led to the supposition that there might be an inverse correlation between iodine intake and cancer prevalence. Asian countries which traditionally have an extremely high iodine intake in daily diet, attributable to seaweed consumption, attract attention by having a lower average cancer incidence rate. Today, based on extensive animal and cell experiments, it has been shown that iodine in form of molecular iodine undoubtedly exerts antitumor effects by inducing apoptosis. Although first analyses were performed with breast cancer cells exclusively, the antitumor effects of molecular iodine were extended by us to a wider range of other tumors, confirming that the antitumor effect is not limited solely to breast cancer.

Keywords: Molecular iodine, iodolactones, malignant cell lines, antiproliferative, apoptotic effects, mitochondria-mediated apoptosis, disruption of the mitochondrial transmembrane potential.

INTRODUCTION

More than 200 years ago, marine sponges and seaweed were used to cure goiter. The operating agent in marine sponges and seaweed was identified as iodine, which exhibited antiproliferative properties. Iodine is a rare element found in soil and most parts of the world are considered to be iodine deficient areas. It is assumed that iodine had been washed out from soil by glacier melt and by weathered rock into the ocean. Therefore, the vast majority of iodine on earth is present in the sea, especially in plants living in salt water, such as seaweed, and in salt water fish [1].

Determinations of chemical species of iodine in seaweed show that the predominant chemical form is iodide ion and iodide bound to biological macromolecules. Iodide ions are used by seaweed as an extracellular inorganic antioxidant, which is released upon oxidative stress to scavenge reactive oxygen species (ROS) and ozone. By reaction with ROS or ozone, iodide ions are oxidized to positively charged molecular iodine. Seaweed contributes hereby to scavenging ozone [2].

EPIDEMIOLOGY

Global breast cancer mortality statistics have shown that Asian regions have the lowest breast cancer mortality rate in the world. Among other reasons, it was thought that dietary factors might be implicated in the etiology of the disease. Traditionally, Asian countries like Japan have a high salt water fish and seaweed consumption in daily diet, so that their daily iodine intake, due to their diet, exceeds the iodine intake in western countries by far [3].

*Address correspondence to this author at the Marktplatz 29 – 88416 Ochsenhausen Germany; Tel: 0049-07352-941736;
Fax; 0049-07352-941738; E-mail: dr.torremante@onlinemed.de

Epidemiological studies indicate that high consumption of iodine-rich seaweed correlates with a lower breast cancer incidence and probably also with a reduction of the cancer rate in general. Iodine-rich extracts of brown seaweed exerts anticarcinogenic, antimutagenic, and antipromotion activities. In cell experiments with 7,12-dimethylbenz(a)anthracene (DMBA), a breast carcinogen, and with 3,2'-dimethyl-4-aminobiphenyl (DMAB), a colon and breast carcinogen, seaweed extract inhibited mutagenicity. So it may be assumed that seaweed consumption could prevent breast cancer [4,5]. A recently published case-control study, examining the amount of daily seaweed consumption related to the risk of breast cancer development, confirmed this hypothesis [6].

UPTAKE AND ROLE OF IODINE IN GROWTH REGULATION OF THE THYROID GLAND

Molecular iodine is incorporated by the digestive tract after being almost reduced to iodide ions and is transferred into the plasma [7]. In animal experiments iodine uptake by the digestive tract was proven to be mediated by the intestinal sodium-iodide symporter (NIS) [8]. The thyroid gland, the most active iodide ion-trapping organ, takes up the iodide ions by the NIS located at the basal membrane of the polarized thyroid cells. Iodide ions drift along the electrical gradient at the apical membrane of the thyroid cells. The efflux through the apical membrane into the follicular lumen is mediated partly by pendrin (PDS) and the chloride channel ClCn5 localized at the apical membrane of thyrocytes possibly in conjunction with other chloride channels, involved in mediating apical iodide efflux or iodide/chloride exchange [9]. Once iodide ions reach the cell-colloid interface, they are oxidized and rapidly organified by incorporation into selected tyrosyl residues of thyroglobulin. This reaction is catalyzed by thyroid peroxidase (TPO) in the presence of hydrogen peroxide which is generated by the thyroid oxidase

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1 and 2 (Duox 1 and 2) [10]. Blocking oxidation of the iodide ions results in a failure of the antiproliferative effect of iodide ions. Studies of the antigoitrogenic effects of iodine led to the detection of δ-iodolactone, which is synthesized by the thyroid cells. δ-iodolactone is an iodinated product of the cell-membrane polyunsaturated fatty acid, achacidonic acid. This iodinated fatty acid has been shown to regulate proliferation of thyroid cells; it can also induce apoptosis [11-13].

In iodine deficiency, thyrocytes produce growth factors which are secreted into the surroundings. These growth factors stimulate the thyrocytes in an autocrine manner and the surrounding connective tissue in a paracrine manner. The most important intrathyroidal growth factors are insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and transforming growth factor beta (TGF-β). These growth factors are not specific to the thyroid gland, but are identical with the growth factors which are formed in other epithelial organs. δ-iodolactone can inhibit these growth factors. Therefore, iodine deficiency leads to intrathyroidal δ-iodolactone deficiency, promoting self-induced hyperplasia of the thyroid gland. Recently, it was demonstrated that the goiter inhibitory action of δ-iodolactone is mediated by the inhibition of cell proliferation and transient stimulation of apoptosis. This action may be related to the stimulation of TGF-β3 but not TGF-β1 [14-16].

UPTAKE AND METABOLISM OF IODINE BY NON-THYROID CELLS AND TISSUES

Although widely observed in context with the thyroid gland, δ-iodolactones can be synthesized by all cells containing peroxidase activity and enzymes to synthesize hydrogen peroxide (H_2O_2) e.g. by neutrophil cells containing myeloperoxidase (MPO) and phagocyte oxidase and by eosinophil cells containing eosinophil peroxidase (EPO), generating H_2O_2 as a result of superoxide production from the reduced nicotinamide adenine dinucleotide phosphate oxidase system [17-19]. Apparently, similar to interferon, they are an integral part of the immunological system with antibiotic and anti-tumor effects. The antibiotic effect is attributed to the physiological halide, chloride, as encountered in biological fluids at concentrations considerably higher than required. Iodide is the most effective halide on a molar basis; however, the concentration of free iodide in biological fluids is very low (< 1 mg %) [20-23]. This constellation might be different when iodide intake is extremely high for example in daily diet as is the case in Japan. From previous studies with eosinophil cells, it has been shown that δ-iodolactones are synthesized by the peroxidase - hydrogen-peroxide - halide system, whereby iodide appeared to be most appropriate, as the transforming reaction of arachidonic acid to δ-iodolactone runs at a pH of 7.0. Eosinophil cells, like neutrophil cells, respond to perturbation of the plasma membrane with a respiratory burst, in which oyxgen is converted to H_2O_2 and released with arachidonic acid into the extracellular fluid to synthesize δ-iodolactone. Since MPO and EPO are strongly basic proteins and thus bind to negatively charged surfaces such as cell membranes, the synthesis of δ-iodolactones could also be performed on the membrane surface of the tumor cell which is to be eliminated. Successful experiments demonstrating the anti-tumor effect of δ-iodolactone were performed with lymphoma cells [23]. Incidentally, hereditary MPO deficiency is a common feature in Europe and USA rating 1:2000-4000. In Japan it is less common rating 1:57,000 for the complete MPO deficiency [24]. This hereditary difference may further contribute to a lower cancer rate in Japan. Naturally occurring Iodolactones were extracted from some kinds of seaweed [25].

IODINE UPTAKE IN NON-THYROIDAL TISSUES

The thyroid gland is the most active organ, which is able to absorb and concentrate iodide ions and iodine deficiency triggers a self-induced machinery by which the thyroid cells begin to proliferate. Besides the thyroid gland, the salivary gland, gastric mucosa, mammary gland and various others non-thyroidal tissues are also capable of taking up iodide ions, although the physiological relevance is still not completely understood [26]. Iodine uptake by non-thyroidal tissues is also predominantly mediated by the NIS. RT-PCR and Southern hybridisation revealed NIS gene expression in the parotid gland, submandibular gland, pituitary gland, pancreas, testis, mammary gland, gastric mucosa, prostate, ovary, adrenal gland, heart, thymus, and lung [27]. NIS expression has been further demonstrated in more than 80 % of breast cancer tissues. Recently, NIS activity was discovered *in vivo* in breast cancer metastases [28]. Functional analysis of the NIS carried out in breast cancers, revealed no correlation between thyroid iodide uptake and breast cancer iodide uptake. This implies a different regulation of iodide uptake in these organs. Expression of the NIS in breast cancer cells can be induced *in vitro* by lactogenic hormones, insulin, peroxisome proliferator-activated receptor-γ (PPAR-γ) ligands, retinoids and glucocorticoids [29]. Inhibition of the MAPK/ERK kinase decreases all trans-retinoic acid and hydrocortisone induced endogenous NIS expression level, as well as exogenous NIS expression level in MCF-7 human breast cancer cells [30]. In addition to the NIS, others iodide transporters have been detected in mammary gland such as PDS and sulfate/iodide exchanger which is inhibited by 4,40-diisothiocyanatostilbene 2,20-disulfonic acid [31,32]. For molecular iodine there appear to exist alternative uptake proteins in mammary cancer cells. Aceves showed that iodine uptake in MCF-7 breast cancer cells was independent of NIS, PDS, Na^+ and energy, but was saturable and dependent on protein synthesis suggesting a facilitated diffusion [5,33]. Additional functional NIS expression was found in a subset of murine colorectal tumors [34].

ROLE OF IODINE IN CANCER PREVENTION *IN VIVO*

Recent investigations suggested that there might also be a correlation between iodine deficiency and proliferative disease of the mammary gland. Furthermore, iodine deficiency could be associated with a higher risk for the development of breast cancer and gastric cancer. Women with breast cancer show a significant decrease in iodine excretion. Therefore, it was supposed that iodine might play a role in breast cell differentiation and integrity. The same applies for patients with gastric cancer. Comparison of the iodine content in gastric cancer and surrounding normal tissue led to the detection of a lower iodine level in gastric cancer tissues. Hence, it was postulated that iodine prophylaxis could be a protective fac-

tor against the development of gastric cancer in iodine deficient areas [35,36].

INDUCED IODINE DEFICIENCY AND SUPRAPHYSIOLOGICAL DOSES OF IODINE

Since the antiproliferative properties of iodine have been known for over a century, it was understandable to test iodine in cancer therapy as well. As early as 1928, the first experiments were conducted and iodine was applied in malignant disease. Animal tests were performed with organic bound iodine using carcinoma and sarcoma transplants. The results revealed that iodine efficiently induced tumor growth inhibition, and it brought about tumor regression [37].

The effects of iodine deficiency and the potential benefit of iodine therapy have been intensely examined in the mammary gland. Extensive animal experiments in which iodine deficiency was induced, resulted in breast tissue hyperplasia and dysplasia. Molecular iodine prevented N-methyl-N-nitrosourea (MNU) and DMBA induced breast cancer tumors in animals [4,5,38,39]. Therefore, iodine is considered as an important element for normal breast development and as a prophylactic agent against proliferative mastopathia [40]. Experimental application of supraphysiological doses of molecular iodine in humans successfully reduced fibrocystic breast disease and cyclic mastalgia [41]. A plethora of published data to date suggests that iodine deficiency results not only in goiter formation but also in breast tissue hyperplasia, perilobular and ductal fibrosis. In conclusion, iodine constitutes an important element for the health of the mammary gland.

Molecular iodine is widely used in the form of polyvidon-iodine as a disinfectant in surgery. Cancer surgery runs the risk for transfering tumor cells into surrounding healthy tissue. In search of appropriate anticancer substances, systematic analyses were performed, resulting in confirmation that molecular iodine preparation such as polyvidon-iodine is an excellent cytotoxic and anticancer agent in breast cancer and in colorectal cancer surgery. The prevention of recurrent colorectal cancer caused by tumor cell seeding and the reduction of intraperitoneal tumor implantation and port-site metastases after laparoscopic interventions were effectively assisted by molecular iodine without causing organ toxicity [42,43].

ANTIPROLIFERATIVE ACTIVITY OF IODINE COMPOUNDS IN CELL CULTURES

There is only sparse data existing on experiments examining molecular iodine and tumors other than breast cancer and sometimes the results were obtained by chance. One ingenious experiment with non-radioactive iodide demonstrated that in genetically modified non-small cell lung cancer cells, apoptosis could be effectively induced. To enhance tumor apoptosis, the non-small lung cancer cells were transduced with retroviral vectors containing a NIS, that along with thyroid peroxidase, oxidizes iodide ions into molecular iodine. These genetically modified cells generated molecular iodine which induced apoptosis. N-acetylcysteine (NAC), a thiol-containing chemical, inhibited the iodide-induced apoptosis. Finally, it was found that iodinated contrast media induced neutrophil apoptosis through a mitochondrial and caspase-mediated pathway [44,45].

To analyze the antiproliferative activity of molecular iodine and δ-iodolactone in a variety of cancer cells including different breast cancer cells, we systematically experimented with twelve different cancer cell lines. For these studies δ-iodolactone was synthesized as described by Boeynaems JM and Hubbard WC [46]. The following cell lines were included: SH-SY5Y (neuroblastoma), MCF-7, MDA-MB-134, MDA-MB-157, MDA-MB-436 (all mammary carcinoma), MCF-10 (mammary epithelial cells), A549, HS24 (both lung carcinoma), SNB19 (glioblastoma), U87M6 (glioblastoma), IPC298 (melanoma), Capan II (pancreas carcinoma), PATU 8902 (pancreas carcinoma), and CCL221 (colon carcinoma). Excepting A549 (lung carcinoma) and the CCL221 (colon carcinoma), we found an inhibition of cell proliferation in all cell lines upon culturing in molecular iodine for two days. The degree of inhibition was, however, highly varied. Among the four mammary carcinoma cell lines tested, MCF-7 cells turned out to be most susceptible, followed by MDA-MB-436 cells [47]. These findings were in line with published data demonstrating a strong antiproliferative activity of molecular iodine in MCF-7 cell cultures [33,40,48].

Although systematic analysis was performed for the first time to our knowledge, these striking results led us to presume that various non-mammary human cancer cell lines could be strongly affected by molecular iodine treatment, too. For example, the inhibition rate of the neuroblastoma cells (SH-SY5Y) was 100 % and these cells proved to be even more susceptible than MCF-7 cells, followed by Capan II (pancreas carcinoma), IPC298 (melanoma), HS24 (lung carcinoma), SNB19 (glioblastoma), and PATU 8902 (pancreas carcinoma). Proliferation of the latter was inhibited by molecular iodine up to 56 % in a dose- and time-dependent manner. We also found a similar antiproliferatve effect in cells that were cultured in the presence of δ–iodolactone for two days. This compound completely inhibited the growth of SH-SY5Y cells and that of MCF-7 cells by 77.7 % [47].

Since δ–iodolactone is synthesized by arachidonic acid derived from cell membranes and molecular iodine, the study results raised the question whether the effects of molecular iodine were secondary to an intracellular generation of δ–iodolactone [33,46]. The possibility that molecular iodine effects may be mediated due to δ–iodolactone formation after being internalized were further supported by findings of Arroyo-Helguera *et al*. [48,49]. In goiter formation, δ–iodolactone inhibits EGF-induced proliferation. Our data obtained from MCF-7 and SH-SY5Y cells indicated that both molecular iodine and likewise δ–iodolactone completely abolished EGF-induced promotion of cell proliferation. These results were in accordance with previous findings showing that δ-iodolactones decreased EGF-induced *in vitro* proliferation of thyroid follicular cells [50]. We further questioned whether iodo-compounds might interfere with EGF-signaling pathways. The experiments performed with SH-SY5Y neuroblastoma cells revealed that neither activation (phosphorylation) of EGF-receptors nor activation of MAPK I ($erk_{1,2}$) was influenced by molecular iodine. Furthermore, we found that an EGF-induced activation of actin-

driven lamellar protrusions was not impaired by molecular iodine. These data indicate that in SH-SY5Y cells, molecular iodine and δ–iodolactone did not interfere with EGF-signaling. In contrast to our results, a recently published paper showed that molecular iodine induced apoptosis in thyroid cancer cells by activation of a MAPKs-related pathway involving the mitochondria [51].

MOLECULAR IODINE AND ITS ROLE IN INDUCING MITOCHONDRIA-MEDIATED APOPTOSIS

In older experiments it was shown that molecular iodine has a marked effect upon the structure and function of mitochondria. Positively charged molecular iodine, e.g. iodine chloride, caused a rapid swelling of the mitochondria and was accompanied by fatty acid release. The explored mechanism showed that molecular iodine as a strong oxidizer inhibits ATPase activity, enormously increases oxygen consumption, uncouples the process of respiration and oxidative phosphorylation, causes a physico-chemical change of the phospholipid membrane by reacting with the sulfhydryl groups, lowering the electric resistance of the mitochondrial membrane and increases the permeability. Furthermore, activity of the mitochondrial malat dehydrogenase was strongly inhibited. This enzyme is essential for cell energy metabolism. It interacts as a metabolic substrate mediator between mitochondria and cytosol. Adding ATP reversed mitochondrial swelling [52-56].

The maintenance of the mitochondrial transmembran potential (MMP) is required for a variety of mitochondrial functions including protein import, ATP production and regulation of metabolite transport. The space between the inner and outer mitochondrial membrane hosts biologically active proteins such as cytochrome c, which plays a key role in caspase 9 activation, and apoptosis-inducing factor (AIF), which causes nuclear degradation, independent of caspase activation. By appropriate stimuli these proteins can be released into the cytosol. In order to release these factors there is the need for mitochondrial outer membrane permeabilization, an event that is considered to be the 'point of no return' during apoptosis. Once mitochondrial outer membrane permeabilization has occurred, the cytosolic machinery responds by activating caspases. If this pathway is inhibited, a caspase-independent cell death process ensures cellular demise by processes which utilize among others ROS. In both situations, mitochondrial outer membrane permeabilization occurs, disrupting mitochondrial function, dissipating the MMP and energy production leaving the cell to die [57].

Regarding our data obtained with MCF-7 and SH-SY5Y cells, the antiproliferative effect of molecular iodine resulting in a several-fold increase of the apoptotic rate was preceded by a disruption of the MMP. These results were consistent with detailed published findings demonstrating iodine-induced caspase-independent and mitochondria-mediated apoptosis of MCF-7 cells involving depletion of the cellular thiol content and dissipation of MMP [58]. In agreement with this study, our observation confirmed that a pre-incubation of the cells in the presence of NAC, a thiol containing agent, prevented the induction of MMP loss by molecular iodine. A case can be made that thiol depletion was the critical event in mitochondria-mediated apoptosis [59,60]. Taking into consideration that in some cell types the disruption of the MMP by molecular iodine was transitory, and proliferation was only diminished without ensuing cell death, this could explain why the effects of iodine were found to be very different in these cell lines tested.

In organic chemistry molecular iodine is used as a highly reactive agent for oxidative coupling of thiols into disulfides [61]. The most abundant intracellular thiol is glutathione (GSH). GSH is synthesized in the cytosol and transferred into the mitochondria. GSH is a component of the cytoplasm and mitochondrial reduction-oxidation (redox) system which can easily remove ROS by generating reduced GSH. Since reduced GSH is cytotoxic, it is rapidly converted back to GSH by the enzyme GSH reductase. As a consequence, the ratio of oxidized GSH to reduced GSH is tightly held at about 100:1 [60]. In a series of *in vitro* experiments it was shown that altering the intracellular redox potential by changing the GSH concentration induced apoptosis by different redox dependent signal transduction [62-64].

A further important thiol containing redox system, whereby molecular iodine can also interact, is thioredoxin (Trx). In mammalian cells there are two isoforms: cytosolic (Trx 1) and mitochondrial (Trx 2) thioredoxin. Mitochondrial thioredoxin has proven to be a special regulator of the mitochondrial permeability transition, a required condition for inducing the dissipation of the MMP, it also acts as an inhibitor of the mitochondria-located ASK1-mediated apoptosis [65-67].

Judging current status, it is most likely that the antiproliferative effect of molecular iodine is mediated by different mechanisms and pathways involving mitochondria-mediated apoptosis and/or by interaction with the estrogen metabolism or with the PPAR. Thus, Aceves *et al*. provided evidence that the apoptotic effect of molecular iodine not only involves an increase of Bcl-2–associated X protein (BAX) and a nuclear translocation of AIF but also a PARP 1 cleavage and an activation of a caspase-7 subunit [68,69].

In respect to a potential therapeutic strategy, it is of interest to note, that normal mammary epithelial cells (MCF-10) turned out to be much less susceptible to iodine-treatment than MCF-7 cells. Keeping in mind that molecular iodine induced total depletion of cellular thiol content, perhaps a key step in mitochondria-mediated apoptosis of MCF-7 cells, it seems that this mechanism does not work in the same way in normal breast cells. This disparity might reflect differences in thiol metabolism and function between non-malignant cells and cancer cells. Indeed, a different susceptibility of MCF-10 and MCF-7 cells to thiol antioxidant-induced G 1–delay and differences in peroxiredoxin mediated resistence to H_2O_2 have been reported [70,71]. Therefore, we propose that a variable susceptibility of MCF-7 and MCF-10 to molecular iodine was related to different mitochondrial properties. This view is supported by studies with isolated mitochondria demonstrating a different action of iodine on mitochondria from human tumoral- and extra-tumoral tissue [72].

In the course of normal metabolism and especially in cancer patients living with a carcinoma-induced exaggerated

energy consumption, oxidizing equivalents or ROS are generated when oxygen is partially reduced as electrons leak out of the electron transport chain during respiration in the mitochondria. These 'activated' oxygen molecules can readily react with organic substances by non-catalytic means. The redox state of the cell is a consequence of the balance between the levels of oxidizing (ROS) and reducing equivalents. Elevation of ROS in excess of the buffering capacity results in potentially cytotoxic 'oxidative stress'. Under these pro-oxidant conditions, highly reactive radicals can damage DNA, RNA, proteins, and lipid components, which may lead to cell death. To counteract the effects of oxidative stress, cells have developed two important defence mechanisms: a thiol reducing buffer consisting of small proteins with redox-active reducing sulfhydryl moieties, e.g. GSH and thioredoxin (TRX) and enzymatic systems, e.g. superoxide dismutase, catalase, and GSH peroxidase [73-75].

Exaggerated tumor metabolism causes intracellular excess of ROS. To defend themselves against inducing a self-killing program, carcinoma cells overexpress higher levels of GSH and TRX and related enzyme systems [73,76-78]. As previously mentioned, cell experiments with non-malignant human breast epithelial cells and breast cancer cells provided evidence of different susceptibility to thiol antioxidant induced G1-delay [70]. Bringing an intracellular imbalance by reducing the thiol content of these cells may therefore result in the induction of apoptosis [79,80].

Another described pathway by which an imbalance of the intracellular redox state could act to induce mitochondrial mediated apoptosis involves the TNFα pathway, which is activated by ligand binding to the TNFα receptor. TNFα inhibits respiration and increases the mitochondrial production of ROS. Several cytosolic proteins can be recruited to the TNFα receptor death domain, which in turn leads to the production of a variety of second messengers. As a result, multiple protein-mediated signaling cascades are activated, including caspase-dependent and caspase-independent pathways, phospholipases, which can release arachidonic acid out of the cell membrane, protein kinases, and protein phosphatases. TNFα receptor engagement causes caspase-dependent cleavage of cytosolic phospholipase A2 generating arachidonic acid which in turn can activate the ceramide pathway by stimulating neutral sphingomyelinase, thereby creating a feed-forward mechanism in the propagation of the apoptotic signal by TNFα. Arachidonic acid is also a potent inducer of mitochondrial swelling, a molecular sign for affecting mitochondrial function [81].

ANTIPROLIFERATIVE EFFECTS OF IODOLACTONES

Our results have further given evidence of potential benefits0 of molecular iodine and iodolactones for treating breast cancer as well as other human cancers such as neuroblastomas, melanomas, lung- and pancreas carcinomas. The need to extend treatment with molecular iodine or δ–iodolactone to other carcinomas is further supported by a newly published experiment demonstrating that δ-iodolactone is effective in apoptosis induction in thyroid cancer cells. Interestingly, pre-incubation of the human thyroid follicles with selenium which induces the glutathion peroxidase, lowered the apoptosis rate. These findings also suggest that free oxygen radicals are involved in the induction of apoptosis [82]. Furthermore, it was found that another iodolacton was extremely effective against the pancreatic adenocarcinoma BXPC-3 cell line [83].

POTENTIAL ANTICANCER THERAPY WITH MOLECULAR IODINE

So far, molecular iodine has only been tested in rats. To elucidate potential adverse effects for humans, an exposition of rats to potable water, which was disinfected with molecular iodine, led to a very different thyroid hormone metabolism. Feeding rats with molecular iodine resulted in an increased serum thyroxine level, since rats are capable of recycling thyroid metabolites in the intestinal tract and to reabsorb this extrathyroidal synthesized thyroxine. In humans, who consumed iodinated water, an increase of the TSH serum level was proven. This indicates a suppressive effect of excess iodine intake (Wolff-Chaikoff effect) [84-88].

Molecular iodine and iodate are highly reactive oxidized forms of iodide. Orally applied molecular iodine must overcome multiple physiological redox systems, which protect the body against oxidative damage. The intestinal tract is lined by extracellular GSH peroxidase and intestinal epithelial cells of the small intestine contain sufficient GSH to reduce molecular iodine [89-91]. Even, if molecular iodine enters the circulation in oxidized form, it is probable that it is rapidly reduced by GSH of red blood cells, which are able to take up iodine [92,93]. Although it has only been tested with iodate and not with molecular iodine, it was shown that intravenously given iodate is immediately reduced to iodide by GSH in rabbits [94]. Since molecular iodine administered orally is presumably reduced to iodide, a parenteral application mode should be considered. The oxidative antimicrobial properties of molecular iodine are used extensively, including preparations containing pathogen-free red blood cells. The pretreatment of red blood cell solutions with iodide, followed by molecular iodine generate a germ-free compound [95,96]. Apparentely, a treatment that combines iodide and molecular iodine, leads to a solution in which the red blood cells do not reduce the total molecular iodine content. Transferred to a possible anticancer therapy, a combination of iodide/iodine should be applied intravenously, beginning with iodide and adding molecular iodine in a coutinuous manner for several days. There is extensive experience with high doses of iodide used in Graves' disease for the pretreatment of the hyperactive thyroid gland, before and during thyrectomy was performed [97,98].

Alternatively, it should be investigated, how molecular iodine can be transferred to the tumor with iodophors using povidon, povidon iodine or nanoparticles, or how molecular iodine can be generated in situ [99-106]. In this context, all subsequent animal experiments should not be carried out with rats.

OUTLOOK

To date it is not clear whether the different pathways by which molecular iodine exerts effects, leading to apoptosis induction, are really different or only different steps of one associated reaction. Nonetheless, there is enough evidence

demonstrating that molecular iodine and iodolactones could be to helpful as potent anticancer agent. Still now, there are no serious efforts to implement molecular iodine or δ-iodolactone in pharmacological anticancer strategies. The most pertinent data on the antiproliferative effects of molecular iodine and δ-iodolactone concerns breast cancer, the most frequent cancer in women in the western world. Considerations must be made about the use of molecular iodine in other frequently more aggressive tumors like pancreatic cancers.

The prognosis of breast cancer, still a matter of fear and stigma in women, has successfully been improved, although there remains much to do. In England, one year breast cancer survival rate has been increased from 82 % for women diagnosed in 1971-1975 to 96 % for women in 2004-2006 [107-109]. For women diagnosed with breast cancer in 2001-2006, the five-year relative survival rate has reached 82 % compared with only 52 % thirty years earlier in 1971-75 [109,110]. Nevertheless, in cases where this form of cancer has metastasised, the disease is still considered incurable.

Pancreatic cancer has a very poor prognosis and is considered largely incurable. According to the American Cancer Society, for all stages of pancreatic cancer combined, the one-year relative survival rate is 20 %, and the five-year rate is 4 %. These low survival rates are attributable to the fact that fewer than 10 % of patients' tumors are confined to the pancreas at the time of diagnosis; in most cases, the malignancy has already progressed to the point where surgical removal is impossible. In those cases where resection can be performed, the average survival rate is 18 to 20 months. The overall five-year survival rate is about 10 %, although this can rise as high as 20 % to 25 % if the tumor is removed completely and if the cancer has not spread to lymph nodes. Keeping in mind the above mentioned effects of molecular iodine and δ-iodolactone against pancreatic cancer, those patients could benefit first by an intensive pharmacological concept with molecular iodine or δ-iodolactone [111].

ABBREVIATIONS

AIF = Apoptosis-inducing factor

ASK 1 = Apoptosis signal-regulating kinase 1

BAX = Bcl-2–associated X protein

bFGF = Basic fibroblast growth factor

DMBA = 7,12-dimethylbenz[a]anthracene

DMAB = 3,2' dimethyl-4-aminobiphenyl

Duox 1 and 2 = Oxidase 1 and 2

EGF = Epidermal growth factor

EPO = Eosinophil peroxidase

GSH = Glutathione

H_2O_2 = Hydrogen peroxide

IGF-1 = Insulin-like growth factor I

MAPKs = Mitogen-activated protein kinases

MAPK/ERK Kinase = Mitogen-activated protein kinase/extracellular signal-regulated kinase

MMP = Mitochondrial transmembran potential

MNU = N-methyl-N-nitrosourea

MPO = Myeloperoxidase

NAC = N-acetylcysteine

NIS = Sodium-iodide symporter

PDS = Pendrin

PPAR-γ = Peroxisome proliferator-activated receptor-γ

Redox = Reduction-oxidation

ROS = Reactive oxygen species

RT-PCR = Reverse transcription-polymerase chain reaction

TGF-β = Transforming growth factor

TPO = Thyroid peroxidase

Trx = Thioredoxin

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